Altitude affects the reproductive performance in monoicous and dioicous bryophytes: examples from a Brazilian Atlantic rainforest

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Abstract

Background and aims	Short life cycles and trade-offs linked to breeding systems make bryophytes good models for the study of plant reproductive strategies. Our aim was to test if differences in sexual reproductive performance of bryophytes in tropical rainforests are driven by the breeding system of the species (monoicous or dioicous) or are mainly affected by the habitat.
Methodology	The reproductive performance (sexual branches, gametangia (sex organs), fertilization and sporophyte production) of 11 species was repeatedly monitored and analysed from populations at sea-level and montane sites of a Brazilian Atlantic rainforest over 15 months.
Principal results	Monoicous species had the highest reproductive performance, particularly for sexual branches, fertilized gametangia and sporophyte production. Species at the sea-level site produced more sexual branches and had more female-biased sex ratios of gametangia than species in the montane site. Fertilizations were more frequent at the montane site, but sporophyte frequency was similar between the two sites. Fertilization tended to occur mostly in the periods of heavy rain (October to December).
Conclusions	Breeding system is not the only major influence on the reproductive performance of bryophytes. We show that habitat is also an important factor determining life-history differentiation. Female-biased sex ratios and low rates of fertilization are seen to be compensated for by high production of reproductive structures at the initial phases of the reproductive cycle.

Introduction

The regenerative phase (Grime 2001) is crucial in the life cycle of plants and, since young individuals must find safe sites to establish and survive (Harper *et al.* 1965), the reproductive phase is subject to strong selective forces. Different reproductive strategies have been developed among plants by means of both asexual and sexual types of reproduction, in accordance with their life histories and the environment in which they live (Bengtsson and Ceplitis 2000; Obeso 2002). In general, the reproductive strategies based on long life-span are related to low growth rate and low reproductive

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effort, while rapid development and high reproductive effort are linked to short lifespan (Grime 2001; García *et al.* 2008). Other traits can be related directly or indirectly to the longevity of plants, such as breeding systems, seasonality of reproduction and formation of diaspore banks (During 1979; Grime 2001), where the probability and frequency of reproductive events generally decrease with increasing lifespan.

Plant life-history traits change along environmental gradients, sometimes within short distances, involving growth and reproductive parameters (Hassel *et al.* 2005; von Arx *et al.* 2006; Hautier *et al.* 2009; Milla *et al.* 2009). Different strategies permit plants to explore a range of distinct habitats, but not all variations in growth and reproductive traits result in different ecotypes (Reynolds and McLetchie 2011). It has been reported that species at high altitude invest more in growth than in reproduction (a conservative approach), while species at low altitude tend to invest more in reproduction (von Arx *et al.* 2006; Hautier *et al.* 2009). These findings are often related to the more severe conditions in the high mountains, with low temperatures, a period of snow cover and low productivity (Körner 2007). We do not know if they apply to altitudinal gradients of tropical areas with less severe conditions at higher altitudes.

Tropical rainforests are extremely threatened environments with high richness and diversity of species (Gómez-Pompa *et al.* 1972; Murray-Smith *et al.* 2009). For instance, the Atlantic forest in Brazil currently has less than 16 % of its initial cover (Ribeiro *et al.* 2009), and is restricted to small forest fragments and a few large nature reserves. Areas with complete altitudinal gradients of forest from sea level to the mountain tops are now very scarce (e.g. Atlantic Forest covering the 'Serra do Mar' in southeastern Brazil). The Brazilian Atlantic forest retains a large number of plant species, with a total of ∼15 800 (7155 endemic species), of which 1230 are bryophytes (Stehmann *et al.* 2009).

Bryophytes are a large and important component in tropical rainforests, covering substrates such as tree bark, leaves and decaying wood, and contributing to the high species richness and diversity (Whitmore *et al.* 1985; Frahm and Gradstein 1991). They also affect ecosystem functions (nutrient and water cycling, and habitat for micro fauna—Nadkarni 1984; Schofield 1985; Veneklaas 1990; Turetsky 2003). Since these plants (liverworts, mosses and hornworts) have a dominant haploid generation, reduced size, similar types of breeding system in common, and in general a shorter life cycle than seed plants (Glime 2007), they are excellent models to study factors affecting sexual reproduction.

Absence of males or female-biased sex ratios are common among dioicous bryophytes (Longton and Schuster 1983; McLetchie 2001; Stark 2002), though population sex ratios around 1 : 1 are also recorded in the literature (*Marchantia inflexa* studied by McLetchie and Puterbaugh 2000). Specifically, they can be used to understand life-history strategies involving the reproductive performance of, for example, monoicous and dioicous species in different habitats of the same ecosystem. Our overall aim was to test whether differences in sexual reproductive performance of bryophytes in tropical rainforests are driven by the breeding system of the species (monoicous or dioicous), or are mainly affected by the habitat.

Since monoicous species can self-fertilize and therefore tend to produce sporophytes more frequently than dioicous ones (Gemmell 1950; Rohrer 1982; Longton and Schuster 1983; Longton 1992), we expected that monoicous species have higher production of gametangia (i.e. sex organs: ♂—antheridia, ♀—archegonia), fertilization rate and sporophyte production than dioicous species. We also expected to find differences in these features within species or between closely related species among sites, since reproduction can be differently triggered by abiotic factors (e.g. light, temperature and humidity; see Chopra and Bhatla 1983; Kumra and Chopra 1983; Longton 1990). We studied the production of sexual branches and gametangia, fertilization and sporophyte production of bryophytes in two contrasting sites of Atlantic tropical rainforests (montane and sea level) in Brazil. We specifically investigated the following questions:

(1) Do monoicous and dioicous species differ in their sexual reproductive performance?
(2) If so, at what stages of reproduction do the differences occur (production of male and female structures, fertilization, sporophyte production)?
(3) Do the same, or closely related, species differ in sexual performance between habitats?
(4) Is there a seasonal variation in reproduction?
(5) Do abiotic factors such as pH and moisture of substrates influence reproduction?

Materials and methods

### Study sites

We selected two different altitudinal sites of an Atlantic ombrophilous dense forest (Veloso *et al.* 1991; Alves *et al.* 2010), located in the 'Serra do Mar' State Park, Brazil. The low-altitude forest is known locally as 'Restinga forest', a close-to-the-sea and seasonally flooded forest, referred to in our study as 'sea-level site' (Núcleo Picinguaba, 23°17′–34′S and 45°02′–11′W).

The high-altitude forest (~1000 m a.s.l.) is referred to as 'montane site' (Núcleo Santa Virgínia, 23°17′–24′S and 45°03′–11′W). For more details regarding the features of the study sites, see Maciel-Silva *et al.* (2012).

Monthly or bimonthly measurements of minimum and maximum temperatures were taken in the understorey of the two sites, using two max–min thermometers at each site. These measurements were taken from September 2007 to December 2009. We used precipitation data from two meteorological stations [Instituto Nacional de Pesquisas Espaciais (INPE)] next to the study sites (~100 m from the sea-level site and ~10 km from the montane site; Plataforma de coleta de dados,

Centro de Previsão de Tempo e Estudos Climáticos—CPTEC/INPE; Projeto 'Estudos da Previsibilidade de Eventos Meteorológicos Extremos na Serra do Mar').

The temperature in the montane site has a mean of 17 °C, and ranges between 4 °C in the winter and 25 °C in the summer, whereas in the sea-level site it has a mean of 23 °C, and ranges between 12 °C in the winter and 32 °C in the summer (Fig. 1A and B). The annual mean precipitation in the montane site is >2000 mm and in the driest months (June to August) the monthly precipitation is about 60 mm (Setzer 1966; Fig. 1A), and the vapour pressure deficit (VPD) of the site is 0.37 kPa (Maciel-Silva *et al.* 2012). In the sea-level site
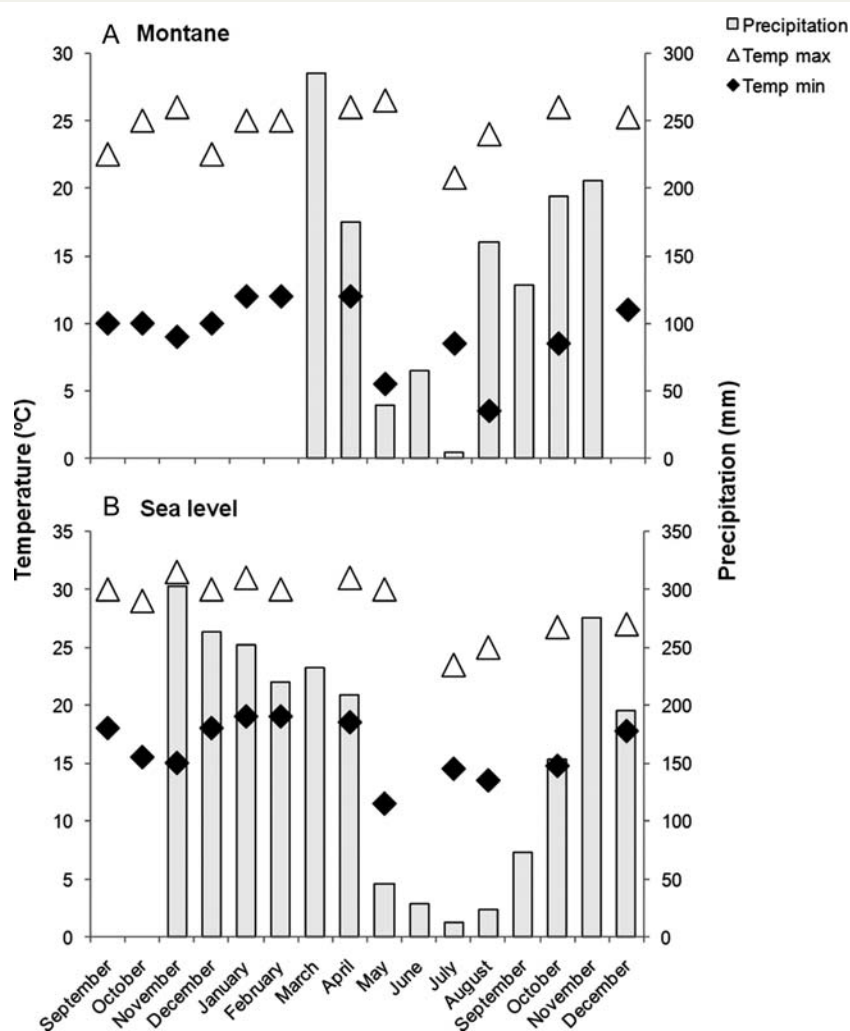
Fig. 1 Maximum and minimum monthly temperatures (°C) and precipitation (in millimetres per month) from montane (A) and sea-level (B) sites (September 2007 to December 2008). Precipitation data are from meteorological stations; note that data were not available for all months at both sites. (Source: 'Plataforma de coleta de dados, Centro de Previsão de Tempo e Estudos Climáticos—CPTEC/INPE' for precipitation data.)

the annual mean precipitation is >2200 mm and in the driest months about 80 mm (Setzer 1966; Fig. 1B), and the VPD is 0.44 kPa (Maciel-Silva *et al.* 2012). The air relative humidity is similar in these two sites, but the montane site is more shaded at the understorey level than the sea-level site, and the montane site has a lower photosynthetically active radiation and red : far red ratio (Maciel-Silva *et al.* 2012).

Sampling and study species

At each forest site (sea-level and montane) we established one area (10 000 m^2) sub-divided into 100 plots of 100 m^2 each. We initially collected 244 and 167 bryophyte samples in the sea-level and montane sites, respectively, from bark and decaying wood in the majority of the 100-m^2 plots. This was used as a basis to select study species according to the occurrence, breeding system (Gradstein *et al.* 2001 and references therein) and phylum (Bryophyta—mosses and Marchantiophyta—liverworts) (Table 1). We characterized the mosses as pleurocarpous (lateral perichaetia) and acrocarpous (terminal perichaetia) according to the growth form (La Farge-England 1996). Here we considered the cladocarpous moss *Pyrrhobryum spiniforme* as pleurocarpous, since it has one or more capsules along the main stem. To cover the variation in these attributes we selected 11 species from seven genera of mosses and liverworts (Table 1). When one species did not appear or was very rare at one of the sites, two congeneric species were chosen with the same breeding system and growth form.

Five to six colonies of each species and site were selected and sampled bimonthly during 15 months (from October 2007 to December 2008). Colony size varied from ~100 cm^2 for liverworts to 1000 cm^2 for mosses. We selected colonies of the same species at least 5 m apart. From each colony, squares of at least 4 cm^2 for liverworts and 9 cm^2 for mosses were collected (put in paper bags and carried to the laboratory). The samples contained both plants and a substrate. At each sampling time, the samples were taken from different parts of the colony to minimize disturbance (Laaka-Lindberg 2005).

We randomly picked out 10 shoots per colony in October 2007, and six shoots in the other months. We carefully cleaned these shoots, removing fragments of other bryophyte species and leaf litter. The shoots were air-dried in the laboratory (temperature around 20–27 °C, air relative humidity 60 %), and kept in paper bags up to the weighing process (to avoid damaging the gametangia we did not oven-dry these plants). The number of sexual branches and sporophytes per mass was assessed from December 2007 to December 2008, while gametangium production and fertilization were also assessed from the first sampling (October 2007). All variables were assessed on the same shoots and in the statistical analyses we used the mean values for the shoots in a colony.

Production of sexual branches

We weighed the shoots collectively from each colony using an analytical balance with an accuracy of 10 μg (Ohaus AP250D-0). With dissecting microscopy we counted their sexual branches (♂—perigonia, ♀—perichaetia and bisexual branches in some species). The production of sexual branches was expressed as number per mass.

Production of gametangia

From each shoot we assessed the number of gametangia (i.e. sex organs: ♂—antheridia, ♀—archegonia) per sexual branch on the actively developing gametophyte part, using dissecting and light microscopes. We also calculated the sex ratio of each species at both sites from

Table 1 Phylum, growth form and breeding system of the bryophyte species studied at two sites of a Brazilian Atlantic rainforest.

Genus	Species		Phylum	Growth form	Breeding system
	Montane	Sea level			
Bazzania	*heterostipa*	*phyllobola*	Liverwort	–	Dioicous
Plagiochila	*martiana*	*disticha*	Liverwort	–	Dioicous
Leucobryum	*crispum*	*clavatum*	Moss	Acrocarpous	Dioicous
Leucoloma	*serrulatum*	*serrulatum*	Moss	Acrocarpous	Dioicous
Phyllogonium	*viride*	*viride*	Moss	Pleurocarpous	Dioicous
Neckeropsis	*undulata*	*disticha*	Moss	Pleurocarpous	Monoicous
Pyrrhobryum	*spiniforme*	*spiniforme*	Moss	Pleurocarpous	Monoicous

the mean number of female and male gametangia produced per sexual branch. We compiled literature data regarding the gametangia number (antheridia and archegonia) per sexual branch in monoicous and dioicous bryophyte species. We assessed gametangial sex ratios of each species in the two breeding systems.

Fertilization and production of sporophytes

We counted the total number of fertilized archegonia (with swollen venter) per sexual branch. To assess sporophyte production we counted the number of sporophytes per mass unit.

pH and moisture of substrates

Samples of substrates (bark and decaying wood) of each species were collected at both sites (only for December 2008—rainy season), put in plastic bags, sealed and brought to the laboratory as rapidly as possible. A part of each sample was separated to measure the pH. We used around 1 g suspended in 5 mL of deionized water overnight at 27 °C (Watson *et al.* 1988; Farmer *et al.* 1990). The other part was immediately weighed (accuracy of 1 mg). These samples were dried for 3 days at 70 °C, and again weighed. The substrate moisture was expressed in per cent of fresh mass. Since preliminary analyses showed that gametangium production was the response most influenced by habitat, we related the gametangium production at both sites to these measurements of pH and moisture.

Statistical analysis

To investigate the effect of site, breeding system, species (nested in breeding system) and time on the reproductive performance of the bryophytes, we performed repeated measures ANOVAs. For the variables 'production of sexual branches' and 'sporophyte production', the data were $\log_{10}$ transformed ($\log x + 1$) to achieve normality of residuals and homogeneity of variances. We performed the analyses considering the between-subjects effect (site, breeding system and species effects), the within-subjects effect (time effect) and interactions. To detect differences in the 'production of male and female gametangia' and 'fertilized female gametangia', the analyses were performed considering the between-subjects effect (site and breeding system effect), the within-subjects effect (time effect) and interactions. However, since some species did not produce gametangia during our study, we were not able to test the effect of species for the variables 'male and female gametangia' and 'fertilized female gametangia'. The post hoc Tukey test was used to detect differences between species within each site. Student's *t*-tests were used to detect differences within species pairs

between sites [see Additional Information—Table 1]. Chi-squared test was used to compare the frequency of female and male gametangia for different species between sites and breeding systems (we only used data in species having both male and female gametangia). In addition, we also performed simple linear regressions to detect possible effects of pH and moisture on the production of female and male gametangia, fertilization and sporophyte production of monoicous and dioicous species in the two sites.

Results

An overview of the results is shown in Table 2, with ANOVA summaries in Table 3. Some aspects of reproduction varied between the sites and breeding system, and among species, and also showed clear phenological patterns (for detailed time variation see Fig. 2).

Production of sexual branches

Significant differences in the production of sexual branches were detected between the two sites (Table 3), with more sexual branches per mass produced at sea level than in the montane site (Table 2). Species and breeding systems differed in their production of sexual branches, and more sexual branches were found in monoicous than in dioicous species (Tables 2 and 3), a difference caused especially by the high number in the monoicous *Neckeropsis* and the low number in the dioicous *Leucobryum* and *Leucoloma* (Table 2). Significant differences were found between some congeneric pairs in the two sites, and except for *Phyllogonium* more sexual branches were produced in the sea-level site (Table 2). Production of sexual branches varied over time among the species (Table 3 and Fig. 2A).

Gametangium production

Sites, species and breeding systems did not differ regarding the number of male and female gametangia produced per sexual branch (Table 3). *Neckeropsis* had more female gametangia in the sea-level site, while *Phyllogonium* had more female gametangia in the montane site (Table 2). Production of female gametangia did not vary over time, but male gametangia tended to vary (Table 3, Fig. 2B and C); *Phyllogonium* and *Pyrrhobryum* mostly produced male gametangia in October to December (Fig. 2C).

Production of archegonia was higher than production of antheridia, especially in the sea-level site (montane: $\chi^2 = 10.16$, df $= 4$, $P = 0.038$; sea level: $\chi^2 = 20.47$, df $= 4$, $P < 0.001$; Table 2). The over-representation of archegonia was more evident in dioicous ($\chi^2 = 22.06$, df $= 5$, $P < 0.001$) than in monoicous species

Table 2 **Reproductive parameters measured in bryophytes in two sites of a Brazilian Atlantic rainforest.** Values are means $\pm$ standard deviation from four to six colonies in each species followed during 15 months regarding the number of sexual branches per mass, gametangia per sexual branch, gametangial sex ratio and number of fertilized gametangia per sexual branch, and number of sporophytes per mass (BS, breeding system; Di, dioicous; Mo, monoicous; M, montane site; SL, sea-level site). Significant differences in reproductive variables between the two sites are shown for all species (Student's *t*-tests, see Additional Information—Table 1; n.s. $P > 0.08$; [†]$P < 0.08$ (marginally significant); *$P < 0.05$; ** $P < 0.001$; n.a., *t*-test not applicable).

Genus	BS	Site	Number of sexual branches (cg^{-1})	Female gametangia per sexual branch	Male gametangia per sexual branch	Gametangial sex ratio F : M	Fertilized gametangia per sexual branch	Number of sporophytes (cg^{-1})
Bazzania	Di	M	2.92 $\pm$ 2.80	8.4 $\pm$ 0.4	4.3 $\pm$ 0.97	1.9	0.06 $\pm$ 0.11	0
		SL	10.21 $\pm$ 9.33[†]	0 n.a.	4.4 $\pm$ 0.3 n.s.	Only ♂	– n.a.	0 n.a.
Plagiochila	Di	M	5.27 $\pm$ 1.31	11.9 $\pm$ 0.6	13.0 $\pm$ 2.7	0.9	0.85 $\pm$ 1.03	0.26 $\pm$ 0.54
		SL	7.85 $\pm$ 3.26 n.s.	21.9 $\pm$ 15.5 n.s.	17.7 $\pm$ 14.1 n.s.	1.2	0.005 $\pm$ 0.01 n.s.	0.21 $\pm$ 0.32 n.s.
Leucobryum	Di	M	0	0	0	No sex	–	0
		SL	0.68 $\pm$ 0.65*	7.4 $\pm$ 0.8 n.a.	0 n.a.	Only ♀	– n.a.	0 n.a.
Leucoloma	Di	M	0.82 $\pm$ 1.39	17.9 $\pm$ 4.9	7.6	2.3	0	0
		SL	1.27 $\pm$ 1.02 n.s.	24.5 $\pm$ 6.7 n.s.	0 n.a.	Only ♀	0.16 $\pm$ 0.41 n.s.	0 n.a.
Phyllogonium	Di	M	4.74 $\pm$ 3.00	15.3 $\pm$ 5.8	9.8 $\pm$ 4.8	1.5	0.03 $\pm$ 0.07	0.23 $\pm$ 0.37
		SL	1.70 $\pm$ 0.81*	7.5 $\pm$ 0.8*	7.9 $\pm$ 2.5 n.s.	0.9	0 n.a.	0 n.a.
Neckeropsis	Mo	M	5.08 $\pm$ 2.05	8.3 $\pm$ 0.3	6.6 $\pm$ 1.3	1.2	0.14 $\pm$ 0.06	0.43 $\pm$ 0.25
		SL	9.32 $\pm$ 4.18[†]	11.4 $\pm$ 1.3**	7.4 $\pm$ 0.7 n.s.	1.5	0.10 $\pm$ 0.07 n.s.	1.42 $\pm$ 0.98[†]
Pyrrhobryum	Mo	M	2.26 $\pm$ 1.36	13.9 $\pm$ 4.3	12.1 $\pm$ 3.8	1.1	0.16 $\pm$ 0.08	0.49 $\pm$ 0.63
		SL	4.09 $\pm$ 1.57[†]	17.0 $\pm$ 2.7 n.s.	9.6 $\pm$ 2.8 n.s.	1.8	0.06 $\pm$ 0.06*	0.38 $\pm$ 0.37 n.s.

($\chi^2 = 8.57$, df $= 3$, $P = 0.035$). The sex ratio of gametangia was female biased for the majority of species (Table 2). *Bazzania*, *Leucobryum* and *Leucoloma* produced only one sex at sea level, and *Leucobryum* had neither male nor female gametangia at the montane site (Table 2).

From the literature data, bryophyte monoicous species generally had more balanced sex ratios of gametangia (M : F sex ratio $= 1.77 \pm 1.08$) compared with dioicous ones (M : F $= 5.28 \pm 6.19$; see Additional Information— Table 2). Overall for bryophyte species, there were more male gametangia than female ones produced per sexual branch (i.e. male-biased sex ratio) in dioicous species ($\chi^2 = 544.82$, df $= 18$, $P < 0.001$), and to some degree in monoicous ones ($\chi^2 = 54.62$, df $= 19$, $P < 0.001$).

Fertilization and sporophyte production

There were effects of site and breeding system on fertilization (Table 3), mostly due to high fertilization in the monoicous *Pyrrhobryum* in the montane site (Table 2). In general, fertilization was high for the monoicous *Pyrrhobryum* and *Neckeropsis* at the two sites, whereas the dioicous *Plagiochila* had notable fertilization only in the montane site. Fertilizations were rarely recorded in

the dioicous *Bazzania*, and not at all in *Leucobryum*. Fertilization time did not vary significantly, but there were tendencies for higher rates in October to December, and sporadically in other months (Fig. 2D, with a peak for *Plagiochila* at the montane site in April caused by one deviating observation).

Overall, the number of sporophytes per mass did not differ between the sites (Table 3) with the exception of *Neckeropsis*, in which the number of sporophytes was higher at sea level (Table 2). Sporophytes were more frequent in monoicous species (*Neckeropsis* and *Pyrrhobryum*), with some dioicous species failing to develop sporophytes (Table 2). Time had no significant effect on sporophyte production, but the dioicous *Phyllogonium* and *Plagiochila* tended to have sporophytes mostly during April, June and December, and the monoicous *Pyrrhobryum* and *Neckeropsis* had sporophytes all year round (Fig. 2E).

Effect of pH and moisture

pH in the sea-level bark was lower than that in the montane site (mean $\pm$ standard deviation: sea level 3.8 $\pm$ 0.61; montane 4.4 $\pm$ 0.16), and moisture was

Table 3 **Summary of the repeated measures ANOVAs for sexual features of bryophyte species in two sites of a Brazilian Atlantic rainforest.** The response variables 'Sexual branches per mass' and 'Sporophytes per mass' were transformed as $\log_{10}(x + 1)$ prior to analysis.

Source	Repeated measures ANOVA			
	df	MS	F	P
Sexual branches per mass				
Between-subjects effects				
Site	1	2.39	7.78	0.007
Species (breeding system)	5	3.14	10.24	<0.001
Breeding system	1	7.45	24.29	<0.001
Error	48	0.30		
Within-subjects effects				
Time	6	0.16	2.12	0.051
Time × site	6	0.07	0.89	0.501
Time × species (breeding system)	30	0.21	2.67	<0.001
Time × breeding system	6	0.06	0.83	0.549
Error	288	0.08		
Male gametangia per sexual branch				
Between-subjects effects				
Site	1	15.00	0.39	0.573
Breeding system	1	15.02	0.40	0.573
Error	3	37.81		
Within-subjects effects				
Time	7	19.59	2.23	0.073
Time × site	7	8.75	0.99	0.461
Time × breeding system	7	3.38	0.38	0.900
Error	21	8.79		
Female gametangia per sexual branch				
Between-subjects effects				
Site	1	45.58	0.16	0.697
Breeding system	1	6.97	0.02	0.878
Error	6	273.88		
Within-subjects effects				
Time	7	4.31	0.36	0.917
Time × site	7	6.24	0.53	0.807
Time × breeding system	7	6.98	0.59	0.758
Error	42	11.79		
Fertilized gametangia per sexual branch				
Between-subjects effects				
Site	1	0.07	7.18	0.036

Continued

Table 3 *Continued*

Source	Repeated measures ANOVA			
	df	MS	F	P
Breeding system	1	0.07	6.87	0.039
Error	6	0.01		
Within-subjects effects				
Time	7	0.01	1.07	0.396
Time × site	7	0.01	0.47	0.846
Time × breeding system	7	0.01	1.24	0.303
Error	42	0.01		
Sporophytes per mass				
Between-subjects effects				
Site	1	0.005	0.09	0.761
Species (breeding system)	5	0.12	1.98	0.098
Breeding system	1	2.55	41.99	<0.001
Error	48	0.06		
Within-subjects effects				
Time	6	0.01	0.97	0.442
Time × site	6	0.006	0.56	0.757
Time × species (breeding system)	30	0.006	0.56	0.972
Time × breeding system	6	0.01	1.15	0.333
Error	288	0.01		

similar in the two sites (sea level 42.1 ± 17.8 %; montane 46.7 ± 16.1 %). Only gametangium production was affected by pH and moisture, albeit the effect was rather weak. pH had a significant but weak negative effect on the number of both male and female gametangia of monoicous species in the sea-level forest (male: $R^2 = 0.06$, $P = 0.050$; female: $R^2 = 0.07$, $P = 0.012$). The gametangial production of both sexes occurred from pH 4.4 to 5.6, decreasing with increasing pH. For dioicous species in the sea-level site, female gametangia increased with increasing pH ($R^2 = 0.31$, $P = 0.0008$). Moisture had a positive effect on the number of male and female gametangia of monoicous species at the sea-level site (male: $R^2 = 0.23$, $P = <0.001$; female: $R^2 = 0.27$, $P = <0.001$); male gametangia of monoicous species in the montane site increased significantly with increasing moisture ($R^2 = 0.07$, $P = 0.029$). For dioicous species in the sea-level site, female gametangia increased with increasing moisture ($R^2 = 0.61$, $P = <0.001$). For regression details see Additional Information (Table 3).

Discussion

Returning to the initial questions, our main findings were as follows. (i) The monoicous species had a generally higher reproductive performance, while (ii) some dioicous species failed in the production of sexual branches, fertilization or sporophyte production. (iii) Especially for sexual branches, several species performed better at sea level compared with the montane site. (iv) Species differed in the time of sexual branch production. (v) pH and moisture explained gametangium production, but not fertilization or sporophyte production, to some degree.

Influence of the breeding system on reproductive performance

Sex expression of bryophytes in tropical rainforests seems to be influenced by the breeding system, where some dioicous species fail to express one or both sexes and consequently fail to produce sporophytes. Some dioicous species in our study had high numbers of sexual branches
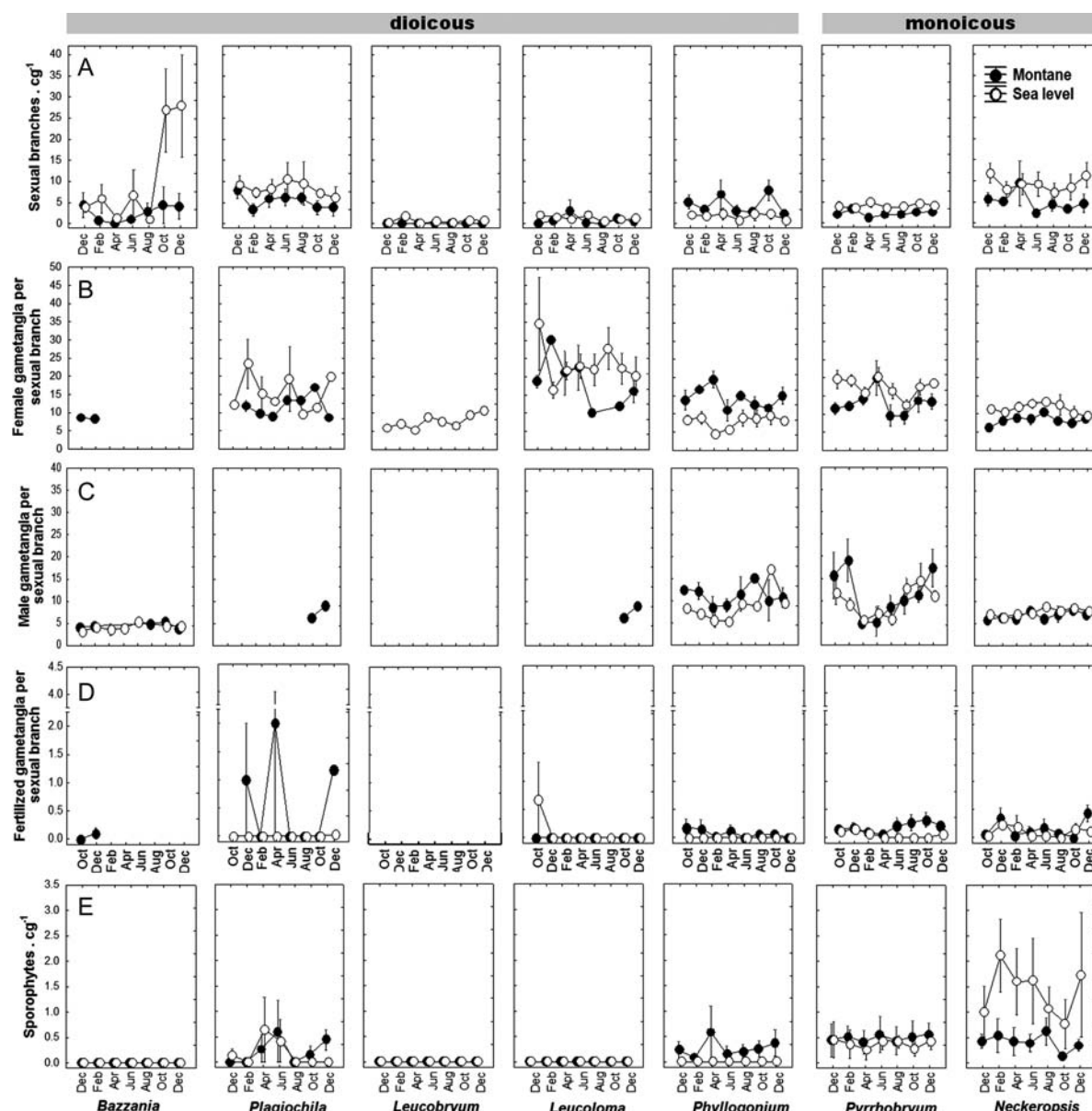
Fig. 2 Number (mean ± standard error) of sexual structures in dioicous and monoicous bryophyte species at two sites of a Brazilian Atlantic rainforest. Sexual structures are the number of sexual branches per mass (A), number of female (B) and male (C) gametangia per sexual branch, number of fertilized female gametangia per sexual branch (D) and number of sporophytes per mass (E). Note that data were not available for all species at both sites.

and gametangia, but still failed to produce large numbers of sporophytes (e.g. *Bazzania* and *Leucoloma* at the montane site). This failure is related to spatial separation among populations of the two sexes and absence of male plants (or failure to express sex among male plants—'shy male hypothesis'), supporting previous suggestions by Gemmell (1950), Longton and Schuster (1983), Bowker

et al. (2000), Oliveira and Pôrto (2002), Bisang and Hedenäs (2005), Stark *et al.* (2005).

Among bryophytes, dioicism is considered ancestral to monoicism, whereas the dioecism in seed plants is derived (Bawa and Beach 1981; Longton and Schuster 1983) (note that the suffix '-oicism' is used instead of '-oecism' to distinguish between gametophytic and

sporophytic breeding systems; Allen and Magill 1987; Mishler 1988). In seed plants, the evolution of the pollination mechanisms selects strategies that favour genetic recombination (Bawa 1980; Bawa and Beach 1981), but in bryophytes the water dependence of the sperm to perform fertilization establishes trade-offs. The relationship of fertilization likelihood (and offspring output) versus genetic recombination is generally opposite for monoicous and dioicous bryophytes. Monoicous species frequently have more sporophytes than dioicous ones, but inbreeding events are more common among monoicous species (Longton and Schuster 1983; Longton 1992, 1997; Eppley *et al.* 2007). Since the breeding likelihoods can be low for dioicous bryophytes, these plants should compensate by a longer lifespan compared with monoicous ones (During 1979; Longton 1997).

Our findings agree with the literature data, with bryophyte monoicous species generally having more balanced sex ratios of gametangia compared with dioicous ones. More male gametangia may guarantee higher fertilization due to many sperm reaching archegonia (Glime 2007). Trade-offs and costs related to production of gametangia, fertilization and sporophyte development may explain the investments in sexual reproduction and vegetative growth of bryophytes (Rydgren *et al.* 2010). The production of male gametangia, compared with female ones, seems to be more expensive for bryophyte shoots (Stark *et al.* 2000, 2005), but sporophyte-bearing shoots have more costs to support developing sporophytes. In our study, monoicous and especially dioicous species have proportionally more female than male gametangia per sexual branch, suggesting possible costs related to different phases in the life cycle.

The monoicous mosses *Pyrrhobryum* and *Neckeropsis* are able to produce many sexual branches compared with the dioicous mosses *Leucobryum* and *Leucoloma*. However, *Leucobryum* and *Leucoloma* have an efficient means of asexual reproduction by regeneration of detached leaves (A. S. Maciel-Silva, unpublished data). Trade-offs between sexually produced spores that are small and easily dispersed and vegetative propagules that are larger and have higher establishment probability have previously been stressed in bryophytes (Löbel and Rydin 2009). Asexual propagules must be important for the propagation of these species, since sexual reproduction is rare in the two forest sites investigated.

The breeding system is not the only factor acting on the sexual reproduction of the studied bryophytes. The growth form seems to be important to characterize different strategies, where some of the dioicous pleurocarpous mosses and liverworts produce several reproductive branches per shoot while acrocarpous species do not. The former strategy is based on increased breeding likelihoods by means of investment in more sexual branches.

The role of the habitat in reproductive performance

Sexual reproductive performance of bryophytes varied between the sites. There is a higher production of sexual branches per mass at sea level, and female gametangia are more numerous than male ones, especially at sea level. Conversely, there is a higher frequency of fertilization in the montane site. The reproductive strategy of bryophytes at sea level is based on more investment in sexual reproduction (sexual branches) than at the montane site, and this could to some extent counteract the negative effects of the female-biased sex ratio of gametangia at sea level.

Different strategies in the same or closely related species along altitudinal ranges are commonly recorded. There is generally an important role of trade-off between reproductive investment and vegetative growth, as, for example, plants at higher altitudes often have low reproductive effort (Hassel *et al.* 2005; von Arx *et al.* 2006; Hedderson and Longton 2008; Hautier *et al.* 2009). Functional explanation involving growth versus reproduction trade-offs and selection to maintain the population growth or to establish new individuals are linked to distinct plant life histories at different altitudes.

In temperate and boreal areas, the abiotic conditions of mountains tend to be harsher than in the lowlands, mostly due to low temperatures (Körner 2007), favouring plants with conservative strategies (i.e. more investment in growth compared with reproduction). The climate of the montane site in the present study cannot be considered harmful for bryophytes, but rather has temperatures and water availability that would favour the growth and reproduction of bryophytes. In contrast to the montane site, the temperatures are elevated at sea level, reaching >30 °C in the summer. During the winter, which is an important period for induction of gametangia (primarily male) (Maciel-Silva and Válio 2011), the temperatures are around 20 °C in the montane and 25 °C at sea level. Since high temperatures can promote the growth of reproductive structures for one or both sexes (Chopra and Bhatla 1981; Chopra and Rahbar 1982), the higher reproductive performance at sea level may be related to the high temperature in addition to water and light availability (Maciel-Silva *et al.* 2012).

Although the timing of fertilization varies, there is an influence of the rainiest period on fertilization at both sites. Other studies in tropical rainforests confirm the importance of the rainiest period for fertilization in

bryophytes (Oliveira and Pôrto 1998, 2001, 2002). Invertebrate-mediated fertilization in bryophytes is possible without a water film (Cronberg *et al.* 2006). Such interactions between bryophytes and invertebrates may also have an influence in tropical rainforests, but unassisted fertilization is probably more important in these generally moist habitats.

pH and moisture may clarify some constraints on gametangium production of bryophytes (albeit the relationships are moderate to weak in this study). The moistest substrates supported high numbers of gametangia compared with the driest substrates, demonstrating the importance of water availability for the success of gametangium formation (Egunyomi 1979). Microhabitats with high water-holding capacity (e.g. decaying wood) may support colonies producing many gametangia in the field. The effect of pH on gametangium production, observed in plants at sea level, is different for monoicous and dioicous species, where monoicous species are favoured by lower pH. Additionally, the spatial separation of sexes in dioicous species may be more important than the effect of moisture on fertilization and sporophyte production (Stark *et al.* 2005),

explaining the low number of sporophytes still in species with high gametangium production in this study.

Different reproductive strategies based on breeding system and habitat

Figure 3 gives a conceptual overview of the differences between breeding systems, the two habitats and over time. Whereas plants in the montane site have a reproductive strategy based on fewer sexual structures, a higher reproductive success (high fertilization) was secured in the species by a rather even gametangial sex ratio. Conversely, plants at sea level invest quantitatively in the initial phases of the sexual reproductive cycle (sexual branches), but have lower fertilization success, mostly due to more female-biased sex ratios of the gametangia. Our results suggest that sea-level plants invest more heavily in sexual branches, but as a result of differently successful fertilization, the final reproductive effort (as reflected by sporophyte production per mass) did not differ between the sites. Monoicous species, being independent of shoot sex ratio or spatial segregation of sexes, had high sporophyte production; some dioicous species fail to produce sporophytes
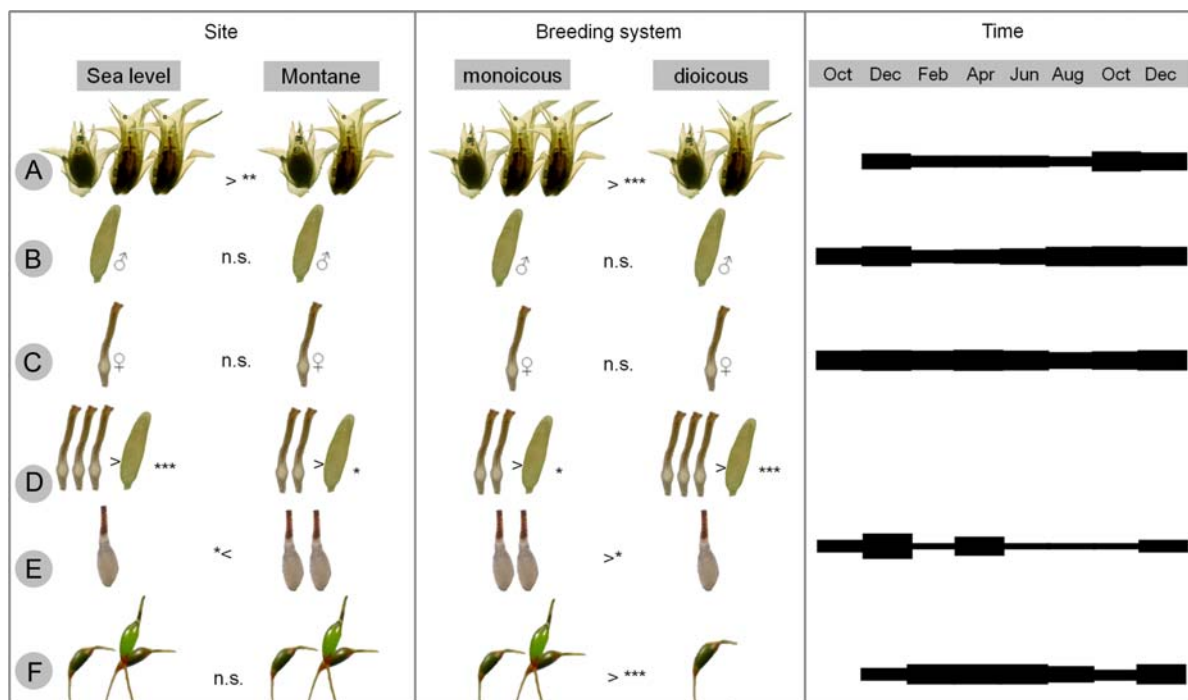
Fig. 3 Summary of the sexual reproductive performance of bryophytes in a tropical rainforest, based on effects of site (Montane and sea level), breeding system (monoicous and dioicous species) and time (different months). (A) Sexual branches; (B) male gametangia; (C) female gametangia; (D) female versus male gametangia; (E) fertilized gametangia; (F) sporophytes. Different numbers of structures denote differences (*P < 0.05, **P < 0.01, ***P < 0.001). Seasonal tendencies are evidenced by different thicknesses of bars.

despite having gametangia. Female gametangia are more numerous than male ones in monoicous and especially dioicous species, contributing to low fertilization rates and few sporophytes.

The difference between the breeding systems can also have secondary population effects, such as a high contribution of monoicous species in the diaspore bank (same study area, see Maciel-Silva *et al.* 2012). Similarly, Caners *et al.* (2009) found that the most frequent species (i.e. high establishment frequency) in a boreal diaspore bank were monoicous mosses.

Seasonal tendencies suggest that the months with heaviest rainfall (increasing moisture) are important for triggering different phases in the life cycle of tropical bryophytes, such as sexual branches and male gametangia, and mostly fertilization.

Conclusions and forward look

Reproductive performance in bryophytes depends on the habitat, including factors such as temperature, moisture, light and pH; and intrinsic traits of the species, such as breeding system (monoicy and dioicy), phylum (mosses and liverworts) and growth form (acrocarpous and pleurocarpous). All these factors are important to determine life-history differentiation. Biased sex ratios of the gametangia are commonly recorded in bryophytes, and mostly in dioicous species. Species with female-biased sex ratios and low rates of fertilization can be compensated for by a large production of reproductive structures at the initial phases of the reproductive cycle.

Future studies with focus on phylogenetic approaches including many species (e.g. related species with different breeding systems), comparisons among sexual and asexual reproduction and clonal growth, and use of common garden conditions, should be encouraged to elucidate breeding system-related trade-offs and evolution of life-history strategies in bryophytes.

Additional information

The following additional information is available in the online version of this article:

Table 1. Summary of Student's *t*-tests for sexual features of bryophyte species between montane and sea-level sites of a Brazilian Atlantic rainforest.

Table 2. Records of female and male gametangia per sexual branch in monoicous and dioicous bryophyte species (values are mean numbers of gametangia per sexual branch; in some cases, when ranges were given, we used the maximum values).

Table 3. Summary of linear regressions for male and female gametangia per sexual branch of monoicous

(mo) and dioicous (di) bryophyte species in montane (M) and sea-level (SL) sites of a Brazilian Atlantic rainforest.

Contributions by the authors

The research idea was developed by A.S.M.-S. and I.F.M.V.; A.SM.-S. collected the data, performed the statistical analyses and was the lead author in collaboration with H.R.

Conflict of interest statement

None declared.

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