Notes on the evolution of dehydration rates in a southern Sweden bryophytes population

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Bryophytes, including mosses, liverworts, and hornworts, represent early diverging lineages of land plants, facing challenges such as water and nutrient transport and desiccation prevention during their evolutionary transition to land. This study aimed to answer two questions: 1) Are moss species growing in dry substrates better at water retention? 2) Are moss species with lower dehydration rates more derived in their evolutionary history? We collected dehydration rate data from ten bryophyte species across diverse substrates and analyzed them using Bayesian phylogenetic mixed effects models. Contrary to our predictions, we found no significant differences in dehydration rates or immediate water loss between substrates. This suggests that substrate type may not significantly influence dehydration rates, with other factors such as structural adaptations and colony density playing more critical roles. Furthermore, we observed no phylogenetic signal for dehydration rates, indicating that these traits are independent of phylogenetic relationships. This suggests that ecological and environmental factors might be more influential in determining dehydration rates in bryophytes. Our findings highlight the complexity of desiccation resistance mechanisms in bryophytes and the need for further research considering microclimate conditions and broader ecological contexts.

Introduction

Bryophytes, including mosses, liverworts, and hornworts, represent some of the earliest diverging lineages of land plants (Bechteler et al., 2023; Boyce & Lee, 2017). During the evolutionary transition to land, plants faced significant challenges such as transporting water and nutrients against gravity and preventing desiccation (Raven & Edwards, 2004; Wang et al., 2022). Vascular plants addressed these challenges by developing specialized conductive tissues, the xylem and phloem (Kumar et al., 2022). In contrast, bryophytes adapted primarily by staying close to water sources and relying on surface water absorption (Charron & Quatrano, 2009). However, some bryophytes are found in environments far from constant water bodies and are prone to desiccation. To cope with these conditions, various bryophyte species have evolved distinct adaptations. Some have altered their branch and leaf structures to reduce water loss (Wang et al., 2022), while others have developed complex life cycles to endure desiccation (Proctor, 2000).

For their ecological importance, the study of bryophytes and their ability to manage hydric stress, it is important to study their ability to retain water. In this sense, this study aimed to answer two questions: 1) Are moss species (Bryophyta) growing in dry substrates better at water retention? 2) Are moss species with lower dehydration rates more derived in the group's evolutionary history? We collected data on the dehydration rates and growing substrates of ten bryophyte species. Using Bayesian phylogenetic mixed effects models, we analyzed the relationships between dehydration rates and growing substrate conditions. We predict that species in drier environments, such as rocks, will have lower dehydration rates due to the need to retain more water (Charron & Quatrano, 2009; Green et al., 2011). Additionally, we expect that species with lower water loss rates are more derived in the phylogeny, reflecting bryophytes' evolutionary transition from aquatic to terrestrial habitats.

Methods

We collected 41 individuals from 10 different bryophyte species in diverse substrates (soil, sand, wood and rock) at Dageberga Stugby (55°49'13.762'' N, 14°4'40.399'' E), Kristianstad commune in Sweden. Each individual underwent a dehydration experiment with the following setup: two 3 cm² pieces of the plant were taken, one intact and the other with individual branches separated and weighed. Both samples were saturated in water for 1 hour to obtain their wet mass. They were then placed on filter paper and weighed at 30-minute intervals over 2 hours, with a final mass taken 11 hours after the water saturation. We defined the rate of dehydration as the slope between the measurements taken at 30 minutes and 120 minutes after saturation. Immediate water loss was defined as the difference between the saturated wet mass and the mass at 30 minutes. The final mass after 11 hours was excluded due to its high correlation

with the dehydration rate (supplementary information).

Rates complete			
Comparison	Posterior Mode	Lower Boundary	Upper Boundary
rock vs sand	-0,507	-1,824	0,644
sand vs soil	0,169	-1,341	1,089
soil vs wood	0,724	-0,063	1,806
rock vs soil	-0,504	-1,234	0,247
rock vs wood	0,349	-0,561	1,337
sand vs wood	1,261	-0,486	2,323
Rates separate			
rock vs sand	-0,597	-1,897	0,598
sand vs soil	-0,315	-1,395	1,025
soil vs wood	0,599	-0,341	1,532
rock vs soil	**-0,822**	**-1,457**	**-0,042**
rock vs wood	-0,324	-1,006	0,863
sand vs wood	0,269	-0,926	1,926
Immediate loss complete			
rock vs sand	-1,159	-2,367	0,178
sand vs soil	0,768	-0,367	2,120
soil vs wood	0,576	-0,529	1,398
rock vs soil	-0,274	-1,051	0,619
rock vs wood	0,293	-0,554	1,511
sand vs wood	1,604	-0,094	2,775
Immediate loss separate			
rock vs sand	-0,119	-2,150	2,191
sand vs soil	-1,027	-2,739	1,659
soil vs wood	0,287	-0,983	2,282
rock vs soil	-0,473	-1,870	0,657
rock vs wood	-0,164	-1,610	1,759
sand vs wood	0,056	-2,458	2,529

To test whether species in drier environments had lower dehydration rates, we used Bayesian generalized mixed effects models with the MCMCglmm R package (Hadfield, 2010). We analyzed both the complete and separate rates, as well as immediate water loss by substrate, setting the phylogenetic relationships between species (Bechteler et al., 2023) as a random factor and assuming an Ornstein-Uhlenbeck model of evolution (Lande, 1976). Model specifications are detailed in the supplementary information. To determine if lower dehydration rates are a derived character in moss phylogeny, we performed ancestral reconstructions for both complete and separate rates and assessed the phylogenetic signal. This analysis was also conducted using the MCMCglmm package as per Hadfield's recommendations (Hadfield, 2017).

Results and Discussion

We found no statistical support for our first question regarding whether bryophyte species growing in drier environments have lower dehydration rates. Contrary to our prediction that species living on dry substrates, such as rocks, would exhibit lower dehydration rates, our data showed no significant differences. Specifically, for both complete and separate rates, as well as immediate water loss for both complete and separate measurements, there were no differences between growing substrates (**¡Error! No se encuentra el origen de la referencia.**). The variation in dehydration rates and immediate water loss was similar across substrates (Figure 2). These results suggest that the substrate may not play a significant role in influencing the dehydration rates of bryophyte species.
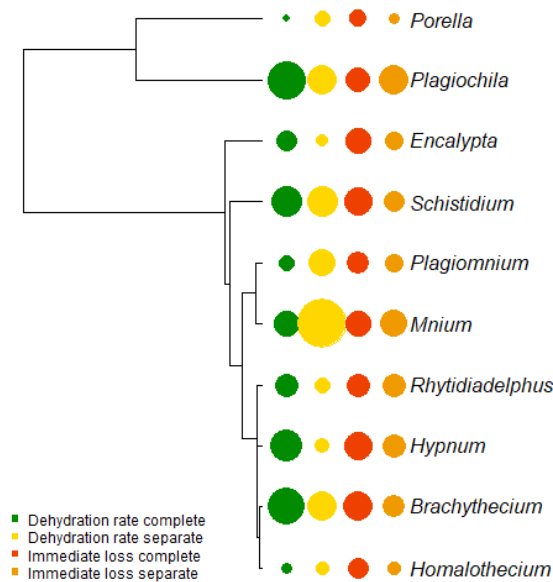

Figure 1. Bryophytes tree with dehydration rates and Immediate water loss values.

Other factors are more critical in avoiding desiccation, such as structural modifications in cells to collect more water from the environment, even in very dry conditions (Jauregui-Lazo et al., 2023). Additionally, to modification in their genomes to alternate the metabolical processes as photosynthesis when the environmental conditions are optimal (Oliver et al., 2004). Alternatively, in more extreme environments the development of mechanisms as dormancy are also important for coping with desiccation (Wang et al., 2022). Moreover, the density of the colonies plays

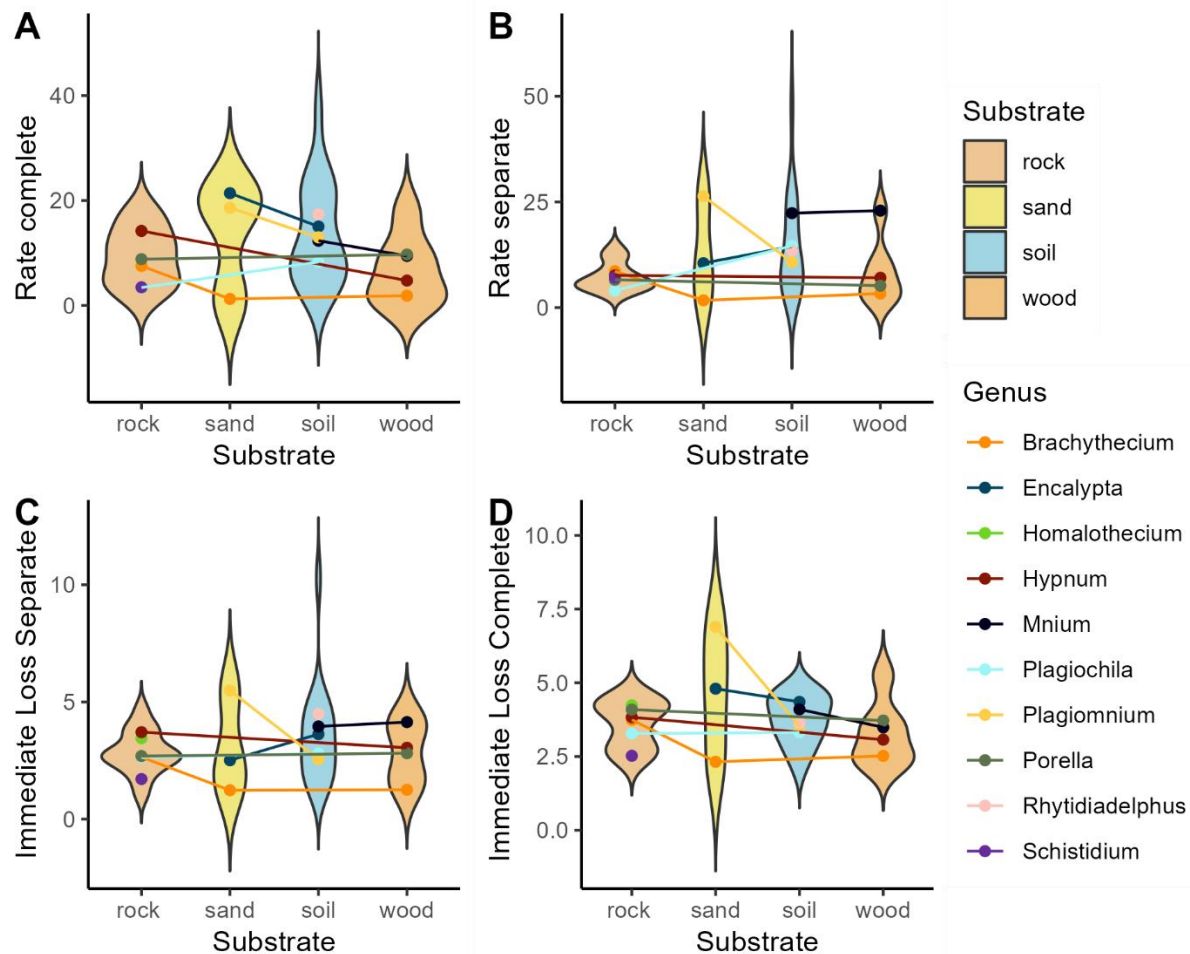an important role when avoiding desiccation (Elumeeva et al., 2011).

Figure 2. Substrate divided data for A. rate of water loss in complete pieces, B rate of water loss in separated pieces, C Immediate water loss in separated pieces, and D immediate water loss in complete pieces.

However, our approach did not account for the humidity of the microenvironment where the bryophytes were found (Wang et al., 2022). It is important to measure microclimate humidity to assess whether differences in dehydration rates are related to substrate choice. For instance, bryophytes in more humid environments might exhibit different dehydration rates irrespective of the substrate.

Interestingly, we found a marginally significant difference in the dehydration rates of separate individuals between rock and soil substrates (Figure 2, **¡Error! No se encuentra el origen de la referencia.**). Bryophytes growing on rocks exhibited less variation in dehydration rates and appeared to have lower values than those growing on soil, partially supporting our predictions. Previous studies suggest

that bryophytes growing on rocks are usually less dense and more resistant to desiccation (Green et al., 2011), also using molecular mechanisms to avoid dehydration as addition of abscisic acid to change their structure and evade desiccation (Charron & Quatrano, 2009; Green et al., 2011). It is important to note that there were no species found on both rock and soil substrates in our study. This lack of overlap could influence our results, and increasing the sample size of species found on both substrates could help clarify whether the statistically significant relationship observed has biological significance. For now, our findings remain speculative.

For our second question, we hypothesized that species with lower water loss rates would be more derived in the phylogeny. However, our analysis

found no phylogenetic signal for either the complete or the separate dehydration rates (Figure 1). The phylogenetic signal, lambda, for both traits was close to zero based on the results of ancestral reconstruction models (lambda < 0.005). This result indicates that the dehydration rates are independent of the phylogenetic relationships among the species studied. Consequently, the ancestral reconstruction does not provide a meaningful measure of dehydration rates in the evolutionary history of these species. This lack of phylogenetic signal suggests that other factors, such as ecological or environmental influences (Wang et al., 2022), might play a more significant role in determining dehydration rates in bryophytes, rather than their evolutionary history.

Supplementary information: All supplementary information as codes and figures can be found in: https://github.com/Emeleme/MossProject

References

Bechteler, J., Peñaloza-Bojacá, G., Bell, D., Gordon Burleigh, J., McDaniel, S. F., Christine Davis, E., Sessa, E. B., Bippus, A., Christine Cargill, D., Chantanoarrapint, S., Draper, I., Endara, L., Forrest, L. L., Garilleti, R., Graham, S. W., Huttunen, S., Lazo, J. J., Lara, F., Larraín, J., … Villarreal A., J. C. (2023). Comprehensive phylogenomic time tree of bryophytes reveals deep relationships and uncovers gene incongruences in the last 500 million years of diversification. *American Journal of Botany*, *110*(11), e16249. https://doi.org/10.1002/ajb2.16249

Boyce, C. K., & Lee, J.-E. (2017). Plant Evolution and Climate Over Geological Timescales. *Annual Review of Earth and Planetary Sciences*, *45*(Volume 45, 2017), 61–87. https://doi.org/10.1146/annurev-earth-063016-015629

Charron, A. J., & Quatrano, R. S. (2009). Between a Rock and a Dry Place: The Water-Stressed Moss. *Molecular Plant*, *2*(3), 478–486. https://doi.org/10.1093/mp/ssp018

Elumeeva, T. G., Soudzilovskaia, N. A., During, H. J., & Cornelissen, J. H. C. (2011). The importance of colony structure versus shoot morphology for the water balance of 22 subarctic bryophyte species. *Journal of Vegetation Science*, *22*(1), 152–164. https://doi.org/10.1111/j.1654-1103.2010.01237.x

Green, T. G. A., Sancho, L. G., & Pintado, A. (2011). Ecophysiology of Desiccation/Rehydration Cycles in Mosses and Lichens. In U. Lüttge, E. Beck, & D. Bartels (Eds.), *Plant Desiccation Tolerance* (pp. 89–120). Springer. https://doi.org/10.1007/978-3-642-19106-0_6

Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software*, *33*(2), 1–22.

Hadfield, J. D. (2017). *MCMCglmm Course Notes*. http://cran.nexr.com/web/packages/MCMCglmm/vignettes/CourseNotes.pdf

Jauregui-Lazo, J., Wilson, M., & Mishler, B. D. (2023). The dynamics of external water conduction in the dryland moss Syntrichia. *AoB PLANTS*, *15*(3), plad025. https://doi.org/10.1093/aobpla/plad025

Kumar, P., Kumar, P., Verma, V., Irfan, M., Sharma, R., & Bhargava, B. (2022). How plants conquered land: Evolution of terrestrial adaptation. *Journal of Evolutionary Biology*, *35*(5), 5–14. https://doi.org/10.1111/jeb.14062

Lande, R. (1976). Natural Selection and Random Genetic Drift in Phenotypic Evolution. *Evolution*, *30*(2), 314–334. https://doi.org/10.2307/2407703

Oliver, M. J., Dowd, S. E., Zaragoza, J., Mauget, S. A., & Payton, P. R. (2004). The rehydration transcriptome of the desiccation-tolerant bryophyte Tortula ruralis: Transcript classification and analysis. *BMC Genomics*, *5*(1), 89. https://doi.org/10.1186/1471-2164-5-89

Proctor, M. C. F. (2000). Mosses and alternative adaptation to life on land. *New Phytologist*, *148*(1), 1–3. https://doi.org/10.1111/j.1469-8137.2000.00751.x

Raven, J. A., & Edwards, D. (2004). 2 - Physiological evolution of lower embryophytes: Adaptations to the terrestrial environment. In A. R. Hemsley & I. Poole (Eds.), *The Evolution of Plant Physiology* (pp. 17–41). Academic Press. https://doi.org/10.1016/B978-012339552-8/50003-2

Wang, Q.-H., Zhang, J., Liu, Y., Jia, Y., Jiao, Y.-N., Xu, B., & Chen, Z.-D. (2022). Diversity, phylogeny, and adaptation of bryophytes: Insights from genomic and transcriptomic data. *Journal of Experimental Botany*, *73*(13), 4306–4322. https://doi.org/10.1093/jxb/erac127