Density dependence on male sexual expression in metapopulations of a tropical moss: a monoicous species almost dioicous

Wagner Luiz dos Santos (wag.lsantos1@gmail.com)
UNICAMP: Universidade Estadual de Campinas https://orcid.org/0000-0002-4434-2542
Katia Pôrto
Universidade Federal de Pernambuco
Juçara Bordin
Universidade Estadual do Rio Grande do Sul
Fabio Pinheiro
Universidade Estadual de Campinas

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Abstract

Life-history variables such as reproductive allocation, sexual expression, sex ratio, and reproductive success, are central aspects of a species' ecology and evolution. For example, biases in male and female sex expression may play a large role in determining the viability of populations in the face of environmental pressures such as population fragmentation, climate change and habitat occupancy. Thus, in this study, we investigated reproductive traits in 10 metapopulations of *Fissidens flaccidus* Mitt. From each metapopulation, 30 patches were randomly selected, and samples were collected from each patch of 1cm^2. A total of 20173 ramets were analyzed and classified into male, non-sporophytic female, sporophytic female, and non-sex expressing. In addition, population density in each patch was quantified. Our results showed that relative reproductive allocation in perigonia and sporophytes is greater than perichaetia. In addition, a trade-off between sexual relative reproductive allocation and asexual gemma production was observed, suggesting an important role of female ramets in asexual reproduction. The number of male ramets does not influence the reproductive success observed in each patch, and ramet density may induce male sex expression. Thus, we concluded that reproductive allocation in male function is efficient, since fewer male ramets can assure a considerable reproductive success. Furthermore, our results suggest that there may be a habitat preference between the sexes, since male ramets are found in patches with high density and mostly below female ramets, suggesting an avoidance of direct sunlight by male ramets.

Introduction

Life history is a schedule of key events in an organism's life cycle, and is usually defined in terms of life-history traits calculated across individuals within a population (Oli and Coulson 2016). This life history theory asserts that vital functions such as growth, reproduction, maintenance and defense compete for every resource produced by an organism (Delph 1999). It is often observed that the allocation of resources in different traits of the organism's life history induces trade-offs (Stearns 1976; Delph et al. 1996). Such trade-offs represent the costs paid when the available resource is allocated differentially among traits (Stearns 1989; Obeso 2002). For example, male expression in dioicous species may be favored in poor soils due to the low energetic requirements of this specific sex, when compared to female of flower plants (Bowker et al. 2000; Segalla et al. 2021). Indeed, trade-offs are commonly observed in reproductive traits, showing a crucial influence in the demography and maintenance of populations (Horsley et al. 2011, Krieg et al. 2018).

Reproductive traits such as sexual expression (proportion of individuals that are expressing sex), and sex ratio (number of males and females) are important variables influencing the reproductive performance of population (Glime and Bisang 2017). In this context, many biotic or abiotic factors can affect development and reproductive traits. For instance, some species need high levels of humidity to express sexually, as during the rainy season, while others do not (Maciel-Silva et al. 2012; Maciel-Silva and De Oliveira 2016). Sexual expression plays an important role in maintaining species, and in some cases, population density is a determining factor of reproductive performance. Density dependence density has been recurrently reported in species of plants and animals (Hanski 1990; Gunton and Kunin 2009). Furthermore, sexual systems have also been linked to the reproductive success of populations, for example in *Fissidens scarious* Mitt., and *Fissidens flaccidus* Mitt., which have reproductive traits differently associated with sexual systems (Stark and Brinda 2013; Santos et al. 2020).

Sexual system is defined by Leonard (2018) as "the pattern of gender allocation that characterizes a species". For plants, we can consider the sexual system as the classification of the distribution of reproductive structures (Leonard 2018). For example, dioicous and monoicous species sexual systems are associated with reproductive allocation in plants, so that a pattern is expected according to sexual system (Bergh et al. 2011; Stark and Brinda 2013). Plants are excellent models for understanding life history; as they are autotrophic and sessile, persistent populations can be followed for long periods of time (Bisang and Ehrlén 2002).

The simple architecture of vegetative and reproductive organs of bryophytes makes them excellent models for ecological studies (Harris et al. 2020). Indeed, bryophytes are considered models to understand the ecology and evolution of sexual systems (Suzuki et al. 2018; Harris et al. 2020), reproductive allocation (Stark and Brinda 2013; Santos et al. 2022), and reproductive cost (Bisang and Ehrlén 2002; Rydgren and Økland 2002). Among the most important traits found in bryophytes are: 1) since fitness can be quantified as the growth rate of a clonal population (Stearns 2000), and bryophytes are highly clonal (Rydgren and Okland 2003), they are considered favorable plants for this quantification; 2) due to the common regeneration of their gametophytes, it is often feasible to cultivate and monitor the development of these plants (Stark et al. 2007); 3) given the small size of bryophytes, one can determine the absolute biomass of the reproductive and vegetative structures to quantify the trade-off between the two functions and reproductive allocation (Ehrlén et al. 2000; Bisang et al. 2006). Evidence suggests that reproductive allocation is strongly related to sexual systems in bryophytes. Sexual systems of bryophytes present a gradient of distance between the sexes, and the more distant the sexes, the greater is the relative reproductive allocation (proportion of resource allocated to reproduction) in the male function (Stark and Brinda 2013).

In this study, we quantified the reproductive allocation (absolute and relative), and the following reproductive traits: sexual expression, sex ratio, reproductive success, and population density for a monoicous species that has similarities with dioicy (dioecy). *Fissidens flaccidus* Mitt. is a species of moss with a rhizautoicous sexual system that reproduces sexually and asexually (by clavate gemma in stem tissues). The rhizautoicous system presents individualized male and female ramets that are connected, at least initially, by rhizoids. This sexual system, therefore, functionally resembles the dioicous system, since the ramets presumably do not compete for resources for their development and formation of reproductive structures. In this context, we investigate the following questions: First, is male reproductive allocation greater than female? This is the usual pattern found in dioicous mosses (Stark and Brinda 2013), and rhizautoicous species have compartmentalization of sexual functions (functionally dioicous). Second, is there a trade-off between sexual and asexual reproduction? Since, according to life history theory, the resources available to individuals are finite, and these resources are subject to competition among different life history features or phases (Oli and Coulson 2016). Third, is the number of male ramets a determinant for greater reproductive success? Since the greater the quantity of male ramets expressing sex, the greater the quantity of male gametes and consequently the chance of fertilization of the female gametes. Fourth, is population density related to the sexual expression of ramets? As density dependence effects are recurrent in many animals and plants, we expect that population density influences the reproduction of the species.

Materials And Methods

Study species, study site, and sampling

Fissidens flaccidus Mitt. is a monoicous acrocarpous moss with a rhizautoicous sexual system, distributed in the Neotropical region, Africa, New Guinea, and Australia (Pursell 2007). In Brazil, the species has a wide distribution, so that they are found in all Brazilian phytogeographic domains (Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, and Wetland) (Bordin and Yano 2013; Flora do Brasil 2020, 2021). *F. flaccidus* produces sporophytes in the rainy season, in addition to presenting clavate gemma in ramets (Santos et al. 2018).

Sampling was carried out in July 2021 in the gardens of the Biosciences Center of the Federal University of Pernambuco (Recife, Brazil). According to Alvares et al. (2013) Recife has a tropical, hot, and humid climate according to the Köppen classification, with an average monthly temperature of 23°C. The rainy season occurs in the autumn-winter period (March-August), with June-July being the rainiest months (Coutinho et al. 1998). The Biosciences Center has isolated gardens, in which metapopulations of *F. flaccidus* are often found. Thus, we selected 10 gardens to collect the material. In each metapopulation, 30 patches were randomly selected. In each selected patche, samples of 1 x 1 cm were collected, totaling 30 x 30 cm per metapopulation. The samples were placed in small Petri dishes to preserve the structures in the ramets.

In the laboratory the samples were analyzed to confirm the species identification. The ramets were then quantified for each sample and classified into the following categories: (1) male – ramets with perigonia; (2) non-sporophytic female – ramets with perichaetia; (3) sporophytic female – ramet with sporophyte, and (4) non expressing sex ramet - without gametoecia or sporophyte.

Reproductive allocation and gemma production

To quantify reproductive allocation and gemma production, 50 ramets were randomly selected from each of the following categories: non-sporophytic female; sporophytic female, and non-sex expressing, and 48 male ramets (male ramet density was lower in metapopulations of *F. flaccidus*). For each ramet, gemma and gametangia were counted. *Fissidens'* simple structure and distichous leaf arrangement makes it possible to quantify gametangia without destroying gametoecia. After the quantification of gametangia and gemmae, each ramet that presented gemmae was excluded and ramets cleaned with distilled water. Then, the perigonia, perichaetia and sporophytes were extracted and stored in small paper envelopes together with ramets.

Envelopes with the ramets were then covered with aluminum foil and placed to dry in an oven for 72H at 70°C. Once removed from the oven, the envelopes were placed in a box sealed with silica gel. Then, reproductive structures (perigonia, perichaetia and sporophytes), and ramets were weighed. To carry out the weighing, an ultramicroanalytical balance model SE2 ultramicrobalance, of Sartorius, Goettingen, Germany, precision of 0.1μg was used.

After weighing, the reproductive allocation was quantified as absolute and relative. Absolute reproductive allocation was quantified by the biomass of the reproductive structures (perigonia, perichaetia, and sporophyte). Relative reproductive allocation was quantified as the proportion of biomass allocated to reproduction. We follow the formula: RA $= \frac{RB}{RB+VB}$ where RA is relative reproductive allocation, RB is reproductive biomass, and VB is ramet biomass (McLetchie and Puterbaugh 2000).

Reproductive traits

Sex expression, sex ratio, reproductive success, and population density

Sex expression was calculated as the proportion of ramets that expressed sex for both the sample (1x1 cm) and for the entire metapopulation. The sex ratio was quantified as the ratio of female to male ramets. Reproductive success rate was calculated by the proportion of female ramets that formed sporophytes. Finally, the population density was quantified as the number of ramets/cm^2. All reproductive traits were calculated for the samples and for the entire metapopulation.

Statistical analysis

Reproductive allocation and gemma production

After transformation, the data did not present a normal disposition, and non-parametric analyzes were used. The Kruskal-Wallis test was used to compare ramet biomass, absolute reproductive allocation, relative reproductive allocation and gemma production between different sexual conditions. To compare gametangia of male and non-sporophytic female ramets we applied a Wilcoxon test.

Once it was clear that gemma allocation varied with sex expression and sex, we tested possible trade-offs between the different functions. To test possible trade-ff among reproductive traits (sexual *versus* asexual), we used Generalized Linear Model (GLM). Firstly, we tested if gemma production is related to ramet biomass and absolute reproductive allocation. To answer this question, we created two models, a complete model in which the amount of gemma produced as response variable and the ramet biomass and the absolute reproductive allocation as predictor variables, with a Poisson distribution along with a model null, which tests the randomness of the data. Then a comparison was carried out between the two models with chi square test. Since there were no differences between the models, no significance was observed. Even so, we checked the complete model summary to see the results.

The second trade-off among reproductive traits was also tested with a GLM. We created two models, one being a full, with response variable as gemma production and predictor variables as relative reproductive allocation and ramet biomass. The model null was created to test the randomness of the data. Since the Chi-square test between null and full model was significantly different, we excluded the null model and analyzed the full model. The model summary was then analyzed for data interpretation, and interactions were tested. The distribution applied in the model was Poisson; since underdispersion was observed, the distribution was changed to quasipoisson.

Reproductive traits

First, sexual expression was analyzed. The normality of the variable was tested, and non-parametric tests were applied, given the non-normality of the data. Thus, the expression rate between metapopulations was compared using the Kruskal-Walli's test. The sex ratio was quantified by ratio of female ramets (sum of sporophytic and non-sporophytic ramets) to male. The Chi-square test was applied to confirm the sexual bias of the metapopulation. We applied the Kruskal-Wallis test to compare sample means by metapopulation. Population density was reported by density graphs.

Once we observed that the fertilization rate was higher in samples with high density and with greater numbers of male ramets, we applied a GLM to determine if the amount of male ramets and the density of the samples influence reproductive success. First, we created a complete model, where the response variable was the reproductive success, and the predictor variables were the amount of male ramets and the sample density. Then, a null model was created to determine if the variation was random. For both models, the distribution was binomial. Both models were then compared via the Chi-square test. Since the result was significant, the null model was excluded. The dispersion of the data was checked, and since it was overdispersion, the model distribution was changed to quasibinomial. The model was analyzed using a test of variance via the F-test.

The GLM was also applied to determine if population density influences sex expression on male ramets. For that, two models were created, namely: complete model, having as response variable the amount of male ramets and as predictor variable the density of samples. For both models, the distribution was Poisson. The complete and null models were compared via Chi-square test. Once the test was significant, the null model was excluded, and the complete model was used to analyze the data. Data dispersion was checked and neither overdispersion nor underdispersion was observed.

Results

Reproductive allocation and gemma production

Concerning vegetative biomass, male ramets, presented a lower mean if compared to other ramets category (non-sex expressing, sporophytic and non-sporophytic females) (Table 1), which in turn did not differ from each other ($X^2 = 73.55$, df = 3, $P < 0.0001$) (Fig. 1. a). Regarding the absolute reproductive allocation, the allocation at the prezygotic level (perigonia and perichaetia) did not differ from each other (Fig. 1b). However, the absolute allocation of the sporophyte was significantly higher ($X^2 = 169.85$, df = 3, $P < 0.0001$) (Table 1). Relative reproductive allocation was similar between male plants and female sporophytic plants, whereas non-sporophytic females had a significantly lower relative reproductive allocation than male and sporophyte plants ($X^2 = 151.26$, df = 3, $P < 0.0001$) (Table 1; Fig. 1. c). Gemma production was significantly lower in male plants ($X^2 = 62.38$, df = 3, $P = 0.0001$), whereas the other ramet categories did not differ from one another (Fig. 1. d). Male and female ramets produced similar numbers of gametangia (W = 948.5, $P = 0.10$) (Table 1).

Regarding the trade-off between gemma production and the ramet and reproductive biomass variables, the complete and null GLM models did not show significant differences, suggesting the model's non-significance; the summary of the complete model indicated no association between the variables. Thus, ramet biomass and absolute reproductive allocation do not determine gemma production according to this model (Table 2). On the other hand, the model that tested whether gemma production is determined by relative reproductive allocation and ramet biomass was significant. According to the results, those plants that allocate a greater amount of resource to relative reproductive allocation, have a lower gemma production rate (Fig. 2). Furthermore, the model showed significance in the interactions between relative reproductive allocation and vegetative biomass, suggesting that the smaller the growth and relative reproductive allocation, the greater the gemma production (Table 3).

Reproductive traits

Regarding sexual expression, the results of the analyses indicated that the proportion of sexual expression is roughly equivalent among metapopulations ($X^2 = 252.11$, df = 218, P = 0.05) (Table 4). Metapopulations 2, 3, 7, and 10 had the highest sexual expression rate (Fig. 3). While metapopulation 6, has a lower rate of sexual expression. Regarding sex ratio, all metapopulations showed a bias towards females (Table 4). However, the exact sex ratio was quantified for metapopulations 1, 3, 5, 7 and 10, as they were the only metapopulations that presented male ramets. Thus, the analyzes showed that all populations that expressed sex were biased towards females (Supplementary Table 1).

Concerning reproductive success (resulting in a sporophyte), the analyses do not show differences in reproductive success ($X^2 = 108.46$, df = 91, P = 0.10). However, the metapopulations that had reproductive success were 1, 3, 5, 7, and 10 (Fig. 4). Finally, population density varied among metapopulations, suggesting that those metapopulations that show the highest density had the greatest reproductive success (Fig. 5).

Regarding the GLM result that we investigated whether reproductive success is higher in samples with high density and with a greater amount of male ramet, it did not show any significant result (Table 5). On the other hand, the model that was applied to investigate if population density entails male sex expression, presented a significance (Table 6). This model showed that population density is an important factor to male ramets express their sex.

Discussion

Sexual systems differ in morphological and reproductive attributes, which confer different mechanisms for each sex. However, the variation in the distance between the sexes can characterize the reproductive allocation and population dynamics of the species. Of the four questions we aimed to answer, three gave the expected results following the pattern observed in similar studies (Stark and Brinda 2013; Santos et al. 2018); the exception was question three, where the density of male ramets does not influence the reproductive success of the population. In general, results from this study indicate a similarity in reproductive patterns observed recurrently in dioicous species. Thus, we can state that rhizautoicous sexual systems are monoicous sexual systems that behave like a dioicous system. Since in this sexual system, male and female ramets are individualized and connected only by rhizoids, that is, each ramet has a resource intended only for a sexual function, male or female, as in dioicous species.

The high relative reproductive allocation in the male function reflects a trade-off

Gender differences were not observed in the absolute reproductive allocation in the prezygotic phases (perigonia and perichaetia formation). On the other hand, for relative reproductive allocation, male ramets had a significantly higher mean. This pattern of greater reproductive allocation in male function compared to females is observed in most dioicous species and some rhizautoicous species (Stark et al. 2000; Horsley et al. 2011; Stark and Brinda 2013; Santos et al. 2018). Stark and Brinda (2013) noted that, as the distances between males and females increase, the greater is the relative reproductive allocation in male function. Thus, in rhizautoicous sexual systems where male and female ramet connections dissolve, plants can be considered functionally dioicous. It is expected that the larger the distance between the male and female ramets, the chances of fertilization decline. Indeed, results reported by several studies confirm this expectation (Glime and Bisang 2017). Thus, we can infer that this pattern may be interpreted as an evolutionary strategy that increases the chance of fertilization in sexual systems showing large distances between sexual functions.

Male ramet biomass is lower than other ramet categories (non-expressing sex, sporophytic and non sporophytic female). This result has been related to this same species (Santos et al. 2018), and a strong sexual dimorphism measured. The lower male ramet biomass may be related to reproductive cost using relative reproductive allocation. According to Obeso (2002) there are many definitions of reproductive cost, among them, direct cost, is defined as somatic costs of reproduction during the current reproductive season. Therefore, if a direct cost is related to the current reproductive season, a trade-off between growth and reproduction is expected. Indeed, male ramets have a much smaller biomass than females, since the proportion of resources allocated to perigonia is relatively high and these resources are not available for growth. In this context, phenological observations can provide clues to the effect of reproductive allocation on species biology. For instance, Santos et al. (2020) reported on a study where the phenology of two species of *Fissidens* with different sexual systems was observed, namely: *Fissidens scarious* Mitt. - rhizautoicous; and *Fissidens submarginatus* Bruch - gonioautoicous. The authors observed that in the species with the rhizautoicous sexual system, protandry occurred, as they allocate more resources to reproduction, compared to the gonioautoicous (with male and female sex structures produced distally along single ramets) species.

Reproductive allocation competes for resources with asexual reproduction

The results showed that reproductive allocation for sexual reproduction (*i.e.*, resource allocated to formation of perigonia, perichaetia, and sporophytes) entail in fewer resources for gemma production. Trade-offs occur because environments are variable, often seasonal, and the plants are fixed organisms, they must adjust to these conditions. In this context, trade-offs can be interpreted as adjustments to environmental variations (Liu et al. 2009). A clear example of a trade-off between sexual and asexual reproduction was reported in a liverwort by Laaka-Lindberg (2001) in the species *Lophozia silvicola* H. Buch, where the author found female plants allocating more resources to sexual reproduction compared to male plants. On the other hand, the amount of gemmae was higher in plants that did not express sex, and the average increased from males to females. The latter finding makes it clear that sexual and asexual reproduction compete for resources produced by the plant. Nonetheless, this trade-off relationship between sexual and sexual functions has been reported recurrently in bryophytes (Kimmerer 1991; Bisang and Ehrlén 2002; Fuselier and Mcletchie 2002; Rydgren and Okland 2003; McLetchie and Stark 2006; Hedderson and Longton 2008; Stark et al. 2009; Horsley et al. 2011).

Population density can influence the reproductive fitness of metapopulations

Our results showed that the density of male ramets is not a factor increasing reproductive success in metapopulations of *F. flaccidus*. Thus, our findings indicate that the large reproductive allocation directed at male sexual function is efficient in bringing about the reproductive success of the population. Reese (1984) showed that in dioicous *Syrrhopodon texanus* Sull. male plants are very rare. However, when males are found, there are also females bearing sporophytes, being like our founds. Regarding sex ratio in dioicous species, commonly populations are found with female bias. For instance, Stark (2002) reports that 2/3rds of the studied dioicous species (N = 30) have populations with a female bias, 5 with a male bias and 5 without a sexual bias. In the same sense Bisang and Hedenäs (2005) shows that approximately 80% of analyzed species (N = 103) presented a female bias. However, although less studied, rhizautoicous species tend to express a female ramet bias, namely: *Atrichum undulatum* (Hedw.) P. Beauv., *Tortula muralis* Hedw. (Longton and Miles 1982),*Weissia controversa* Nees & Hornsch. (Anderson and Lemmon 1972), and *Fissidens scarious* Mitt. (Santos et al. 2020). Deviating from this expected pattern is the moss *Aloina bifrons* (De Not.) Delgad. (Stark and Brinda 2013).

Population density was associated with male sexual expression induction in the studied metapopulations, that is, the denser the population, the greater the chance of male ramets expressing sex. According to phenological patterns, usually in those plants where there is greater reproductive allocation in a function, they are developed first. Indeed, protandry is commonly observed in these plants with greater reproductive allocation in male function. For example, in *Aloina bifrons* (De Not.) Delgad., Stark and Brinda (2013) showed that the development of the male plants preceded the female ramets and, at the end of the experiment, a greater amount of male ramets was observed. Similar to the findings by Santos et al. (2020), however, the number of female ramets was higher in rhizautoicous *Fissidens scarious*. Thus, we can suggest that population density may create alternative microhabitat that favors male ramets expression, which may take advantage from specific conditions created by higher or lower densities.

The preference for microhabitat in relation to sex has been reported in some bryophyte studies. For example, Bowker et al. (2000) found habitat preferences by sex in the widely distributed desert dioicous moss *Syntrichia caninervis* Mitt. in populations from the Mojave Desert in Nevada (USA). It was observed that male plants tend to establish and persist in shade, compared to female plants that are found across a span of shaded and open microhabitats. In the same sense, Cameron and Wyatt (1990) reported for the monoicous species *Splachnum ampullaceum* Hedw., *Splachnum sphaericum* Hedw., and dioicous *Splachnum rubrum* Hedw. that low light intensity and other factors favor the formation of male ramets. Thus, an explanation that population density is associated with male sexual expression is suggested, since the greater density of patches leads to a favorable microclimate for the ramets to express the male sex. Furthermore, the male ramets have a reduced size that are mostly protected near the female plants.

Thus, we can conclude that the studied metapopulations present characteristics of dioicous species, such as: greater reproductive allocation in the male sexual function. In addition, the reproductive allocation in the male function is sufficient to generate greater reproductive success. Since the number of male ramets is not correlated with reproductive success. Thus, few male ramets have the potential to induce higher reproductive success compared to metapopulations that have many male ramets. Finally, our results suggest a possible preference for habitat in relation to male ramets, since these are always present in metapopulations that present higher density.

Declarations

This paper deserves to be honored as a Highlighted Student Paper: *This study has the potential to be an article highlight, as we investigate a central question of ecology, the theory of life history. Our findings reinforce the predictions of the theory studied, in addition to showing that the resource relationship is associated with various stages of the life of organisms. Furthermore, density dependent is important in male sexual expression.*

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Author contribution statement WLS and KCP conceived the ideas. WLS, KCP, and FP designed the methodology. WLS conducted field work supported by KCP. WLS, FP, KP and JB analyzed the data. WLS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

We investigated the life history theory in 10 metapopulations of a monoecious moss that presents the segregation of the sexes, that is, it is functionally dioecious. We proved that reproduction is expensive, and the resource is finite, generating reproductive cost in different reproductive traits of the organism's life history. In addition, other population parameters indicated relationships with the life history theory.

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Ethics approval (This article does not contain any studies with human participants or animals performed by any of the authors.)

Consent to participate (Not applicable)

Consent for publication (Not applicable)

Availability of data and material (The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.)

Code availability (Not applicable)

References

1. Alvares CA, Stape JL, Sentelhas PC et al (2013) Köppen's climate classification map for Brazil. Meteorol Z 22:711–728. https://doi.org/10.1127/0941-2948/2013/0507
2. Anderson LE, Lemmon BE (1972) Cytological Studies of Natural Intergeneric Hybrids and Their Parental Species in the Moss. Source Ann Missouri Bot Gard 59:382–416
3. Bergh NG, Anthony Verboom G, Verboom GA (2011) Anomalous capitulum structure and monoecy may confer flexibility in sex allocation and life history evolution in the Ifloga lineage of paper daisies (Compositae: Gnaphalieae). Am J Bot 98:1113–1127. https://doi.org/10.3732/ajb.1000457
4. Bisang I, Ehrlén J (2002) Reproductive Effort and Cost of Sexual Reproduction in Female Dicranum polysetum. Bryologist 105:384–397. https://doi.org/https://doi.org/10.1639/0007-2745(2002)105[0384:REACOS]2.0.CO;2
5. Bisang I, Ehrlén J, Hedenäs L (2006) Reproductive effort and costs of reproduction do not explain female-biased sex ratios in the moss Pseudocalliergon trifarium (Amblystegiaceae). Am J Bot 93:1313–1319. https://doi.org/10.3732/ajb.93.9.1313
6. Bisang I, Hedenäs L (2005) Sex ratio patterns in dioicous bryophytes re-visited. J Bryol 27:207–219. https://doi.org/10.1179/174328205X69959
7. Brazil Flora Group (2021) Brazilian Flora 2020 project - Projeto Flora do Brasil 2020. v393.274. Instituto de Pesquisas Jardim Botanico do Rio de Janeiro. Dataset/Checklist. doi:10.15468/1mtkaw

8. Bordin J, Yano O (2013) Fissidentaceae (Bryophyta) do Brasil. Inst Botânica São Paulo 22:351
9. Bowker MA, Stark LR, McLetchie DN, Mishler BD (2000) Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss Syntrichia caninervis (Pottiaceae). Am J Bot 87:517–526. https://doi.org/10.2307/2656595
10. Cameron RG, Wyatt R (1990) Spatial Patterns and Sex Ratios in Dioecious and Monoecious Mosses of the Genus Splachnum. Bryologist 93:161–166
11. Coutinho RQ, Lima-Filho MF, Souza-Neto JB, Silva ED (1998) Características climáticas, geológicas, geomorfológicas e geotécnicas da Reserva Ecológica de Dois Irmãos. In: Machado IC, Lopes AV, Pôrto KC (eds) Reserva ecológica de Dois ırmãos: estudos em um remanescente de mata atlântica em área urbana, 1st edn. Editora Universitária, Universidade Federal de Pernambuco, Recife, pp 21–50
12. Delph LF (1999) Sexual Dimorphism in Life History. Gender and Sexual Dimorphism in Flowering Plants. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 149–173
13. Delph LF, Galloway LF, Stanton ML (1996) Sexual dimorphism in flower size. Am Nat 148:299–320
14. Ehrlén J, Bisang I, Hedenas L (2000) Costs of sporophyte production in the moss, Dicranum polysetum. Plant Ecol 149:207–217. https://doi.org/10.1023/A:1026531122302
15. Fuselier L, Mcletchie N (2002) Maintenance of sexually dimorphic preadult traits in Marchantia inflexa (Marchantiacae). Am J Bot 89:592–601
16. Glime JM, Bisang I (2017) Chap. 3 – 2 Sexuality: Sex Ratio and Sex Expression Chap. 3 – 2 Sexuality : Sex Ratio. pp 1–30
17. Gunton RM, Kunin WE (2009) Density-dependence at multiple scales in experimental and natural plant populations. J Ecol 97:567–580. https://doi.org/10.1111/j.1365-2745.2009.01492.x
18. Hanski I (1990) Density dependence, regulation and variability in animal populations. Philos Trans R Soc London Ser B Biol Sci 330:141–150. https://doi.org/10.1098/rstb.1990.0188
19. Harris BJ, Harrison CJ, Hetherington AM, Williams TA (2020) Phylogenomic Evidence for the Monophyly of Bryophytes and the Reductive Evolution of Stomata. Curr Biol 30:2001–2012e2. https://doi.org/10.1016/j.cub.2020.03.048
20. Hedderson TA, Longton RE (2008) Local adaptation in moss life histories: population-level variation and a reciprocal transplant experiment. J Bryol 30:1–11. https://doi.org/10.1179/174328208X282175
21. Horsley K, Stark LR, McLetchie DN (2011) Does the silver moss Bryum argenteum exhibit sex-specific patterns in vegetative growth rate, asexual fitness or prezygotic reproductive investment? Ann Bot 107:897–907. https://doi.org/10.1093/aob/mcr027
22. Kimmerer RW (1991) Reproductive Ecology of Tetraphis pellucida. I. Population Density and Reproductive Mode. Bryologist 94:255–260
23. Laaka-Lindberg S (2001) Biomass allocation to sexual and asexual reproduction in a leafy hepatic Lophozia silvicola Buch. J Bryol 23:3–8. https://doi.org/10.1179/jbr.2001.23.1.3
24. Leonard JL (2018) Transitions Between Sexual Systems. Springer International Publishing, Cham
25. Liu F, Chen JM, Wang QF (2009) Trade-offs between sexual and asexual reproduction in a monoecious species Sagittaria pygmaea (Alismataceae): The effect of different nutrient levels. Plant Syst Evol 277:61–65. https://doi.org/10.1007/s00606-008-0103-2
26. Longton RE, Miles CJ (1982) Studies on the Reproductive Biology of Mosses. Journ Hattori Bot Lab 52:219–240
27. Maciel-Silva AS, De Oliveira MP (2016) How Tropical Moss Sporophytes Respond to Seasonality: Examples from a Semi-Deciduous Ecosystem in Brazil. Cryptogam Bryol 37:227–239. https://doi.org/10.7872/cryb/v37.iss3.2016.227
28. Maciel-Silva AS, Valio IFM, Rydin H (2012) Altitude affects the reproductive performance in monoicous and dioicous bryophytes: Examples from a Brazilian Atlantic rainforest. AoB Plants 12:1–14. https://doi.org/10.1093/aobpla/pls016
29. McLetchie DN, Puterbaugh MN (2000) Population sex ratios, sex-specific clonal traits and tradeoffs among these traits in the liverwort Marchantia inflexa. Oikos 90:227–237. https://doi.org/10.1034/j.1600-0706.2000.900203.x
30. McLetchie DN, Stark LR (2006) Sporophyte and gametophyte generations differ in their thermotolerance response in the moss Microbryum. Ann Bot 97:505–511. https://doi.org/10.1093/aob/mcl011
31. Obeso JRR (2002) The costs of reproduction in plants. New Phytol 155:321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x
32. Oli MK, Coulson T (2016) Life History, What is? Encyclopedia of Evolutionary Biology. Elsevier, pp 394–399
33. Pursell RA (2007) Fissidentaceae, Flora Neotropica Monograph 101, 1st edn. New York
34. Reese WD (1984) Reproductivity, fertility and range of Syrrhopodon texanus Sull. (Musci; Calymperaceae), a North American endemic. Bryologist 87:217–222. https://doi.org/10.2307/3242794
35. Rydgren K, Okland RH (2003) Short-Term Costs of Sexual Reproduction in the Clonal Moss Hylocomium Splendens. Bryologist 106:212–220. https://doi.org/10.1639/0007-2745(2003)106[0212:SCOSRI]2.0.CO;2
36. Rydgren K, Økland RH (2002) Ultimate costs of sporophyte production in the clonal moss Hylocomium splendens. Ecology 83:1573–1579. https://doi.org/10.1890/0012-9658(2002)083[1573:UCOSPI]2.0.CO;2
37. Santos WL, Dos, Pereira Alvarenga LD, Pôrto KC (2018) Sexual Dimorphism, Vegetative Growth and Reproductive Investment in the Rhizautoicous Moss Fissidens flaccidus (Fissidentaceae, Bryopsida). Cryptogam Bryol 39:271–281. https://doi.org/10.7872/cryb/v39.iss2.2018.271
38. Santos WL, Maciel-Silva AS, Pôrto KC (2020) How do sexual expression, reproductive phenology and reproductive success relate to sexual systems in Fissidens Hedw. (Fissidentaceae)? A case study comparing two different sexual systems in mosses. Plant Biol 22:573–581. https://doi.org/10.1111/plb.13122
39. Santos WL, Pôrto KC, Pinheiro F (2022) Sex-specific differences in reproductive life-history traits of the moss Weissia jamaicensis. Am J Bot 109:645–654. https://doi.org/10.1002/ajb2.1840

40. Segalla R, Pinheiro F, Barônio GJ, Morellato LPC (2021) Male-biased effective sex ratio across populations of the threatened Zamia boliviana (Zamiaceae). https://doi.org/10.1007/s11258-021-01127-3. Plant Ecol 3:
41. Stark LR (2002) Phenology and its Repercussions on the Reproductive Ecology of Mosses. Bryologist 105:204–218. https://doi.org/10.1639/0007-2745(2002)105[0204:PAIROT]2.0.CO;2
42. Stark LR, Brinda JC (2013) An experimental demonstration of rhizautoicy, self-incompatibility, and reproductive investment in Aloina bifrons (Pottiaceae). Bryologist 116:43–52. https://doi.org/10.1639/0007-2745-116.1.043
43. Stark LR, Brinda JC, McLetchie DN (2009) An Experimental demonstration of the cost of sex and a potential resource limitation on reproduction in the moss Pterygoneurum (Pottiaceae). Am J Bot 96:1712–1721. https://doi.org/10.3732/ajb.0900084
44. Stark LR, Mishler BD, McLetchie DN (2000) The Cost of Realized Sexual Reproduction: and Sporophyte Abortion in a Desert Moss. Am J Bot 87:1599–1608
45. Stark LR, Oliver MJ, Mishler BD, McLetchie DN (2007) Generational differences in response to desiccation stress in the Desert Moss Tortula inermis. Ann Bot 99:53–60. https://doi.org/10.1093/aob/mcl238
46. Stearns SC (1989) Trade-Offs in Life-History Evolution. Funct Ecol 3:259. https://doi.org/10.2307/2389364
47. Stearns SC (1976) Life-History Tactics: A Review of the Ideas. Q Rev Biol 51:3–47. https://doi.org/10.1086/409052
48. Stearns SC (2000) Life history evolution: Successes, limitations, and prospects. Naturwissenschaften 87:476–486. https://doi.org/10.1007/s001140050763
49. Suzuki T, Inoue Y, Tsubota H (2018) Molecular phylogeny of the genus Fissidens (Fissidentaceae, Bryophyta) and a refinement of the infrageneric classification. Mol Phylogenet Evol 127:190–202. https://doi.org/10.1016/j.ympev.2018.05.020

Tables

Tab 1 – The table reports the mean and standard deviation of ramet mass, absolute reproductive allocation, relative reproductive allocation, and gemma production. Kruskal-Wallis parameters are represented in the last columns of the table.

	Sexual condition				**Kruskal-Wallis**		
Variables	**Male** (X ± SD)	**Non-sporophytic female** (X ± SD)	**Sporophytic female** (X ± SD)	**Non expressing sex** (X ± SD)	X^2	**Df**	***P***
Vegetative mass (mg)	0.0139 ± 0.0137	0.0639 ±0.0315	0.0568 ± 0.0324	0.0655 ± 0.0377	73.55	3	< 0.01
Absolute reproductive allocation (mg)	0.0046 ± 0.0030	0.0070 ± 0.0028	0.0223 ± 0.0085	_	169.76	2	< 0.01
Relative reproductive allocation (%)	30.32 ± 14.22	11.88 ± 7.87	30.49 ± 8.19	_	150.98	2	< 0.001
Gemma amount (n)	2.44 ± 4.71	18.66 ±14.15	14.84 ± 12.41	12.04 ± 9.67	62.38	3	< 0.001

Tab 2 – Results of Generalized Linear Models (GLM). Response variable (gemma production), predictor variables (gametangia biomass, and ramet biomass). n.s. = not significant.

Generalized Linear Models (GLM) – Gemma production		
	Df	**Deviance**
Gametangia biomass	1	$20.10^{n.s}$
Ramet biomass	1	$2.42^{n.s}$
Observations	197	
Akaike Information Criterion	NA	
Residual Deviance	2506.5 (Df = 194)	
Null Deviance	2529 (Df = 196)	

Tab 3 – Results of Generalized Linear Models (GLM). Response variable (gemma production), predictor variables (Relative reproductive allocation, and ramet biomass). ** = 0.001; *** = 0.0001; n.s. = not significant.

Generalized Linear Models (GLM) – Gemma production

	Df	Deviance
Relative reproductive allocation	1	20.10**
Vegetative biomass	1	117.44$^{n.s}$
Reproductive relative allocation *versus* Vegetative biomass	1	215.24***
Observations		197
Akaike Information Criterion		NA
Residual Deviance		2176.2 (Df = 193)
Null Deviance		2529.0 (Df = 196)

Tab 4 – Table reports reproductive trait data by patches and for the entire metapopulation. The sexual expression, reproductive success, and density traits calculated for patches are being reported with mean and standard deviation. While the data reported for metapopulations is being represented by the raw data.

Metapopulation	Ramets				Mean of reproductive traits to samples				Absolute reprodu	
	Male	Non-sporophytic female	Sporophytic female	Non Expressing sex	Sexual expression X ± SD	Sexual proportion ▯:▯	Reproductive success per sample X ± SD	Density per sample 1 x 1 cm X ± SD	Sexual expression	Se pr ▯:
1	29	304	648	2747	28.25 ± 18.76	32.14:1	69.70 ± 25.25	124.3 ± 30.31	26.31	32
2	0	530	0	1071	31.91 ± 12.60	-	0	31.91 ± 13.33	33.10	-*
3	83	263	704	1852	37.31 ± 13.14	11.70:1	69.66 ± 18.20	69.66 ± 22.98	36.18	11
4	0	127	0	767	12.26 ± 13.02	-	0	29.80 ± 8.79	14.21	-
5	35	348	481	2810	26.46 ± 16.87	23.69:1	55.05 ± 32.26	122.46 ± 37.08	23.52	23
6	0	0	0	1182	0	-	0	39.40 ± 9.86	0.00	-*
7	74	269	745	1945	35.60 ± 11.18	13.70:1	69.90 ± 22.01	101.10 ± 26.87	35.87	13
8	0	250	0	827	21.96 ± 9.94	-	0	35.90 ± 9.65	23.21	-*
9	0	216	0	819	19.82 ± 13.85	-	0	34.5 ± 6.76	20.87	-*
10	14	229	162	642	38.40 ± 27.08	27.93:1	21.74 ± 37.16	35.56 ± 9.13	38.68	27

Tab 5 – Results of Generalized Linear Models (GLM). Response variable (reproductive success), predictor variables (Male ramets, and total ramets). n.s. = not significant.

Generalized Linear Models (GLM) **Response variable as reproductive success**		
	Df	**Deviance**
Male ramets	1	0.0026 $^{n.s}$
Total ramets	1	11.5135$^{n.s}$
Observations	300	
Akaike Information Criterion	NA	
Residual Deviance	283.31 (Df = 27)	
Null Deviance	294.82 (Df = 29)	

Tab 6 – Results of Generalized Linear Models (GLM). Response variable (male sexual expression), predictor variables (Population density). *** < 0.0001.

Generalized Linear Models (GLM) **Response variable as male sexual expression**		
	Df	**Deviance**
Populational density	1	298***
Observations	300	
Log Likelihood	-1,356.703	
Akaike Information Criterion	NA	
Residual Deviance	644.59 (Df = 298)	
Null Deviance	877.09 (Df = 299)	

Figures

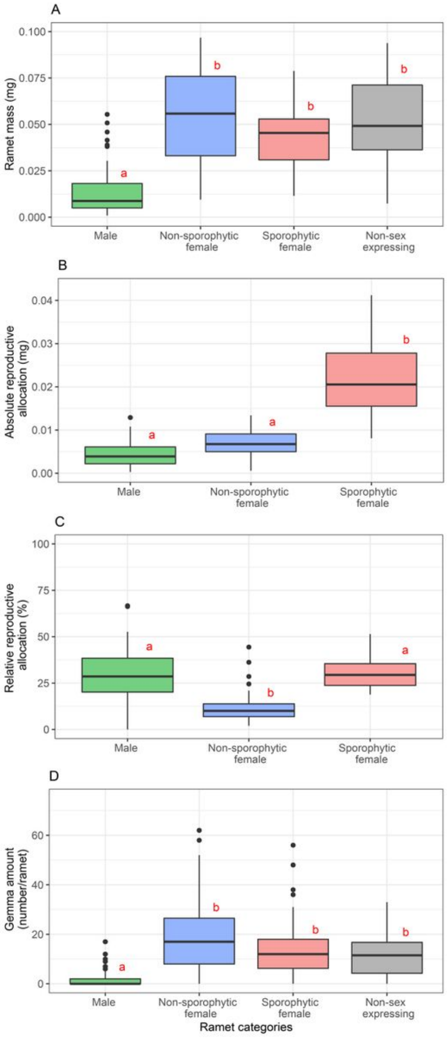

Figure 1

Graphs reporting A - ramets biomass; B - absolute reproductive allocation; C - relative reproductive allocation and D - amount of gemma produced. The letters indicate the significance parameters.

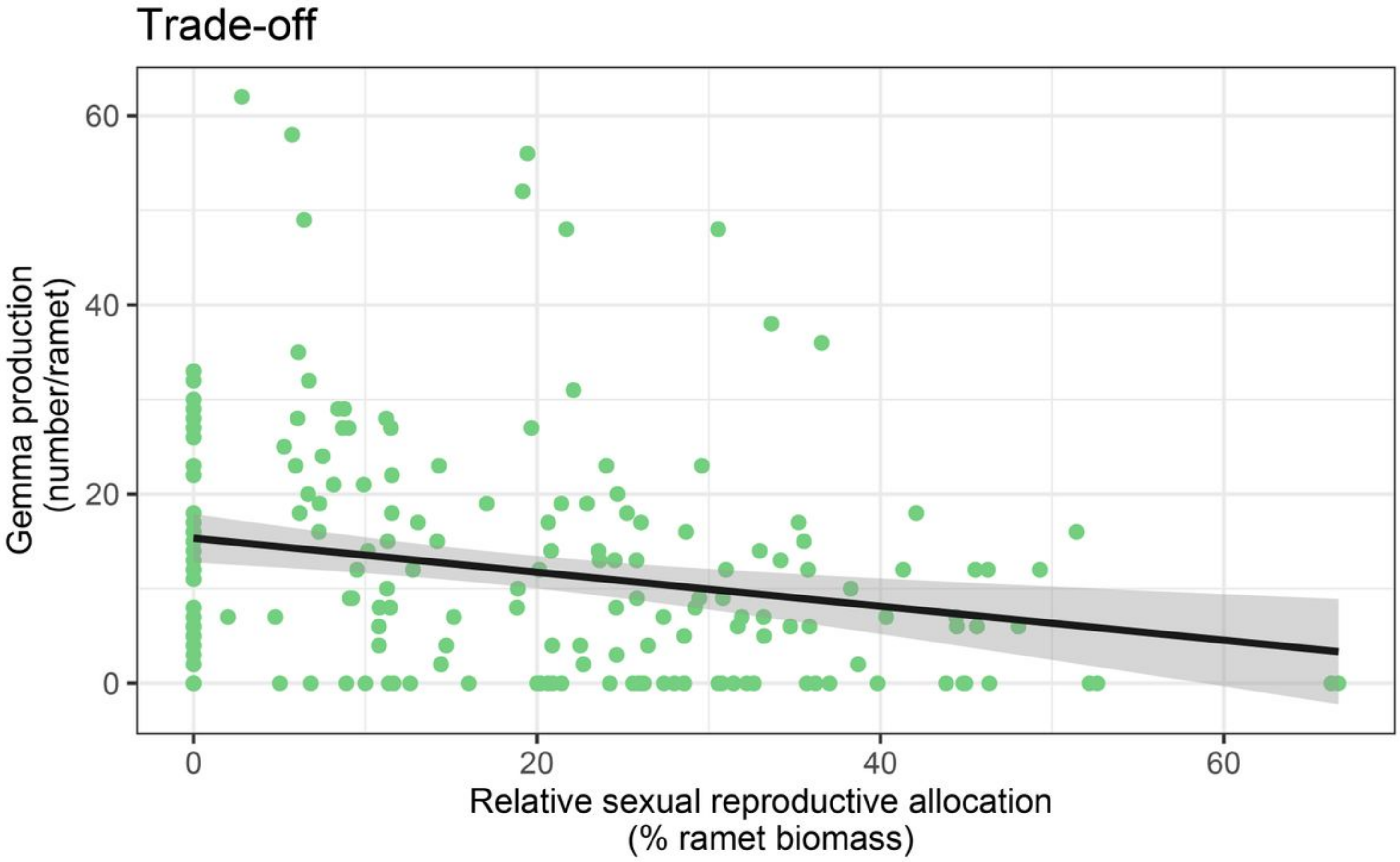

Figure 2

Graphs reporting A - ramets biomass; B - absolute reproductive allocation; C - relative reproductive allocation and D - amount of gemma produced. The letters indicate the significance parameters.

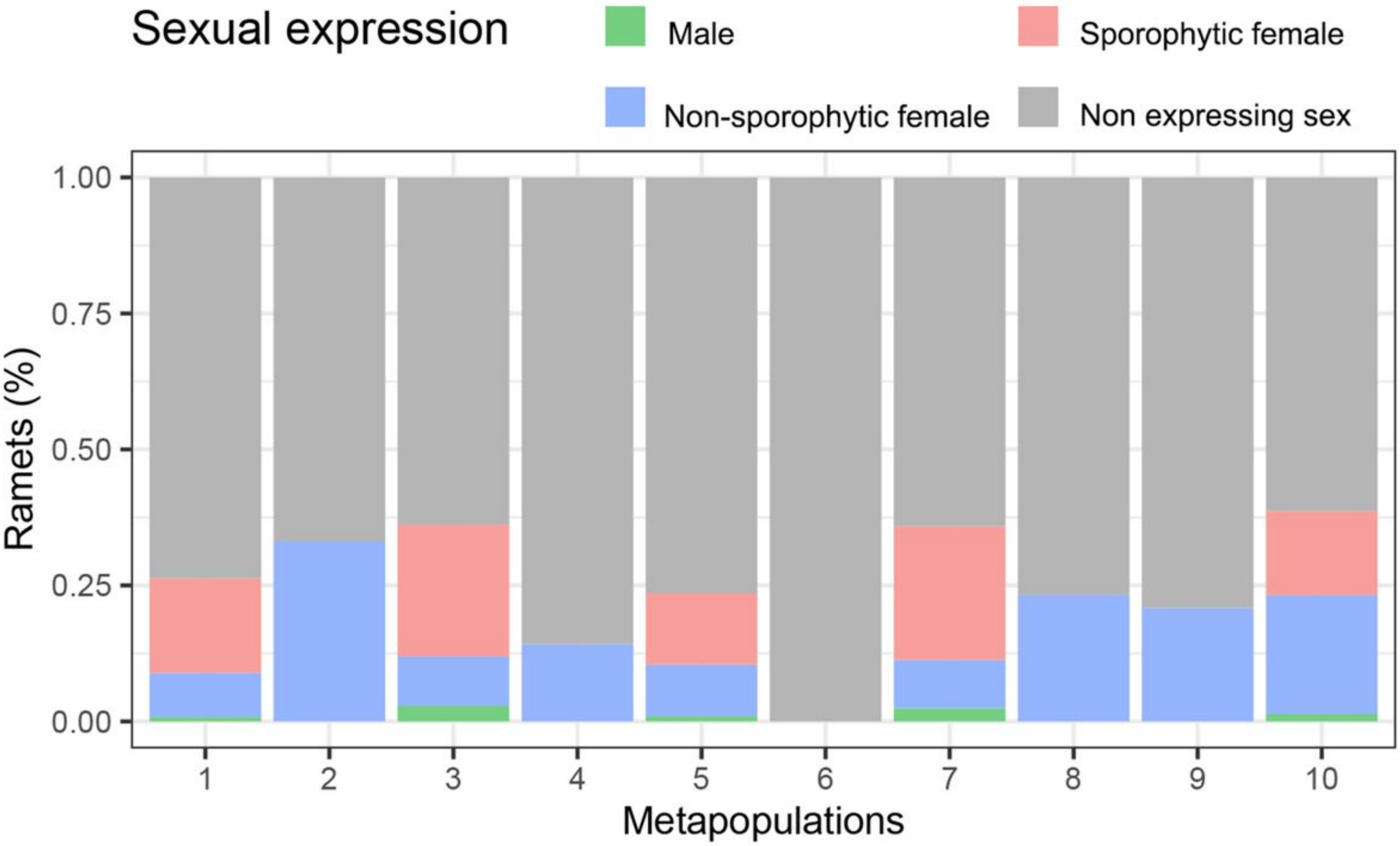

Figure 3

Sexual expression of rhizoautoicous moss *Fissidens flaccidus*. Percentage of ramets male, non-sporophytic female, sporophytic female, and non-expressing sex.

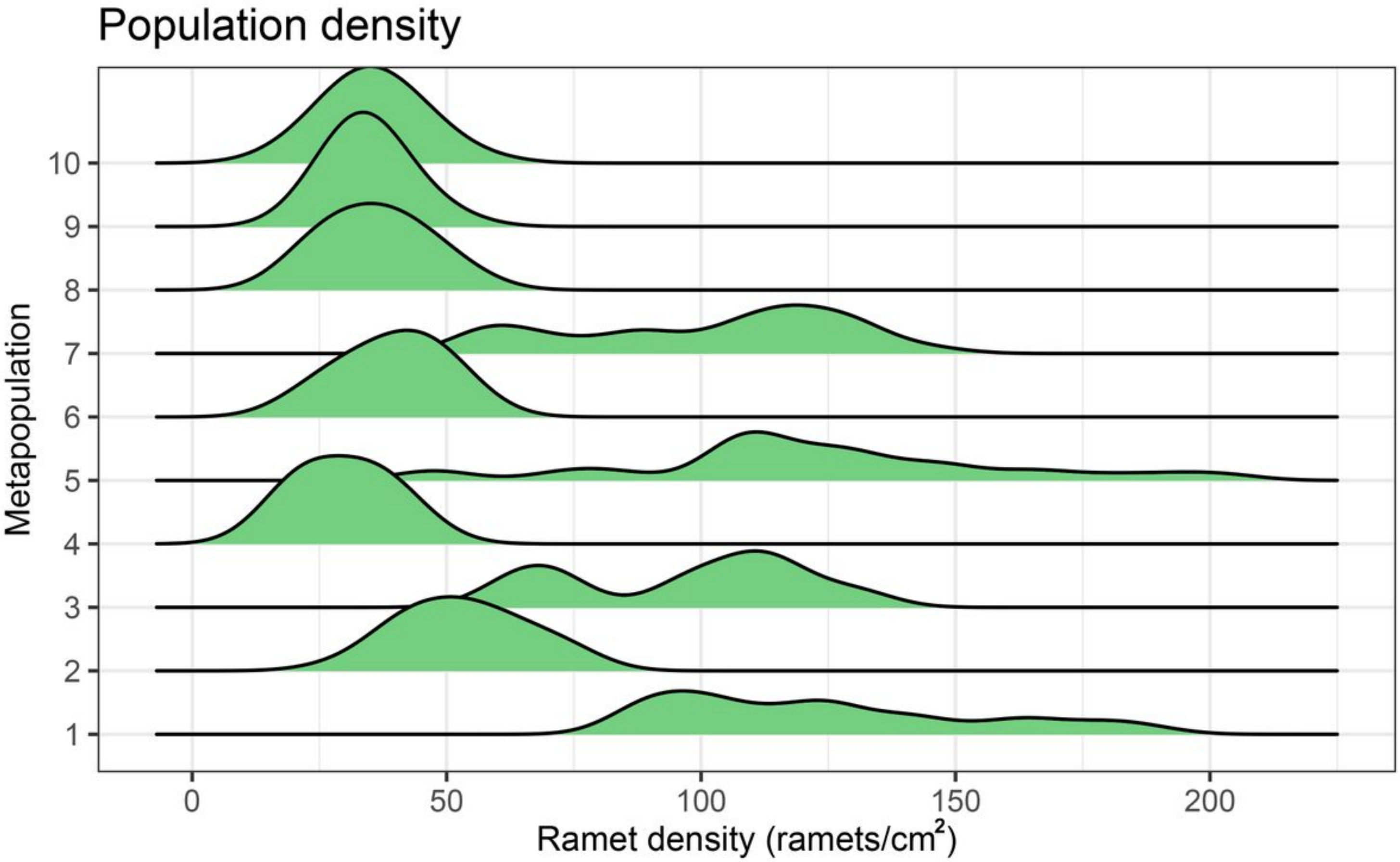

Figure 4

Density graphic show ramet density per metapopulation.

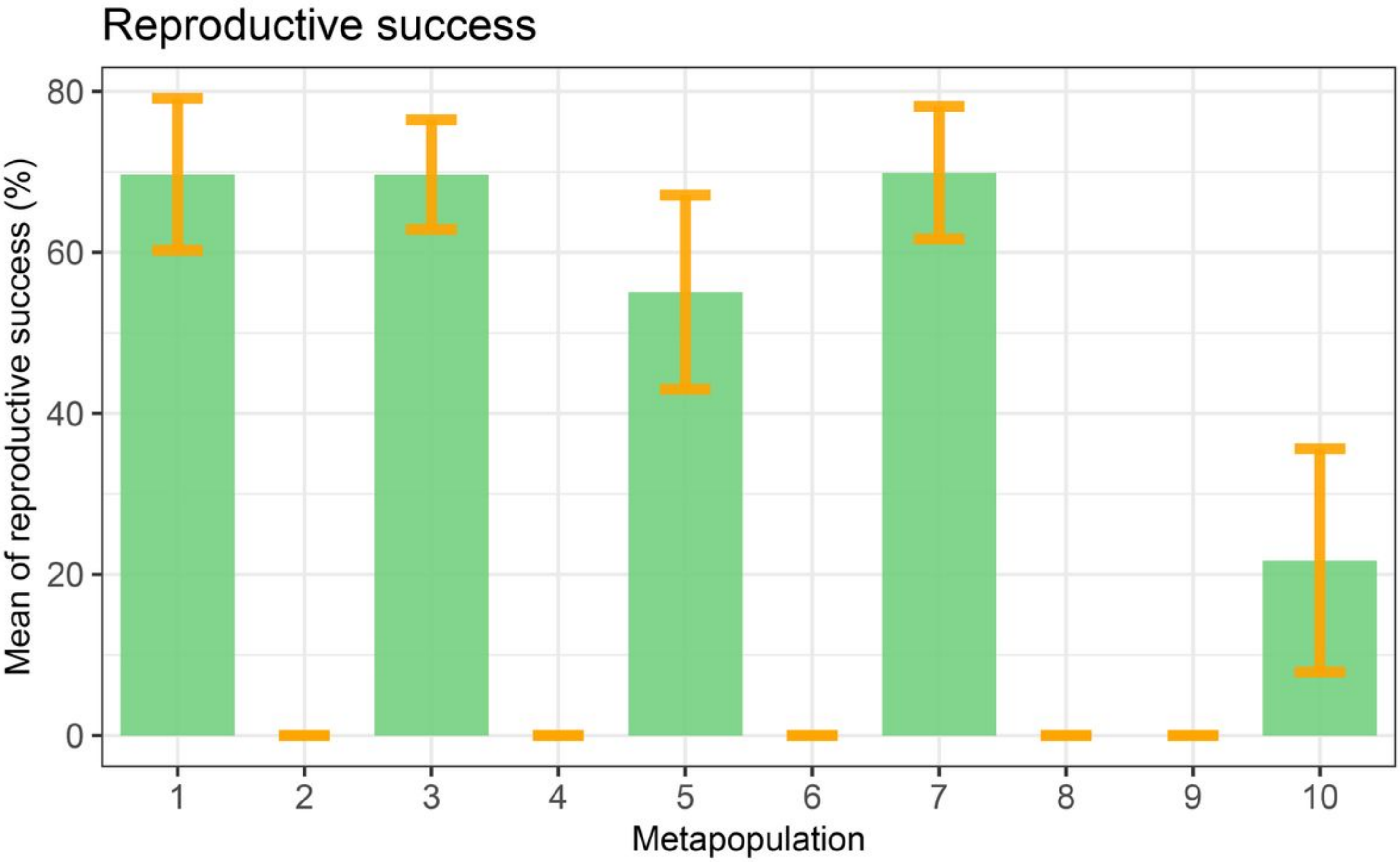

Figure 5

Barplot represent reproductive success by metapopulation with error bar.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable1Chisquere.docx