

1 **Phylogeny of docks and sorrels (*Rumex*, Polygonaceae) reveals plasticity of reproductive**
2 **systems**

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24 **Abstract**

25 The genus *Rumex* is a unique member of the Polygonaceae (Buckwheat) family of plants. A
26 source of intrigue for *Rumex* lies in the diversity of the reproductive systems associated with the
27 subgenera, species, and subspecies within this genus. Four previously circumscribed subgenera,
28 some 200 species, and a number of subspecies comprise the collective *Rumex* genus. These
29 species exhibit monoecious, dioecious, synoecious (hermaphroditic), and polygamous
30 reproductive systems. Moreover, some of the dioecious species contain sex chromosomes, a
31 phenomenon that is very rare in angiosperms. Apart from the confirmed morphological and
32 phytogeographical distinctions, two of the four described subgenera, *Acetosa* and *Acetosella*, are
33 distinctive in their exhibited sex chromosome systems. For this study, we used three chloroplast
34 markers, *rbcL*, *trnH-psbA*, *trnL-F*, and dense taxon sampling, to reconstruct a molecular
35 phylogeny for *Rumex*. The reconstructed phylogeny for this work resolves six major clades and
36 one large grade in *Rumex*. In addition, the species with known dioecious reproductive systems
37 derived from unique sex chromosome systems are resolved in two different clades nested within
38 “the dioecious clade”. These results suggest that the species with divergent sexual systems are
39 more closely related to each other than to other species comprising the rest of the *Rumex* genus.
40 Furthermore, some species with known synoecious reproductive systems are resolved in a single
41 clade which is also nested within “the dioecious clade”. These results imply a possible reversal
42 occurring over time which suggests the highly plastic nature of reproductive systems among
43 *Rumex* species.

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47 **Introduction**

48 Commonly known as docks and sorrels, *Rumex* L. (Polygonaceae) is a relatively large
49 genus. *Rumex* encompasses four circumscribed subgenera, approximately 200 species, and
50 hundreds of described subspecies or varieties. Many species in *Rumex* are cosmopolitan in
51 nature, spanning six continents of the world. However, many individual species are either
52 regionally endemic, native, or introduced on particular continents (Rechinger, 1937). The
53 cosmopolitan attributes of *Rumex* species are indicative of their ability to thrive in a wide variety
54 of environmental conditions. In addition to being distributed globally, plants of the genus inhabit
55 a wide range of habitats and ecotypes.

56 *Rumex* species are among the most ubiquitous plants in the world. Described species are
57 just as recurrent in dry and sandy soils as they are in marshes and cultivated fields spanning the
58 arctic, subarctic, boreal, temperate, tropical, and subtropical localities (Löve & Kapoor, 1967).
59 Although several biological species demonstrate little to no niche preference (*e.g.*, *Rumex*
60 *crispus*, *Rumex obtusifolius*), there are others that exhibit exceedingly precise ecological
61 requirements (*e.g.*, *Rumex bipinnatus*, *Rumex pictus*). The large variation in the distribution of
62 *Rumex* species might also account for the large deviation observed in the morphology of these
63 species, whereby some reach almost seven meters in height, and others rarely exceed a few
64 centimeters (Rechinger, 1949; Löve & Kapoor, 1967; Rechinger, 1990).

65 The broad variation in both the morphology and phytogeography of *Rumex* species is also
66 indicative of the substantial taxonomic classification interest in these species. Documented
67 descriptions of plants in the genus date back to the time of classical Greece. Species of *Rumex*
68 are first noted by Hippocrates (greek physician) and Theophrastus (Greek philosopher) under the
69 name *Lapathum* (Campderá, 1819). The first formal monograph of *Rumex* species was

70 completed in 1819 (Campderá, 1819), documenting 110 species of *Rumex* proper and delineating
71 three genera of "Rumices" in the broad sense: *Emex* (L.) Campd., *Rumex*, and *Oxyria* Auth.

72 Its second formal monograph was completed in 1856 (Meisner, 1856), documenting 134
73 species of *Rumex*, dividing it into three sections: *Acetosa* ('sorrels'), *Acetosella* ('sorrels'), and
74 *Lapathum* ('docks') (Meisner, 1856; Löve, 1967). In the 20th Century, progress in the taxonomic
75 and cytological study of *Rumex* was largely accomplished by two researchers: Áskell Löve and
76 Karl Heinz Rechinger (Rechinger, 1937; Rechinger, 1954; Löve, 1967). Löve extensively
77 documented the cytological diversity of *Rumex*, and he proposed a generic status for *Acetosa* and
78 *Acetosella* (the groups with species bearing heteromorphic sex chromosomes) and subgeneric
79 status for *Axillares* and *Platypodium*. Löve also considered *Rumex* to be composed of several
80 smaller genera corresponding to a number of cytotypes (Löve 1957; Löve & Kapoor 1967;
81 Mariotti *et al.*, 2006, 2009).

82 Over the course of his long career, Rechinger effectively monographed *Rumex*, using
83 plant morphology and geographic distribution (Rechinger 1933, 1937, 1939, 1949, 1954a,
84 1954b, 1984, 1990; Brandbyge & Rechinger, 1989). It was not until the mid-1900's that
85 Rechinger proposed a subgeneric status for *Platypodium* and maintained *Acetosa*, *Acetosella*,
86 and *Lapathum* as comparable subgenera (Rechinger, 1954). In important respects, Rechinger's
87 morphological classification mirrored Löve's cytological classification. Löve's cytotypes were
88 largely reflected in Rechinger's subgeneric sectional system. Rechinger, however, chose to retain
89 *Rumex* as a single genus.

90 Recent molecular phylogenetic work has sought to resolve the placement of *Rumex* in the
91 Polygonaceae more broadly (Sanchez & Kron, 2008, Sanchez *et al.*, 2009; Burke *et al.*, 2010;
92 Burke & Sanchez, 2011; Sanchez *et al.*, 2011; Schuster *et al.*, 2011; Schuster *et al.*, 2013;

93 Schuster *et al.*, 2015). These studies have placed *Rumex* alongside the other Rumices of
94 Campderá (*Emex* and *Oxyria*), with the addition of *Rheum* as either sister to *Oxyria* (Burke *et al.*,
95 2010; Schuster *et al.*, 2011) or to *Rumex* + *Emex* (Schuster *et al.*, 2013; Schuster *et al.*, 2015).
96 One area that lacks clarity has been the placement of *Emex*, which sometimes appears to be
97 nested within *Rumex* (*e.g.*, Sanchez *et al.*, 2011) and is sometimes placed as sister to *Rumex* (*e.g.*
98 Burke *et al.*, 2010). The relationships of species within *Rumex*, including the relationship
99 between *Rumex* and *Emex*, continue to be poorly understood due to insufficient sampling and
100 paucity of data. To date, the relationships among species placed within Rechinger's subgenus
101 *Rumex* are particularly obscure.

102 The reproductive systems of *Rumex* species vary just as much, or more, than their studied
103 morphologies and geographical distributions. The high degrees of variation in the reproductive
104 systems of *Rumex* species can also speak to the macroevolutionary significance of circumscribed
105 *Rumex* subgenera, another attribute that accounts for much of the longstanding interest in this
106 genus. Species of *Rumex* demonstrate synoecious (hermaphroditic), monoecious, dioecious, and
107 polygamous reproductive systems (Rechinger 1949; Rechinger 1954a; Löve & Kapoor, 1967;
108 Mosyakin, 2005; Navajas-Pérez *et al.*, 2005). Most of the reproductive system diversity has been
109 described in subgenera *Acetosa* or *Acetosella*. In particular, most species in those subgenera are
110 dioecious (Rechinger 1937, 1949, 1954a, 1984). A few species in subgenus *Rumex* have variable
111 systems, especially between synoecy and monoecy (*e.g.*, *Rumex crispus*, pers. obs.). Also
112 noteworthy are the three species of *Rumex* endemic to the Hawaiian islands (*Rumex albescens*, *R.*
113 *giganteus* and *R. skottsbergii*), which are all monoecious (Wagner *et al.*, 1999).

114 Heteromorphic sex chromosomes are extraordinarily uncommon in plants, occurring in
115 <1% of all land plants (Ming *et al.*, 2011). Chromosomal sex determination systems are

116 restricted to the angiosperms, bryophytes, and gymnosperms, but have evolved repeatedly and
117 independently within these groups (Charlesworth, 2002; Ming *et al.*, 2011; Renner, 2014). In
118 plants with sex chromosomes, a variety of sex determining mechanisms are present (Ming *et al.*,
119 2011). In *Rumex*, the documented sex chromosomes are heteromorphic. Two sex determining
120 chromosomal mechanisms are known: XX/XY and XX/XY₁Y₂ (Löve, 1940; Löve, 1942; Löve,
121 1943; Löve, 1944; Löve & Löve, 1948; Shibata *et al.*, 1999; Shibata *et al.*, 2000; Navajas-Pérez
122 *et al.*, 2005; Cunado *et al.*, 2007; Ming *et al.* 2011). The XX/XY (male heterogamy) system bears
123 more than a passing resemblance to the mammalian sex determination system, and studies of the
124 formation of this chromosomal arrangement in plants may give insights into the historical
125 formation of the analogous system in mammals (Charlesworth, 2002). The XX/XY₁Y₂ system is
126 dosage-dependent, and plant sex is based on the autosome to sex-chromosome ratio. In this
127 system, female individuals have 14 chromosomes, and male individuals have 15 chromosomes
128 (Löve, 1940; Löve, 1944; Löve, & Kapoor, 1967; Navajas-Pérez *et al.*, 2005).

129 The vast majority of plants are synoecious with at least morphologically hermaphroditic
130 flowers (Ming *et al.*, 2011) and this has been considered the ancestral state for land plants
131 (Navajas-Pérez *et al.*, 2005; Ming *et al.*, 2011, but see also Renner, 2014). Holding true to the
132 same synoecious ancestral state, the vast majority of *Rumex* species, particularly those nested
133 within subgenus *Rumex*, reveal predominantly synoecious reproductive systems (Rechinger,
134 1937; Rechinger, 1954; Świetlińska, 1963). Dioecious plants have been proposed to be derived
135 from synoecious ancestors via two possible pathways, with either gynodioecy as a transitional
136 state or monoecy as a transitional state (Figure 1; Charlesworth & Charlesworth, 1978;
137 Lewis, 1942; Lloyd, 1980; Lloyd & Webb, 1986; Renner & Won, 2001; Barrett, 2013; Crossman
138 & Charlesworth, 2013).

139 Both monoecy and dioecy have evolved multiple times in plants (Charlesworth 2002,
140 Renner & Won 2001; Ming *et al.*, 2011). Historically, it had been suggested that dioecy emerged
141 multiple times in *Rumex* (Fox, 1985). Navajas-Pérez *et al.* (2005), however, concluded that
142 heteromorphic sex chromosomes evolved only once in *Rumex*, while dioecy (without
143 heteromorphic sex chromosomes) evolved twice. For some time, dioecy appeared to be
144 evolutionarily terminal since many dioecious taxa are embedded in clades of hermaphroditic taxa
145 and purely dioecious clades are usually species-poor (Heilbuth, 2000; Barrett, 2013).

146 More recently, however, several cases of reversals from dioecy to monoecy have been
147 recorded (Barrett, 2013). In all of these cases, monoecy was derived from a group of “leaky”
148 dioecious plants. At the population level of these supposedly dioecious taxa, some
149 hermaphroditic and monoecious individuals are always present (Świetlińska, 1963; Lloyd 1980,
150 Charlesworth, 2002; Barrett, 2013; Crossman & Charlesworth, 2013). The exact conditions
151 under which dioecy, especially dioecy controlled by sex chromosomes, transitions back to
152 monoecy or synoecy are unknown and only a few cases have been well studied (Heilbuth, 2000;
153 Schaefer & Renner, 2011; Crossman & Charlesworth, 2013).

154 The purpose of this study was to provide a molecular phylogeny of *Rumex*, test the
155 placement and monophyly of its circumscribed subgenera, and to elucidate the evolution of
156 reproductive systems in *Rumex*. We here present a new phylogeny of *Rumex* constructed using
157 three plastid gene regions (*trnH-psbA*, *rbcL*, and *trnL-F*) and 67 *Rumex* species. One objective is
158 to discover whether Rechinger's subgeneric delineations based on morphology and
159 phytogeography are supported by our phylogeny based on molecular data. In addition, we
160 address whether large scale patterns can be discerned in the reproductive systems exhibited by
161 *Rumex*.

162

163 **Materials and Methods**

164 *Taxon Sampling and DNA Isolation*

165 DNA was isolated from 109 accessions, representing 67 *Rumex* species. Of the 109 included
166 accessions, a total of 99 *Rumex* accessions, 6 *Rheum* species, 3 *Emex* accessions, and 1 species of
167 *Persicaria* are represented. *Persicaria virginiana*, *Rheum alexandrae*, *Rheum emodii*, *Rheum*
168 *nobile*, *Rheum officinarum*, *Rheum palmatum*, and *Rheum rhabarbarum*, were included as
169 outgroup species. Additional plant samples were obtained through the GenBank sequence
170 database (Appendix 1). Samples were taken from a combination of herbarium specimens (K,
171 NY, OSC, RAB, US), field collections, and cultivated samples from collaborators. Herbarium
172 acronyms follow the Index Herbariorum (Thiers, 2019).

173 All fresh leaf samples were dried using silica gel. Plant tissue was homogenized using the
174 FastPrep-24TM 5G Sample Preparation System (M. P. Biomedicals, LLC Santa Ana CA, USA).
175 Total genomic DNA was extracted from herbarium specimen-sampled and silica-dried leaf
176 tissues using a BIOLINE ISOLATE II Plant DNA Kit (Cat No. BIO-52070). Modification for
177 herbarium material proceeded as follows: Cell lysis was carried out using 300 μ L of buffer (PA1
178 or PA2) and 30 μ L of proteinase K (20 μ g/mL) and incubated for 18 hours at 65 $^{\circ}$ on an orbital
179 shaker).

180 *Marker Selection*

181 For this first comprehensive phylogeny for the genus, we focused on plastid marker selection.
182 Previous authors of recently reconstructed Polygonaceae phylogenies have used nrITS as a
183 nuclear marker (Schuster *et al.*, 2011; Schuster *et al.*, 2015). However, we avoided nrITS for this
184 phylogeny due to a number of issues that would interfere with accurate reconstruction of

185 evolutionary relationships: 1) nrITS is extremely variable and difficult to align (66% of nrITS
186 sequence data was excluded in Schuster *et al.* (2015) publication, and 2) Due to widespread
187 polyploidy documented in multiple *Rumex* species, sequences of nrITS would not necessarily be
188 low copy, and there would be substantial issues with paralogy and orthology across multiple
189 polyploidy events.

190 For plastid marker selection, we screened multiple markers that had previously been used
191 in Polygonaceae reconstruction (Burke *et al.*, 2010; Burke and Sanchez, 2011; Koenemann and
192 Burke, 2020). We selected markers that both showed sufficient variation across the genus, and
193 were easily amplified for most taxa.

194

195 *PCR Amplification and Sequencing*

196 Amplification of DNA markers was completed for three plastid regions: *rbcL*, *trnH-psbA* and
197 *trnL-F*. The first amplified region was the plastid large subunit of ribulose-bisphosphate
198 carboxylase (*rbcL*) using the primers *rbcLaF* (5'-ATG TCA CCA CAA ACA GAG ACT AAA
199 GC-3') and *rbcLaR* (5'-GTA AAA TCA AGT CCA CCR CG-3') (Table 1). PCR conditions
200 were as follows: 94°C for 1 min, followed by 34 cycles of 94°C/15 s, 54°C/15 s, and 72°C/30 s, and
201 a final extension period of 5 min at 72°C. The second region was analyzed using primers *trnH*
202 (5'-ACT GCC TTG ATC CAC TTG GC-3') and *psbA* (5'-CGA AGC TCC ATC TAC AAA
203 TGG-3') as an intergenic spacer. PCR conditions were as follows: 94°C for 2 min, followed by
204 34 cycles of 94°C/30 s, 55°C/30 s, and 72°C/30 s, and a final extension period of 7 min at 72°C.
205 Compared to the *rbcL* gene and the *trnH-psbA* intergenic spacer, a second, much shorter
206 intergenic spacer was examined. This intergenic spacer was amplified using primers 3'*trnL*^{UAA}*F*
207 (5'-GGT TCA AGT CCC TCT ATC CC-3') exon and the *trnF*^{GAA} (5'-ATT TGA ACT GGT

208 GAC ACG AG-3') gene which were used as primers. PCR conditions were as follows: 80□ for 5
209 min, followed by 34 cycles of 94□/1 min, 55□/1 min, and 72□/2 min, and a final extension
210 period of 5 min at 72□. PCR was performed to amplify all target gene regions using a BIOLINE
211 MyTaqTM Red Mix, 2X (Cat. No. BIO-25044) with no special PCR conditions. PCR samples
212 were then visualized on 1% agarose gels and run at 100V for 15 - 30 min against the BIOLINE
213 100bp – 2000bp EasyLadder I (Cat. No. BIO-33045) to observe the bands of specified gene
214 regions. PCR experiments were segregated to contain amplification of only fresh or only
215 herbarium material to help prevent cross-contamination.

216 PCR amplicons were sent to Eurofins Genomics (Louisville, KY) for Sanger sequencing.
217 Sequences were edited using Geneious v. 10 (Biomatters Ltd.). Reviewed sequences were
218 aligned with MUSCLE (Edgar, 2004), and concatenated using MESQUITE (Maddison, 2005).

219

220 *Phylogeny Reconstruction*

221 The final dataset contained 92 *rbcL*, 93 *trnL-F*, and 95 *trnH-psbA* accessions (Grant, 2020). All
222 phylogenetic analyses were completed using the CIPRES Science Gateway V 3.3 (Miller *et al.*,
223 2010). Prior to the phylogenetic reconstructions, we performed ModelTest-NG (Darriba *et al.*,
224 2019) for the concatenated matrix to determine the suggested model of evolution. ModelTest-NG
225 indicated that the best fit was the General time reversible (GTR) model.

226 We performed Maximum likelihood (ML) phylogeny reconstruction using GARLI v.
227 2.01.1067 (Zwickl, 2006). We used the default GARLI parameters with the following
228 exceptions. We performed 1000 search replications (10 iterations of 100 search replicates). In
229 order to better search tree space, we increased the attachments per taxon setting to 150 and
230 extended the generations without improvement parameter to 50000. To evaluate support for

231 phylogenetic relationships, statistical bootstrapping was performed, specifying only one search
232 replicate per bootstrap iteration for 100 iterations. All bootstrap trees were downloaded and used
233 to generate a majority rule consensus tree in MESQUITE (Maddison, 2005). The consensus tree
234 was visualized in FigTree version 1.4.3 (Rambaut, 2014).

235 We performed Bayesian Inference phylogeny reconstruction in MrBayes 3.2.7a
236 (Ronquist *et al.*, 2012). The priors were set to the defaults (Dirichlet). We set the seed number at
237 123. We conducted two independent Markov Chain Monte Carlo (MCMC) runs, each with four
238 chains employing BEAGLE library acceleration (as recommended by CIPRES). Each MCMC
239 run was set to complete 5 million generations, with trees sampled every 1,000 generations. The
240 first 25% of trees in each run were discarded as burn-in. MrBayes then synthesized the two
241 independent runs and we extracted the majority rule consensus tree with posterior probabilities.

242 Posterior probability and bootstrap values were visualized using FigTree version 1.4.3
243 (Rambaut, 2014) and MESQUITE (Maddison, 2005). Posterior probabilities above 90% and
244 bootstrap support values above 70% were considered significant and annotated in the final
245 phylogeny.

246

247 **Results**

248 The recovered most likely tree was generated using 109 specimen accessions. This included 7
249 outgroup species, 3 accessions of *Emex*, and 99 accessions of *Rumex*. The present phylogeny
250 represents 67 *Rumex* species, more than twice the number of species of *Rumex* sampled in
251 previous phylogenies (31 species in Navajas-Pérez *et al.*, 2005; 13 species in Schuster *et al.*,
252 2015). A total of 47 sequences were missing from the final matrix, yielding 14.4% missing data
253 in the final analysis (Grant, 2020). Table 1 summarizes the variability of each of the gene

254 regions. The most variable region was *trnH-psbA*, which consisted of 22.1% parsimony
255 informative characters. The least variable region was *rbcL* which consisted of 4.5% parsimony
256 informative characters. The most likely tree recovered by GARLI received a likelihood score of
257 $Ln = -5767.548440$.

258 The genus *Rumex* was recovered as monophyletic with strong support (100 Bayesian
259 Posterior Probability/98 Maximum Likelihood Bootstrap) (Figure 2). The analysis did not
260 recover *Rumex* subgenus *Rumex*, the subgenus with the most species diversity, as monophyletic.
261 In our phylogeny, species of subgenus *Rumex* form a grade at the base of the tree (“Basal Grade”
262 - Figure 2). Above the *Rumex* grade, *Emex* (Clade 1), was recovered as monophyletic and sister
263 to “the dioecious clade” (Figure 2). While the results indicate strong support for the relationship
264 between the known *Emex* species, *E. australis* and *E. spinosa* (100/98), they are conflicting and
265 show poor support for the placement of *Emex* within *Rumex*. Posterior probability support for the
266 placement of *Emex* as sister to the Dioecious Clade is only 52% and the most likely GARLI tree
267 placed *Emex* within the Basal Grade of subgenus *Rumex*. Furthermore, different gene regions
268 reconstructed conflicting topologies for the placement of *Emex*. The *rbcL* phylogeny placed
269 *Emex* within *Rumex* subgenus *Rumex* (50% bootstrap support). Both *trnh-psbA* and *trnL-F*
270 placed *Emex* as sister to the *Rumex* genus (*trnh-psbA* <50% bootstrap support, and *trnL-F* 91%
271 bootstrap support) (results not shown).

272 The remaining taxa, comprising the subgenera *Acetosa*, *Acetosella*, and *Platypodium*
273 form a highly supported (99/80) monophyletic group (Figure 2). This group is denoted as “the
274 dioecious clade” because the known dioecious *Rumex* species were resolved in this group. The
275 relationships of the clades within this group are also well-supported. Our recovered phylogenetic
276 tree did not recover subgenus *Acetosa* as monophyletic. Within the dioecious clade, subgenus

277 *Acetosa* is comprised of three well-supported, monophyletic groups, Clade 2 (100/97), Clade 3
278 (100/78), and Clade 4 (100/97), and is nested below a pair of clades, represented by subgenus
279 *Platypodium* (Clade 5) and subgenus *Acetosella* (Clade 6). The pair is also well supported
280 (100/81). Subgenus *Platypodium* was recovered as monophyletic with strong support (100/100),
281 and consists of four accessions of its only circumscribed species: *Rumex bucephalophorus*.
282 Species in subgenus *Acetosella* were recovered together with strong support (100/89), but the
283 inclusion of *Rumex hastatulus* means the subgenus was not recovered as monophyletic (Figure
284 2).

285 Beginning at its basal lineages, the recovered topology largely corresponds to the
286 diversity of the reproductive and sex chromosome systems present in *Rumex*. Species in
287 subgenus *Rumex* are hermaphroditic with no documented heteromorphic sex chromosomes.
288 These species, while not recovered as a clade, are recovered together in the basal grade. Also
289 with no documented heteromorphic sex chromosomes, *Emex* is represented as a clade that
290 consists of purely monoecious species. Within “the dioecious clade”, subgenus *Acetosa* consists
291 entirely of dioecious species, with some members exhibiting the sex chromosome system
292 XX/XY₁Y₂ represented in Clade 4 (Dioecious Species, Complex Sex Chromosome System)
293 (Figure 2). Subgenus *Platypodium*, another hermaphroditic group with no reported sex
294 chromosomes is nested between subgenera *Acetosa* and *Acetosella*. Subgenus *Acetosella* (Clade
295 6) consists of species that are both dioecious and have the sex chromosome system XX/XY
296 (Figure 2).

297

298 **Discussion**

299 Our results produced a phylogeny of *Rumex*, with six clades and one grade, largely
300 congruent with Rechinger's subgeneric classification and the evolution of reproductive and sex
301 chromosome systems present in *Rumex*. Moreover, the species with divergent sex chromosome
302 systems in *Rumex* are resolved as two separate clades, with the simple sex chromosome system,
303 XX/XY, being more derived. Where the basal grade contains most of the synoecious species, the
304 larger clade in the phylogeny, designated "the dioecious clade", represents most of the diversity
305 and variation across reproductive systems in the genus.

306 Within the phylogeny: the basal grade is mostly made up of synoecious species from
307 *Rumex* subgenus *Rumex*. In this portion of the study, we observed a peculiar finding where
308 subgenus *Rumex* was recovered as a grade instead of the anticipated clade. Given this finding,
309 we suspect that potentially, with even more taxon sampling, subgenus *Rumex* would have been
310 recovered as a monophyletic clade. Although dioecious, the species included in Clade 2 and
311 Clade 3 have no reported sex chromosome systems. The species included in Clade 4 exhibit a
312 complex sex chromosome system (XX/XY₁Y₂). This placement suggests that this heteromorphic
313 sex chromosome system was derived from dioecious ancestors. The genetic origin of
314 heteromorphic sex chromosomes in *Rumex* is beyond the scope of this manuscript, but we provide a
315 framework to investigate potentially intermediary taxa that may contain homomorphic or
316 transitional sex chromosome systems.

317 Subgenus *Platypodium* (Clade 5) was resolved as monophyletic and nested within "the
318 dioecious clade". Based on its plant and chromosome morphology, earlier studies concerning
319 *Rumex bucephalophorus* have referred to it as the link between subgenus *Rumex*, which is
320 predominantly synoecious, and subgenus *Acetosella*, which is predominantly dioecious (Löve,
321 1944). Although morphologically variable, *R. bucephalophorus* consistently exhibits a

322 synoecious reproductive system. Its derivation from among the dioecious species in this
323 phylogeny suggests a reversal from a dioecious condition.

324 The appearance of *R. bucephalophorus* within “the dioecious clade” can also speak to (1)
325 the high degree of reproductive system plasticity and (2) the possibility of a reversal from dioecy
326 to hermaphroditism. This is significant, because it goes against the evolutionary theory which
327 suggests that dioecy may be an evolutionary ‘dead-end’ (Heilbuth, 2000). Reversals from dioecy
328 back to hermaphroditism that typically occur at or near the onset of sex chromosome fluctuations
329 have been documented in some plants (Ming, 2011; Bachtrog *et al.*, 2014). This finding is one
330 that would support the placement of *R. bucephalophorus* as it is in the current phylogeny,
331 between subgenera *Acetosa* (XX/X₁Y₂) and *Acetosella* (XX/X₁Y).

332 Subgenus *Acetosella* (Clade 6), was not recovered as monophyletic. Known dioecious
333 species, *R. hastatulus*, of subgenus *Acetosa* is nested within subgenus *Acetosella*. *Rumex*
334 *hastatulus* is documented to exhibit two chromosomal races: a complex sex chromosome system
335 (XX/X₁Y₂, North Carolina Race) which is characteristic of subgenus *Acetosa* and the simple
336 sex chromosome system (XX/X₁Y, Texas Race) which is characteristic of subgenus *Acetosella*
337 (Navajas-Pérez *et al.*, 2005; Mariotti *et al.*, 2009; Hough *et al.*, 2014). In addition, Rechinger’s
338 1937 treatment indicates a polygamous reproductive system for *R. hastatulus* (Rechinger, 1937).
339 Given the variability found within this species, *R. hastatulus* could have theoretically evolved in
340 either subgenus (*Acetosa* or *Acetosella*), where species appear to have diversified according to
341 the type of sex chromosome system they exhibit. Again, this speaks to the plasticity of
342 reproductive and sex chromosome systems within *Rumex*, as a single species can exhibit two
343 different chromosomal ‘races’.

344 In all, this work has provided a reconstructed phylogeny that differs from those currently
345 published (Navajas-Pérez *et al.*, 2012; Schuster *et al.*, 2015) and tests the placement and
346 monophyly of its circumscribed subgenera. Additionally, this work has begun to elucidate the
347 evolution of reproductive systems in *Rumex* by way of its proposed schematic for the pathway
348 from synoecy (hermaphroditism) to dioecy. Simultaneously, the reconstructed phylogeny
349 emphasizes the high degree of reproductive system plasticity of *Rumex* species. Where lacking,
350 we have increased taxon density which has given rise to a more comprehensive evolutionary
351 history of *Rumex* where the taxa are concerned. Future directions in *Rumex* research include the
352 identification and application of nuclear markers that will allow for a more robust phylogeny to
353 increase the strength of support for molecular inferences.

354

355 **Acknowledgements**

356 This study was largely funded by award NSF-HRD #1601031 to JMB. The authors would like to
357 thank staff at the following herbaria for facilitating access to specimens for sampling: K, NY,
358 OSC, RAB, and US. Special thanks to Spencer Barrett and Joanna Rifkin for access to cultivated
359 *Rumex* material. We also thank Daniel Atha for providing us with a record of his *Rumex*
360 collections, and facilitating access to silica-dried material. Special thanks to Mr. Ibrahim El Hafid
361 for his assistance with transportation during the Morocco expedition. We also thank Ms. Maria
362 Ramos for her assistance with plant cultivation at the Howard University greenhouse.

363

364 **List of Tables**

365 **Table 1**

366 Gene regions used: name of primers, total length of region, % parsimony informative characters

| Gene Region | Reference | Primer Names | Total Aligned Length | PIC (%) |
|------------------|-----------------------------|--|----------------------|------------|
| <i>rbcL</i> | Fazekas <i>et al</i> , 2008 | rbcLF, rbcLR | 539 | 24 (4.5) |
| <i>trnH-psbA</i> | Shaw, 2007 | psbA, trnH | 596 | 132 (22.1) |
| <i>3trnL-F</i> | Shaw, 2005 | 3'trnL ^{UAA} F, trnF ^{GAA} | 442 | 65 (14.7) |
| Combined | | | 1577 | 221 (14.0) |

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374 **List of Figures**

375 **Figure 1.**

376 Hypothesis I proposed gynodioecy as the most likely intermediary state in the pathway to dioecy
377 (Charlesworth & Charlesworth, 1978).

378 Hypothesis II proposed monoecy as the most likely intermediary state in the pathway to dioecy
379 (Lewis, 1942).

380

381 **Figure 2.** Bayesian phylogenetic reconstruction for *Rumex* species using three chloroplast
382 sequences (*rbcL*, *trnH-psbA*, and *trnL-F*). Thickened branch indicates simultaneous posterior
383 probability above 90% and bootstrap support above 70%. Exact support values are indicated at
384 important nodes (Bayesian Posterior Probability / Maximum Likelihood Bootstrap). Outgroup
385 species (*Rheum* and *Persicaria*) are shown in blue. *Rumex* species traditionally placed in
386 subgenus *Rumex* are shown in red. Species traditionally placed in the sister genus *Emex* are
387 shown in green. *Rumex* species traditionally placed in subgenus *Acetosa* are shown in gold.
388 *Rumex* species traditionally placed in subgenus *Platypodium* are shown in pink. *Rumex* species
389 traditionally placed in subgenus *Acetosella* are shown in purple. The arrow denotes the
390 “Dioecious Clade” referenced in the text. Parenthetical abbreviations following the taxa
391 represent collection localities: NA = North America, ASIA = Asia, AFR = Africa, EU = Europe,
392 PAC = Pacific, SA = South America, AU = Australia. Accessions that lack locality information
393 were composed of GenBank sequences, where collection locality could not be determined.

394

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- 529

530 **Appendix 1:** List of taxa sampled, GenBank Accession Numbers, and Sequence Vouchers

531 Parenthetical values following the voucher are institutional barcodes or accession numbers when

532 available. Chloroplast region order is *rbcLa*, *trnH-psbA*, and *3trnL-F* unless otherwise indicated.

533 For sequences that we did not generate, accession information is given as found on GenBank.

534 **GenBank Sequences Used for this Study**

535 *rbcL*: *Rumex pamiricus* Rech. f. - JF944139.1, *Rumex sibiricus* Hulten- KC483892.1

536 *trnH-psbA*: *Rumex pamiricus*- JN047053.1

537

538 **DNA Sequences Generated for this Study**

| Scientific Name | Voucher | Extraction # | trnH-psbA | rbcL | trnL-F |
|---|---------------------------|--------------|-----------|------|--------|
| <i>Emex australis</i> Steinh. | P.C. Zietsma 4053, NY | 180 | | | |
| <i>Emex spinosa</i> (L.) Campd. | Schuhwerk 90/328, NY | 183 | | | |
| <i>Emex spinosa</i> (L.) Campd. | J.M. Burke 302, HUDC | 371 | | | |
| <i>Persicaria virginiana</i> (L.) Gaertn. | J.M. Burke s.n., BH | 106 | | | |
| <i>Rheum alexandrae</i> Batalin | Cultivated Material, HUDC | 204 | | | |
| <i>Rheum emodii</i> Wall. | Cultivated Material, HUDC | 205 | | | |
| <i>Rheum officinale</i> Baill. | Cultivated Material, HUDC | 207 | | | |
| <i>Rheum palmatum</i> var. <i>taguticaum</i> L. | Cultivated Material, HUDC | 208 | | | |
| <i>Rheum rhabarbarum</i> L. | Cultivated Material, HUDC | 206 | | | |
| <i>Rheum nobile</i> Hook. f. & Thomson | Pradham 820581, BH | 104 | | | |
| <i>Rumex abyssinicus</i> Jacq. | Burke 251, HUDC | 137 | | | |
| <i>Rumex acetosa</i> L. | Grant s.n., HUDC | 219 | | | |
| <i>Rumex acetosella</i> L. | R. Brand 1336, NY | 187 | | | |
| <i>Rumex acetosella</i> L. | Atha 10521, NY | 199 | | | |
| <i>Rumex acetosella</i> L. | Grant s.n., HUDC | 209 | | | |

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|--|-----------------------|-----|
| <i>Rumex acetosella</i> L. | J.M. Burke 309, HUDC | 383 |
| <i>Rumex albescens</i> Hillebr. | Lorence 5224, K | 141 |
| <i>Rumex albescens</i> Hillebr. | Wood 14959, US | 216 |
| <i>Rumex alpinus</i> L. | Larsen 20708, US | 164 |
| <i>Rumex alpinus</i> L. | D. E. Atha 5114, NY | 193 |
| <i>Rumex altissimus</i> Alph, Wood | Shultz 8717, US | 173 |
| <i>Rumex altissimus</i> Alph. Wood | D. E. Atha 10857, NY | 198 |
| <i>Rumex alveolatus</i> Los.-Losinsk. | Rechinger 48318, US | 159 |
| <i>Rumex amurensis</i> F. Schmidt ex Maxim. | Barrett Lilan22p | 227 |
| <i>Rumex aquaticus</i> L. | Elias 7251, US | 220 |
| <i>Rumex arcticus</i> Trautv. | Shetler 4560, US | 221 |
| <i>Rumex arifolius</i> All. | K. Deguchi 4023, NY | 189 |
| <i>Rumex bequaertii</i> De Wild. | Germishuizen 3447, US | 222 |
| <i>Rumex berlandieri</i> Meisn. | Thieret 17178, US | 155 |
| <i>Rumex brachypodus</i> Rech. f. | J.M. Burke 312, HUDC | 377 |
| <i>Rumex brasiliensis</i> Link | R. Wasum 1655, NY | 190 |
| <i>Rumex brownii</i> Campd. | Wilson 10250, NY | 191 |
| <i>Rumex brownii</i> Campd. | Wilson 10250, US | 223 |
| <i>Rumex bucephalophorus</i> L. | Barrett 17RBTA5 | 229 |
| <i>Rumex bucephalophorus</i> L. | J.M. Burke 293, HUDC | 363 |
| <i>Rumex bucephalophorus</i> L. | J.M. Burke 301, HUDC | 370 |
| <i>Rumex bucephalophorus</i> L. | J.M. Burke 304, HUDC | 372 |
| <i>Rumex chrysocarpus</i> Moris | D. E. Atha 13012, NY | 196 |
| <i>Rumex conglomeratus</i> Murray | D. E. Atha 10045, NY | 202 |
| <i>Rumex conglomeratus</i> Murray | J.M. Burke 271, HUDC | 212 |
| <i>Rumex conglomeratus</i> Murray | J.M. Burke 298, HUDC | 367 |

| | | |
|---|---------------------------|-----|
| <i>Rumex conglomeratus</i> Murray | J.M. Burke 299, HUDC | 368 |
| <i>Rumex crispus</i> L. | J.M. Burke 268, HUDC | 213 |
| <i>Rumex cuneifolius</i> Campd. | J.C. Solomon 13044, US | 224 |
| <i>Rumex cyprius</i> Murb. | Kocher B-273, US | 167 |
| <i>Rumex densiflorus</i> Osterh. | Pinkava P12626, US | 160 |
| <i>Rumex dentatus</i> L. | D.G. Kelch 07.328, OSC | 406 |
| <i>Rumex giganteus</i> Aiton | K. Thorne 6736, NY | 184 |
| <i>Rumex giganteus</i> Aiton | Canfield 1304, US | 217 |
| <i>Rumex graminifolius</i> Gerogi ex Lamb. | Petrosky 1811, US | 175 |
| <i>Rumex hastatulus</i> Baldwin | D. E. Atha 10503, NY | 203 |
| <i>Rumex hastatus</i> D. Don | MacArthur 1291, US | 172 |
| <i>Rumex hastatus</i> D. Don | Barrett s.n. | 230 |
| <i>Rumex hymenosepalus</i> Torr. | Cultivated material, HUDC | 210 |
| <i>Rumex hymenosepalus</i> Torr. | A. Tiehm 15727, OSC | 407 |
| <i>Rumex induratus</i> Bioss. et Reut. | M.W. Chase 925, K | 140 |
| <i>Rumex induratus</i> Bioss. et Reut. | Barrett s.n. | 231 |
| <i>Rumex induratus</i> Bioss. et Reut. | J.M. Burke 310, HUDC | 375 |
| <i>Rumex intermedius</i> DC. | Rainha 5270, US | 169 |
| <i>Rumex japonicus</i> Houtt. | Bai-Zhang 4049, US | 225 |
| <i>Rumex kernerii</i> Borbás | Barta 2004-390, US | 168 |
| <i>Rumex lanceolatus</i> Thunb. | H.J. Venter 10295, NY | 177 |
| <i>Rumex longifolius</i> DC. | D. E. Atha 8858, NY | 195 |
| <i>Rumex lunaria</i> L. | NR. 8879, NY | 178 |
| <i>Rumex lunaria</i> L. | Barrett 17RLLM1 | 232 |
| <i>Rumex lunaria</i> L. | Barrett 17RLTF1 | 233 |
| <i>Rumex maritimus</i> L. | Shiu Ying Hu 13127, US | 154 |

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| <i>Rumex mexicanus</i> Meisn. | D.E. Breedlove 13305, US | 226 |
| <i>Rumex microcarpus</i> Campd. | Barrett MJ-P40 (Seed) | 234 |
| <i>Rumex nepalensis</i> Spreng. | J.M. Burke 248, HUDC | 135 |
| <i>Rumex nervosus</i> Vahl | J.M. Burke 252, HUDC | 139 |
| <i>Rumex obtusifolius</i> L. | J.M. Burke s.n., BH | 93 |
| <i>Rumex obtusifolius</i> L. | J.M. Burke 270, HUDC | 214 |
| <i>Rumex orbiculatus</i> A. Gray | Ruee 43716, US | 163 |
| <i>Rumex orbiculatus</i> A. Gray | D.E. Atha et al 8883/2010, NY | 211 |
| <i>Rumex pallidus</i> Bigelow | D.E. Atha 13922, NY | 192 |
| <i>Rumex palustris</i> Sm. | J.M. Burke 306, HUDC | 374 |
| <i>Rumex papilio</i> Coss. & Balansa, | S.L. Jury 13659, K | 144 |
| <i>Rumex papilio</i> Coss. & Balansa | J.M. Burke 303, HUDc | 380 |
| <i>Rumex patientia</i> L. | D. E. Atha 10674, NY | 197 |
| <i>Rumex paucifolius</i> Nutt. | Barrett 17RpCOT3.2 | 235 |
| <i>Rumex paucifolius</i> Nutt. | Barrett 17RpCMC15.2 | 236 |
| <i>Rumex peruanus</i> Rech. f. | V. Quipuscoa 1349, NY | 185 |
| <i>Rumex pictus</i> Forssk. | Barrett 17Rp.AR1 | 237 |
| <i>Rumex pulcher</i> L. | J.M. Burke 294, HUDC | 364 |
| <i>Rumex pulcher</i> L. | J.M. Burke 295, HUDC | 365 |
| <i>Rumex pulcher</i> L. | J.M. Burke 296, HUDC | 366 |
| <i>Rumex rothschildianus</i> Aarons. ex Evenari | Barrett 17Rrs3.2 | 238 |
| <i>Rumex sagittatus</i> Thunb. | Strobach B55575, US | 166 |
| <i>Rumex sagittatus</i> Thunb. | H.J. Venter 9995, NY | 181 |
| <i>Rumex salicifolius</i> Weinm. | W. Wood s.n., OSC | 410 |
| <i>Rumex sanguineus</i> L. | J.M. Burke s.n., HUDC | 215 |

| | | |
|---|--------------------------|-----|
| <i>Rumex scutatus</i> L. | Barrett s.n. | 239 |
| <i>Rumex skottsbergii</i> O.Deg. & I.Deg. | Degener 35050, US | 218 |
| <i>Rumex spiralis</i> Small. | D. E. Atha 9727, NY | 200 |
| <i>Rumex stenophyllus</i> Ledeb. | D. E. Atha 11389, NY | 201 |
| <i>Rumex stenophyllus</i> Ledeb. | R.L. McGregor 40643, OSC | 411 |
| <i>Rumex tianschanicus</i> Losinsk. | Barrett SH1-A-2007454 | 240 |
| <i>Rumex thyrsoiflorus</i> Fingerh. | Ollegard 261, US | 153 |
| <i>Rumex thyrsoiflorus</i> Fingerh. | Elias 7282, US | 165 |
| <i>Rumex thyrsoides</i> Desf. | J.M. Burke 305, HUDC | 373 |
| <i>Rumex thyrsoides</i> Desf. | J.M. Burke 313, HUDC | 378 |
| <i>Rumex thyrsoides</i> Desf. | J.M. Burke 307, HUDC | 381 |
| <i>Rumex tuberosus</i> L. | S. Omar et al 52591, K | 147 |
| <i>Rumex tuberosus</i> subsp. <i>nov</i> | J.M. Burke 308, HUDC | 382 |
| <i>Rumex usambarensis</i> (Dammer) | | |
| Dammer | Ellemann 889, NY | 186 |
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