Molecular Phylogenetics and Evolution 182 (2023) 107755

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

A plastome phylogeny of *Rumex* (Polygonaceae) illuminates the divergent evolutionary histories of docks and sorrels

Daniel M. Koenemann [a,*], Logan Kistler [b], Janelle M. Burke [c]

[a] Claflin University, Department of Biology, 400 Magnolia Street, Orangeburg, SC 29115, USA
[b] National Museum of Natural History, Anthropology Department, 10th Street & Constitution Avenue NW, Washington, DC 20560, USA
[c] Howard University, Department of Biology, EE Just Hall, 415 College Street NW, Washington, DC 20059, USA

ARTICLE INFO

Keywords:
PAML
RAxML
Nucleotide diversity
Pi

ABSTRACT

The genus *Rumex* L. (Polygonaceae) provides a unique system for investigating the evolutionary development of sex determination and molecular rate evolution. Historically, *Rumex* has been divided, both taxonomically and colloquially into two groups: 'docks' and 'sorrels'. A well-resolved phylogeny can help evaluate a genetic basis for this division. Here we present a plastome phylogeny for 34 species of *Rumex*, inferred using maximum likelihood criteria. The historical 'docks' (*Rumex* subgenus *Rumex*) were resolved as monophyletic. The historical 'sorrels' (*Rumex* subgenera *Acetosa* and *Acetosella*) were resolved together, though not monophyletic due to the inclusion of *R. bucephalophorus* (*Rumex* subgenus *Platypodium*). *Emex* is supported as its own subgenus within *Rumex,* instead of resolved as sister taxa. We found remarkably low nucleotide diversity among the docks, consistent with recent diversification in that group, especially as compared to the sorrels. Fossil calibration of the phylogeny suggested that the common ancestor for *Rumex* (including *Emex*) has origins in the lower Miocene (22.13 MYA). The sorrels appear to have subsequently diversified at a relatively constant rate. The origin of the docks, however, was placed in the upper Miocene, but with most speciation occurring in the Plio-Pleistocene.

1. Introduction

Commonly known as docks and sorrels, *Rumex* L. (Polygonaceae) is a relatively large genus. *Rumex* encompasses four circumscribed subgenera, approximately 200 species, and hundreds of described subspecies and varieties. *Rumex* has been formally monographed twice (Campderá, 1819; Meisner, 1856), and continued to be of taxonomic interest through the mid-20th Century (Rechinger 1933, 1937, 1939, 1949, 1954a, 1954b, 1984, 1990; Brandbyge and Rechinger, 1989). The 20th Century, however, also brought new tools and therefore a new perspective to studies in *Rumex*. Áskell Löve applied advances in cytology, specifically karyotyping, to *Rumex* and discovered a wealth of variation, sometimes documenting multiple cytotypes within recognized species (Löve, 1957, 1967; Löve and Kapoor, 1967).

Most workers recognize four subgenera in *Rumex*: *Rumex*, *Acetosa*, *Acetosella* and *Platypodium* (Mosyakin, 2005). Recently, a fifth subgenus *Emex,* was proposed (Schuster et al., 2015), based on its inclusion from molecular evidence. The distinction among subgenera is based on morphological characters such as outer tepal type, habit, and base chromosome number. Some workers, in particular, Löve and colleagues, argued that the cytological and morphological differences are so great, that four segregate genera should be recognized (Löve and Kapoor, 1967). Dioecy is largely associated with the occurrence of sex chromosomes, found in *Rumex* subg. *Acetosa* and *Acetosella* (See Table 1 in Grant et al., 2022). Among the dioecious species, there is a documented female-bias (Korpelainen, 2002; Stehlik and Barrett, 2005; Stehlik et al., 2007; Stehlik et al., 2008). Monoecy has been described in the Hawaiin endemics (Navajas-Pérez, 2012), though monoecy, andromonoecy or 'polygamomonoecy' appears to be widespread, at least in North America (Dean and Mitchell, 1979; Mosyakin, 2005). The taxonomic complexity in *Rumex* also presents nomenclatural challenges. There are almost 1200 described taxa within the genus, including species, hybrids, infrageneric and infraspecific taxa.

Several groups have published phylogenies of *Rumex* utilizing molecular data, though none with clear resolution among all species. Navajas-Pérez et al. (2005) were able to outline many of the major relationships in the genus, recovering a phylogenetic topology roughly congruent with the morphological subgeneric classification system

Abbreviations: NMNH, Smithsonian National Museum of Natural History; MYA, Million Years Ago; SRA, Sequence Read Archive.

* Corresponding author.
E-mail addresses: DKoenemann@claflin.edu (D.M. Koenemann), KistlerL@si.edu (L. Kistler), Janelle.Burke@howard.edu (J.M. Burke).

https://doi.org/10.1016/j.ympev.2023.107755
Received 1 November 2022; Received in revised form 7 February 2023; Accepted 5 March 2023
Available online 10 March 2023

proposed in the 20th Century (Rechinger, 1933, 1937, 1939, 1949, 1954a, 1954b, 1984, 1990; Brandbyge and Rechinger, 1989). Yet, this phylogeny did not sample widely in the genus and left many relationships, especially within subgenus *Rumex* ('docks'), poorly resolved. This work was improved upon by Grant et al. (2022), who were able to greatly increase the sampling of species within *Rumex*. At the same time, their work also failed to resolve the relationships among species in subgenus *Rumex*.

In addition to questions of phylogenetic topology, there exist related questions of evolutionary processes within *Rumex*. One such area of interest is understanding why relationships within subgenus *Rumex*, the largest of the subgenera, have been so difficult to resolve. There are a number of possible explanations; It may be that the lineages within subgenus *Rumex* are very old, and that the long branches of these lineages have accumulated conflicting genetic information, making phylogenetic resolution difficult. Alternatively, it might be that the lineages within subgenus *Rumex* are very young and, as a result, there are few accumulated genetic differences among them, making phylogenetic resolution difficult. It is also well documented that species of subgenus *Rumex* commonly form hybrids among themselves (*e.g.* Ziburski et al., 1986; Rechinger, 1990; Kitchener, 2002; Takahashi and Hanyu, 2015). Indeed, by our count, Rechinger documented some 32 hybrid taxa within subgenus *Rumex*. Frequent hybridization can increase the likelihood and accelerate the process of so-called "chloroplast capture", contributing to phylogenetic ambiguity in plastid-informed phylogenetic reconstructions.

Phylogenetic work in *Rumex* to date, at least with respect to the chloroplast genome, has largely exhausted the capacity of first-generation (Sanger) sequencing technologies to resolve relationships within the genus. Second generation (massively parallel) sequencing technologies have the capacity to generate the many more informative characters that are likely necessary both to resolve relationships within *Rumex*, as well as to elucidate the underlying processes driving those relationships (Zhang et al., 2022). Such genome-scale datasets, combined with contemporary phylogenetic algorithms and fossil data, allow us to begin to answer questions of evolutionary process by assessing genetic similarity among collections of phylogenetically well-resolved taxa set in the context of an absolute timescale (Soltis et al., 2010; Leebens-Mack et al., 2019; Wong et al., 2020).

Therefore, as part of our ongoing efforts to elucidate the origin and evolution of heteromorphic sex chromosomes in *Rumex*, we hoped to 1) more clearly resolve the cladistic status (monophyletic/ paraphyletic) for the subgenera of *Rumex*, 2) better resolve species relationships within those subgenera, 3) more clearly resolve the placement of the genus *Emex* relative to *Rumex*, and 4) obtain a better understanding of evolutionary processes in *Rumex*, particularly subgenus *Rumex*, through fossil calibration of the *Rumex* phylogeny and the calculation of genetic diversity metrics.

We here present the results of this work: a maximum likelihood plastome phylogeny for 34 species of Rumex (including one species of Emex) plus 14 taxa within the Polygonaceae to provide temporal constraint, time-calibrated using three calibration points, and set in the context of average pairwise nucleotide diversity (π) among the 'docks' (subg. *Rumex*) and the 'sorrels' (subg. *Acetosa* and subg. *Acetosella* together).

2. Methods

Infiles and code can be found in the supplemental files (S1–S16). Assemblies and annotations are available on Dryad (Koenemann et al., 2023)

2.1. Sampling

We sampled 34 species of *Rumex,* representatively among the subgenera: 12/41 Subgenus *Acetosa*, 3/5 Subgenus *Acetosella*, 1/2 Subgenus *Emex*, 1/1 Subgenus *Platypodium*, 17/126 Subgenus *Rumex* - species estimates from Grant et al. (2022). We obtained 15 plastome sequences preassembled from GenBank (Benson et al., 1999), especially the outgroups (Table 1). The majority of the *Rumex* plastomes we assembled for this work. In some cases, these assemblies were generated from Illumina read archives that are publicly available from the Sequence Read Archive (Leinonen et al., 2011) (Table 1). We assembled the remainder of the plastomes from Illumina read archives that we generated for this study (Table 1).

For Illumina read archives generated for this study, we extracted whole-genomic DNA from herbarium or silica-preserved leaf material using methods described in Grant et al. (2022). Library preparation and Illumina HiSeq whole genome sequencing (KAPA PCR free library preparation, 500 bp insert, paired-end sequencing, 740 M total reads over 25 pooled samples) were conducted by Admera Health (South Plainfield, NJ).

2.2. Sequence intake and quality control

We removed the second inverted repeat from all GenBank plastomes and plastomes assembled for this study. We used the default settings in FastQC v0.11.9 (Wingett and Andrews, 2018) to identify potential problems in all Illumina read archives (no problems were identified). We used AdapterRemoval2 (Scubert et al., 2016) to remove adapter fragments from reads, trim reads past the first occurrence of a base call with quality score <20, enforce a minimum fragment length of 35 bp, and split the reads into two files consisting of complementary read pairs (in preparation for input into GetOrganelle).

2.3. Assembly

We assembled all read archives into whole plastid genomes (plastomes) *de novo* using GetOrganelle v1.6.2d (Jin et al. 2020). We input read pairs as separate files. We set the number of rounds of extension to 15 and provided the *Rumex nepalensis* GenBank plastome as the initial seed in the case of *Rumex* and *Emex* species, or the *Rheum palmatum* GenBank plastome as the initial seed in the case of *Rheum* species (Table 1). Otherwise, all parameters were set to the defaults. GFA and log files were examined to confirm proper assembly of each plastome.

GetOrganelle was not able to assemble a circular plastid genome for *Rumex thyrsoides*. Rather, it was assemble into seven scaffold sequences (32,810 bp, 30,390 bp, 26,285 bp, 13,095 bp, 4,815 bp, 4,410 bp, 3,964 bp). We used Geneious Prime v2023.0.1 (https://www.geneious.com; Kearse et al., 2012) to align these scaffolds to the plastome of the nearest evolutionary neighbor of *R. thyrsoides* (*R. acetosa*). The scaffolds aligned to the reference as non-overlapping and nearly contiguous. We generated the consensus sequence, coding the gaps between the scaffolds and on the ends of the alignment as ambiguous (N).

2.4. Annotation and feature analysis

We generated annotations for all plastomes included in this study using GeSeq through the CHLOROBOX web platform (Tillich et al., 2017). We used the program defaults for plastid genomes, with the exception of adding the optional annotation search functionality of tRNAscan-SE v2.0.7 (Chan and Lowe, 2019).

Using the feature coordinates from the GeSeq output, we used BEDTools (Quinlan and Hall, 2010) to extract the sequences of each of the features (exons, introns, rRNA, tRNA) from the unaligned plastome of each taxon, as well as the spacer regions bridging each of the features (we only carried forward spacers at least 100 base pairs long). We then used MAFFT v7.505 (Katoh and Standley, 2013) to align each feature and spacer across relevant taxa. We used the nuc.div() function in R from the pegas v1.1 package (Paradis, 2010) to calculate the average pairwise per-site nucleotide diversity (π) for each aligned feature and each aligned spacer. Alignment and calculation of π were done three

Table 1
Sampling and source material for the taxa utilized in this study.

Taxon	Source	GenBank Reference ID or DOI	Data Type	Subfamily Classification
Atraphaxis bracteata Losinsk.	GenBank	NC_059952.1	Assembled Plastome	NA (Outgroup)
Calligonum colubrinum I.G.Borshch.	GenBank	NC_049142.1	Assembled Plastome	NA (Outgroup)
Calligonum pumilum Losinsk.	GenBank	NC_053262.1	Assembled Plastome	NA (Outgroup)
Fagopyrum dibotrys (D.Don) Hara	GenBank	NC_037705.1	Assembled Plastome	NA (Outgroup)
Fagopyrum leptopodum (Diels) Hedberg	GenBank	NC_056984.1	Assembled Plastome	NA (Outgroup)
Fallopia multiflora (Thunb.) Haraldson	GenBank	NC_041239.1	Assembled Plastome	NA (Outgroup)
Fallopia sachalinensis (F.Schmidt) Ronse Decr.	GenBank	NC_047446.1	Assembled Plastome	NA (Outgroup)
Muehlenbeckia platyclada (F.Muell.) Meisn.	GenBank	NC_062330.1	Assembled Plastome	NA (Outgroup)
Oxyria sinensis Hemsl.	GenBank	NC_032031.1	Assembled Plastome	NA (Outgroup)
Persicaria filiformis (Thunb.) Nakai	GenBank	NC_058319.1	Assembled Plastome	NA (Outgroup)
Persicaria japonica (Meisn.) Nakai	GenBank	NC_056952.1	Assembled Plastome	NA (Outgroup)
Rheum nobile Hook.f. & Thomson	GenBank	NC_046506.1	Assembled Plastome	NA (Outgroup)
Rheum palmatum L.	GenBank	NC_027728.1	Assembled Plastome	NA (Outgroup)
Rheum rhabarbarum L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	NA (Outgroup)
Rumex abyssinicus Jacq.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex acetosa L.	SRA Illumina Read Archive	ERR5554750	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex acetosella L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosella* ("Sorrel")
Rumex albescens Hillebr.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex alpinus L.	SRA Illumina Read Archive	ERR5554590	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex altissimus Alph.Wood	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex aquaticus L.	SRA Illumina Read Archive	ERR5555389	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex arcticus Trautv.	SRA Illumina Read Archive	ERR5529493	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex bucephalophorus L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Platypodium*
Rumex conglomeratus Murray	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex crispus L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex dentatus L.	SRA Illumina Read Archive	SRR15698557	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex graminifolius Georgi ex Lamb	SRA Illumina Read Archive	ERR5555126	Illumina Read Archive	Subgenus Acetosella ("Sorrel")
Rumex hastatulus Baldwin (North Carolina Genotype)	SRA Illumina Read Archive	SRR6294518	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex hastatus D.Don	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex hymenosepalus Torr.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex hypogaeus T.M.Schust. & Reveal (*Emex australis* Steinh.)	GenBank	NC_050054.1	Assembled Plastome	Subgenus *Emex*
Rumex induratus Boiss. & Reut.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex longifolius DC.	SRA Illumina Read Archive	ERR5555327	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex lunaria L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex mexicanus Meisn.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex nepalensis Spreng.	GenBank	NC_057504.1	Assembled Plastome	Subgenus *Rumex* ("Dock")
Rumex obtusifolius L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")

(*continued on next page*)

Table 1 (*continued*)

Taxon	Source	GenBank Reference ID or DOI	Data Type	Subfamily Classification
Rumex papilio Coss.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex paucifolius Nutt.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosella* ("Sorrel")
Rumex peruanus Rech.f.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex rothschildianus Aarons.	SRA Illumina Read Archive	SRR6294517	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex sagittatus Thunb.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex sanguineus L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex scutatus L.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex sibiricus Hultén	SRA Illumina Read Archive	ERR5529374	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex stenophyllus Ledeb.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Rumex* ("Dock")
Rumex thyrsiflorus Fingerh.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")
Rumex thyrsoides Desf.	Illumina Read Archive Generated for this Study	https://doi.org/10.5061/dryad.mkkwh714r	Illumina Read Archive	Subgenus *Acetosa* ("Sorrel")

times for each feature and spacer, using three different groups of species: sorrels only, docks only, and all *Rumex* (Table 1). For these calculations we included *Rumex bucephalophorus* in the sorrels, but excluded *R. hypogaeus* (*Emex australis*) from any of the calculations.

Preliminary π calculations revealed that elements of the features associated with *rps*12 displayed anomalously inflated π values. The various elements of *rps*12 are known to be naturally separated onto different parts of the plastome, with some exons located in the inverted repeat region and some in the large single copy region. mRNA splicing brings these elements together after transcription (Hildebrand et al., 1988). We believe that the plastome annotator attempted to map all elements of *rps*12 to both locations and generated, as a result, an inaccurate mapping. We have therefore excluded all features associated with *rps*12 from the final figure (Fig. 1) and any calculated metrics.

The placement of features and spacers inside of the major plastid regions (LSC, SSC, IR) followed the results of the annotation coupled with reference to recent plastome mapping in the *Rumex* sister genus *Rheum* (Zhou et al., 2018).

2.5. Phylogeny reconstruction

We reconstructed the *Rumex* phylogeny using a maximum likelihood criterion (RAxML v8.2.4: Stamatakis, 2014). The input alignment was generated from whole plastomes (one of the repeats having been removed, as outlined above) using MAFFT. This alignment was manually adjusted to remove an artificial indel produced by the ambiguous characters in the *R. thyrsoides* consensus sequence. We ran ModelTest-NG v0.1.7 (Darriba et al., 2019), which revealed the GTR nucleotide substitution model to be the best fit model that was available for use in the RAxML phylogenetic algorithm. As a result, we specified the RAxML reconstruction conditions to be a nucleotide substitution model of GTR + Γ for two separate analyses. In the first analysis we conducted 1000 search replicates with no bootstrap iterations. In the second analysis we conducted a single search replicate with 1000 bootstrap iterations. We used FigTree v1.4.4 (Rambaut, 2018) to visualize the phylogenetic output. We constructed the bootstrap consensus tree using MESQUITE (Maddison and Maddison, 2021).

We also reconstructed the *Rumex* phylogeny using a Bayesian criterion (MrBayes v3.2.7a: Ronquist et al., 2012). We specified a single partition with a nucleotide substitution model of GTR + Γ for two runs, each with four chains. Each run lasted 5 million generations, sampling every 1000 generations. We used the RWTY v1.0.2 (R We There Yet?: Warren et al., 2017) package in R to assess the convergence of the MrBayes runs (S12). This analysis suggested that the runs did converge and that a 20 percent burn-in fraction was appropriate when summarizing the runs. We then summarized the two runs with a burn-in fraction of 20 percent, using *Sump* and *Sumt*, with all other parameters set to the default.

We used the *mcmctree* function in PAML (PAML v.4.9j: Yang, 2007, 2021) to time-calibrate the *Rumex* phylogeny. We analyzed the data as a single partition. We used the correlated-rates evolutionary clock model (evolutionary rates may vary across the branches, but are correlated with rates on other local branches). We set the nucleotide substitution model to HKY85 (the model most similar to the GTR recommended by ModelTest, and also available in *mcmctree*). The topology was fixed to that of the best RAxML tree. The other parameters were set with the aid of the *mcmctree* section of the PAML manual (pamlDOC, 2020). Our *mcmctree* control file is available as a supplement (S2). We ran the analysis for 20,000 generations, sampling every 10 generations, with a burn-in of 1,000 generations (following Cai et al., 2015).The RAxML, MrBayes, and PAML analyses were conducted on the Smithsonian Institution high performance computing cluster (https://doi.org/10.25572/SIHPC).

Three date parameters were used to calibrate the phylogeny: two primary node constraint (common ancestor) priors based on fossil information, and one secondary node constraint prior based on the findings of previous research. PAML *mcmctree* only uses "soft" node constraints (pamlDOC, 2020). That is, *mcmctree* may reconstruct node ages outside of the ranges specified by the user. As such, all three of our node constraints were soft constraints. These age constraint priors are also uniform in shape, that is, all node ages within the limits of the constraint were regarded as equally probable.

The first primary node constraint was placed at the base node (common ancestor) of the genus *Rumex*. This node was set to a minimum of age of 3.6 MYA (mid-Pliocene) and a maximum age of 23 MYA (lower Miocene). The age of this node was informed by fossils of *Rumex* fruits that can be dated to this age range (Huang et al., 2021), as well as pollen records of *Rumex* dating into the late Miocene (5–10 MYA) but not further back than the end-Oligocene (23 MYA) (*e.g.* Muller, 1981; Barrón et al., 2006; Jiménez-Moreno et al., 2007; Manchester and O'Leary, 2010).

The second primary node constraint was placed at the base node of the genus *Persicaria*. This node was set to a minimum age of 2.5 MYA (end-Pliocene) and a maximum age of 59 MYA (lower Paleogene). The

age of this node was informed by fossilized *Persicaria* pollen dating to this range of ages (Schuster et al., 2013, *et hoc cit.*).

The secondary node constraint prior was placed at the base of the Polygonaceae (all taxa in the tree). This node was set to a maximum age of 122 MYA (lower Cretaceous). The age of this node was informed by previous studies that dated the common ancestor of the Polygonaceae family to this age (Soltis et al., 2008; Forest and Chase, 2009; Schuster et al., 2013; Kostikova et al., 2014).

3. Results

3.1. Dataset

In total, we sampled plastomes from 14 outgroup species and 34 species of *Rumex* (including *Emex*), including species from all of the subfamilies of *Rumex* (Table 1). Of the 34 *Rumex* plastomes sampled, 32 were assembled by us for this work. The total aligned matrix length, including both *Rumex* and the outgroups, was 162,918 base pairs, with 27,021 variant patterns (S8).

3.2. Plastome assembly

In most cases (32 of 33) GetOrganelle was able to assemble a fully circular plastome *de novo*. In one case (*Rumex thyrsoides*), GetOrganelle was able to assemble seven scaffold sequences *de novo* (32,810 bp, 30,390 bp, 26,285 bp, 13,095 bp, 4,815 bp, 4,410 bp, 3,964 bp), but was not able to connect them. We used these scaffolds to generate a partial sequence for *R. thyrsoides* as described in the methods.

Across the 33 plastomes assembled for this study, the median coverage depth for *de novo* assembled regions was 393.8X, and the mean coverage depth for *de novo* assembled regions was 385.3X (Table 2).

Table 2
Metrics for plastomes assembled in this study.

Taxon	SRA Reference ID	Average (Mean) Coverage Depth
Rheum rhabarbarum	PRJNA935754	471.5
Rumex abyssinicus	PRJNA935754	357.1
Rumex acetosa	ERR5554750	107.8
Rumex acetosella	PRJNA935754	330
Rumex albescens	PRJNA935754	352.4
Rumex alpinus	ERR5554590	237.8
Rumex altissimus	PRJNA935754	392.4
Rumex aquaticus	ERR5555389	346.9
Rumex arcticus	ERR5529493	346.4
Rumex bucephalophorus	PRJNA935754	375.4
Rumex conglomeratus	PRJNA935754	401.1
Rumex crispus	PRJNA935754	404.7
Rumex dentatus	SRR15698557	407.5
Rumex graminifolius	ERR5555126	162
Rumex hastatulus	SRR6294518	494.6
Rumex hastatus	PRJNA935754	372.8
Rumex hymenosepalus	PRJNA935754	410.8
Rumex induratus	PRJNA935754	323.9
Rumex longifolius	ERR5555327	181.7
Rumex lunaria	PRJNA935754	549.6
Rumex mexicanus	PRJNA935754	427.2
Rumex obtusifolius	PRJNA935754	510.9
Rumex papilio	PRJNA935754	508.4
Rumex paucifolius	PRJNA935754	489.5
Rumex peruanus	PRJNA935754	505.2
Rumex rothschildianus	SRR6294517	416.4
Rumex sagittatus	PRJNA935754	465.5
Rumex sanguineus	PRJNA935754	393.8
Rumex scutatus	PRJNA935754	369.9
Rumex sibiricus	ERR5529374	505.4
Rumex stenophyllus	PRJNA935754	393.5
Rumex thyrsiflorus	PRJNA935754	436
Rumex thyrsoides	PRJNA935754	266.7 (across the 7 *de novo* assembled scaffolds)

3.3. Plastome annotation

The annotation of the *Rumex* plastomes identified 127 genetic features: 88 coding regions (exons), 10 introns, 5 rRNAs, and 24 tRNAs (Fig. 1). Additionally, we identified 83 spacers at least 100 bp long, bridging these genetic features.

The nucleotide diversity among the docks was notably lower than among the sorrels (Fig. 1). The mean π value averaged across all features for docks was 0.006488413, and for sorrels was 0.019003196. The mean π value averaged across all spacers was 0.0251772658, across all coding regions (exons) was 0.0061026585, across all introns was 0.0137102818, across all rRNA was 0.0002471127, and across all tRNA was 0.0182284642.

3.4. Phylogeny reconstruction

The best RAxML tree was fully resolved (all nodes represent bifurcations; Fig. 2). Additionally, subgenus *Rumex* ('docks') was resolved as monophyletic. Subgenus *Acetosa* was not resolved as monophyletic, however, with subgenera *Acetosella* and *Platypodium* being nested inside of it. The sorrels in their historical sense (subgenera *Acetosa* and *Acetosella* together) were not resolved as monophyletic through the inclusion of the monotypic subgenus *Platypodium*. *Rumex hypogaeus* (*Emex australis*) was resolved as sister to the sorrels.

The bootstrap consensus tree recapitulated the topology of the best RAxML tree. Most clades received strong support (bootstrap support >90). The exceptions to this were the clade uniting the Rumiceae (the genera *Oxyria, Rheum*, *Emex*, and *Rumex*), the clade uniting the docks *Rumex mexicanus and Ru. altissimus,* and the clade uniting the docks *Ru. alpinus*, *Ru. dentatus*, and *Ru. albescens*.

The MrBayes tree was able to fully resolve all relationships with high posterior probability support (PP > 95). The topology of this tree was identical to that of the best RAxML tree and the RAxML bootstrap consensus tree. The results of the RWTY analysis showed that the independent runs each adequately searched tree space, and adequately converged on solutions that were similar and becoming more similar over time.

3.5. PAML fossil calibration

The fossil calibration placed the most recent common ancestor for *Rumex* (including *Emex*) in the lower Miocene (22.13 MYA, 95% Highest Posterior Density Interval [HPD] 18.56–25.58 MYA) and the most recent common ancestor for the tribe Rumiceae (*Rumex*, *Emex*, *Rheum*, and *Oxyria*) in the upper Eocene (35.17 MYA, 95% HPD 27.38–44.13 MYA). The origin of the docks (subgenus *Rumex*) was dated to 11.45 MYA (95% HPD 7.99–14.90 MYA), and the origin of the sorrels (subgenera *Acetosa*, *Acetosella*, and *Platypodium* together) was dated to 13.77 MYA (95% HPD 10.22–17.42 MYA). Species within the subgenera *Acetosa* and *Acetosella* appear to have subsequently diversified at a relatively constant rate. Most diversification in subgenus *Rumex*, however, occurred beginning in the mid-Pliocene (approx. 3.6 MYA) and Pleistocene (Fig. 2).

4. Discussion

We observed a marked difference in π values between the docks and the sorrels. Specifically, the docks displayed little variation among their plastid genomes. This low nucleotide diversity among the docks is consistent with the recent divergence times of its lineages. The low nucleotide diversity observed here in the docks was also observed in the plastid genomes of *Rheum*, the sister genus of *Rumex*, whose lineages are interpreted to be very young (Zhang et al., 2021). What makes this comparison more remarkable, however, is that most of the species of

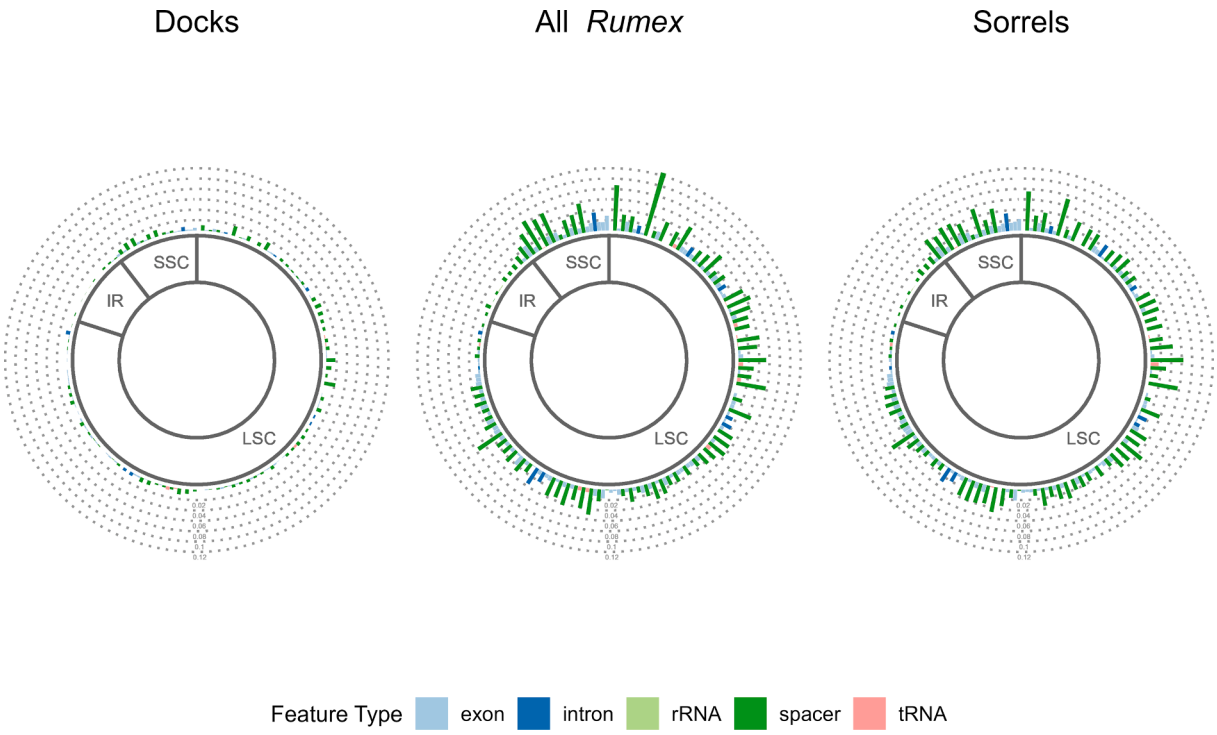

Fig. 1. Nucleotide Diversity Among Docks and Sorrels. Bars represent π (average pairwise per-site nucleotide diversity) values for each feature and spacer. Features and spacers are presented in syntenic order and color-coded, according to the results of the annotation. The inner circle depicts the major regions of the plastid: Large Single Copy Region (LSC), Small Single Copy Region (SSC), and one of the Inverted Repeats (IR). The gray, dashed concentric circles are the y-axis scale and indicate different values of π. Labels for these circles can be found at the bottom of each subchart. An alternate version of this figure, with each feature labeled, can be found in the supplemental files (S6).

Rheum are restricted to Central Asia, whereas the docks are cosmopolitan in distribution.

Together, these pieces of information suggest a recent and rapid collection of speciation events within *Rumex* subgenus *Rumex*, ultimately accounting for most of the taxonomic diversity in the genus. This result serves to explain much of the taxonomic and phylogenetic

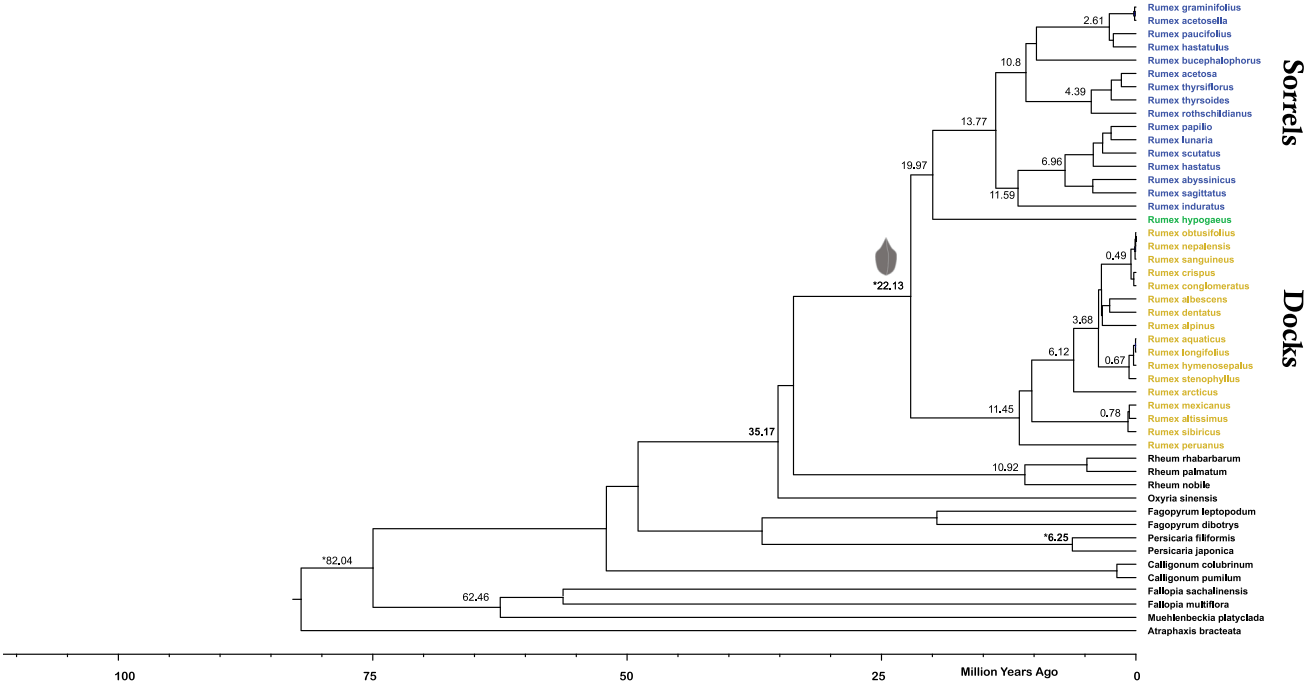

Fig. 2. RAxML Phylogeny of *Rumex* Fossil-Calibrated with PAML. All nodes have high support (bootstrap and posterior probability over 90) except where indicated in the results section. Key nodes have been annotated with the reconstructed date from PAML. Node ages with an asterisk were constrained during the PAML calibration. The achene is located at the base of *Rumex*, to indicate the node for which we constrained using data from a fossilized achene.

difficulty encountered in this subgenus over the past decades. Recent divergence of the docks would serve as an explanation for the poor species boundaries and frequent hybridization observed among many of the species in the subgenus. Recent divergence of the docks, coupled with frequent hybridization, would serve as an explanation for taxonomic confusion in the subgenus. Finally, recent divergence of the docks, coupled with frequent hybridization, would serve to explain the historical lack of phylogenetic resolution in the subgenus.

Our results demonstrate that the sorrels represent an evolutionary lineage distinct from the docks. These two major groups in *Rumex* have long been recognized as morphologically divergent but previous molecular work had produced mixed results regarding the monophyly of subgenus *Rumex* (*e.g.* Navajas-Pérez et al., 2005; Grant et al., 2022). This work resolves the docks (subgenus *Rumex*) as monophyletic with high confidence using molecular data. The paraphyly of the sorrels is not unexpected. Previous work (Grant et al., 2022) had shown the monotypic subgenus *Platypodium* (*R. bucephalophorus*), which is not historically considered as one of the sorrels, to be nested inside the sorrels. That placement is confirmed here.

The final major finding with respect to topology is the placement of *Rumex hypogaeus* (*Emex australis*). For centuries it had been recognized that *Emex* had an affinity to *Rumex* (Campderá, 1819), but was thought to be distinct from *Rumex* due to differences in tepal morphology. Subsequent molecular work suggested that *Emex* was nested within *Rumex* (*e.g.* Sanchez et al., 2011; Schuster et al., 2015), with Schuster et al. (2015) proposing that *Emex* should be included in *Rumex*. However, there was some controversy over this taxonomic act due to the differences in tepal morphology, and the fact that other work had shown the placement of *Emex* to be ambiguous (*e.g.* Burke et al., 2010; Grant et al., 2022). Our work has again resolved *Emex* inside of *Rumex* and sister to the sorrels, further justifying that *Emex* should be recognized as a subgenus of *Rumex*.

Our estimates for the origins of the Rumiceae are younger than estimates from previous work (Schuster et al., 2013). That study, however, neither employed genomic data nor included any fossil priors within the Rumiceae. Our dating of the common ancestor of *Rumex* is older than that found in a recent dated plastome phylogeny of the sister genus *Rheum* (Zhang et al., 2021), where *Rumex* was used as an outgroup. That study, however, included only two species of *Rumex* and no *Rumex* fossils. As a result, we consider our dating here to be likely improvements on previous estimates.

The relatively recent origins (Plio-Pleistocene) for the majority of species in the docks, is a striking finding of the paper. It is interesting in no small part because the majority of the species in the genus (ca. 130 of ca. 200) are placed in the docks (*Rumex* subg. *Rumex*). The large number of species also makes the interpretation of this finding difficult. A truly universal or generalized explanation for speciation in a group of plants with a cosmopolitan distribution is probably asking too much. One possible unifying theme, however, could be Pleistocene glaciation. While the species in the docks are distributed globally, most still inhabit colder regions, either North or South temperate regions, or montane tropical regions. It is possible that repeated glaciation, and repeated retreat of *Rumex* populations into refugia, was a critical driver of evolution in this group. The result has been a large number of morphologically identifiable groups, many with incomplete reproductive isolation.

Given that the sorrels occupy similar habitats (temperate/montane) as the docks, why have they not speciated at the same rate as the docks? One possible explanation is that Pleistocene evolution in the sorrels has been largely cytological rather than morphological. It is possible that during that same Plio-Pleistocene period the various sex chromosomes and sex chromosome systems of the sorrels, as well as their differences in chromosome numbers, were evolving. Future studies focusing on the nuclear genome are planned in order to examine this.

5. Conclusions

Our results show a striking difference in the rates of molecular evolution within one genus. The docks and sorrels within genus *Rumex* not only represent different taxonomic subgenera with different morphology, but clearly the rates of molecular change are different among these groups as well. Our study only demonstrated the variability of the plastid genome. Future phylogenomic studies that include the nuclear genome will be able to further investigate the genomic features which may be driving this change, especially comparisons among different homomorphic or heteromorphic sex chromosomes within the sorrel clade.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Science Foundation [NSF-DEB #2101101 to JMB and LK, 2021]. Some of the computations in this paper were conducted on the Smithsonian High Performance Cluster (SI/HPC), Smithsonian Institution. Doi: 10.25572/SIHPC.

The authors would like to thank A. Lin (National Museum of Natural History) for help with phylogenetic time calibration, R. Everly (NMNH Botany Library) for help in obtaining fossil citations, R. Dikow and M. Kweskin (Smithsonian HPC) for help with computing, and A. Vaughan, H. Barnes, and N. Karunajeewa (Royal Botanic Gardens Victoria: RBGV, MEL) for help verifying vouchers for preassembled GenBank plastomes.

The authors would also like to thank two anonymous reviewers for their helpful comments that improved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2023.107755 and https://doi.org/10.5061/dryad.mkkwh714r.

References

Barrón, E., Lassaletta, L., Alcalde-Olivares, C., 2006. Changes in the Early Miocene palynoflora and vegetation in the east of the Rubielos de Mora Basin (SE Iberian Ranges, Spain). N. Jb. Geol. Palaont. Abh. 242, 171–204.

Benson, D.A., Boguski, M.S., Lipman, D.J., Ostell, J., Ouellette, B.F.F., Rapp, B.A., Wheeler, D.L., 1999. GenBank. Nucleic Acids Res. 26, 1–7. https://doi.org/10.1093/nar/27.1.12.

Brandbyge, J., Rechinger, K.H., 1989. A new *Rumex* from Ecuador. Nord. J. Bot. 9, 203–204. https://doi.org/10.1111/j.1756-1051.1989.tb02115.x.

Burke, J.M., Sanchez, A., Kron, K.A., Luckow, M.A., 2010. Placing the woody tropical genera of Polygonaceae: a hypothesis of character evolution and phylogeny. Am. J. Bot. 97, 1377–1390. https://doi.org/10.3732/ajb.1000022.

Cai, J., Liu, X., Vanneste, K., Proost, S., Tsai, W.-C., Liu, K.-W., Chen, L.-J., He, Y., Xu, Q., Bian, C., Zheng, Z., Sun, F., Liu, W., Hsiao, Y.-Y., Pan, Z.-J., Hsu, C.-C., Yang, Y.-P., Hsu, Y.-C., Chuang, Y.-C., Dievart, A., Dufayard, J.-F., Xu, X., Wang, J.-Y., Wang, J., Xiao, X.-J., Zhao, X.-M., Du, R., Zhang, G.-Q., Wang, M., Su, Y.-Y., Xie, G.-C., Liu, G.-H., Li, L.-Q., Huang, L.-Q., Luo, Y.-B., Chen, H.-H., Van de Peer, Y., Liu, Z.-J., 2015. The genome sequence of the orchid *Phalaenopsis equestris*. Nat. Genet. 47, 65–72. https://doi.org/10.1038/ng.3149.

Campderá y Camin, F., 1819. Monographie des rumex, précédée de quelques vues générales sur la famille des polygonées, Treuttel et Würtz, Paris.

Chan, P.P., Lowe, T.M., 2019. tRNAscan-SE: searching for tRNA genes in genomic sequences. Methods Mol. Biol. 1962, 1–14. https://doi.org/10.1007/978-1-4939-9173-0_1.

Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B., Flouri, T., 2019. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. Mol. Biol. Evol. 37, 291–294. https://doi.org/10.1093/molbev/msz189.

Dean, J.K., Mitchell, R.,1979. Polygonaceae of New York State. New York State Museum Bulletin no. 431. State Education Department, Albany, NY.

Forest, F., Chase, M.W., 2009. Eudicots. In: Hedges, S., Kumar, S. (Eds.), Timetree of Life. Oxford University Press, Oxford, pp. 169–176.

Grant, K.D., Koenemann, D., Mansaray, J., Ahmed, A., Khamar, H., El Oualidi, J., Burke, J.M., 2022. A new phylogeny of *Rumex* (Polygonaceae) adds evolutionary context to the diversity of reproductive systems present in the genus. PhytoKeys 204, 57–72.

Hildebrand, M., Hallick, R.B., Passavant, C.W., Bourque, D.P., 1988. Trans-splicing in chloroplasts: the rps 12 loci of Nicotiana tabacum. Proc. Natl. Acad. Sci. USA 85, 372–376. https://doi.org/10.1073/pnas.85.2.372.

Huang, Y.-J., Zhu, H., Su, T., Spicer, R.A., Hu, J.-J., Jia, L.-B., Zhou, Z.-K., 2021. Rise of herbaceous diversity at the southeastern margin of the Tibetan Plateau: first insight from fossils. J. Syst. Evol. In Press. https://doi.org/10.1111/jse.12755.

Jiménez-Moreno, G., Aziz, H.A., Rodríguez-Tovar, F.J., Pardo-Igúzquiza, E., Suc, J.-P., 2007. Palynological evidence for astronomical forcing in Early Miocene lacustrine deposits from Rubielos de Mora Basin (NE Spain). Palaeogeogr. Palaeoclimatol. Palaeoecol. 252, 601–616. https://doi.org/10.1016/j.palaeo.2007.05.013.

Jin, J.J., Yu, W.B., Yang, J.B., Song, Y., dePamphilis, C.W., Yi, T.-S., Li, D.Z., 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 21, 241. https://doi.org/10.1186/s13059-020-02154-5.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Kitchener, G.D., 2002. *Rumex* × *xenogenus* Rech. fil. (Polygonaceae), the hybrid between Greek and Patience Docks, found in Britain. Watsonia 24, 209–213.

Koenemann, D., Kistler, L., Burke, J.. A plastome phylogeny of Rumex (Polygonaceae) illuminates the divergent evolutionary histories of docks and sorrels, Dryad, Dataset. https://doi.org/10.5061/dryad.mkkwh714r.

Korpelainen, H., 2002. A genetic method to resolve gender complements investigations on sex ratios in *Rumex acetosa*. Mol. Evol. 11, 2151–2156.

Kostikova, A., Salamin, N., Pearman, P.B., 2014. The role of climatic tolerances and seed traits in reduced extinction rates of temperate Polygonaceae. Evolution 68, 1856–1870. https://doi.org/10.1111/evo.12400.

Leebens-Mack, J.H., Barker, M.S., Carpenter, E.J., Deyholos, M.K., Gitzendanner, M.A., Graham, S.W., Grosse, I., Li, Z., Melkonian, M., Mirarab, S., Porsch, M., Quint, M., Rensing, S.A., Soltis, D.E., Soltis, P.S., Stevenson, D.W., Ullrich, K.K., Wickett, N.J., DeGironimo, L., Edger, P.P., Jordon-Thaden, I.E., Joya, S., Liu, T., Melkonian, B., Miles, N.W., Pokorny, L., Quigley, C., Thomas, P., Villarreal, J.C., Augustin, M.M., Barrett, M.D., Baucom, R.S., Beerling, D.J., Benstein, R.M., Biffin, E., Brockington, S. F., Burge, D.O., Burris, J.N., Burris, K.P., Burtet-Sarramegna, V., Caicedo, A.L., Cannon, S.B., Çebi, Z., Chang, Y., Chater, C., Cheeseman, J.M., Chen, T., Clarke, N. D., Clayton, H., Covshoff, S., Crandall-Stotler, B.J., Cross, H., dePamphilis, C.W., Der, J.P., Determann, R., Dickson, R.C., Di Stilio, V.S., Ellis, S., Fast, E., Feja, N., Field, K.J., Filatov, D.A., Finnegan, P.M., Floyd, S.K., Fogliani, B., García, N., Gâteblé, G., Godden, G.T., Goh, F., Greiner, S., Harkess, A., Heaney, J.M., Helliwell, K.E., Heyduk, K., Hibberd, J.M., Hodel, R.G.J., Hollingsworth, P.M., Johnson, M.T.J., Jost, R., Joyce, B., Kapralov, M.V., Kazamia, E., Kellogg, E.A., Koch, M.A., Von Konrat, M., Könyves, K., Kutchan, T.M., Lam, V., Larsson, A., Leitch, A.R., Lentz, R., Li, F.-W., Lowe, A.J., Ludwig, M., Manos, P.S., Mavrodiev, E., McCormick, M.K., McKain, M., McLellan, T., McNeal, J.R., Miller, R.E., Nelson, M.N., Peng, Y., Ralph, P., Real, D., Riggins, C.W., Ruhsam, M., Sage, R.F., Sakai, A.K., Scascitella, M., Schilling, E.E., Schlösser, E.-M., Sederoff, H., Servick, S., Sessa, E.B., Shaw, A.J., Shaw, S.W., Sigel, E.M., Skema, C., Smith, A.G., Smithson, A., Stewart, C. N., Stinchcombe, J.R., Szövényi, P., Tate, J.A., Tiebel, H., Trapnell, D., Villegente, M., Wang, C.-N., Weller, S.G., Wenzel, M., Weststrand, S., Westwood, J. H., Whigham, D.F., Wu, S., Wulff, A.S., Yang, Y., Zhu, D., Zhuang, C., Zuidof, J., Chase, M.W., Pires, J.C., Rothfels, C.J., Yu, J., Chen, C., Chen, L., Cheng, S., Li, J., Li, R., Li, X., Lu, H., Ou, Y., Sun, X., Tan, X., Tang, J., Tian, Z., Wang, F., Wang, J., Wei, X., Xu, X., Yan, Z., Yang, F., Zhong, X., Zhou, F., Zhu, Y., Zhang, Y., Ayyampalayam, S., Barkman, T.J., Nguyen, N., Matasci, N., Nelson, D.R., Sayyari, E., Wafula, E.K., Walls, R.L., Warnow, T., An, H., Arrigo, N., Baniaga, A.E., Galuska, S., Jorgensen, S.A., Kidder, T.I., Kong, H., Lu-Irving, P., Marx, H.E., Qi, X., Reardon, C. R., Sutherland, B.L., Tiley, G.P., Welles, S.R., Yu, R., Zhan, S., Gramzow, L., Theißen, G., Wong, G.K., 2019. One thousand plant transcriptomes and the phylogenomics of green plants. Nature 574, 679–685. https://doi.org/10.1038/s41586-019-1693-2.

Leinonen, R., Sugawara, H., Shumway, M., 2011. The sequence read archive. Nucleic Acids Res. 39, D19–D21. https://doi.org/10.1093/nar/gkq1019.

Löve, Á., 1957. Sex determination in *Rumex*. Proc. Genet. Soc. Canada 2, 31–36.

Löve, Á., 1967. IOPB chromosome number reports. XIII. Taxon 16, 445–461.

Löve, Á., Kapoor, B.M., 1967. A chromosome atlas of the collective genus *Rumex*. Cytologia 32, 328–342.

Maddison, W.P., Maddison, D.R., 2021. Mesquite: A Modular System for Evolutionary Analysis. Version 3.70. http://www.mesquiteproject.org.

Manchester, S.R., O'Leary, E.L., 2010. Phylogenetic distribution and identification of fin-winged fruits. Bot. Rev. 76, 1–82. https://doi.org/10.1007/s12229-010-9041-0.

Mosyakin A., 2005. *Rumex*. In: Flora of North America Editorial Committee, Vol. 5. eds. 1993+. *Flora of North America North of Mexico*. 16+ vols. New York and Oxford.

Meisner, C.F., 1856. Prodromus Systematis Naturalis Regni Vegetabilis 14, 1–186.

Muller, J., 1981. Fossil pollen records of extant angiosperms. Bot. Rev. 47, 1–142.

Navajas-Pérez, R., 2012. The genus Rumex: a plant model to study sex-chromosome evolution. In: New insights on plant sex chromosomes. Nova Science Publishers Inc, New York, pp. 91–109.

Navajas-Pérez, R., de la Herrán, R., González, G.L., Jamilena, M., Lozano, R., Rejón, C.R., Rejón, M.R., Garrido-Ramos, M.A., 2005. The evolution of reproductive systems and sex-determining mechanisms within *Rumex* (Polygonaceae) inferred from nuclear and chloroplastidial sequence data. Mol. Biol. Evol. 22, 1929–1939. https://doi.org/10.1093/molbev/msi186.

pamlDOC, 2020. User Guide, PAML: Phylogenetic Analysis by Maximum Likelihood. http://abacus.gene.ucl.ac.uk/software/pamlDOC.pdf (accessed 24 June 2022).

Paradis, E., 2010. pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26, 419–420. https://doi.org/10.1093/bioinformatics/btp696.

Quinlan, A.R., Hall, I.M., 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841–842. https://doi.org/10.1093/bioinformatics/btq033.

Rambaut, A., 2018. *FigTree*. Github. https://github.com/rambaut/figtree.

Rechinger, K.H., 1933. Vorarbeiten zu einer Monographie der Gattung *Rumex*, III. Die Süd-und zentralamerikanischen Arten der Gattung *Rumex*. Ark. Bot. 26A, 1–58.

Rechinger, K.H., 1937. The North American species of *Rumex*. Field Mus. Nat. Hist. 1–151.

Rechinger, K.H., 1939. Polygonaceae. In: Hegi, G., Hanser, C. (Eds.), Illustrierte flora von Mittel-Europa. Verlag, Munich, pp. 352–436.

Rechinger, K.H., 1949. Rumices asiatici: Vorarbeiten zu einer Monographie der Gattung *Rumex* VII. Candollea 12, 9–162. https://www.jstor.org/stable/43336109.

Rechinger, K.H., 1954b. Beiträge zur kenntnis von *Rumex* XII: Some new American species of *Rumex*. Leafl. West. Bot. 7, 133–135.

Rechinger, K.H., 1984. *Rumex* (Polygonaceae) in Australia: a reconsideration. Nuytsia 5, 75–122. https://florabase.dpaw.wa.gov.au/nuytsia/article/98.

Rechinger, K.H., 1990. *Rumex* subgen. *Rumex* sect. *Axillares* (Polygonaceae) in South America. Plant Syst. Evol. 172, 151–192. https://doi.org/10.1007/BF00937805.

Rechinger, K.H., 1954a. Monograph of the genus *Rumex* in Africa: vorarbeiten zu einer Monographie der Gattung *Rumex* VIII. Gleerup, Lund.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029.

Sanchez, A., Schuster, T.M., Burke, J.M., Kron, K.A., 2011. Taxonomy of Polygonoideae (Polygonaceae): a new tribal classification. Taxon 60, 151–160. https://doi.org/10.1002/tax.601013.

Schuster, T.M., Setaro, S.D., Kron, K.A., 2013. Age estimates for the buckwheat family Polygonaceae based on sequence data calibrated by fossils and with a focus on the Amphi-Pacific *Muehlenbeckia*. PLoS One 8, e61261.

Schuster, T.M., Reveal, J.L., Bayly, M.J., Kron, K.A., 2015. An updated molecular phylogeny of Polygonoideae (Polygonaceae): Relationships of *Oxygonum*, *Pteroxygonum*, and *Rumex*, and a new circumscription of *Koenigia*. Taxon 64, 1188–1208. https://doi.org/10.12705/646.5.

Scubert, M., Lindgreen, S., Orlando, L., 2016. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Res. Notes 9, 88. https://doi.org/10.1186/s13104-016-1900-2.

Soltis, D.E., Bell, C.D., Kim, S., Soltis, P.S., 2008. Origin and early evolution of angiosperms. Ann. N. Y. Acad. Sci. 1133, 3–25. https://doi.org/10.1196/annals.1438.005.

Soltis, D.E., Moore, M.J., Burleigh, J.G., Bell, C.D., Soltis, P.S., 2010. Assembling the angiosperm tree of life: progress and future prospects. Ann. Mo. Bot. Gard. 97, 514–526. https://doi.org/10.3417/2009136.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Stehlik, I., Barrett, S.C., 2005. Mechanisms governing sex-ratio variation in dioecious *Rumex nivalis*. Evolution 59, 814–825.

Stehlik, I., Friedman, J., Barrett, S.C., 2008. Environmental influence on primary sex ratio in a dioecious plant. Proc. Natl. Acad. Sci 105, 10847–10852.

Stehlik, I., Kron, P., Barrett, S.C., Husband, B.C., 2007. Sexing pollen reveals female bias in a dioecious plant. New Phytol. 175, 185–194.

Takahashi, K., Hanyu, M., 2015. Hybridization between alien species *Rumex obtusifolius* and closely related native vulnerable species *R. longifolius* in a mountain tourist destination. Sci. Rep. 5, 13898. https://doi.org/10.1038/srep13898.

Tillich, M., Lehwark, P., Pellizzer, T., Ulbricht-Jones, E.S., Fischer, A., Bock, R., Greiner, S., 2017. GeSeq – versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 45, W6–W11.

Warren, D.L., Geneva, A.J., Lanfear, R., 2017. RWTY (R We There Yet): an R package for examining convergence of Bayesian phylogenetic analyses. Mol. Biol. Evol. 34, 1016–1020. https://doi.org/10.1093/molbev/msw279.

Wingett, S.W., Andrews, S., 2018. FastQ Screen: a tool for multi-genome mapping and quality control. F1000Research 7, 1338. https://doi.org/10.12688/f1000research.15931.2.

Wong, G.-K.-S., Soltis, D.E., Leebens-Mack, J., Wickett, N.J., Barker, M.S., Van de Peer, Y., Graham, S.W., Melkonian, M., 2020. Sequencing and analyzing the transcriptomes of a thousand species across the tree of life for green Plants. Annu. Rev. Plant Biol. 71, 741–765. https://doi.org/10.1146/annurev-arplant-042916-041040.

Yang, Z., 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Mol. Biol. Evol. 24, 1586–1591. https://doi.org/10.1093/molbev/msm088.

Yang, Z., 2021. Phylogenetic Analysis by Maximum Likelihood (PAML) v4.9j (version 4.9j). Github. https://github.com/abacus-gene/paml.

Zhang, H.-J., Zhang, X., Landis, J.B., Sun, Y.-X., Sun, J., Kuang, T.-H., Li, L.-J., Tiamiyu, B.B., Deng, T., Sun, H., Wang, H.C., 2021. Phylogenomic and comparative analyses of *Rheum* (Polygonaceae, Polygonoideae). J. Syst. Evol. In Press. 10.1111/jse.12814.

Zhang, S., Guo, B., Ho, S., Ho, Y., 2022. Characterization of the complete chloroplast genome of *Rumex dentatus* L. (Polygonaceae). Mitochondrial DNA Part B 7, 546–547. https://doi.org/10.1080/23802359.2022.2054380.

Zhou, Y., Nie, J., Xiao, L., Hu, Z., Wang, B., 2018. Comparative chloroplast genome analysis of rhubarb botanical origins and the development of specific identification markers. Molecules 23, 2811. https://doi.org/10.3390/molecules23112811.

Ziburski, A., Kadereit, J.W., Leins, P., 1986. Quantitative aspects of hybridization in mixed populations of *Rumex obtusifolius* L. and *R. crispus* L. (Polygonaceae). Flora 178, 233–242. https://doi.org/10.1016/S0367-2530(17)31501-3.