

# Prezygotic resource-allocation dynamics and reproductive trade-offs in Calymperaceae (Bryophyta)<sup>1</sup>

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**PREMISE OF THE STUDY:** Resource allocation is difficult to characterize in plants because of the challenges of quantifying gametes and propagules. We surveyed six sympatric, unisexual species in the family Calymperaceae (Bryophyta) to test for trade-offs in prezygotic sexual and asexual expression and density-dependent survivorship of female gametangia.

**METHODS:** We tallied gametangial and asexual propagule output for 1820 shoots from 17 populations of six species at monthly intervals during one year (2010–2011) in a central Amazonian forest. Generalized linear mixed models were used to test for trade-offs in sexual and asexual expression and density-dependent senescence probability of gametangia. Precipitation and microsite variables were also included in the model.

**KEY RESULTS:** For all species, sexual and asexual expression were positively correlated with mean monthly precipitation. Asexually expressing shoots produced significantly fewer gametangia than nonexpressing ones, and the probability of senescence increased with shoot density. Archegonium density per shoot was also consistently lower than the modeled optimum to maximize the number of receptive archegonia.

**CONCLUSIONS:** Trade-offs among reproductive strategies and positive density-dependent senescence of female gametangia suggest that prezygotic sexual and asexual expression come at a tangible investment. However, the apparently inefficient resource-allocation dynamics in the production of female gametangia makes the possible advantages of squandering such investments unclear. One possibility is that the study populations, like those of many dioicous mosses, are skewed toward expressing females with low sporophyte production, which would suggest that asexual reproduction predominates and upstages efficient resource allocation in prezygotic investment.

**KEY WORDS** Amazon; gametangia; moss; rainforest; reproduction; resource allocation

The distribution and proportion of sexual and asexual individuals influence reproductive success and genetic structure within and among plant populations (Loveless and Hamrick, 1984; Barrett and Eckert, 1990; Hock et al., 2009). In unisexual populations, the functional sex ratio (SR) is determined by a combination of the relative frequencies, spatial distribution, and rates of sex expression at temporal and spatial scales. Allocation theory predicts that accelerated returns on resource investment in sex expression will favor dioicy (Charlesworth and Charlesworth, 1981; Campbell, 2000). Although this trait is found in a small percentage (4–6%) of angiosperms

(de Jong and Klinkhamer, 2005), more than half of all bryophyte taxa are dioicous (Wyatt, 1982).

Bryophytes (mosses, hornworts, and liverworts) are ideal candidates for mating ecology studies in plants because of their haploid-dominated life cycle, diverse sexual strategies (Wyatt, 1985), and reliance on asexual reproduction (Longton and Schuster, 1983). In seed plants, sex is determined at syngamy rather than meiosis. As such, quantifying investment in gametes requires estimating pollen and ovule production, a nearly intractable endeavor with most angiosperms. In dioicous bryophytes, sex is determined following meiosis by the formation of independent male or female gametophytes. Likewise, the reproductive success of a seed-plant gametophyte is binary, whereas it is potentially unlimited for bryophyte gametophytes because most reproduce asexually.

Expressed SRs are variable in angiosperms (Barrett et al., 2010) and bryophytes alike (Wyatt and Anderson, 1984), but they are more heavily skewed toward females in populations of the latter (Bowker et al., 2000; Bisang and Hedenäs, 2005; Stark et al., 2010).

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Nearly one-fifth of bryophyte taxa exhibit either a balanced or a male-biased SR, including rare cases of male-skewed populations, which typically inhabit ecologically extreme substrates such as leaves (e.g., epiphylls; Alvarenga et al., 2013) and rocks in fast-flowing streams (Hola et al., 2014). Nonetheless, reproductive-allocation studies in bryophytes have been principally focused on mechanisms that promote female-bias. Sex-specific differences in prezygotic reproductive investment (McLetchie, 1992; Stark et al., 2000), interactions among life history and environmental gradients (Bowker et al., 2000; McLetchie and Puterbaugh, 2000; Benassi et al., 2011), and progeny SR (Shaw and Gaughan, 1993; Stark et al., 2010; Bisang and Hedenäs, 2013) have all been cited as possible reasons for lopsided SRs in bryophytes.

For example, expressed male rarity in the dioicous desert moss *Syntrichia caninervis* is assumed to be the result of increased per shoot investment in male, compared to female, gametangia (Stark et al., 2000). However, a study of the pleurocarp *Pseudocalliergon trifarium*, also characterized by expressed male rarity, reported greater biomass investment in entire female structures (perichaetia) than in entire male structures (perigonia), which suggests that sex-specific differences in prezygotic investment are not a universal predictor of female-biased SR in bryophytes (Bisang et al., 2006). The cost of prezygotic investment for future reproductive success has yet to be empirically demonstrated in bryophytes (but see Rydgren et al. [2010] for a modeling approach).

Life history theory predicts trade-offs in resource investment in sexual and asexual reproduction (Seeger and Eckhart, 1997); however, few studies have empirically tested this expectation in plant populations. In bryophytes, investigations have been focused on postzygotic reproductive investment and asexual reproduction (reviewed in Stark et al., 2009), with few demonstrations of trade-offs between prezygotic rates of sexual expression and investment in production of asexual propagules (Fuselier and McLetchie, 2002). Dioicous bryophyte populations range from no sex-specific differences in gemma frequency (Hóla et al., 2014) to sharp ones (Laaka-Lindberg, 2001), suggesting that gender-biased investment in asexual reproduction is not consistent in bryophytes.

Macroevolutionary patterns show no correlation between separate sexes and asexual reproduction, suggesting that asexual reproduction was not a pivotal adaptation in the evolution of unisexuality in mosses (Crawford et al., 2009). Moreover, comparative phylogenetic analysis among liverworts has shown that production of asexual propagules, rather than sexual system (e.g., monoicy vs. dioicy), is correlated with geographic range size, suggesting that the former is more crucial to long-distance dispersal than previously considered (Laenen et al., 2016). Nonetheless, spores and asexual propagules are considered to play different roles in the ecology and life history of bryophytes (Longton and Schuster, 1983; Kimmerer, 1991; Hock et al., 2008).

The Calymperaceae (C.Müll.) Kind. is a pantropical family of 191 species from seven genera (Shaw and Goffinet, 2000; Missouri Botanical Garden, 2015). In the Neotropics, the family is represented by 56 species distributed among four genera, *Calymperes* Sw. ex F. Weber, *Syrrhopodon* Schw., *Octoblepharum* Hedw., and *Leucophanes* Brid. Its center of richness in the Americas is the lowland tropical forests of the Amazon Basin and neighboring Guyana Shield of northern South America, where more than one-third of the Neotropical taxa occur (Reese, 1993). Central Amazonia, in particular, harbors both Amazonian endemics and widespread taxa, which makes the region a crossroads of Calymperaceae's richness.

An infrafamilial phylogenetic study revealed that *Syrrhopodon* is paraphyletic (Fisher et al., 2007). Calymperaceae are dioicous, and all species produce specialized asexual propagules along the mid-vein on either the adaxial or abaxial leaf faces (Reese, 1993).

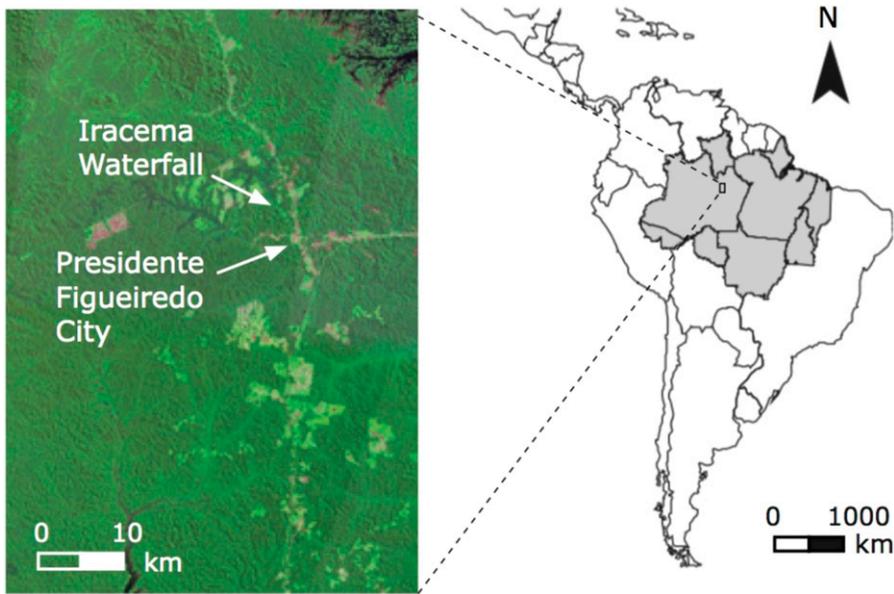
We conducted a year-long phenological census of multiple populations from six sympatric species of Calymperaceae in a central Amazonian forest to test two predictions. Considering that the production of archegonia and asexual propagules represents quantifiable investments from the mother shoot, we predicted trade-offs in reproductive strategies among the prezygotic expression of female gametangia and the production of asexual propagules (prediction 1). Under the similar assumption that investment in archegonial production also incurs a quantifiable prezygotic investment for the mother shoot, we predicted that the *proportion* of senescent archegonia is independent of their total production per shoot (prediction 2) because positive density-dependent senescence of archegonia would result in a squandering of resources. In light of these predictions, we attempt to disentangle effects of variation among species, populations, and seasonality (as defined by mean monthly precipitation) on rates of prezygotic sexual expression, asexual expression, and patterns of archegonial senescence.

## MATERIALS AND METHODS

**Study area**—The Iracema Ecological Reserve is located along the Urubuí River near the Municipality of Presidente Figueiredo, Amazonas, Brazil (01°59'13"S; 60°03'34"W; Fig. 1). Situated along the transition between the erosion-resistant Precambrian Guyana shield and lowland Amazonia, the region is characterized by rocky outcrops and grottoes scattered throughout the rainforest (de Granville, 1988). The reserve is dominated by upland *terra firme* forest (Veloso et al., 1991), which grades into isolated patches of Amazonian white-sand forests (*campinarana*; Esteves and Vicentini, 2013). Mean annual temperature at the study site ranges from 19°C to 39°C (mean = 26°C), and annual rainfall ranges from 1900 to 3500 mm, with a distinct dry season from July to November.

**Focal species**—Six species within Calymperaceae (Bryophyta) were selected for study. Four species are considered endemic to the Amazon basin (*Syrrhopodon helicophyllum* Mitt., *S. annotinus* W.D. Reese & D.G. Griffin, *S. simmondsii* Steere, and *S. fimbriatus* Mitt.), and two species are widely distributed across northern South America (*S. leprieurii* Mont. and *Calymperes mitrafugax* Florsch.; Fig. 2). The Amazonian endemics are typical of, but not entirely restricted to, white-sand forests. *Syrrhopodon helicophyllum* is epiphytic on twigs and trunks of small understory trees, whereas *S. annotinus* and *S. simmondsii* occur on the forest floor on mixed sand- and clay-based substrates, and *S. fimbriatus* occurs on both trees and rocks in proximity to water courses. The two widespread Neotropical taxa, *S. leprieurii* and *C. mitrafugax*, are obligatorily saxicolous and epiphytic, respectively. The study species were selected because of their unisexual reproduction, high abundance, and diverse representation of substrates in the study area.

**Field sampling and data collection**—In September 2010, three populations of four species and two populations of two species (*S. fimbriatus* and *S. simmondsii*;  $n = 16$  populations) were marked for study along a 5 km trail in the reserve. During the course of the study, two populations of *S. helicophyllum* went extinct. The



**FIGURE 1** Location of the study site: Iracema Ecological Reserve, Presidente Figueiredo, Amazonas, Brazil.

14 remaining populations were censused over the entire study. The trail begins in *terra firme* forest and passes through high-canopy white-sand forests, boulder gardens, and riparian forests (along the Urubuí River), ending in low-canopy white-sand forests. Study populations were located  $\geq 10$  m from the access trail to avoid anthropogenic disturbance and were marked with aluminum stakes and tags. Populations (i.e., cushions) with a minimum size of 1000 cm<sup>2</sup> were selected so that monthly sampling, which involved removing shoots, would have a limited impact on the remaining ones.

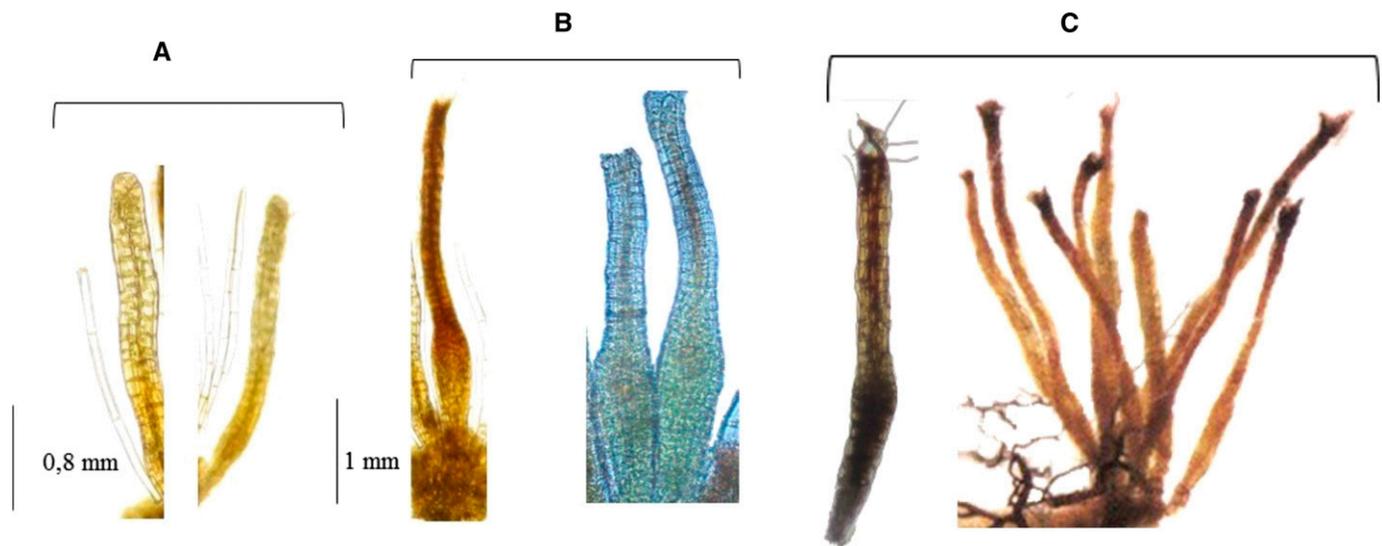
From September 2010 to September 2011, we conducted 12 censuses during the third week of each month. In each census, 10 shoots from each study population were randomly selected and taken to

the Cryptogamic Laboratory at the National Institute for Amazonian Research in Manaus, where the presence of specialized asexual propagules (gemmae) and male (perigonium) or female (perichaetium) gametangia was tallied. For female-expressing individuals, the perichaetia from the present year were removed, dissected, and scored. All female gametangia (archegonia) for each shoot were characterized as either immature, receptive, or senescent, based on the visual condition of the neck and ventral canal cells and the size and/or coloration of the egg (Fig. 2). We were unable to determine whether senescent female gametangia had been fertilized and subsequently aborted; however, the paucity of male gametangium and sporophyte production for most of the species would suggest that a majority of the senescent archegonia were unfertilized. For male-expressing shoots, the total number of antheridia was tallied.

During the census, sporophyte production was quantified in four 25 cm<sup>2</sup> quadrats randomly placed monthly within each population.

Excluding *C. mitrafugax*, sporophyte production in all species was rare both within and outside the quadrats (M. R. Periera, personal observation). Mean monthly precipitation was obtained from the National Meteorological Institute of Brazil (INMET), Manaus, from a single weather station (Lat:  $-2^{\circ}3'23.61''$ , Long:  $-60^{\circ}1'32.72''$ ) located in the Municipality of Presidente Figueiredo, Amazonas, Brazil (<http://www.inmet.gov.br>).

**Data analysis**—*Prediction 1: Trade-offs in prezygotic sexual and asexual expression*—To disentangle the effects of rainfall on prezygotic sexual and asexual expression, we developed generalized linear mixed models (GLMMs) using the probability of gametangium



**FIGURE 2** Examples of archegonial stages from Calymperaceae at the study site: (A) immature, (B) receptive, and (C) senescent. Immature archegonia are identified by a closed neck canal; receptive archegonia are identified by an open and transparent neck canal with a visible egg; and senescent archegonia are identified by a closed, darkened, and shriveled neck canal.

production and the number of gametangia produced per shoot as response variables. In all models, we included mean monthly rainfall and the presence of gemmae as predictor variables. Mixed models are used to investigate central trends (fixed effects) in light of how model parameters, such as intercept and slope, vary among groups (random effects). Because the nesting of samples within groups is controlled, these models can also be used to cope with lack of independence (Millar and Anderson, 2004). As such, we included population as a grouping factor. We also investigated overall differences in gametangium production among species and populations (random intercept), and their response to rainfall (random slope). Species were treated as a second grouping factor in the GLMMs, and populations were nested within species, so that the models incorporated the three hierarchical levels in the data. To investigate the effect of gemma production on gametangium output, we also included the presence of gemmae as a covariate; for model simplicity, “gemma” was treated as a fixed variable only. There were no temporal or spatial trends in the residuals, so no corrections for autocorrelation were conducted.

Environmental conditions affect both gemma and gametangium production in tropical bryophyte populations (Oliveira and Pôrto, 2001; Maciel-Silva and Valio, 2011; Zartman et al., 2015), making it difficult to disentangle the association between gemma and gametangium production (e.g., trade-offs in expression probability per shoot) from measured (e.g., rainfall) or unmeasured environmental variables. If environmental conditions simultaneously influence gemma and gametangium production, their probability of expression will be correlated. By contrast, a negative correlation between these reproductive strategies could indicate either negative trade-offs in gemma and gametangium production or phenological separation in their expression. A further complication is that non-expressing shoots may be under other unaccounted-for environmental stresses or genetic constraints. These potential factors, which inflate zero values in the dataset, may spuriously reduce the number of shoots producing gemmae and gametangia, compared to that predicted by trade-offs. Since trade-offs are more easily detected by excluding nonexpressing shoots, we removed them from this analysis.

We tested the effects of rainfall and gemma expression on (1) the probability of gametangium production, using a logistic regression (GLMM with a binomial distribution of errors and a logit link); and (2) the number of gametangia produced per shoot, using a Poisson regression (GLMM with Poisson distribution of errors and a log link). This model was run by using the number of gametangia per shoot as the response variable.

To test for variation in the probability of gametangium production per shoot (GLMM<sub>binom</sub>) and the number of gametangia produced per shoot (GLMM<sub>poisson</sub>) among populations and species, we created subsets of the GLMMs. In each subset, one model parameter was removed (e.g., species identity), and the subset was compared with the full model by using a likelihood ratio test. Logistic and Poisson regressions using individual species (without random effects), including gemma as a predictor variable, generated results similar to those of the GLMMs. Because *S. annotinus* shoots did not express gemmae, that species was omitted from this analysis.

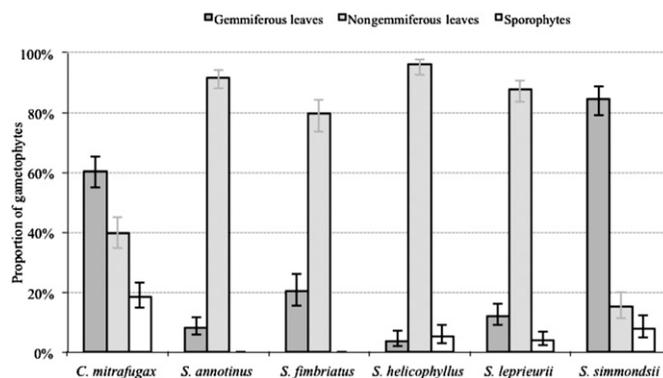
**Prediction 2: Density independent survivorship of archegonia per shoot**—To test for the association between senescent archegonia and their respective density per shoot, we ran a logistic regression

model (general linear model) using the number of viable archegonia as the response variable and the density of archegonia per shoot as the predictor variable. We then used the results from the logistic regression to calculate the expected number of receptive archegonia per shoot (expected number of archegonia = probability of receptive archegonia × total number of archegonia). All statistical analyses were conducted in R (R Development Core Team, 2015) using the package lme4 (Bates et al., 2014).

## RESULTS

**Reproductive biology**—More than half (59%) of the 1820 shoots studied were without sexual and asexual expression. Of the expressing shoots, a vast majority (92%) were female. For three of the four Amazonian endemics, *S. helicophyllus*, *S. annotinus*, and *S. fimbriatus*, no expressing males were observed in >800 examined shoots. Sporophytes were observed in *S. helicophyllus*, indicating that expressing males were present, but not sampled, in the study area. By contrast, no sporophytes were observed in *S. annotinus* and *S. fimbriatus*. *Syrrhopodon simmondsii* was the only Amazonian endemic in which expressed males and sporophytes were sampled, and this species was characterized by the greatest proportion of gemmiferous shoots among the six studied species (Fig. 3). Both expressing males and sporophytes were observed in the two widespread Neotropical species, *S. lepreurii* and *C. mitrafugax*, and the latter had the highest numbers of male-expressing shoots and sporophytes in the study area (Table 1). The proportion of gemmiferous vs. nongemmiferous leaves varied substantially among species, and sporophyte presence, except for *C. mitrafugax*, was consistently rare (Fig. 3).

**Trade-offs in reproductive strategies (prediction 1)**—The presence of gametangia was more common in shoots producing gemmae ( $b = 0.44$ ,  $z = 1.98$ ,  $P = 0.047$ ; Table 2) and when rainfall was high ( $b = 0.55$ ,  $z = 2.46$ ,  $P = 0.013$ ; Table 2). However, the absolute number of gametangia (archegonia) produced per shoot was negatively correlated with gemma production when only shoots producing at least one gametangium or gemma were included in the analyses



**FIGURE 3** Histogram of reproductive patterns of the six study species. Presence of gemmiferous leaves (asexual reproduction) is indicated by dark gray bars. Absence of asexual expression (nongemmiferous leaves) is presented by light gray bars, and presence of sporophytes (sexual reproduction) by white bars. Proportions were used instead of absolute values to facilitate comparisons among species.

**TABLE 1.** Total number of shoots ( $N_{\text{shoots}}$ ) for each studied species of bryophyte, with a summary of the numbers of shoots collected during the year in parentheses. Note that two species, *Syrrophodon fimbriatus* and *S. simmondsii*, had only two populations surveyed from the beginning of the study; and that two populations of *S. helicophyllus* went extinct during the course of the study—reducing the numbers of shoots in these populations (see text for details). Sex ratio (SR) was calculated as the overall number of shoots producing antheridia divided by the overall number of shoots producing archegonia ( $SR_{\text{shoot}}$ ); and as the mean number of shoots producing antheridia per population divided by the mean number of shoots producing archegonia per population ( $SR_{\text{pop}}$ ). Chi-square tests were performed to test the null hypothesis of no difference in sex proportions ( $P_{\text{male}} = P_{\text{female}} = 0.5$ ).

Species	$n_{\text{shoots}}$	Shoots expressing gemmae	Shoots expressing archegonia	Shoots expressing antheridia	Total archegonia	Total antheridia	$SR_{\text{shoot}}$	$SR_{\text{pop}}$
<i>Calymperes mitrafugax</i>	360 (120; 120; 120)	215 (60%)	44 (12%)	18 (5%)	476	328	0.41***	0.59***
<i>S. annotinus</i>	360 (120; 120; 120)	0	39 (11%)	0	572	0	0***	0***
<i>S. fimbriatus</i>	240 (120; 120)	68 (28%)	13 (5%)	0	87	0	0***	0***
<i>S. helicophyllus</i>	260 (120; 70; 70)	11 (4%)	32 (12%)	0	443	0	0***	0***
<i>S. lepreurii</i>	360 (120; 120; 120)	45 (12%)	53 (15%)	3 (<1%)	701	52	0.06***	0.07***
<i>S. simmondsii</i>	240 (120; 120)	203 (86%)	80 (33%)	1 (<1%)	1150	10	0.01***	0.01***

Note:  $P < 0.001$ \*\*\*;  $P < 0.01$ \*\*;  $P < 0.05$ \*;  $P < 0.1$ †.

(prediction 1 not rejected:  $b = -1.58$ ,  $z = -28.26$ ,  $P < 0.001$ ; Table 2). When analyzing individual species separately, gametangium presence was weakly associated with gemma production in most species ( $-0.42 < b < -0.16$ ). *Syrrophodon simmondsii* and *S. lepreurii* were the only species in which gametangia were more common in shoots producing gemmae ( $b = 0.51$  and  $b = 1.62$ , respectively). Overall, the number of gametangia per shoot was lower in those with gemmae ( $-4.21 < b < -0.57$ ).

The probability of a shoot producing perichaetia was higher in those expressing gemmae ( $b = 0.61$ ,  $z = 3.26$ ,  $P = 0.001$ ; Table 2) and in months of greater precipitation ( $b = 0.54$ ,  $z = 4.2$ ,  $P < 0.001$ ; Table 2). However, when only female-expressing shoots were analyzed, the number of perichaetia produced per shoot was also negatively correlated with gemma production ( $b = -1.59$ ,  $z = -11.78$ ,  $P < 0.001$ ; Table 2). The number of archegonia produced per shoot was also positively associated with rainfall ( $b = 0.41$ ,  $z = 2.37$ ,  $P = 0.018$ ; Table 2), whereas the number of perichaetia was only marginally associated ( $b = 0.20$ ,  $z = 0.63$ ,  $P = 0.08$ ). Furthermore, rainfall was more strongly associated with expression of females than with expression of males or gemmae (Fig. 4A–C).

Despite an overall increase in production of gametangia with rainfall, that production was variable among species and populations (note high SD in Table 2). When all shoots were included in the analyses, the production of gametangia varied more among populations than among species (note high SD in first two columns

of Table 2). However, when nonexpressing shoots were removed, the number of gametangia produced per shoot was more variable among species than among populations (note high SD in the last column of Table 2). Statistical tests for the species SD were mostly nonsignificant, likely a result of the low number of study species and the difficulties of estimating  $P$  values for mixed models (Pinheiro and Bates, 2000).

**Shoot-density-independent archegonial senescence (prediction 2)**—A majority (79%) of the 3594 observed female gametangia (archegonia) were senesced, and the probability of senescence increased with per shoot archegonial density (prediction 2 rejected:  $b_{\text{GLM}} = -0.02$ ,  $P < 0.001$ ; Fig. 5A). The modeled maximum number of receptive archegonia is 43 (five of which would remain receptive; Fig. 5B). Per shoot expression  $>43$  would result in increased rates of senescence due to the observed density-dependent effects. However, average production of archegonia per shoot was 13, of which three, on average, are predicted to remain receptive throughout the longevity of the perichaetia (Fig. 5B).

## DISCUSSION

As reported of other dioicous bryophytes, the study populations were dominated by nonexpressing shoots, unbalanced SR, little

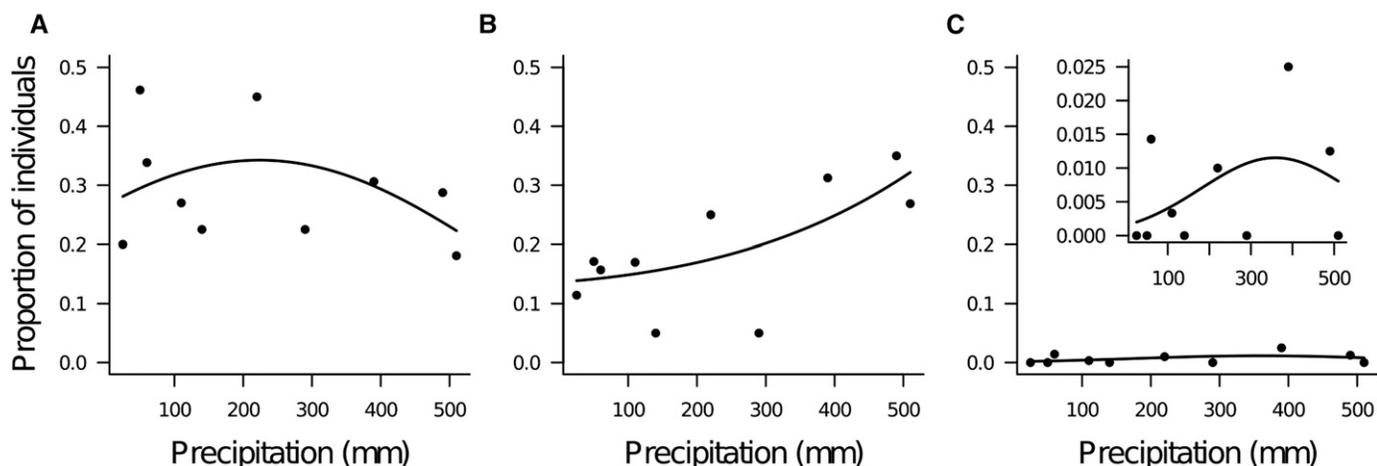
**TABLE 2.** Coefficients from logistic and Poisson regression models testing for the association of gametangia and perichaetia with rainfall and presence of gemmae. Variation among populations ( $SD_{\text{population}}$ ) and species ( $SD_{\text{species}}$ ) in their overall gametangium and perichaetium expression, and in their response to rainfall, was investigated by using mixed-effect models. To assess the variation around the average intercept and slope in the regression models, intercept and rainfall slope were treated as both fixed and random effects. The variation around the estimated coefficients was assumed to have a normal distribution. For the Poisson regression models, only shoots with at least one gametangium or gemma were included. Values for rainfall were standardized prior to analyses. When shoots not producing reproductive structures are removed from the analyses (last column), most of the variation in overall gametangia expression results from interspecific variation ( $SD_{\text{species}} > SD_{\text{population}}$ ).

	Response variable			
	Perichaetia <sub>PA</sub> (binomial)	Gametangia <sub>PA</sub> (binomial)	Perichaetia <sub>AB</sub> (Poisson)	Gametangia <sub>AB</sub> (Poisson)
Intercept	-1.81***	-2.20***	0.39**	2.04***
$SD_{\text{population}}$	0.74***	0.65***	0.27	0.44***
$SD_{\text{species}}$	0.14	0.33	0.25†	0.95
Rainfall	0.54***	0.55*	0.20†	0.41*
$SD_{\text{population}}$	0.39***	0.39*	0.20**	0.33***
$SD_{\text{species}}$	0.10	0.45	0.19*	0.40
Gemma	0.61**	0.44*	-1.59***	-1.58***

$P < 0.001$ \*\*\*;  $P < 0.01$ \*\*;  $P < 0.05$ \*;  $P < 0.1$ †.

or no sporophyte production, and wide interspecific variation in asexual expression. However, our results provide novel evidence of a trade-off between asexual and prezygotic sexual expression (prediction 1) and positive density-dependent senescence of female gametangia (prediction 2).

The negative correlation between asexual and prezygotic sexual expression (prediction 1) was not an artifact of microsite differences nor of seasonal partitioning among the reproductive strategies; rather, it suggests a tangible investment in alternative reproductive strategies. The proportional increase in senesced female gametangia per shoot (prediction 2), furthermore, suggests that factors other than



**FIGURE 4** Association of rainfall with (A) asexual, (B) archegonial, and (C) antheridial expression. In all graphs (except inset in C) the y-axis was scaled between 0 and 1. Inset graph C shows the same data on archegonial expression probability in relation to precipitation at a smaller scale as well, to show comparison among sexes. The graphs summarize monthly probability of prezygotic sexual and asexual expression for >1800 shoots from the six species investigated. The x-axis shows (nonstandardized) mean monthly rainfall.

prezygotic resource-allocation dynamics influence archegonium production. One possibility, as evidenced by the extremely low per shoot density of archegonium production compared to the modeled optimum, is that this inefficient prezygotic investment in gametangial output is an artifact of genotypes maintained by asexual reproduction. However, future studies on population structure are necessary to shed light on this hypothesis.

**Sex ratios**—A vast majority of the examined shoots were nonexpressing, making it impossible to accurately assess the primary SR without use of sex markers. Sex-specific genetic markers have been found in other bryophytes (McDaniel et al., 2007), and their utilization has shown, in a few cases, that expressed SR approximates primary SR (Hedenäs et al., 2010; Bisang and Hedenäs, 2013). A purported explanation for the lopsided expressed SR of bryophytes is that physiological stress, which populations may experience at the extremes of their geographic distributions, may be due to sex-specific differences in environmental tolerances (Stark, 2002; Stark and McLetchie, 2006; Benassi et al., 2011). However, for the three endemics, *S. helicophyllus*, *S. annotinus*, and *S. fimbratus*, in which no expressed males were observed, the study area is located in the center of their ranges (Reese and Griffin, 1976; Reese, 1993).

Demographic models have also attempted to explain skewed SR in bryophytes, pointing to the possibilities of sex-specific differences in tolerance to environmental heterogeneity (McLetchie et al., 2002), metapopulation structure (García-Ramos et al., 2007), and/or vegetative growth (Rydgren et al., 2010). For example, Rydgren et al. (2010) demonstrated for the pleurocarp *Hylocomnium splendens* that female-biased SR would be maintained under the model of limited sporophyte production and male shoot growth intermediate to that of postreproductive and sterile females. However, *H. splendens* does not reproduce via specialized asexual propagules and is also characterized by higher sporophyte production than observed in the study species.

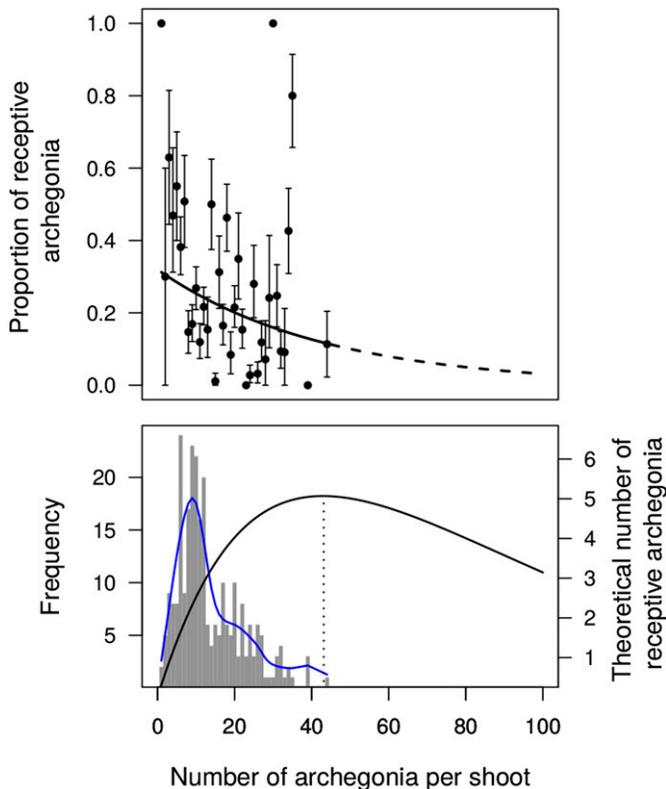
In unisexual plants, equilibrium SR should be maintained by negative frequency-dependent processes (Barrett et al., 2010). This has been demonstrated in seed plants in which female-determining

microgametophytes from male-skewed populations have increased fertilization success (Stehlik et al., 2008). The unbalanced SR found in the present study could also be an effect of sampling scale, given that the study area was confined to a small area. However, assessment of sex expression patterns in Calymperaceae would suggest that this is unlikely, because male expression does not apparently exhibit geographic structure in some studied taxa in the family (Fisher, 2011). However, many dioicous bryophytes exhibit extreme patterns of sexual allopamy (Schuster, 1983; Renzaglia and McFarland, 1999), even including the global absence of one of the two sexes (Longton and Schuster, 1983). To our knowledge, mechanisms contributing to possible negative frequency-dependent processes have yet to be investigated in bryophytes.

**Prediction 1: Trade-offs in asexual and prezygotic sexual expression**

—In bryophytes, the cost of sexual reproduction is generally considered to be higher than that of asexual reproduction. For example, sporophyte production has been correlated with higher shoot mortality among females (Pohjamo and Laaka-Lindberg, 2003), reduced size of vegetative offshoots, reduced branching (Rydgren and Okland, 2003), and reduced production of new reproductive organs (Bisang and Ehrlen, 2002). Such demonstrable costs in postreproductive growth and asexual output have been documented for bryophyte species in both the field and laboratory (reviewed in Stark et al., 2009). However, studies on reproductive-strategy trade-offs as inferred from differences in gametangial production and asexual expression and sporophyte success are scarce (Stark and Stephenson, 1983).

Trade-offs among prezygotic sexual and asexual expression may be of genetic origin and not necessarily evidence of resource-allocation dynamics (Horsley et al., 2011; Brzyski et al., 2014). The negative correlation between prezygotic sexual and asexual expression may be the result of genotypic segregation of alternative reproductive strategies. For example, genotypes expressing for high archegonial loads are unlinked to those expressing for asexual propagule production. Our results, however, offer equivocal support for this hypothesis, in that sexual and asexual expression were more likely to be observed on the same, rather than on separate,



**FIGURE 5** (Top) Positive density-dependent probabilities of senescence of archegonia per shoot. Proportion of receptive (nonsenescent) archegonia is plotted on the y-axis, and density of female gametangia per shoot on the x-axis. (Bottom) Theoretical optimum of archegonial production to maximize nonsenescent archegonia per shoot. Gray bars represent the observed size-class distributions of archegonia per shoot. Blue is a smoothed line of the observed frequency distribution. The black line is the modeled prediction for optimal per shoot archegonial production. The vertical dashed line (crossing the x-axis at 43) represents the modeled number of archegonia per shoot to maximize the receptive (nonsenescent) archegonia. The black line decreases following the optimum because of positive density-dependent senescence of archegonia.

shoots. Only in expressing shoots was a trade-off in reproductive strategies detected. Experimental studies, such as those of Horsley et al. (2011), Stark et al. (2009), and Brzyski et al. (2014), may disentangle such ultimate causes of this trade-off; nonetheless, asexual expression and archegonial output are shown to be inversely correlated in these species.

**Prediction 2: Density-dependent archegonial senescence**—Bryophyte shoots frequently produce multiple archegonia per perichaetium (Longton and Schuster, 1983), even though it is rare for more than one sporophyte to reach maturity (Hughes, 1980; Longton and Schuster, 1983; Szövényi et al., 2009). However, we found evidence that the proportion of senescent archegonia increases significantly with per shoot density. Although the proximate cause of this pattern may be resource limitation, the ultimate reason for it remains unclear. Assuming that the production of an archegonium represents a quantifiable prezygotic investment (whether fertilized or not), the advantage derived from the squandered resources is unclear, given that the positive density-dependent probability of

archegonial senescence per shoot suggests inefficient prezygotic resource-allocation dynamics. One possibility is that overproduction would swamp out the high numbers of unfertilized archegonia to ensure the presence of receptive archegonia. However, a vast majority of the expressed shoots harbored numbers of archegonia well below the modeled optimum.

An alternative explanation is that perichaetia with more archegonia are relatively older. In such a case, more densely packed ones would be expected to host a greater number of older archegonia, thus resulting in a positive density-dependent pattern of senescence. However, our analyses were conducted by summing the number of archegonia per female shoot, of which nearly half (48%) produced more than one perichaetium, so positive density dependence in archegonial senescence was detected at the scale of the entire female gametophyte, not at the scale of an individual perichaetium.

Two possible patterns exist in the development of archegonial mother cells: (1) predeterminant, in which mother cells initiate growth in synchronous development; or (2) sequential, resulting in a range of archegonial age classes/phenophases in a perichaetium at any given moment. Personal observations by M. R. Pereira have suggested that Calymperaceae follow the predeterminant strategy, as evidenced by the fact that archegonia occupying larger perichaetia were largely of a similar phenophase. In such a case, the possibility that positive density-dependent senescence is a result of successional waves of old and senescing archegonia is also unlikely.

**Broader implications**—In seed plants, factors promoting positive density dependence in production of sexual structures are generally attributed to animal-mediated interactions that offer selective advantages in pollination success or predation avoidance (Stephenson, 1981; Rapp et al., 2013). However, evidence for the importance of animal vectors in fertilization events in bryophytes is limited (Cronberg et al., 2006) and, therefore, that is not a likely explanation for the overabundant production of gametangia. An alternative possibility may be that overproduction increases the window of opportunity for sperm to fertilize the egg. Individual archegonia may be constrained to short periods of receptivity. A plant producing few archegonia over longer intervals may maintain an extended level of viability. Nonetheless, the marked seasonality in rainfall at the study area would select against such a strategy in archegonial production.

Macroevolutionary patterns of plant sexual strategies have been investigated in angiosperms (Barrett, 2010) and bryophytes (McDaniel et al., 2012) alike, yet little is known of the processes that influence their reproductive strategies. The ultimate cause of trade-offs among reproductive strategies in these Amazonian endemics, despite apparently inefficient resource allocation in prezygotic investment, remains unclear. To disentangle how geographic structure, sex-specific variation in environmental tolerance, and/or genetic effects influence sexual expression, integrative studies of reproductive biology and genetic structure are necessary.

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