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Title: Phylogenetic Inference of Trap Evolution and Dispersal in Carnivorous Plants Using Custom Discrete Trait and Biogeographic Models

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Background:

Ancestral character estimation is a fundamental method in evolutionary biology, allowing researchers to infer historical trait states using phylogenies and observed tip data (Harmon, 2019). The complexity of such analyses depends on the number of character states, the parameterization of models, and the inclusion of additional evolutionary processes. While standard models are widely used across phylogenetic comparative methods and historical biogeography (Harmon, 2019), relatively few studies employ custom models tailored to specific hypotheses. These bespoke approaches—modifying transition rates or allowed changes—require more effort but can yield deeper biological insights.

This thesis aims to design, deploy and test custom evolutionary and biogeographic models to advance understanding of the evolution and distribution of carnivorous plants. These plants have intrigued scientists since Darwin's *Insectivorous Plants* (1875), and exhibit remarkable adaptations of trapping mechanisms for life in nutrient-poor environments. Despite physiological specialization, carnivorous plants inhabit a wide range of habitats worldwide, often with disjunct distributions (Brewer & Schlauer, 2017). While the hypothesis for the evolutionary origin of the Venus flytrap from adhesive traps (Darwin, 1875; Juniper et al., 1989), is well-supported (e.g., Cameron et al., 2002), others—such as the bladder traps of *Utricularia*, the most diverse carnivorous genus—remain poorly understood. This thesis will devise a number of custom models describing different hypotheses about the major stages by which complex carnivorous plant traps evolve from simpler ancestral traps.

Custom models may also be deployed in biogeography. The field of historical biogeography seeks to understand how species and clades have come to occupy their present-day distributions by reconstructing where species or lineages were located geographically and when those movements or changes happened (Ronquist & Sanmartín, 2011). A central challenge in this field is long-distance dispersal (LDD), where species colonize geographically distant regions across barriers such as oceans (Jordano, 2017). Long-distance dispersal (LDD) has often been viewed with skepticism, with some critics arguing that it resembles a "miracle" rather than a scientific explanation due to its reliance on rare and complex sequences of events (Nathan, 2006), such as the presence of a specific seed-dispersing bird, the survival of seeds during transit, and suitable environmental conditions for germination upon arrival.

Modern phylogenetic tools such as BioGeoBEARS (Matzke, 2018) have advanced the historical biogeography field by making a number of basic models accessible for estimation and statistical model comparison. These enable researchers to estimate ancestral ranges, infer

dispersal pathways, and compare different hypotheses about range evolution and the mechanisms behind it. However, their capacity to realistically model LDD is limited, as they often lack the complexity needed to incorporate processes such as extinction, dynamic paleogeography, and environmental factors (e.g., winds, ocean currents, or transient island chains) that vary through time. Here, carnivorous plants again provide a useful case study, as they have a global but patchy distribution that has been affected by extinction and long-distance dispersal. This thesis aims to devise and deploy custom biogeographical models where dispersal and extinction rates are informed by changing paleogeography (derived from the GPlates plate tectonics package), to attempt to estimate when and where key events in carnivorous plant trap evolution may have occurred.

Aim & Objectives:

My main aims are to understand the evolution, the distribution pattern, and the diversification of carnivorous plants by constructing phylogenetic models that utilises discrete characteristics, geographic distance, and region to modify speciation and extinction in biogeographical SSE model (state-dependent speciation/extinction) framework.

> **Chapter 1 - Introduction:** Introduction to carnivorous plants and a review of the history and development of discrete character models in evolutionary biology.
>
> **Chapter 2 - Phylogenetic model for the evolution of carnivorous plant traps:** How can the evolutionary transitions between carnivorous plant trap types be statistically modeled using discrete trait evolution on phylogenies, and does the "pitcher hypothesis" model for the origin of *Utricularia*'s bladder traps better explain the trap evolution than alternative scenarios?
>
> **Chapter 3 - Carnivorous Plant Biogeography:** What is the biogeographical history of carnivorous plant lineages, and in particular, what is the most likely time and region for the extinct transitional forms postulated by the Chapter 2's pitcher hypothesis?
>
> **Chapter 4 - LDD (long distance dispersal) on large phylogenies:** Do carnivorous plants exhibit higher or lower rates of long-distance dispersal (LDD) compared to other plant and vertebrate lineages, and how do dispersal syndromes (e.g. wind, water, animal-mediated) influence these patterns?
>
> **Chapter 5 - SSE (state-dependent speciation and extinction) models on Carnivorous Plants:** How does carnivory influence diversification rates across angiosperms, and are carnivorous plant lineages associated with elevated speciation or extinction compared to non-carnivorous relatives? In addition, are regional factors—such as occurrences in tropical versus temperate regions—modify these evolutionary dynamics?
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> **Chapter 6 - Conclusion and future steps:** This chapter reviews limitations of the work done to date, and suggests future steps.

Research Design:

The first research chapter (Chapter 2) is largely complete and focuses on the evolution of trap types in carnivorous plants. Published genus-level phylogenies (e.g., Ellison et al., 2012; Fleischmann et al., 2010; Jobson et al., 2017) were digitized using WebPlotDigitizer (Rohatgi, 2021) and integrated into a comprehensive angiosperm framework using the *V.PhyloMaker* R package (Jin & Qian, 2019). Trap traits—such as type, subtype, size, and shape—were coded from the literature to define discrete character states (Figure 1a) These were used to construct Markov models, including a custom Complex Trap Evolution (CTE) model reflecting the "pitcher hypothesis" for the origin of *Utricularia* traps. The CTE model treats 11 trap types as ordered evolutionary states, with transitions governed by a custom rate matrix (Figure 1b, c). This model framework enables ancestral state estimation, comparison of alternative scenarios, and statistical evaluation of competing hypotheses. By quantifying previously narrative evolutionary models, this chapter provides a rigorous test of complex trap evolution in carnivorous plants.

Chapter 3 will examine the historical biogeography of carnivorous plants. Using the fixed tree developed in Chapter 2, I will perform ancestral range estimation to identify broad-scale geographic patterns across major clades. For key groups lacking recent dated phylogenies— such as the Lentibulariaceae—I will generate new time-calibrated trees using Bayesian phylogenetic methods in BEAST2 (Bouckaert et al., 2019). This analysis will incorporate curated molecular datasets and fossil-based calibration points (Tables 1 & 2). Species occurrence data from GBIF will be used for range coding, ensuring broad taxonomic and geographic coverage. These analyses will provide a temporal and spatial framework for understanding the diversification and dispersal of carnivorous plant lineages.

Chapter 4 will examine whether carnivorous plants exhibit different rates of long-distance dispersal (LDD) compared to other plant and vertebrate lineages. Using dated phylogeny and species distribution data, I will apply biogeographic models to infer dispersal events and evaluate how dispersal syndromes (e.g. wind, water, animal-mediated) influence these patterns. To support this, I will develop an R package that integrates with GPlates to calculate modern and paleogeographic distances through time, enabling tests of whether inferred LDD events correspond with historical continental configurations and how syndromes may have facilitated dispersal across shifting landmasses.

Chapter 5 will focus on state-dependent diversification models (e.g. BiSSE, HiSSE, GeoHiSSE) to evaluate whether the trait "carnivory", or particular trap types, are associated with shifts in speciation and extinction rates. I will also examine whether these effects vary geographically, particularly between tropical and temperate regions. This analysis will utilize a time-calibrated angiosperm phylogeny incorporating both carnivorous and non-carnivorous lineages, alongside species occurrence data. Competing models, including null models assuming no trait-dependent diversification, will be statistically compared to test the significance and explanatory power of trait- and region-dependent diversification hypotheses.

a

Trapping Specialization	Adhesive/snap traps	Adhesive/Pitcher Intermediate traps	Pitcher and pitcher-derived traps
Aerial	**2** Thin, filiform, adhesive leaves — *Triphyophyllum peltatum*, *Drosophyllum lusitanicum*, *Drosera regina*, *Pinguicula heterophylla*, *Roridula gorgonias*	**6** Simple funnel-shaped pitchers, adhesive inner walls — *Nepenthes aristolochioides*, *Nepenthes talangensis*, *Nepenthes eymae*	**8** Erect, trumpet-shaped pitchers — *Nepenthes mirabilis*, *Nepenthes fusiatiana*, *Sarracenia leucophylla*, *Sarracenia alata*, *Heliamphora tatei*, *Heliamphora heterodoxa*
Ground	**3** Broad adhesive leaves — *Pinguicula vulgaris*, *Pinguicula acuminata*, *Pinguicula ibarroe*, *Drosera prostratoscaposa*, *Drosera schizandra*, *Drosera cuneifolia*	**7** Highly curled adhesive leaf — *Pinguicula lutea*, *Pinguicula grandiflora*, *Pinguicula villosa* (and many other *Pinguicula*)	**9** Short, squat pitchers — *Nepenthes ampullaria*, *Nepenthes rowanae*, *Sarracenia purpurea*, *Sarracenia rosea*, *Cephalotus follicularis*, Extinct pitcher plants*
Amphibious	**4** Rapidly-closing traps — *Drosera burmannii*, *Drosera sessilifolia*, *Dionaea muscipula*	- - - Gain of Carnivory; Pitcher Hypothesis for the Origin of *Utricularia*	**10** Pitchers with eel-trap — *Darlingtonia californica* (juvenile), *Sarracenia psittacina*, Extinct pitcher plants*, Genus *Genlisea* (e.g. *G. aurea*)
Aquatic	**5** Aquatic snap trap — *Aldrovanda vesiculosa*	—— Darwin Hypothesis for the Origin of Dionea/Aldrovanda	**11** Aquatic "pitchers" — *Genlisea aurea*, *Genlisea repens*, *Utricularia wannani*, *Utricularia dichotoma* (and many other *Genlisea* and *Utricularia*)

b — Complex trap evolution model for the origin of bladder traps

States	1	2	3	4	5	6	7	8	9	10	11
1	0	2	2								
2	1	0	3			11					
3	1	3	0	4			9				
4	1			0	5						
5	1				0						
6	1					0		12			
7	1						0		10		
8	1							0	6		
9	1							6	0	7	
10	1									0	8
11	1										0

c

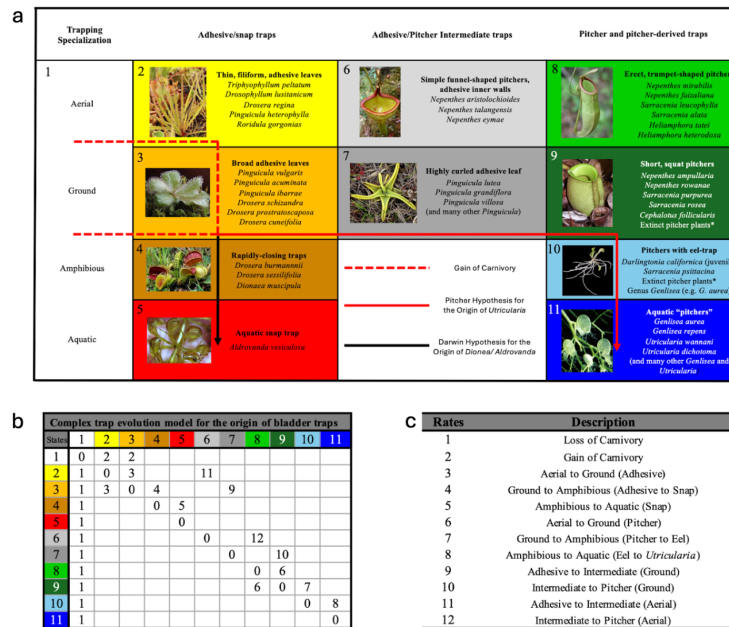| Rates	Description
1	Loss of Carnivory
2	Gain of Carnivory
3	Aerial to Ground (Adhesive)
4	Ground to Amphibious (Adhesive to Snap)
5	Amphibious to Aquatic (Snap)
6	Aerial to Ground (Pitcher)
7	Ground to Amphibious (Pitcher to Eel)
8	Amphibious to Aquatic (Eel to *Utricularia*)
9	Adhesive to Intermediate (Ground)
10	Intermediate to Pitcher (Ground)
11	Adhesive to Intermediate (Aerial)
12	Intermediate to Pitcher (Aerial)

Figure 1. The pitcher model for the origin of *Utricularia*'s bladder traps is presented as a transition between a series of stages, within the overall context of convergent evolution of carnivorous plant trapping mechanisms. (**a**) There are 11 states that are represented by numbers, and color-coded to match the color codes in phylogeny/character mapping figures. Character states are explained as following: (1) non-carnivorous, (2) aerial adhesive/ flypaper trap, (3) ground adhesive/ flypaper trap, (4) amphibious snap trap, (5) aquatic snap trap, (6) aerial adhesive/pitcher intermediate trap, (7) ground adhesive/pitcher intermediate trap, (8) aerial pitchers, (9) ground pitchers, (10) amphibious pitchers, and (11) aquatic pitchers. (**b**) Transition matrix for the Complex Trap Evolution (CTE) model postulated in (**a**), which includes the pitcher hypothesis for the origin of the *Utricularia* trap. (**c**) Each number represents a different free transition rate parameter in the model.

Family	Genus	# of species (matk gene)	# of species (GBIF)
Lentibulariaceae	*Utricularia*	61	287
	Genlisea	24	29
	Pinguicula	81	131
Droseraceae	*Aldrovanda*	1	1
	Dionaea	1	1
	Drosera	18	246
Nepenthaceae	*Nepenthes*	85	210
Sarraceniaceae	*Sarracenia*	11	54
	Heliamphora	6	21
	Darlingtonia	1	1

Table 1. The list of each carnivorous plant clade shows the available number of species on data source (GenBank, https://www.ncbi.nlm.nih.gov/genbank/ & GBIF, https://www.gbif.org/) for molecular data and their biogeography.

Family	Age (mya)	Type	Reference
Droseraceae	55–38	Fossil	Degreef, J. D. (1989). Early history Drosera and Drosophyllum. Carnivorous Plant Newsletter, 18(3), 86–89. Degreef, J. D. (1997). Fossil Aldrovanda. Carnivorous Plant Newsletter, 26(3), 93–97. Schlauer, J. (1997). Fossil Aldrovanda—Additions. Carnivorous Plant Newsletter, 26(3), 98–98.
Droseraceae	53.4	Secondary	Fleischmann, A., Schlauer, J., Smith, S. A., & Givnish, T. J. (2018). Evolution of carnivory in angiosperms (Vol. 1). Oxford University Press. https://doi.org/10.1093/oso/9780198779841.003.0003
Nepenthaceae	84.8	Secondary	Fleischmann, A., Schlauer, J., Smith, S. A., & Givnish, T. J. (2018). Evolution of carnivory in angiosperms (Vol. 1). Oxford University Press. https://doi.org/10.1093/oso/9780198779841.003.0003
Nepenthaceae	76.8	Secondary	Magallón, S., Gómez-Acevedo, S., Sánchez-Reyes, L. L., & Hernández-Hernández, T. (2015). A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. The New Phytologist, 207(2), 437–453.
Nepenthaceae (or Droseraceae)	129~113	Fossil	https://www.mindat.org/taxon-4890151.html
Sarraceniaceae	34.9	Secondary	Liu, S., & Smith, S. D. (2021). Phylogeny and biogeography of South American marsh pitcher plant genus Heliamphora (Sarraceniaceae) endemic to the Guiana Highlands. Molecular Phylogenetics and Evolution, 154, 106961. https://doi.org/10.1016/j.ympev.2020.106961
Lentibulariaceae (Utricularia + Genlisea)	39	Secondary	Silva, S. R., Gibson, R., Adamec, L., Domínguez, Y., & Miranda, V. F. O. (2018). Molecular phylogeny of bladderworts: A wide approach of Utricularia (Lentibulariaceae) species relationships based on six plastidial and nuclear DNA sequences. Molecular Phylogenetics and Evolution, 118, 244–264. https://doi.org/10.1016/j.ympev.2017.10.010
Lentibulariaceae (Pinguicula)	65~54	Secondary	Cieslak, T., Polepalli, J. S., White, A., Müller, K., Borsch, T., Barthlott, W., Steiger, J., Marchant, A., & Legendre, L. (2005). Phylogenetic analysis of Pinguicula (Lentibulariaceae): Chloroplast DNA sequences and morphology support several geographically distinct radiations. American Journal of Botany, 92(10), 1723–1736. https://doi.org/10.3732/ajb.92.10.1723
Lentibulariaceae (Utricularia + Genlisea)	40	Secondary	Ibarra-Laclette, E., Lyons, E., Hernández-Guzmán, G., Pérez-Torres, C. A., Carretero-Paulet, L., Chang, T.-H., Lan, T., Welch, A. J., Abraham Juárez, M. J., Simpson, J., Fernández-Cortés, A., Arteaga-Vázquez, M., Góngora-Castillo, E., Acevedo-Hernández, G., Schuster, S. C., Himmelbauer, H., Minoche, A. E., Xu, S., Lynch, M., … Herrera-Estrella, L. (2013). Architecture and evolution of a minute plant genome. Nature, 498(7452), 94–98. https://doi.org/10.1038/nature12132

Table 2. The list of each carnivorous plant clade shows the available time calibrations for BEAST analysis.

Ethics approval: Not required; however, all data are from public sources (e.g., GenBank) and analyzed using open-source tools (e.g., R, BEAST2), with proper citation throughout.

Resources: I plan to attend an international evolution or biogeography conference, supported by a Rutherford grant, to present my work, receive feedback, and build research networks.

Potential problems: I do not foresee any potential problems.

Revised timeline of research:
Year 1: (+ beginning of Year 2)
    Create more alternative trait evolution models for statistical comparison - Done
    Revise character states for each carnivorous plant species - Done
    Conduct statistical comparison of trap evolution models - Done
    Draft manuscript - Done
    Get comments, and submit to journal - In Progress
    Create GPlates R package to test modern distance vs paleodistance - Begun
    Gather biogeographic data + molecular data for BEAST analysis - Begun
Year 2:
    BEAST2 phylogenetic dating and generate MCC trees
    BioGeoBEARS analysis on fixed and BEAST-dated trees
    Generate ancestral range reconstructions
    Large-scale LDD analysis on seed plants + gymnosperm vs vertebrate group
    Build trait dataset for dispersal syndrome classification (wind, water, etc…)
Year 3:
    Complete LDD statistical comparison between carnivorous plants and other lineages
    Test effect of dispersal syndromes on LDD probabilities using custom models
    Begin SSE modelling to compare diversification rates of carnivorous plants vs others
    Test for interaction between carnivory trait and tropical vs temperate distributions

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Signatures required:

Candidate _____

Main Supervisor _____

Please indicate if the proposal is to be treated confidentially (tick). Yes _____ No _____

Co-Supervisor/s _____
(Advisors need not sign)

SBS Graduate Committee _____