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Sex-specific differences in reproductive life-history traits of the moss *Weissia jamaicensis*

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Abstract

Premise: We investigated sex-specific differences in the life-history traits of a metapopulation of the dioicous moss *Weissia jamaicensis*. Field observations revealed high rates of fertilization, which is uncommon for most dioicous bryophytes. We raised four hypotheses associated with the way the reproductive traits are related to the fertilization rate in this metapopulation.

Methods: We sampled 10 patches of the metapopulation and quantified sexual expression, sex ratio, reproductive success, and reproductive allocation. The ramets were classified as male, non-sporophytic female, sporophytic female, or non-sex-expressing. Thirty ramets from each of the categories expressing sex were placed for regeneration to test the effect of reproductive allocation on this trait.

Results: We found greater expression of the female function in all patches, implying a female bias in the metapopulation. The number of male ramets was variable in each patch and did not affect reproductive success. At the prezygotic level, the allocation of resources to the male function was higher. However, the large allocation of resources to sporophyte development in sporophytic females, which exceeded allocations at prezygotic levels, was related to the higher mortality rate of these ramets, suggesting reproductive cost.

Conclusions: The prezygotic ramets that allocated the greatest amount of resources to reproduction expressed sex less frequently, biasing the sex ratio toward the sex that allocated the least amount of resources to reproduction. Overall, the ramets that allocated the greatest amount of resources to reproduction had the lowest regeneration rate, suggesting reproductive cost.

KEYWORDS

bryophyte ecology, cost of reproduction, fertilization rate, metapopulation, regeneration, reproductive allocation, reproductive success, sex ratio, sexual expression, trade-off

Life history is the timeline of key events in the life cycle of an organism, defined in terms of life-history traits calculated among individuals in a population (Oli and Coulson, 2016). The life-history theory predicts that every resource available to an organism is directed to its vital functions, namely growth, reproduction, maintenance, and defense (Delph, 1999). The allocation of resources in these different functions usually induces trade-offs (Stearns, 1976; Delph et al., 1996), which are the costs paid when a beneficial change in one trait resulting from resource allocation is linked to a detrimental change in another (Stearns, 1989; Obeso, 2002).

Among trade-offs, those that involve reproduction are defined as sexual reproductive costs. The reproductive cost has been the object of several studies due to its complex effects on vegetative development and future performance of organisms (Stearns, 1989; Ehrlén et al., 2000; Stark et al., 2000; Obeso, 2002). The reproductive cost has been found to be different for the female and male function, and this brings consequences to population parameters such as sexual expression, sex ratio, and reproductive success (Stark et al., 2000). According to the theory of reproductive cost, the more expensive sex—the one in which the formation of sex organs requires more energy—is expected to appear in smaller quantities in populations, originating a sexual bias toward the less expensive sex (Bowker et al., 2000; Stark et al., 2000; Obeso, 2002).

Bryophytes are a monophyletic group of land plants (Harris et al., 2020) that comprises the mosses, liverworts,

and hornworts. Due to the simple architecture of their vegetative and reproductive organs, bryophytes are ideal models to test ecological theories concerning resource allocation in different vital functions (Vanderpoorten and Goffinet, 2009). Many traits can be easily recorded in bryophyte populations, increasing the explanatory power of ecological studies, for the following reasons. First, since fitness is measured as the growth rate of a population composed of identical phenotypes (Stearns, 2000), and bryophytes have a high rate of clonal growth, these plants can be used as an excellent model to quantify fitness. Second, due to the typical regenerative potential of gametophytes, the reproductive cost incurred by the survival of this life stage can be easily quantified (Stark et al., 2007). And third, given the small size of bryophytes, the total biomass of reproductive and vegetative structures can be determined to quantify the trade-off between the reproductive and the vegetative function (Ehrlén et al., 2000; Bisang et al., 2006).

Studies investigating different life-history features of bryophytes suggest that the sexual systems (dioicy and monoicy) are associated with the reproductive traits of each species (Glime and Bisang, 2017a). For example, species with separate sexes—that is, rhizautoicous (i.e., monoicous species in which antheridia and archegonia are produced in different ramets connected through rhizoids) and dioicous species—show greater reproductive allocation to the male function (Stark and Brinda, 2013; dos Santos et al., 2018). Besides, populations of bryophytes with separate sexes present fewer sex-expressing male ramets, lower rates of sexual expression, and a trend toward lower reproductive success (Horsley et al., 2011; dos Santos et al., 2020). On the other hand, populations of cosexual monoicous species present higher rates of sexual expression, shorter distances between the sexes, and greater fertilization rates (de Oliveira and Pôrto, 1998; Pôrto and de Oliveira, 2002; dos Santos et al., 2020). However, some species of the two sexual systems—dioicous and rhizautoicous—have been observed to deviate from the expected pattern (e.g., dioicous species with male biased populations; Alvarenga et al., 2013; Holá et al., 2014).

Several studies have found unbalanced sex ratios in unisexual species of different land plants (Field et al., 2013; Segalla et al., 2021). In unisexual bryophytes, the rarity or absence of male sex expression is recurrently observed (Bisang and Hedenäs, 2005). One potential explanation for this phenomenon is the reproductive cost, since resource allocation to male sex expression is often more expensive than that to the female function (Stark et al., 2000). Reproductive allocation is often calculated in absolute (biomass) and relative (proportion of resources allocated to reproduction) levels (Obeso, 2002), but other metrics are also used, such as the number of gametangia produced and the density of gametoecia per square area (Stark and Brinda, 2013; Pereira et al., 2016). Trade-offs are usually quantified on the basis of reproductive allocation, since it is this parameter that reveals the real amount of resources allocated to reproduction (Laaka-Lindberg, 2001; dos Santos et al., 2018).

Weissia jamaicensis (Mitt.) Grout. is a dioicous moss and, as expected for dioicous species, sporophytes are recurrently absent in the field (Müller et al., 2018). However, we found a metapopulation of *W. jamaicensis* with unexpectedly high levels of sporophyte formation. Thus, in order to investigate the underlying causes of such an exceptional condition, we investigated the following hypotheses, which suggest situations that increase the chances of fertilization: (1) the sex ratios of the metapopulation and within each colony (patch) are not biased (1:1); (2) there is a high rate of sexual expression among males, which enhances the reproductive success because the presence of more male ramets expressing sex increases the amount of gametes for fertilization; (3) reproductive allocation is higher in the male than in the prezygotic female function, such that the greater amount of resources allocated to the male function generates a greater amount of male gametes; and (4) reproduction and regeneration of ramets trade off each other, such that greater reproductive allocation is associated with lower regeneration rates of ramets.

MATERIALS AND METHODS

Study site

The study was conducted in a remnant of Cerrado *s.s.* in the municipality of Itaú de Minas, state of Minas Gerais, Brazil (20°46′20.8″S, 46°45′09.9″W). The weather in Itaú de Minas is tropically hot and dry according to Koppen classification, with an average monthly temperature of 23°C (Alvares et al., 2013) (INMET, https://portal.inmet.gov.br). The rainy season occurs in November–February (spring–summer), and December and January are the rainiest months (INMET).

Study species and sampling

Weissia jamaicensis is a dioicous moss found in Africa, North America, and the Neotropics, recurrently without sporophytes (Müller et al., 2018). In Brazil, *W. jamaicensis* is found on roadside rocks and riverbanks, in altitudes of 100–800 m, in Savanna and Atlantic Rainforest (Costa, 2015) (Figure 1).

The metapopulation of *W. jamaicensis* selected for this study was found in a rural area of the municipality of Itaú de Minas with an approximate size of 20 × 15 m. The plants were growing in patches on a thin layer of sandy soil above a limestone rock. In July 2020, one 12 × 12 cm sample was collected from each of ten of these patches. To extract the maximum amount of information about reproductive parameters, samples were collected randomly with a minimum sampling distance of one meter. The samples were collected by placing a 12 × 12 cm germination box on the colony and pressing it against the ground to remove the plants (Appendix S1).
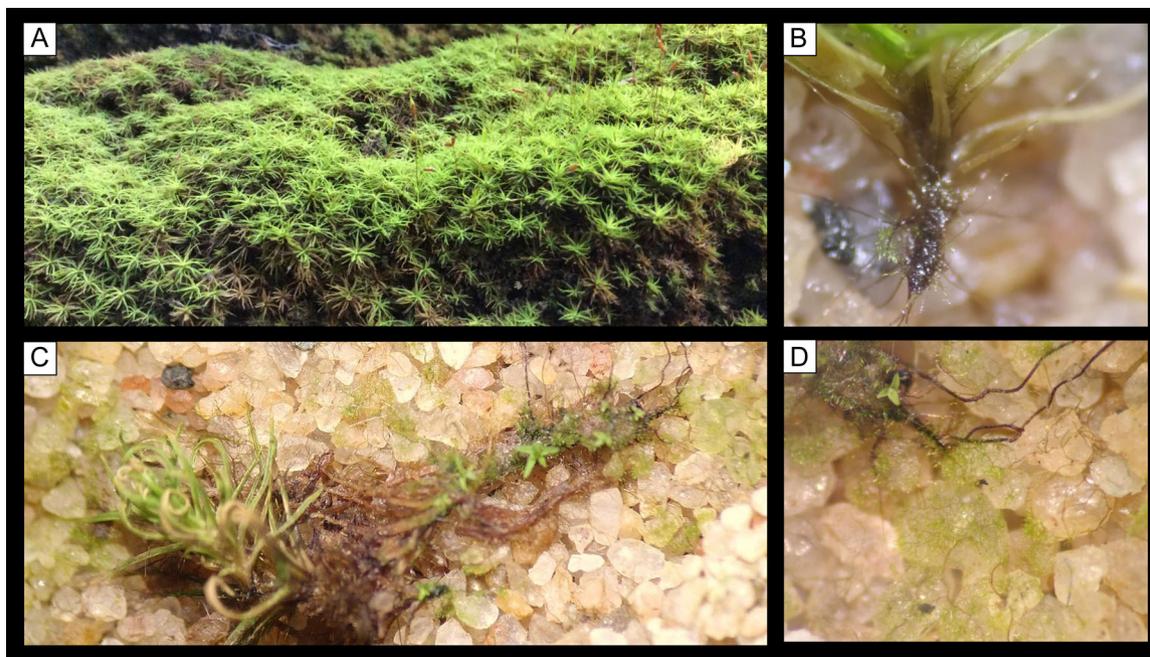
FIGURE 1 (A) *Weissia jamaicensis* in its natural habitat. (B) Protonema being formed at the base of the stem. (C) Ramets with protonemata and new ramets being formed. (D) Protonemata and rhizoids with a juvenile ramet.

Sexual expression, sex ratio, reproductive success

In the laboratory, each box was divided into nine quadrants of 4 × 4 cm, and five of these quadrants (the central one and the four of the corners) were selected for harvesting ramets (Appendix S1). Ten ramets were collected in each of the five quadrants, totaling 50 ramets per box. Thus, 50 ramets were collected from each patch, for 500 in total. These ramets were used to quantify sexual expression, sex ratio, and reproductive success.

To quantify sexual expression, each ramet was classified into one of the following sexual conditions: (a) male—ramet with perigonia; (b) non-sporophytic female—ramet with perichaetia, without sporophytes; (c) sporophytic female—ramet with sporophytes; and (d) non-sex-expressing—ramet without sexual expression. Sexual expression and sex ratios (Segalla et al., 2021) were calculated for the metapopulation (all patches) and for each patch. Sexual expression was calculated as the proportion of ramets expressing sex (male, non-sporophytic female, and sporophytic female), and sex ratios were calculated on the basis of numbers of male and female ramets (calculation of sex ratios in this study is based on ramets expressing sex). Reproductive success was calculated as the proportion of female ramets that produced sporophytes.

Reproductive allocation

To quantify reproductive allocation, 30 male, 30 non-sporophytic, and 30 sporophytic female ramets were randomly selected. The methodology proposed by Bisang and Ehrlén (2002) was followed: each ramet was cleaned and the biomass of brown portions of the gametophytes was removed, leaving only the photosynthetically active parts to be weighed. This is because the brown parts may be dead, and even if they are alive (when they can regenerate), they do not affect the allocation of resources destined for reproduction. Thus, the reproductive parts (perigonia, perichaetia, and sporophytes) were separated from the ramets, placed in small paper envelopes wrapped in aluminum foil, and dried in an oven for 72 h at 70°C.

After drying, each ramet and its respective reproductive structures were weighed on an ultramicrobalance (SE2 ultramicrobalance; Sartorius, Goettingen, Germany; precision ± 0.1 µg). The vegetative biomass and the absolute and proportional reproductive biomass (absolute reproductive allocation [ARA] and relative reproductive allocation [RRA], respectively) were measured.

Regeneration

To investigate the sex-specific regeneration rates in *W. jamaicensis*, 30 male, 30 non-sporophytic female, and 30 sporophytic female ramets were selected. Each male and non-sporophytic female ramet had the gametoecia excised and the number of gametangia counted. Then, each ramet was cleaned with 10% sodium hypochlorite solution for 20 s and sterile mineral water. After drying at room temperature, each ramet was placed in a Petri dish with sand from the collection site and each Petri dish was hydrated weekly with 10 mL of water collected in a waterfall next to the collection

site. The Petri dishes were kept in a greenhouse at room temperature and 50% shade and monitored weekly for 7 wk. The following parameters were quantified for each ramet: week in which protonema formation started; week in which the protonema formed the first ramets; and total number of ramets produced.

Statistical analyses

The rate of sexual expression was calculated for each patch and the metapopulation. Generalized linear models (GLMs) were applied to determine whether sexual expression varied according to patch and sex. First, a full model was created in which the response variable was sexual expression and the predictor variables were the patch, the numbers of male and female ramets, and the interaction between these variables. A null model was created to test whether the observed variation occurred at random. Since the response variable was a proportion, the binomial family was used in the model. The models were compared using the chi-square test, and since the observed difference was significant ($P < 0.001$), the null model was rejected and the full model was adopted to obtain our answers. Interactions were not significant and, therefore, excluded from the model. The full model was run to investigate which of the variables were important for sexual expression. The model was subjected to a residual analysis to assess the adequacy of the error distribution, and subdispersion was found. Thus, the distribution family was changed to quasibinomial so that subdispersion was addressed.

To investigate our first hypothesis of nonbiased sex ratios, a matrix with the numbers of male and female (sporophytic and non-sporophytic) ramets per patch was created and then the chi-square test was applied to analyze whether there was sex bias in each patch and in the metapopulation.

To test our second hypothesis, GLMs were used to investigate whether greater numbers of male ramets induced greater reproductive success. Two models were created: a full model with reproductive success as response variable and number of male ramets and sex ratio as predictor variables, and a null model with reproductive success as response variable to test whether the probability of reproductive success was explained by chance (i.e., not influenced by any of the factors). The logit link function and a binomial distribution were used in both models. The chi-square test was applied to compare the models and, since a significant difference was found, the null model was rejected and the full model was used. The summary of the first model was checked to determine whether the predictor variables and their interactions influenced sexual expression in *W. jamaicensis*. The model was submitted to a residual analysis to assess the adequacy of the error distribution, and overdispersion was detected; thus, a quasibinomial distribution was used. To test our third hypothesis of greater male than prezygotic female reproductive allocation, the three

categories of ramets studied had their absolute and relative reproductive allocation compared through the Kruskal-Wallis test with post hoc Dunn's test with Bonferroni correction, since data were not normally distributed even after transformations.

The numbers of gametangia produced by male and non-sporophytic female ramets were compared using a *t*-test; for that, all the assumptions were analyzed. The numbers of gametoecia were also compared between male and non-sporophytic female ramets using the Mann-Whitney *U*-test, since data were not normally distributed even after transformations. The absolute and relative reproductive allocation differed between male, non-sporophytic female, and sporophytic female ramets, being smaller in non-sporophytic female ramets and greater in sporophytic female ramets. Thus, the following life-history traits were compared between ramets with low (non-sporophytic female ramets), medium (male ramets), and high (sporophytic female ramets) reproductive allocation: (1) start of protonema formation, (2) formation of the first ramet, and (3) total number of ramets produced. The Kruskal-Wallis test with post hoc Dunn's test with Bonferroni correction was used to this end because the variables presented nonparametric distribution even after transformations.

RESULTS

Sexual expression, sex ratio, and reproductive success

The rate of sexual expression in the metapopulation was 57.2% (Table 1), and only three of the ten patches analyzed presented sexual expression below 50%. Sexual expression rates varied nonsignificantly across patches, but the proportion of male and female ramets varied significantly between patches (Table 2). The interaction term between sex and patch was not significant.

Sex ratios, which are based on sexually expressing ramets, were skewed toward the female sex in seven of the 10 patches, toward males in two patches, and balanced in one patch (Table 1). The metapopulation was female biased, with a sex ratio of 1.83♀:1♂ (Table 1).

Reproductive success was not significantly influenced by the variables tested in the GLM (Table 3). The number of male ramets, the sex ratios, and the interaction between these variables showed no influence on the number of fertilized female ramets in the patches of the metapopulation.

There was a significant difference between male and prezygotic female reproductive allocation ($t = -7.83$, df = 58, $P < 0.001$): male ramets produced a greater number of gametangia and biomass of gametoecia than female ramets ($U = 240$, $P < 0.001$).

The vegetative biomass and the absolute and proportional reproductive biomass differed between sexes. Sporophytic and non-sporophytic female ramets had higher average vegetative biomass, and a significant difference

TABLE 1 Numbers of male, non-sporophytic female, and sporophytic female ramets, absolute and proportional numbers of sex-expressing ramets, and sex ratio per patch. Significance indicates occurrence of sexual bias ([ns]nonsignificant; *$P < 0.05$, ***$P < 0.01$, ***$P < 0.001$).

| Patch | Category of ramets | | | | Sex-expressing ramets | | Population parameters | | |
	Male	Non-sporophytic female	Sporophytic female	Non-sex-expressing	Total female	Total male	Sexual expression (%)	Sex ratio (♀/♂)	Sex characterization
1	8	18	7	17	25	8	66	3.13:1**	Female biased
2	2	6	4	38	10	2	24	5.00:1*	Female biased
3	30	0	12	8	12	30	84	0.40:1**	Male biased
4	1	6	23	20	29	1	60	29.00:1***	Female biased
5	0	6	0	44	6	0	12	–	Female biased
6	4	3	12	31	15	4	38	3.75:1*	Female biased
7	34	0	8	8	8	34	84	0.24:1***	Male biased
8	13	9	15	13	24	13	74	1.85:1[ns]	Unbiased
9	0	12	24	14	36	0	72	–	Female biased
10	9	5	15	21	20	9	58	2.22:1*	Female biased
Metapopulation	**101**	**65**	**120**	**214**	**185**	**101**	**57.2**	**1.83:1*****	**Female biased**

TABLE 2 Generalized linear model effects of sex and patch on sexual expression without interaction term.

	Response variable (sexual expression)
Patch	−0.003 (0.010)
	$t = -0.260$
	$P = 0.804$
Female ramet	0.091 (0.004)
	$t = 25.906$
	$P < 0.001$
Male ramet	0.096 (0.003)
	$t = 32.129$
	$P < 0.001$
Constant	−2.328 (0.092)
	$t = -25.193$
	$P < 0.001$
Observations	10
Residual deviance	0.449 (df = 6)
Null deviance	120.567 (df = 9)

TABLE 3 Generalized linear model effects of number of male ramets, sex ratio, and their interaction on the reproductive success of the studied metapopulation.

	Response variable (reproductive success)
Male	0.257 (0.197)
	$t = 1.306$
	$P = 0.240$
Sex ratio	0.159 (0.096)
	$t = 1.667$
	$P = 0.147$
Male: Sex ratio	−0.135 (0.099)
	$t = -1.372$
	$P = 0.220$
Constant	0.410 (0.545)
	$t = 0.753$
	$P = 0.481$
Observations	10
Residual deviance	23.082 (df = 6)
Null deviance	52.097 (df = 9)

was observed only between these two categories of ramets ($\chi^2 = 14.509$, df = 2, $P < 0.001$; Figure 2A). Absolute reproductive allocation differed between the three categories of ramets ($\chi^2 = 65.278$, df = 2, $P < 0.001$; Figure 2B). Finally, the relative reproductive allocation differed between the three categories ($\chi^2 = 45.628$, df = 2, $P < 0.001$; Figure 2C). The inversely proportional relationship between relative

reproductive allocation and survival of ramets supports the existence of reproductive cost (Figure 3).

The initiation of protonema formation did not differ significantly between sexes ($\chi^2 = 4.61$, df = 2, $P = 0.10$; Table 4), indicating that, regardless of sex, when conditions for regeneration are established, protonemata will form in
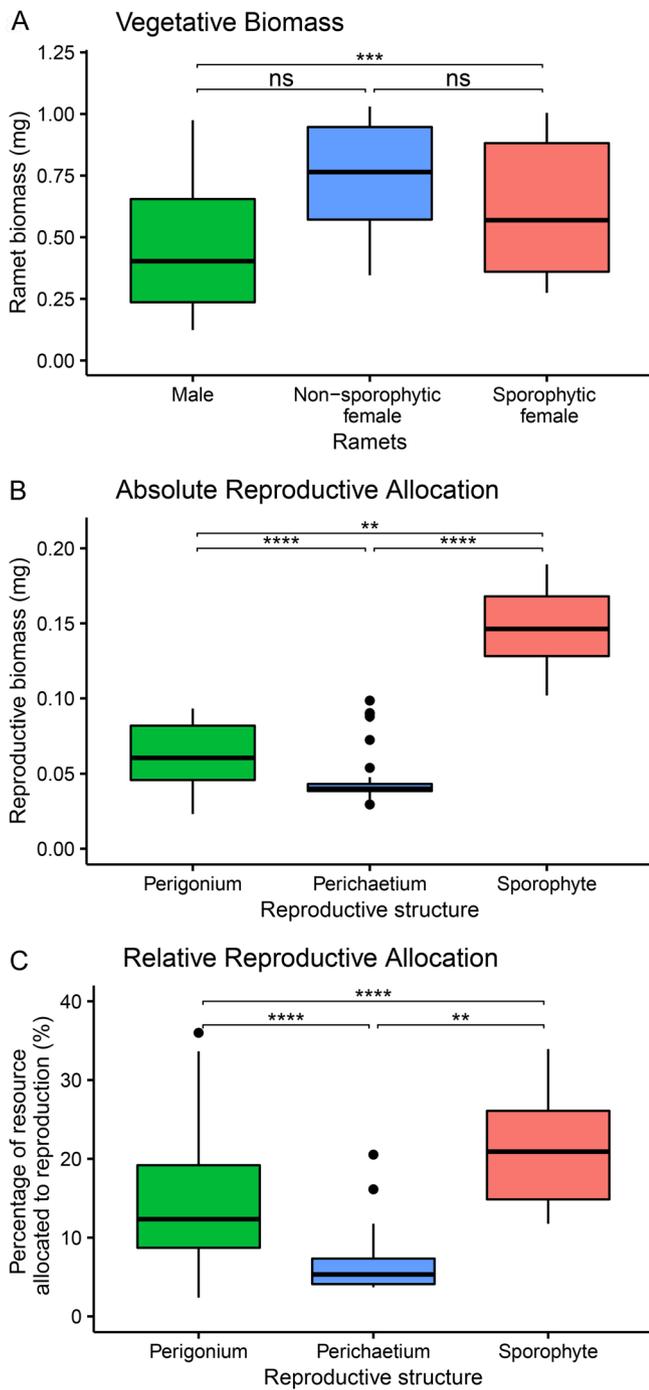
FIGURE 2 (A) Vegetative biomass, (B) absolute reproductive allocation, and (C) relative reproductive allocation of male, non-sporophytic female, and sporophytic female ramets of *Weissia jamaicensis* (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ ****$P < 0.0001$; [ns]nonsignificant).

due time. No sex-specific differences were observed in the formation of the first ramets either ($\chi^2 = 3.77$, df = 2, $P = 0.15$; Table 4). In turn, the number of ramets produced after seven weeks of the experiment varied between sexes ($\chi^2 = 20.88$, df = 2, $P < 0.001$; Table 4), and the ramets that allocated a larger amount of resources in reproduction presented lower regeneration rates, suggesting reproductive cost.

DISCUSSION

Dioicy is commonly associated with a suite of life histories and reproductive traits that may involve genetic, demographic, and ecological costs (Barrett et al., 2010; Renner, 2014). For instance, the obligate outcrossing mechanism imposed by dioicy means that when potential mates of the opposite sex are absent, individuals will remain effectively sterile (Käfer et al., 2017). Populations of dioicous mosses often show a sexual bias that contributes to decrease the levels of fertilization, mainly when compared to monoicous species (Longton, 1992). In *W. jamaicensis*, high levels of fertilization were found despite the significant female bias found in most sampled patches, contradicting the expected patterns for dioicous species showing strong sexual bias (Bisang and Hedenäs, 2005). We found that reproductive allocation was higher in males than in prezygotic females, and this negatively affected the growth of male ramets. Thus, our results revealed a trade-off in allocation associated with sex-differential reproductive costs (Freeman et al., 1997; Zhang et al., 2014). The combination of detailed data from field observations with the regeneration rates obtained in the greenhouse experiment in this study provides information not only about the impacts of sexual bias in the reproduction of mosses, but also about the origin of the specialization and competitive ability in resource allocation between sexes.

Sex-specific variation in reproductive traits and its effects on population parameters

Our results showed that sexual expression did not vary across patches in the study metapopulation, but sex expression varied between sexes. In dioicous mosses, female sex expression is observed more frequently than male sex expression (Glime and Bisang, 2017a). The "shy male hypothesis," which predicts that male ramets fail to sexually express, has been proposed and tested in some studies in the attempt to explain this pattern. Stark et al. (2010) tested it with the dioicous moss *Bryum argenteum* Hedw. They quantified the sexual expression rates in sex-expressing ramets and non-sex-expressing ramets grown until sexual expression. They observed that >80% of the ramets that expressed sex were female and that male expression was rarer in xeric sites. Furthermore, the authors observed that the calculated sex ratio of the non-expressing ramets did not differ from the sex ratio of those expressing sex. The same pattern was found for the moss *Drepanocladus lycopodioides* (Brid.) Warnst. by Bisang and Hedenäs (2013), in which the sex ratio was calculated using sex-specific molecular markers. These authors found a female-skewed sex ratio in the population of 2.6:1, so that the shy male hypothesis was refuted for this species.

To further test the shy male hypothesis, it is essential to think about spore sex ratios. These ratios have been examined so far in only a few species of bryophytes through

FIGURE 3 Illustration of the trade-off between relative reproductive allocation and numbers of live male, sporophytic female, and non-sporophytic female ramets of *Weissia jamaicensis.*
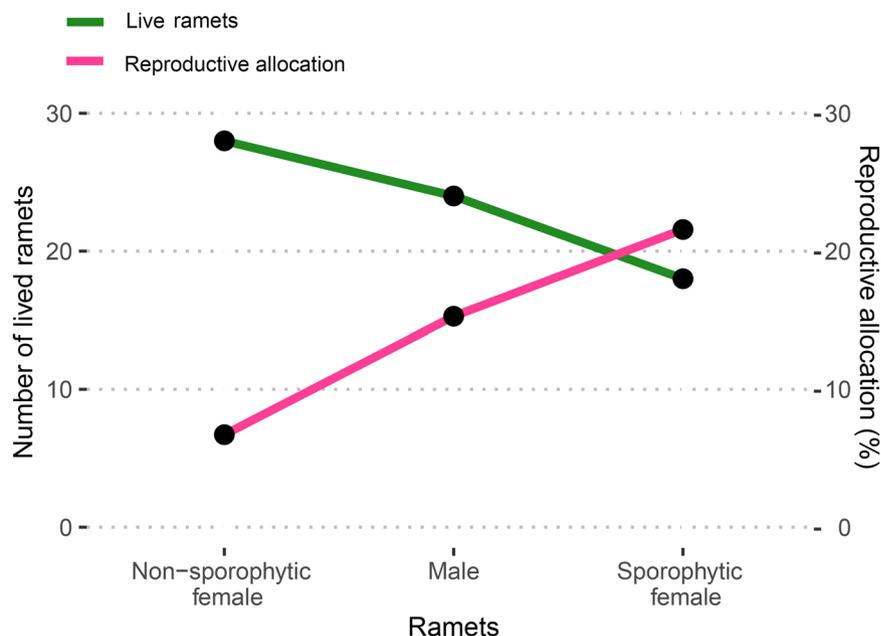

TABLE 4 Descriptive statistics (means ± SD) of the life-history traits initiation of protonema formation, start of ramet formation, and number ramets formed (superscript letters signify the parameters of significance; matching letters indicate equal averages) and results of Kruskal-Wallis test.

Trait	Ramet category			Kruskal-Wallis		
	Male	Non-sporophytic female	Sporophytic female	χ^2	df	P
Initiation of protonema formation	0.80 ± 0.40^a	0.93 ± 0.25^a	0.70 ± 0.65^a	4.61	2	0.10
Start of ramet production	3 ± 1.76^a	3.66 ± 1.02^a	2.56 ± 2.22^a	3.77	2	0.15
Number of ramets formed	3.40 ± 2.02^a	4.66 ± 1.74^b	2.23 ± 1.27^c	20.88	2	0.001

cytological evidence and spore germination until sexual maturity (Glime and Bisang, 2017a). According to Glime and Bisang (2017b), in dioicous bryophyte species, at the beginning of meiosis, the sporocyte (the cell that gives rise to spores) presents a set of chromosomes containing a male chromosome and a set containing a female chromosome. Thus, if the process occurs normally during meiosis, one sporocyte will produce four daughter cells—two female and two male—consequently generating a 1:1 ratio. Among the species in which spore sex ratios have been quantified through germination, the liverwort *Sphaerocarpos texanus* Austin. had a sex ratio of 1:1, but higher mortality rates of male spores caused populations to become female biased (McLetchie, 1992). Likewise, the spore sex ratio of the dioicous moss *Ceratodon purpureus* (Hedw.) Brid. did not differ significantly from 1:1, but sexual dimorphism in life-history traits was observed (Shaw and Gaughan, 1993; Shaw and Beer, 1999). Norrell et al. (2014) reported a variation in sporophyte production and spore viability for *C. purpureus* that could bias the sex ratio of the population. Finally, more recently, Bisang et al. (2017) investigated the spore sex ratio in the dioicous moss *D. lycopodioides* and also observed a balanced sex ratio (1:1), suggesting that the sexual dimorphism during the formation of the ramets favors a female bias. Furthermore, Eppley et al. (2018) demonstrated

that female-biased ramet ratios in *C. purpureus* were established in the gametophytic phase, during protonemal or ramet formation. Based on the above considerations, we can entertain the possibility that biotic (spore mortality, germination, and growth rate) and abiotic (availability of nutrients and harshness of the habitat) factors may differentially affect the development of male spores of *W. jamaicensis*, leading to female-biased sex ratios. However, conclusive evidence for this hypothesis depends on further investigation.

It is logical to think that the functional sex ratio influences the reproductive success because more male ramets imply a greater number of antherozoids for fertilization. Our results refuted our first and second hypotheses: the sex ratios were not balanced and there was no higher male sex expression in the metapopulation. Yet reproductive success was high despite the fact that male ramets were less abundant than female ramets in most patches and in the metapopulation. Two possible reasons for this are that (1) the male ramets were homogeneously distributed in the population, such that they were always close to female ramets, resulting in high fertilization rates; and (2) the antherozoids dispersed over longer distances than typically assumed, favored by moisture availability/water flow or even microarthropod dispersal. We consider

the second reason particularly plausible because male ramets were absent in some patches where sporophyte formation was observed. Also, the number of male ramets did not significantly influence the reproductive success. It has been suggested that the segregation of sexes (Wyatt, 1977) and the availability of male ramets (Hugonnot et al., 2014) in dioicous mosses lead to lower fertilization rates, but our results did not support either of these assumptions in the studied metapopulation. Similarly to what we observed in *W. jamaicensis*, the distance from male ramets did not influence fertilization rates in two species of Pottiaceae, *Hyophila involuta* (Hook.) Jaeg. and *Barbula agraria* Hedw. (de Oliveira and Pôrto, 2005), or in the liverwort *Marchantia polymorpha* L. (McAdam et al., 2021). In the latter case, all female plants were fertilized. The authors concluded that these results were explained by the amount of antherozoids produced and their ability to "swim" around by flagella. However, in the species *Dicranum flagellare* Hedw., Kimmerer (1994) observed that the fertilization rate was influenced by population density: an optimal density enhanced the production of sporophytes, and when the density deviated upward or downward from the expected optimum, the fertilization rate decreased. Therefore, it can be suggested that the density of ramets in *W. jamaicensis* may affect fertilization rate.

Higher male reproductive allocation leads to greater reproductive success

Regarding the reproductive allocation by male and pre-zygotic females, *W. jamaicensis* followed the pattern for dioicous mosses of greater allocation to the male function. Thus, our third hypothesis was corroborated. This is likely related to the goal of enhancing reproductive success (dos Santos et al., 2020). Male ramets were also less abundant in the studied metapopulation, skewing the sex ratio toward females, which is in line with the reproductive cost theory (Obeso, 2002), in that the most expensive sex is found in less quantity. However, we suggest that this greater allocation of resources to the male function may reflect a strategy to save resources, because a single male plant is capable of fertilizing several female plants. If male plants had high rates of sexual expression and high reproductive allocation, there would be an excess of male gametes in relation to female gametes to be fertilized. Stark et al. (2000) found that the male reproductive allocation in *Syntrichia caninervis* Mitt., the model plant of the family Pottiaceae used in several of their studies, was more costly than the female prezygotic reproductive allocation, and they found evidence of higher reproductive cost for the male function in several ontogenetic stages, in terms of leaf regeneration (Stark and McLetchie, 2006), population parameters (Stark et al., 2001, 2004), and tolerance to abiotic stresses (Stark et al., 2005; Stark and McLetchie, 2006).

Another aspect that we can associate with the results on reproductive allocation and reproductive cost at the

prezygotic level is the production of new ramets. Our results showed that regeneration rates did not differ between male and non-sporophytic female ramets in terms of time for onset of protonema formation and of ramet formation. However, the total number of ramets produced was higher among females (Figure 2C). Thus, the greater regeneration rates of female ramets may explain the skewed sex ratios. This is in line with the findings of Stark et al. (2004), who quantified leaf regeneration rates according to leaf age and sex in *S. caninervis* and found no differences in the time until protonemal emergence, linear extension of the protonemata, or rate of biomass accumulation. Nevertheless, those authors concluded that the faster proliferation of female ramets may help explain the rarity of males in this species. Following the same line of reasoning, we consider it very likely that female ramets of *W. jamaicensis* are more frequent in the studied metapopulation due to their higher regeneration rates.

Offspring costs impact the life of sporophytic female ramets

It is reasonable to think that postzygotic reproductive costs exceed prezygotic costs (Glime and Bisang, 2017a). The large number of sporophytic female ramets that died after reproduction and that consequently generated the lowest number of new ramets may be indicative of the cost of sporophyte production. The first bryophyte species to have the cost of sporophyte production estimated was the moss *Dicranum polysetum* Sw. (Ehrlén et al., 2000). The higher biomass allocated to the vegetative function in plants that aborted all the sporophytes in this species suggested that sporophyte formation led to lower vegetative growth. A negative correlation between the development of mature sporophytes and new annual segments was also detected in *D. polysetum* (Bisang and Ehrlén, 2002). Similarly, in our study, greater reproductive allocation was associated with lower post-reproduction development (regeneration), indicating sex-specific differences in the life-history traits of *W. jamaicensis*.

CONCLUSIONS

Despite the consistent female bias observed in the metapopulation studied, most patches showed high levels of fertilization. In each patch, the number of male ramets did not influence the formation of sporophytes, suggesting that female bias has a positive effect in the reproduction of *W. jamaicensis*. Additionally, reproductive cost was indicated by the fact that reproductive allocation was inversely related to regeneration rates (Haig, 2016). Since only one metapopulation was investigated here, our results should be interpreted with caution. Future studies should focus on (1) identifying the sex of ramets using molecular methods so as to quantify the genetic sex ratio of the populations and not

only the sex ratio of sex-expressing ramets; (2) analyzing whether the different sexual conditions of the ramets show different tolerance to environmental factors such as temperature and water availability, in order to investigate whether female ramets are more tolerant and consequently better competitors, justifying their greater abundance in the population; and (3) investigating whether population density can influence population parameters (sex ratios, sexual expression, and reproductive success).

AUTHOR CONTRIBUTIONS

W.L.S. originally formulated the idea, developed the methodology, and conducted fieldwork. W.L.S., K.C.P., and F.P. collaborated in imaging analysis. W.L.S. performed the statistical analyses and wrote the manuscript. K.C.P. and F.P. corrected and discussed the manuscript.

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DATA AVAILABILITY STATEMENT

The data used for this study are accessible in the Figshare repository at: https://figshare.com/s/8d8db58974aeaf5f9f02.

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SUPPORTING INFORMATION

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

Appendix S1. Scheme of the five 4×4 cm quadrants (highlighted in gray) selected in each germination box for collection of ramets of *Weissia jamaicensis*. Black dots indicate the points where ramets were intentionally collected.