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The spatial arrangement of sexes is related to reproductive allocation in mosses: a comparative study of reproductive allocation in three different monoicous sexual systems

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• **Background and Aims** We examined the relationship between reproductive allocation and vegetative growth in three monoicous sexual systems of bryophytes. The sexual systems show a gradient of increasing distance between the sexes, from gonioautoicous to cladautoicous to rhizautoicous. Here, we investigated the following two hypotheses: (1) reproductive allocation differs between sexes and sexual systems, and male reproductive allocation increases with increasing distance between male and female gametangia; and (2) reproductive allocation is negatively related to vegetative growth.

• **Methods** We sampled the three sexual systems, represented by three moss species of the genus *Fissidens* in the Atlantic Forest of Southeastern Brazil. Ramets were washed in the laboratory; the reproductive structures were detached from the vegetative ramets and sorted regarding sex and individual, dried at 70 °C for 72 h, and weighed in an ultramicrobalance. We calculated the mean reproductive and vegetative mass and reproductive allocation and used generalized linear models to test our predictions.

• **Key Results** Reproductive allocation differed between species and sexes. It was higher in the rhizautoicous than in the cladautoicous and gonioautoicous species. Mean reproductive allocation was greater in males than in females of the rhizautoicous species, greater in females than males of the cladautoicous species, and did not differ between the sexes in the gonioautoicous species. Estimates of reproductive and vegetative mass were positively related in females of the rhizautoicous species. Vegetative mass was not related to reproductive allocation in the gonioautoicous species, but negatively related to reproductive allocation in the male and female branchlets of the cladautoicous species and in the female ramets of the rhizautoicous species.

• **Conclusions** The reproductive allocation patterns differ between the rhizautoicous species and the 'truly' monoicous species, with shorter intersexual distances, which implies that our hypotheses were supported only in part. We suggest that the hypotheses should be reformulated and tested further by comparing 'truly' monoicous species with dioicous species and by including other genera.

Key words: Cladautoicous, *Fissidens pseudoplurisetus*, *Fissidens scariosus*, *Fissidens weirii*, gonioautoicous, monoicy, reproductive allocation, reproductive mass, reproductive investment, reproductive trade-off, rhizautoicous, sexual systems.

INTRODUCTION

Life-history theory predicts that the three essential functions of life, namely reproduction, growth and defence, compete for resources (Delph, 1999; Oli and Coulson, 2016). Thus, understanding the patterns of resource allocation to different functions in organisms is crucial to understanding their life-history traits (Charlesworth and Morgan, 1991; Söderström and Gunnarsson, 2003). Reproductive allocation (RA) is commonly quantified as the proportion of the total dry mass allocated to reproductive structures (Ashman, 1994; Karlsson and Méndez, 2005; Suzuki, 2005). Following the 'principle of allocation', which deals with the allocation of resources that are available in limited amounts, allocation to one function should have negative consequences for other functions (Karlsson and Méndez, 2005). This is commonly referred to as 'trade-offs', which eventually influence the performance of populations (Charlesworth and Morgan, 1991). Plant allocation patterns have a genetic component and are influenced by plant traits (e.g. size) and environmental factors, such as competition, environmental stress or stand density (Rice *et al.*, 2001; Karlsson and Méndez, 2005; Friedman *et al.*, 2020).

Studies of RA in plants have a long history and have been focused mainly on angiosperms (Darwin, 1877; Fox, 1993; Chiang and Lin, 2001; Suzuki, 2005; Hautier *et al.*, 2009; Zhang *et al.*, 2021). In bryophytes, RA has attracted attention only in recent years. The diversity of sexual systems exhibited by bryophytes (Wyatt, 1985) coupled with their relatively simple structure, largely lacking storage organs, makes these plants suitable organisms in which to understand the trade-offs

© The Author(s) 2023. Published by Oxford University Press on behalf of the Annals of Botany Company. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com. involving RA in plants (Convey and Smith, 1993; Convey, 1994*a*, *b*; González-Mancebo and During, 1997; Ehrlén *et al.*, 2000; Stark *et al.*, 2000; Laaka-Lindberg, 2001; Bisang and Ehrlén, 2002; Horsley *et al.*, 2011; Stark and Brinda, 2013; Santos *et al.*, 2018, 2022*a*, *b*).

Unlike seed plants, the bryophyte life cycle is dominated by the haploid phase, and the bryophyte reproductive structures are formed on the gametophyte (Parihar, 1963). Thus, a distinction is generally made between sex expression in the diploid sporophyte phase of angiosperms (monoecious, dioecious) and sex expression in the haploid gametophyte phase of bryophytes (monoicous, dioicous) (Glime and Bisang, 2017). More than half of all bryophyte species in the world are dioicous [i.e. they carry male (antheridia) and female (archegonia) sexual structures on separate individuals], and the rest are monoicous, with male and female sexual structures on the same individual (Fig. 1; and see below in the subsection 'Studied species, reproductive structures and sample collection') (Parihar, 1963; Wyatt, 1982; Bisang and Hedenäs, 2005). The diploid sporophyte develops on the female gametophyte from a zygote after fertilization (Fig. 1). Fertilization can fail owing to lack of water, spatial distance between the male and female reproductive organs, or unsuitable environmental conditions (e.g. Bisang et al., 2004; Haig, 2016; Hedenäs and Bisang, 2019). This implies that RA in female bryophytes occurs both before (archegonia and related structures) and after (sporophyte) fertilization and might be restricted to the former if fertilization fails. In male bryophytes, however, RA is always restricted to the pre-fertilization (prezygotic) stage. Different spatial positions of male and female gametangia are found in the monoicous sexual system, with increasing distance between sexual organs from the synoicous to the rhizautoicous arrangement (Fig. 1). The few studies conducted to date on RA in monoicous bryophytes have investigated species with a rhizautoicous sexual system (Stark and Brinda, 2013; Santos et al., 2018, 2022b).

Sexual systems affect mating success in plants in many ways (Longton and Schuster, 1983; Bergh and Verboom, 2011; Stark and Brinda, 2013; Santos *et al.*, 2020). For instance, according to Longton and Schuster (1983), outcrossing relative to self-fertilization is expected to increase as the distance between antheridia and archegonia increases in self-compatible

monoicous bryophyte species (i.e. from the synoicous to the rhizautoicous mating system). In flowering plants, Bergh and Verboom (2011) suggested that the prezygotic allocation to female function in hermaphroditic species should increase proportionally to the degree of self-fertilization. Given that higher selfing rates in bryophyte species with short intersexual distances seem plausible, Stark and Brinda (2013) applied the notion of hermaphrodite flowering plants of Bergh and Verboom (2011) to bryophytes, namely that prezygotic RA to the male relative to the female function should increase with decreasing selfing (i.e. along the axis from synoicy to dioicy). Stark and Brinda (2013) found a higher RA per culture surface area and per shoot in males than in females in the rhizautoicous Aloina bifrons (De Not.) Delgad., yet they reported that their postulation was inconsistent with the allocation pattern observed in three other rhizautoicous species. Later, Santos et al. (2018) found that male RA exceeded female RA on a shoot basis in the rhizautoicous Fissidens flaccidus Mitt.

In this study, we test the prediction postulated by Stark and Brinda (2013) in three Fissidens species. This is a suitable genus in which to investigate the theme because it comprises species with all sexual systems observed in bryophytes, thus encompassing the whole range of intersexual distances (Fig. 1). We compare RA in Fissidens scariosus Mitt. (rhizautoicous), Fissidens pseudoplurisetus Bordin, Pursell & O. Yano (cladautoicous) and Fissidens weirii Mitt. (gonioautoicous) and investigate the possible relationships between RA and vegetative growth. We test the following hypotheses: (1) prezygiotic RA differs between sexes and sexual systems, and male RA increases with increasing distance between male and female gametangia (Fig. 2); and (2) the investment in reproductive structures, measured as absolute values and proportional values relative to vegetative mass, is negatively related to vegetative growth (Fig. 2).

MATERIALS AND METHODS

Study area

We collected samples at Parque das Neblinas in the limits of Bertioga and Mogi das Cruzes municipally in the state of São



FIG. 1. Schematic drawing of the sexual systems of bryophytes. Definitions: cladautoicy, perigonia and perichaetia on separate branches but both branches connected to one main branch; dioicy, male and female gametoecia on different plants; gonioautoicy, terminal perichaetia and axillary perigonia in the same ramet; paroicy, antheridia adjacent to archegonia in the same gametoecia; rhizautoicy, male ramet (with perigonia) attached to the female ramet (with perichaetia) by rhizoids; and synoicy, antheridia and archegonia mixed in the same gametoecia. Definitions are adapted from Wyatt (1985) and Stark and Brinda (2013).



FIG. 2. Schematic diagram of the two hypotheses addressed in this study: (1) reproductive allocation differs between sexes and sexual systems and male reproductive allocation increases with increasing distance between male and female gametangia; and (2) allocation to reproductive structures is negatively related to vegetative growth. Abbreviations: Clad, cladautoicous; Gon, gonioautoicous; Rhi, rhizautoicous.

Paulo (23°44′04.6″S, 46°10′53.0″W). With an area of 7000 ha of dense rainforest, the park is part of the largest continuum of Brazilian Atlantic Forest in the Serra do Mar State Park (Onofre *et al.*, 2010). The Bertioga region has a 'Tropical rainforest climate', according to the Köppen classification, with an average rainfall of \geq 60 mm every month (Malhi and Wright, 2004). The annual average temperature is ~24 °C, and the average rainfall amounts to 3207 mm (Alvares *et al.*, 2013).

Studied species, reproductive structures and sample collection

We chose the genus *Fissidens* (Fissidentaceae, Bryophyta) because it presents a great diversity of sexual systems. Three species from the same subgenus (Aloma) were selected in order to reduce the potential effect of phylogenetic distance (Pursell, 2007; Bordin and Yano, 2013). The rhizautoicous Fissidens scariosus Mitt. (Figs 1 and 3A) is widely distributed in Brazil and occurs in the most diverse phytogeographical domains, such as the Amazon, Cerrado and Atlantic Forest, but predominantly in the last of these (Pursell, 2007; Bordin and Yano, 2013). We sampled Fissidens pseudoplurisetus Bordin, Pursell & O. Yano (Figs 1 and 3B), described as rhizautoicous or gonioautoicous by Bordin et al. (2011) but observed only as cladautoicous in our samples. In our collections, the rhizautoicous condition pointed out by Bordin et al. (2011) was not confirmed. Fissidens pseudoplurisetus has a very restricted distribution and occurs in mixed ombrophilous forests in South and Southeast Brazil. The gonioautoicous Fissidens weirii Mitt. (Figs 1 and 3C) is distributed in the Americas and Africa. In Brazil, it occurs in the Atlantic Forest of the South and Southeast regions (Pursell, 2007).

In bryophytes, sexual organs are formed on the gametophyte, and their position is used to define different mating systems (Haig, 2016; Fig. 3). Each antheridium produces numerous spermatozoids (male gametes), and each archegonium produces one egg cell (female gamete). Antheridia and archegonia are collectively termed gametangia. In mosses, the gametangia and the surrounding specialized photosynthetic leaves form reduced sexual branches called perichaetia in females and perigonia in males (collectively termed gametoecia; Fig. 3C). They are prezygotic reproductive structures. Here, we refer to male or female ramets (and male or female branchlets) when the gametophyte portion carries, respectively, male or female sexual structures (Fig. 3). The motile spermatozoid fertilizes the sessile egg cell in the perichaetium. The sporophyte develops on the maternal ramet after fertilization (postzygotic) and remains attached to it during its lifetime.

Phenology of tropical bryophyte species is largely governed by precipitation, with high rates of sexual reproduction during the rainy period (Maciel-Silva and Válio, 2011; Glime and Bisang, 2017). Thus, we sampled all species during the rainy season in February 2021 from the same study area (7000 ha), which is covered by dense rainforest vegetation. For all three species, we collected colonies with ramets in the same phenophase (Greene, 1960). We therefore believe that our sampling design allows for interspecific comparison. We placed the samples in paper bags and noted the geographical location. Species identification took place in the laboratory, using specialized literature (Pursell, 2007; Bordin *et al.*, 2011; Bordin and Yano, 2013).

Reproductive allocation and association with vegetative mass

After identification, we cleaned and separated the ramets and classified them according to their sexual expression using a stereomicroscope and microscope. We selected 30 male ramets (with perigonia) and 30 non-sporophytic female ramets (with unfertilized perichaetia) of *F. scariosus* (rhizautoicous) and 30 ramets that bore both perigonia and perichaetia but no sporophytes of each of the other two species, *F. pseudoplurisetus* (cladautoicous) and *F. weirii*



FIG. 3. Species studied, with their respective sexual systems. (A) *Fissidens scariosus* (rhizautoicous): on the left, male ramet attached by rhizoids to the female ramet on the right. (B) *Fissidens pseudoplurisetus* (cladautoicous): male and female branchlets on a common main ramet. (C) *Fissidens weirii* (gonioautoicous): ramet with perichaetia, with juvenile sporophyte in the apex and perigonia in the leaf axils.

(gonioautoicous). We picked gametangia to represent the phenophase 'immature', implying gametangia of half to full length, green and with intact cap cells (Greene, 1960). We separated the perigonia and perichaetia from each ramet using fine-tipped forceps and a needle. In cladautoicous species, the perigonia and perichaetia sit on separate small branchlets of the main ramet (Fig. 3B). We quantified the vegetative mass of these branchlets, and not the vegetative mass of the main ramet, with respect to sex. For the vegetative parts of the ramet, we followed the methodology proposed by Bisang and Ehrlén (2002) and removed the basal brownish parts from the green photosynthetically active parts. We placed the vegetative parts of each ramet and the reproductive structures pooled separately according to each sex in small envelopes, wrapped them in aluminium foil and dried them for 72 h at 70 °C. The perichaetia and perigonia of each ramet were placed into a single small envelope, resulting in 30 envelopes of each sex per sexual system and a total of 180 envelopes. The vegetative part of each ramet was also placed into a separate envelope, resulting in 30, 60 and 60 envelopes of the gonioautoicous, cladautoicous and rhizautoicous species, respectively, and a total of 150 envelopes.

After drying, the content of each envelope with perigonia and perichaetia, the vegetative parts of the ramets of the gonioautoicous and rhizautoicous species, and the vegetative parts of the male and female branchlets of the cladautoicous species were weighed separately using an ultramicrobalance (SE2 ultramicrobalance; Sartorius, Goettingen, Germany; precision of 0.1 μ g). For the gonioautoicous species, the perigonia found in the same ramet were weighed together.

Statistical analyses

Reproductive allocation (the proportion of resources allocated to reproduction; as a percentage) was calculated for each ramet separately based on the dry mass of its vegetative structures (VB; in milligrams) and reproductive structures (RB, in milligrams) as follows: RA = RB/(RB + VB) (McLetchie and Puterbaugh, 2000). We calculated the mean and s.d. of vegetative mass, reproductive mass and RA at ramet level for each species and sex. We compared the vegetative and reproductive mass among species and sexes by modelling each of them as the response variable of two separate general linear models (GLMs) with a Gaussian distribution and identity link function. We used species and sex as predictor variables except in the gonioautoicous species, in which it is not possible to model vegetative mass as a function of sex because both perigonia and perichaetia are produced on the same ramet; hence, in this case, species was the only predictor variable (Fig. 3). We applied F-tests to compare each model with a null model. We then applied F-tests to each full model separately to test whether the response variables varied between sexes. Error distributions were analysed, and neither of the two models showed underdispersion or overdispersion. We used Tukey's post hoc tests to check the differences between the three sexual systems.

We tested the first hypothesis using a GLM with a binomial distribution and logit link function. Initially, we created a full model, with RA as the response variable and with sex, sexual systems and their interactions as predictor variables. We compared the full model with a null model without a predictor, using a χ^2 test. The difference was significant, and thus we rejected the null model. We also removed the non-significant interactions from the model. A subsequent residual analysis of the error distribution revealed subdispersion, and thus we used a GLM with quasibinomial distribution to adjust the error distribution. We applied *F*-tests to check the model parameters and Tukey's post hoc comparisons between sexual systems.

We tested the second hypothesis with separate GLMs with a Gaussian distribution and identity link function to infer the effects of reproductive mass and sex on vegetative mass and the effects of RA and sex on vegetative mass. These effects were investigated in each sexual system separately. As explained above, we ran the models for the gonioautoicous species without sex, and thus without the interaction term as a predictor. In the models for the cladautoicous and rhizautoicous species, we removed non-significant interactions from the models. In the model testing the relationship between reproductive and vegetative mass in the rhizautoicous species, we encountered Simpson's paradox (Dunn and Smyth, 2018); that is, a statistical phenomenon in which a trend appears in multiple groups of data but disappears or reverses when these groups are combined. Thus, to solve this problem, models were fitted to each sex separately. For all models, we applied F-tests to compare the full and null models. Full models that were significantly different from the null models were submitted to residual analysis to evaluate the error distribution.

We used RStudio (RStudio Team, 2021) to perform the statistical analyses. The MULTICOMP package (Hothorn *et al.*, 2016) was used to make the pairwise comparisons, and the GGPLOT2 package was used to produce the graphics (Wickham *et al.*, 2016).

RESULTS

The vegetative mass differed among species with different sexual systems (d.f. = 4, F = 53.46, P < 0.001; Table 1). The mean vegetative mass of the gonioautoicous species was greater than that of the cladautoicous and rhizautoicous species in males and females (Fig. 4A). Mean vegetative mass between the cladautoicous and rhizautoicous species differed only in males, being greater in the rhizautoicous species. Intraspecifically, female and male vegetative mass in the cladautoicous species dif not differ from each other, but females had a greater mean vegetative mass than males in the rhizautoicous species (Fig. 4A).

Overall, reproductive mass also differed among sexual systems (d.f. = 5, F = 5.59, P < 0.001). The difference was attributable mainly to lower male reproductive mass in the cladautoicous species relative to the others, whereas female reproductive mass did not differ among the three species (Table 1; Fig. 4B). Intraspecifically, the mean reproductive mass did not differ between sexes in the gonioautoicous and rhizautoicous species, whereas it was greater in females than in males in the cladautoicous species (Fig. 4B; Table 1).

Reproductive allocation differed between sexes and among sexual systems (Table 2) and showed considerable variation within species, particularly in the rhizautoicous *F. scariosus* (Fig. 5). The mean RA of *F. scariosus* was higher than that of the gonioautoicous and cladautoicous species in both sexes (Fig. 5). Intraspecifically, mean RA did not differ between

TABLE I. Mean and s.d. of vegetative and reproductive mass (in milligrams) in male and female plants of three Fissidens species with different sexual systems from Atlantic Forest in São Paulo State, Brazil: Fissidens weirii, gonioautoicous; F. pseudoplurisetus, cladautoicous; and F. scariosus, rhizautoicous; see Fig. 1 for sexual systems

Sexual system	Sex	Vegetative mass	Reproductive mass
Gonioautoicous	Female	0.102 ± 0.015^{a}	$0.0081 \pm 0.006^{a,b}$
	Male		0.0050 ± 0.0012^{a}
Cladautoicous	Female	$0.0273 \pm 0.0358^{b,c,d}$	$0.0123 \pm 0.0169^{\text{b}}$
	Male	$0.0175 \pm 0.0111^{\text{b}}$	0.0024 ± 0.0014^{d}
Rhizautoicous	Female	$0.0466 \pm 0.0207^{\circ}$	$0.0090 \pm 0.0038^{a,b}$
	Male	0.0283 ± 0.0342^{d}	$0.0087 \pm 0.0060^{a,b}$

Different superscript letters indicate significant differences at P < 0.05 based on separate generalized linear models for vegetative and reproductive mass.

sexes in the gonioautoicous species, whereas it was higher in females than in males in the cladautoicous species, and higher in males than in females in the rhizautoicous species (Fig. 5).

Vegetative mass was related to reproductive mass, and there was an effect of sex on reproductive mass (Table 3). Female reproductive mass increased with increasing vegetative mass in the rhizautoicous species, whereas we found a 'Simpson effect' in male ramets (Supplementary data, Supplementary material 1). When analysed in separate models for each sex, a positive association between vegetative and reproductive mass was confirmed for females (parameter estimate 3.62 ± 0.76 , P < 0.001) but not for males (parameter estimate 0.55 ± 1.02 , P = 0.51) (Table 3; Fig. 6C). No association between reproductive and vegetative mass was found in the gonioautoicous and cladautoicous species (Table 3; Fig. 6A, B). Vegetative mass decreased with increasing RA in female ramets of the rhizautoicous species and in male and female branchlets of the cladautoicous species (Table 4; Fig. 6E). In the gonioautoicous species, vegetative mass was unrelated to RA (Table 4; Figs 5 and 6A, D). The results of the comparison of the full and null GLMs are presented in the Supplementary data (Supplementary material 2).

DISCUSSION

This study is the first to quantify prezygotic RA in terms of relative resources devoted to reproduction in monoicous mosses with a sexual system other than rhizautoicous. We tested the predictions that RA to the male function increases with the distance between male and female sexual structures in monoicous mosses, as proposed by Stark and Brinda (2013), and that vegetative growth is negatively related to reproductive mass and RA. Neither of the two hypotheses could be supported fully. Instead, our results revealed a difference between species with 'truly' monoicous sexual systems and species that perform as functionally dioicous species. In gonioautoicous and cladautoicous systems, the sexual structures of both sexes are carried by a common vegetative ramet. In the rhizautoicous



FIG. 4. Boxplot diagrams of reproductive and vegetative mass of *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo State, Brazil: *Fissidens weirii*, gonioautoicous; *F. pseudoplurisetus*, cladautoicous; and *F. scariosus*, rhizoautoicus. (A) See Figure 3 for depiction of vegetative ramet or ramet parts. Abbreviations: Clad, total mass of male and female branchlets without the common ramet; Gon, ramet mass excluding perigonia and perichaetium; Rhiz, total mass of female and males ramets excluding sexual branches. (B) Reproductive mass of sexual branches (perigonia, perichaetia). Different letters denote statistically significant differences based on Tukey's post hoc test. Black dots represent outliers.

system, the male and female organs sit on individual ramets that are connected by rhizoids, which can eventually break apart. The male and female ramets still form a genet, but the distance is usually considerably longer than in the other systems (Fig. 1).

Reproductive allocation in the rhizautoicous sexual system

Reproductive allocation in the rhizautoicous system was higher in males than in females, contrary to the observations TABLE 2. Effects of sex and sexual system on reproductive allocation in three Fissidens species with different sexual systems from Atlantic Forest in São Paulo state, Brazil, based on a generalized linear model with a binomial distribution and logit link function

d.f.	Deviance	<i>P</i> -value
1	0.292	<0.01
2	8.406	< 0.001
	180	
	20.034 (d.f. = 17	79)
	11.334 (d.f. = 17	76)
	d.f. 1 2	d.f. Deviance 1 0.292 2 8.406 180 20.034 (d.f. = 17) 11.334 (d.f. = 17)



FIG. 5. Boxplot diagrams of reproductive allocation in males and females in *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo State, Brazil: *Fissidens weirii*, gonioautoicous (Gon); *F. pseudoplurisetus*, cladautoicous (Clad); and *F. scariosus*, rhizautoicous (Rhiz). Different letters denote statistically significant differences based on Tukey's post hoc test. Black dots represent outliers.

in the cladautoicous species. Previous studies that quantified prezygotic RA on a shoot basis in the rhizautoicous mosses A. bifrons (Stark and Brinda, 2013) and F. flaccidus (Santos et al., 2018) also found higher RA in males than in females. However, when estimated as the ratio between the number of male and female sexual branches (antheridia and archegonia) in earlier studies, RA was found to be higher in females than males in the rhizautoicous Tortula muralis Hedw., Atrichum undulatum (Hedw.) P. Beauv and Weissia controversa Hedw. (Anderson and Lemmon, 1972; Longton and Miles, 1982). We argue that estimates in terms of proportional mass devoted to reproduction provide a more accurate picture of resource distribution in a plant. This is basically because when we calculate the proportion of resources that a plant allocates to reproduction, we are measuring the real effort that the plant makes to reproduce (Karlsson and Méndez, 2005). In this way, we can exclude the non-photosynthetic parts, as suggested by Bisang and Ehrlén (2002), because they do not contribute to the production of resources allocated to reproduction.

The rhizautoicous sexual system can be considered a functionally dioicous system because the distance between male and female ramets can be in the same order of magnitude as in mixed populations of dioicous species (Stark and Delgadillo, 2001). Although genetically identical (because they emerge from a single spore or asexual propagule), the ramets can become physiologically independent if the rhizoid connection breaks up (Stark and Delgadillo, 2001; Stark and Brinda, 2013). Such separation might reduce competition for shared resources within an individual plant, but still offers benefits in terms of the maintenance of moisture within a patch of close neighbouring ramets (Rice et al., 2001: positive density dependence). This reduction of resource competition has been reported in monoecious angiosperms with sexual systems that promote some segregation of the sexes. For example, in gynodioecious species, in which plants bear either female or bisexual flowers (Bawa and Beach, 1981), the resource allocated to reproduction is higher in unisexual than in bisexual plants (Ashman, 1994; Vaughton and Ramsey, 2011). Our study adds valuable information on prezygotic reproductive allocation patterns in bryophytes, which is also scarce in dioicous species, in particular when it comes to estimates of RA based on relative mass. Prezygotic RA in terms of proportional mass allocated to sexual structures was higher in males than in females in the dioicous mosses Weissia jamaicensis (Mitt.) Grout. and Syntrichia caninervis Mitt. (Stark et al., 2000; Santos et al., 2022a). In the dioicous moss Bryum argenteum Hedw., individual perigonia weighed more than perichaetia, and RA was greater to the male than to the female function when calculated per unit area of culture media by Horsley et al. (2011). The latter method was also applied by Stark and Brinda (2013) for rhizautoicous A. bifrons. In Drepanocladus trifarius (F. Weber & D. Mohr) Broth., reproductive mass per sexual branch and per annual ramet and the RA in terms of proportional mass were higher in females than in males (Bisang et al., 2006). In contrast to the species mentioned earlier, D. trifarius is a pleurocarpous moss growing in wetlands. Prezygotic RA in D. trifarius did not result in decreased vegetative growth in males or females, indicating the lack of prezygotic reproductive costs (Bisang et al., 2006). No evidence of a negative relationship between RA in a given year and growth ability in the subsequent year was detected in Polytrichum juniperinum Hedw. either (Hedderson and Longton, 2008). Finally, in a study with the liverwort Lophozia silvicola H. Buch., female RA, including both pre- and postzygotic structures, and male RA were quantified by Laaka-Lindberg (2001).

Although the lack of a standardized methodology in the abovementioned examples makes data interpretation and comparisons difficult, they strongly suggest that reproductive allocation patterns depend on life-history traits, such as growth form, and environmental conditions, which could explain some of the differences observed among the species. Nevertheless, it is reasonable to think that in certain circumstances, an enhanced reproductive allocation to the male function in dioicous bryophytes increases the chance that female plants will be fertilized. Higher allocation to the male sex might eventually result in more male ramets, more male sexual structures and/ or more sperm available for fertilization. Dioicous bryophytes commonly exhibit female-dominated ramet sex ratios (Bisang and Hedenäs, 2005). It is assumed that a single male plant can fertilize more than one female plant, but fertilization success in dioicous species has been shown to be limited by male mate

availability and intersexual distances (Rydgren and Økland, 2001; Bisang *et al.*, 2004). Although fertilization distances are an order of magnitude longer than commonly claimed (Bisang *et al.*, 2004), investment in sperm without a guarantee that an archegonium will be fertilized might come at a cost (Haig, 2016). A premise is put forward by several authors that high allocation to reproductive structures might trade off with investment in other functions and eventually lead to higher mortality in the sex with higher RA (Stark *et al.*, 2000; Rydgren *et al.*, 2010; Haig, 2016). Indeed, the trade-off between investments in different functions might be one explanation for the low level of sexual expression of males in many populations of dioicous bryophyte species or even in the sexual dimorphism of the species, as in *Ceratodon purpureus* (Hedw.) Brid (Kollar *et al.*, 2021).

Reproductive allocation in the gonioautoicous or cladautoicous species

The intraspecific pattern of RA differed between the three species (Figs 2 and 3). Although male RA was higher than female RA in the rhizautoicous species, we found the opposite in the cladautoicous species and no difference in the gonioautoicous species. Thus, a clear increasing trend in RA to the male function from synoicy to rhizautoicy, as predicted by Stark and Brinda (2013), was not evidenced (Fig. 1). Thus, we infer that RA differs between two main groups: (1) monoicous; and (2) dioicous and functionally dioicous (rhizautoicous) species. Further relationships between RA and sexual systems have been indicated in other studies. For example, in a comparative study with Fissidens submarginatus Bruch. (gonioautoicous) and F. scariosus (rhizautoicous), Santos et al. (2020) found: (1) higher sexual expression in the gonioautoicous species; (2) a greater number of gametangia produced per female archegonia than per male perigonia in both species; and (3) a significantly longer time for maturation of male gametangia in the rhizautoicous (4 months) than in the gonioautoicous species (3 months). Given that higher sexual expression is a striking feature of monoicous bryophytes (Glime and Bisang, 2017), the fact that sexual expression was higher in the gonioautoicous F. submarginatus and lower in the rhizautoicous F. scariosus indicates a trend towards dioicous functionality in the latter. In turn, although traits such as the timing and duration of maturation of sexual organs have not had their association with sexual systems and RA explored, evidence suggests that such relationships might exist. For example, in the rhizautoicous species F. scariosus, reproductive allocation was greater to the male function, and male sexual organs took longer to mature, indicating that the male sex might be the more expensive, requiring more resources and longer developmental time.

The gonioautoicous *F. submarginatus*, conversely, had a lower reproductive allocation and shorter developmental time. This protogynous development has been observed in monoicous species without a spatial separation between sexes, as, for example, in gonioautoicous species such as *Forsstroemia trichomitria* (Hedw.) Lindb. (Stark, 1985), *Trichostomum perligulatum* (Flowers) R.H. Zander (Stark and Castetter, 1995), *Syntrichia inermis* (Brid.) Bayrh. (Stark, 1997), and in the autoicous Octoblepharum albidum Hedw. (Egunyomi, 1978). In turn,

 TABLE 3. Effects of reproductive mass and sex on vegetative mass in three Fissidens species with different sexual systems from Atlantic Forest in São Paulo state, Brazil, based on separate generalized linear models with a Gaussian distribution and identity link function. Sex was excluded as a predictor variable in the gonioautoicous species (see Table 1 for sexual systems)

Gonioautoicous				
		d.f.	Deviance	<i>P</i> -value
Reproductive mass		1	2.90 ⁻⁰⁶	>0.05
GLM, summary				
	Estimate	s.e.	<i>t</i> -value	<i>P</i> -value
Reproductive mass	-0.048	0.44	-0.11	>0.05
Observations			30	
Akaike information criterion			-160.97	
Null deviance			0.007 (d.f. = 29)	
Residual deviance		0.007 (d.f. = 28)		
Cladautoicous				
		d.f.	Deviance	P-value
Reproductive mass		1	0.0001	>0.05
Sex		1	0.0013	>0.05
GLM, summary				
	Estimate	s.e.	<i>t</i> -value	<i>P</i> -value
Female	-0.034	0.292	-0.118	>0.05
Male	-0.01	0.007	-1.347	>0.05
Observations			60	
Akaike information criterion			-259.17	
Null deviance			0.042 (d.f. = 59)	
Residual deviance			0.041 (d.f. = 57)	
Rhizautoicous, female				
		d.f.	Deviance	<i>P</i> -value
Reproductive mass		1	-0.005	< 0.001
GLM, summary				
	Estimate	s.e.	<i>t</i> -value	<i>P</i> -value
Female	3.624	0.764	4.740*	< 0.001
Observations			30	
Akaike information criterion			-159.95	
Null deviance			0.012 (d.f. = 29)	
Rhizautoicous, male				
		d.f.	Deviance	<i>P</i> -value
Reproductive mass		1	0.001	>0.05
GLM, summary				
	Estimate	s.e.	t value	<i>P</i> -value
Male	0.554	1.092	0.508	>0.05
Observations			30	
Akaike information criterion			-112.58	
Null deviance			0.034 (d.f. = 29)	
Residual deviance			0.033 (d.f. = 28)	

*Significant at P < 0.05.



FIG. 6. Relationships between reproductive mass (A–C) or reproductive allocation (D–F) and vegetative mass. Male and female structures were pooled in the gonioautoicous sexual system and separated in the rhizautoicous and cladautoicous systems. Note the different scales between species on the *y*-axes.

protandry has been observed in other sexual systems, such as dioicous and rhizautoicous, such as in *Barbula lambarenensis* P. de la Varde (Fotoba, 1998), *L. silvicola* (Laaka-Lindberg, 2005) and *A. bifrons* (Stark and Brinda, 2013). The comparatively lower investment in the male function in gonioautoicous and cladouticous species can be explained by the short intersexual distances of another plant, which implies that there is no need for dedication of a large amount of resources to sperm production, contrary to what occurs in the rhizautoicous system.

Relationship between reproduction and vegetative growth

Estimates of reproductive and vegetative mass were positively associated only in female ramets of the rhizautoicous species, whereas no relationship between reproductive and vegetative mass was found in male plants or in other sexual systems. Again, a difference seems to be evident between functionally dioicous (rhizautoicous) and 'truly' monoicous species. A positive association between vegetative and reproductive mass has previously also been reported in female plants of the dioicous *Dicranum polysetum* Sw. (Ehrlén *et al.*, 2000; Bisang and Ehrlén, 2002). This indicates that, in absolute values, a vegetatively strong female plant invests more in reproduction than a weaker plant, but only in the rhizautoicous species, and that the resources allocated to the formation of perichaetia do not trade off with vegetative growth. Rhizautoicous ramets supply resources to reproductive structures of only one sex, because they are likely to be physiologically independent, and in females the resources will then also be invested in the future development of sporophytes. In the dioicous moss *Ceratodon purpureus* (Hedw.), however, allocations to vegetative and reproductive gametophyte tissues were unrelated in females, while a negative association was evidenced in males (McDaniel, 2005).

Vegetative mass was negatively related to RA in female ramets of the rhizautoicous species and male and female branchlets of the cladautoicous species in the present study.

TABLE 4. Effects of reproductive allocation and sex o	n vegetative mass in three	e Fissidens species with diff	ferent sexual systems from
Atlantic Forest in São Paulo state, Brazil, based on sepa	arate generalized linear mo	odels with Gaussian distribu	tion and identity link func-
tion. Sex was excluded as	a predictor variable in the	e gonioautoicous species	

Gonioautoicous				
	d.f.		Deviance	P -value
Reproductive allocation	1		0.0008	>0.05
GLM, summary				
	Estimate	s.e.	<i>t</i> -value	P-value
Reproductive allocation	-0.11	0.056	-1.96	>0.05
Observations			30	
Akaike information criterion			-160.97	
Null deviance			0.007 (d.f. = 29)	
Residual deviance			0.006 (d.f. = 28)	
Cladautoicous				
	d.f.		Deviance	P-value
Reproductive allocation	1		0.001	< 0.05
Sex	1		0.005	>0.05
GLM, summary				
Sex	Estimate	s.e.	<i>t</i> -value	P-value
Female	-0.074	0.028	-2.617	< 0.05
Male	-0.022	0.008	-2.758	< 0.01
Observations			60	
Akaike information criterion			-265.96	
Null deviance			0.042 (d.f. = 59)	
Residual deviance			0.037 (d.f. = 57)	
Rhizautoicous				
	d.f.		Deviance	P-value
Reproductive allocation	1		0.014	< 0.001
Sex	1		0.0001	>0.05
GLM, summary				
Sex	Estimate	s.e.	<i>t</i> -value	P-value
Female	-0.145	0.037	-3.850	< 0.001
Male	-0.003	0.007	-0.394	>0.05
Observations			60	
Akaike information criterion			-265.26	
Null deviance			0.052 (d.f. = 59)	
Residual deviance			0.037 (d.f. = 56)	

This can be interpreted in different ways depending on the direction of the relationship. It might reflect the shortage of resources for vegetative growth resulting from RA in females (Convey and Smith, 1993; Ehrlén *et al.*, 2000; Bisang and Ehrlén, 2002). Or, in contrast, RA decreases in vegetatively larger plants. However, this pattern is somewhat unexpected, because one would anticipate a perennial plant to ensure survival by investing in vegetative growth if it is small, and increasing the relative investment to reproduction if it is larger (Convey and Smith, 1993; Ehrlén *et al.*, 2000; Bisang and Ehrlén, 2002). Interestingly, male RA in the rhizautoicous species was not related to vegetative mass. This could be

explained by the phenology of this species, in that the production of male gametangia starts early and continues for relatively long time intervals, and resources can be supplied over a longer time span (Santos *et al.*, 2020). In contrast, Santos *et al.* (2018) reported a negative association between RA and vegetative mass in all sexual morphs (male, non-sporophytic and female sporophytic) of the rhizautoicous moss *F. flaccidus*. Thus, reproductive allocation patterns seem to vary among species. More species with different sexual systems and other life histories need to be investigated and more comprehensive predictions need to be tested before a more general pattern can emerge.

Conclusion

Our data suggest a possible trade-off between reproductive and vegetative allocation and that the allocation patterns vary among bryophyte species with different sexual systems. Our original hypotheses on RA were supported only in part. Rather than showing a gradient of increasing allocation to male reproduction in monoicous sexual systems with increasing levels of intersexual distance, the data revealed a distinction in different aspects of RA between 'truly' monoicous and monoicous but functionally dioicous sexual systems. To explore further the conditions in which males allocate more to reproduction than females in (functionally) dioicous bryophytes, as postulated by Stark and Brinda (2013), the life-history traits of the species and environmental factors need to be taken into account. For example, it is possible that trade-offs between reproduction and vegetative growth are associated with other reproductive traits in these sexual systems. We suggest that the hypotheses should be rephrased and tested further by comparing monoicous and dioicous species from different genera and environments. In addition, the postzygotic resource allocation in females in species with different sexual systems must be investigated to deepen our understanding of resource allocation patterns in bryophytes.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Supplementary 1 - "Simpson effect" in male ramets of rhizautoicous species.

Supplementary 2 - comparation of null and full models.

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