Cis-regulation of gene expression between sexes and life stages in *Rumex hastatulus*

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Abstract

The potential for conflict between sexes and life stages while sharing predominantly the same genome has important evolutionary consequences. In dioecious angiosperms, genes beneficial for the haploid pollen stage may reduce the fitness of diploid offspring of both males and females. However, we still lack an understanding of the extent of shared genetic architecture for gene expression between the sexes or life stages in plants, a key component for predicting the scope for conflict. We performed expression quantitative trait loci (eQTL) mapping to test if standing variation affects sexes and life stages differently using a population sample of the dioecious outcrossing plant *Rumex hastatulus*. We compared effect sizes and allele frequencies of *cis*-eQTLs in male and female leaf tissues and pollen and tested for genotype-by-sex interactions for gene expression. We found stronger shared genetic architecture between sexes than between the life stages. In addition, comparisons of the site frequency spectra for *cis*-eQTLs to a null distribution found no evidence for a genome-wide pattern consistent with purifying selection. Rare alleles had both up- and down-regulation of gene expression. Our results suggest that any conflict over optimal gene expression between pollen and leaves may be easily resolved due to their distinct genetic architectures, whereas there is more scope for conflict between the sexes in gene expression for leaves, which earlier studies indicate are sexually dimorphic. Our study highlights the use of eQTLs to investigate selection on gene expression and the evolution of conflict between sexes and life stages in dioecious species.

Keywords: *cis*-regulatory variation, eQTL, selective pressure, antagonistic pleiotropy

Introduction

Evolutionary conflict may arise when different sexes or life-cycle phases of an organism have different fitness optima while sharing a common genome (Ewing 1977; Arnqvist and Rowe 2005; Haig and Wilczek 2006; Schärer et al. 2015). For example, sexual conflict occurs when males and females in dioecious species have different fitness optima involving reproduction, and this can drive sexual dimorphism in phenotypic traits (Lande 1980; Bonduriansky and Chenoweth 2009). In plants, the diploid and haploid phases of the life cycle (i.e., sporophytes and gametophytes respectively, we use "life stages" to refer to these phases) are distinct in their morphology, physiology, and development despite sharing extensive overlap in gene expression, creating potential for genetic trade-offs (Haig and Wilczek 2006; Beaudry et al. 2020). Both sexual and life-stage conflict have been suggested to generate balancing selection and stable genetic variation under some conditions (Kidwell et al. 1977; Immler et al. 2012). However, we lack comprehensive understanding of the traits and genetic architecture underlying the conflict between sexes or life stages. Here, we use expression quantitative trait locus (eQTL) mapping to identify genome-wide *cis*-regulatory variation and investigate the extent of shared genetic architecture between sexes and life stages and the potential for sexual and life-stage conflict in a dioecious plant.

Current empirical examples of life-stage conflict often involve both sexes (i.e., a conflict both between the sexes and life stages). For example, alleles beneficial for haploid gametophytic competition were shown to reduce the fitness of female diploid offspring in the hermaphroditic annuals *Clarkia* (Travers and Mazer 2001) and *Collinsia* (Lankinen and Kiboi 2007; Lankinen et al. 2017). Sexual conflict independent of life stage differences has been observed in several plant species (e.g., Delph et al. 2011; Duffy et al. 2021; Wang et al. 2021), but genomic tests of sexual conflict in plants remain limited compared to animals (but see Delph et al. 2022). Gene expression is an important molecular trait underlying phenotypic variation. While direct tests of conflict require measurements of fitness which is often laborious and challenging, sexual or life-stage dimorphism in gene expression has been widely used to indirectly study conflict (Mank 2017; Yuan et al. 2025). With the considerable potential for both sexual and life-stage conflict in plants, whether there is a greater likelihood for one type of conflict than the other, or whether one type of conflict is more resolved than the other, remains unanswered. Thus, plants offer

excellent systems to study the evolution of conflict between sexes and life-cycle phases in a single species.

The potential for future conflict, i.e., whether new mutations and standing variation are more likely to be pleiotropic, depends on the degree of shared genetic architecture underlying phenotypic traits between sexes or life stages. Genetic architecture describes how genetic variation shapes phenotypic differences, including the number and effect sizes of contributing genes. When both sexes share most of the genetic architecture underlying a trait (i.e., a positive and high intersexual genetic correlation r_{mf}), there is strong potential for ongoing conflict in the presence of antagonistic selection, and the evolution of sexual dimorphism is constrained (Lande 1980; Poissant et al. 2010; Wyman et al. 2013). Genetic covariances between sexes inferred through gene expression studies in *Drosophila* suggest considerable scope for ongoing conflict (Allen et al. 20182018; Houle and Cheng 2021; Singh and Agrawal 2023; Grieshop et al. 2025). In contrast, when there is distinct genetic architecture between sexes or life stages, traits can evolve towards the same optima under concordant selection, while dimorphisms can evolve under antagonistic selection. Testing the extent of shared genetic architecture genome-wide is an important first step to understand the potential for ongoing of conflict between sexes and life stages.

Expression quantitative trait loci (eQTL) mapping is useful for identifying loci underlying phenotypic variation or those subject to selection (Kudaravalli et al. 2009; Lee et al. 2014; Josephs et al. 2015). Local eQTLs, i.e., genetic variants that are significantly associated with the expression level of a closely linked focal gene, are differentiated from distant eQTLs by a distance threshold in eQTL mapping studies. Most local regulatory variation acts in *cis* and has allele-specific effects; we refer to local eQTLs as *cis*-eQTLs or *cis*-regulatory variation. *Cis*-regulatory variation has a relatively large effect on gene expression, making it easier to map *cis*-eQTLs in association studies (Josephs et al. 2017; Signor and Nuzhdin 2018). Whether *cis*-eQTLs affect different sexes or life stages concordantly can be used to infer the extent of shared genetic architecture between sexes and life stages. For example, a study on eQTLs across human tissues has shown limited standing variation with sex-specific effects (Genotype × Sex interaction) in expression, suggesting high potential for sexual conflict under sexually antagonistic selection (Oliva et al. 2020). Furthermore, selective pressure on eQTLs can be

95 inferred from their distribution of allele frequencies when compared to the neutral expectation. In
96 the leaf tissues of *Capsella grandiflora*, *cis*-eQTLs showed excessive rare alleles consistent with
97 purifying selection (Josephs et al. 2015); whereas in floral tissues of *Mimulus guttatus*, *cis*-
98 eQTLs exhibited more common than rare alleles (Brown and Kelly 2021). These studies of two
99 plant species suggest that eQTLs can experience different selective pressures, and that potentially
100 differential selection can act on genes expressed in different sexes and/or life stages.
101
102 We investigated *cis*-regulation of gene expression through *cis*-eQTL mapping in the dioecious,
103 wind-pollinated and obligately outcrossing annual *Rumex hastatulus* (Polygonaceae). Several
104 previous findings in *R. hastatulus* indicated the potential for antagonism between sex and life
105 stages, especially during pollen competition. Sexual dimorphism in phenotypic and life history
106 traits of this species is likely associated with differential reproductive roles between sexes during
107 wind pollination, e.g., males are taller and produce more inflorescences during peak flowering to
108 maximize pollen dispersal while females are taller and have more inflorescences at reproductive
109 maturity to maximize seed dispersal (Puixeu et al. 2019). Vegetative traits such as leaf
110 morphology are tightly linked to survival and reproductive success (e.g., Delph et al. 2011;
111 Scharmann et al. 2021). In *R. hastatulus*, leaf size is positively correlated with plant height in
112 both sexes, and both leaf number and size are sexually dimorphic (Puixeu et al. 2019). The high
113 intensity of pollen competition in wind pollinated plants provides an opportunity to test
114 gametophytic selection during pollen competition (Friedman and Barrett 2009; Field et al. 2012)
115 and its pleiotropic effects on diploid fitness, e.g., a potential trade-off between pollen success and
116 diploid offspring fitness. Selection during pollen competition has been shown to affect sex-ratio
117 bias (Field et al. 2012) and sex chromosome evolution (Sandler et al. 2018) in *R. hastatulus,* e.g.,
118 female biased sex ratios are likely caused by greater competitive ability of X-bearing pollen
119 following Y chromosome degeneration (Hough et al. 2014; Crowson et al. 2017). The potential
120 conflict between sexes and life stages in *R. hastatulus* makes it a promising experimental system
121 to test the direction of selection on gene expression between sexes and life stages and also to
122 compare the potential for sexual and life-stage conflict.
123
124 We tested differential gene expression between sexes and life stages and mapped *cis*-eQTLs
125 separately in leaf and pollen tissues to compare their effect sizes and allele frequencies. We
126 found significantly fewer differentially expressed genes between sexes than between life stages,

127 and most of the highly sex-biased genes were on the sex chromosomes. There was a greater
128 overlap in eGenes (i.e., genes with their expression level affected by eQTLs) and eQTLs
129 between sexes than life stages. Effect sizes of eQTLs were positively correlated between sexes
130 and life stages, with a much stronger correlation between sexes, indicating more shared genetic
131 architecture between sexes. Consistent with this result, we found limited Genotype × Sex
132 interaction in gene expression. Lastly, we found similar selective pressures in eQTLs between
133 sexes and life stages based on their allele frequency distributions but with no excess of rare
134 alleles among eQTLs in any tissues when compared to a null distribution. Rare genetic variation
135 upstream of each gene exhibited both up- and down-regulated gene expression, presumably
136 reflecting stabilizing selection on gene expression (Kremling et al. 2018; Uzunović et al. 2019).
137

138 **Results**

139 *Differential expression between sexes and life stages*

140 We generated leaf DNA and RNA sequences and pollen RNA sequences from 78 male-female
141 sibling pairs in a single large population (see Methods). We used male and female leaves to
142 compare the genetic architecture of gene expression between the sexes and used male leaves and
143 pollen to compare diploid and haploid life stages, respectively. We performed differential gene
144 expression analyses to identify sex-biased genes in leaves and life stage-biased genes between
145 pollen and male leaf tissues (Table S2). Among the 37,659 annotated genes across the genome,
146 we first filtered for genes with evidence of expression in at least one of the two tissues being
147 compared, resulting in 19,494 genes in the between-sex comparison and 21,337 genes in the
148 between-life-stage comparison. In a principal component analysis (PCA) of RNA read counts,
149 pollen and male leaf samples were separated by PC1 that explained 99% variance both before
150 and after excluding sex-specific regions on sex chromosomes (Fig S1). Male and female leaf
151 samples were separated by PC1 which explained 52% of the variance when sex-specific regions
152 were included but were not separated based on autosomal and pseudoautosomal (PAR) genes
153 (Fig S1). We identified significantly differentially expressed (DE) genes based on fold change
154 (FC, the ratio of expression level between two tissues) and the false discovery rate corrected *p*-
155 value (adjusted *p*), unless stated otherwise, the cutoffs for DE genes are $|\log_2 FC| > 1$ and adjusted
156 $p < 0.05$. Consistent with the PCA results, there were over 10-fold more DE genes between life
157 stages (16,649) than between sexes (1,496) (Table S2).
158

159 We tested for enrichment of DE genes on sex chromosomes and the overlap between sex-biased
160 and life stage-biased genes. Sex-biased genes were highly enriched on sex chromosomes (Table
161 S2), with female-biased genes enriched on the X-specific regions (378/405; Chi-squared test, $p <$
162 2.2×10^{-16}) and male-biased genes enriched on the Y-specific regions (1,026/1,091; Chi-squared
163 test, $p < 2.2 \times 10^{-16}$).$^{-16}$) Among 6,702 pollen-biased genes, there was an excess of over-
164 expression of these genes in male leaves compared to female leaves (30 on autosomes and PAR,
165 1 on X, 269 on Y) tissue compared to female-biased genes (7 on autosomes and PAR, 63 on X, 3
166 on Y) (Fisher's exact test, $p = 0.0028$). Although it was a relatively small subset of pollen-based
167 genes, the larger overlap between male leaf-biased and pollen-biased genes was mostly driven by
168 the Y-specific regions. This finding implies that selection for higher gametophytic expression in
169 pollen could have pleiotropic effects on expression in male leaves (and *vice versa*), which could
170 lead to antagonistic or synergistic pleiotropy for gene expression depending on the direction of
171 selection on expression between life stages.
172
173 A previous study in two *Rumex* species found an enrichment of high pollen expression relative to
174 leaves on sex chromosomes compared to autosomes, suggesting a role of male gametophytic
175 selection in Y chromosome evolution (Sandler et al. 2018). With a new PacBio genome
176 assembly containing X and Y phased assemblies (Sacchi et al. 2025), we attempted to replicate
177 these results with our gene expression data in *R. hastatulus*. In accord with the earlier results, we
178 found a significant enrichment of pollen overexpression relative to leaf expression on the Y
179 chromosome compared to autosomes or the X chromosome (Fig S2, Fisher's exact test, $p =$
180 0.007 for autosomes, $p = 0.001$ for X) specifically for highly pollen-biased genes ($|\log_2 FC| > 2$,
181 adjusted $p < 0.05$). Among moderately pollen-biased genes ($|\log_2 FC| > 1$, adjusted $p < 0.05$), we
182 found a significant enrichment of pollen-biased genes on the Y chromosome only when
183 compared to the X chromosome (Fig S2, Fisher's exact test, $p = 0.031$ for X, $p > 0.1$ for
184 autosomes).
185
186 *eQTLs between sexes and life stages*
187 To test whether *cis*-eQTLs affect expression concordantly between sexes or life stages, we
188 performed *cis*-eQTL mapping separately in male leaves, female leaves and pollen. For each
189 gene, we tested the nearby SNPs within 20 kb to the transcription start site for significant
190 associations between their genotypes and the focal gene's expression level, significant

191 associations of gene-SNP pairs after false discovery rate corrections would be considered as *cis*-
192 eQTLs (see Methods). For leaf samples, we mapped *cis*-eQTLs using leaf expression and leaf
193 genotypes in males and females separately. For pollen samples, we used pollen expression and
194 male leaf genotypes as they were from the same male individual and shared the same genotypes.
195 We obtained 4,509,904 and 3,288,995 SNPs on autosomes from 74 female and 69 male leaf
196 DNA samples, respectively, after removing variants with low minor allele frequency or strong
197 Hardy–Weinberg deviations. Based on Tracy-Widom tests, we found 5 and 6 significant PCs for
198 female and male samples explaining 6.53% and 7.7% of genetic variation, respectively (TW
199 statistic ≥ 1.068, $p \leq 0.0441$ for females; TW statistic ≥ 2.288, $p \leq 0.00634$ for males); we
200 included the significant PCs as covariates in eQTL mapping.
201

202 We identified *cis*-eQTLs and genes whose expression was influenced by eQTLs ("eGenes") in
203 male leaves, female leaves and pollen (Table 1, Fig S3). We found more shared eGenes and
204 eQTLs between male and female leaves than between male leaves and pollen (Fig 1a-b). The
205 number of eQTLs per eGene ranged from 1 to 126 in pollen, 1 to 170 in male leaves, and 1 to
206 268 in female leaves, with a median of 2 eQTLs per eGene in both male leaves and pollen, and 3
207 in female leaves. We found eQTLs were located closer to their associated eGenes than the SNPs
208 tested in all tissues (median distance to the transcription start site: 7718 - 7893 bp for eQTLs vs.
209 8982 - 9254 bp for tested SNPs). There were similar numbers of eQTLs upstream and
210 downstream of the transcription start site (Fig S4). When purifying selection reduces the
211 frequencies of large-effect alleles in expression, there will be a negative correlation between
212 effect sizes and allele frequencies of eQTLs (Josephs et al. 2015). We found a strong negative
213 correlation between minor allele frequencies and effect sizes of eQTLs in all tissues when
214 selecting the most significant eQTL (top eQTL) per eGene (Spearman correlation, $\rho = -0.61$,
215 -0.60, -0.66 for male leaves, female leaves, and pollen, respectively; $p < 10^{-16}$) and when
216 selecting a random eQTL per eGene ($\rho =$ -0.71, -0.72, -0.75 for male leaves, female leaves, and
217 pollen, respectively; $p < 10^{-16}$) (Fig S5). However, this pattern of negative correlation could also
218 be caused by confirmation bias.

219

220 **Table 1**. eQTL mapping in male leaves, female leaves and pollen on autosomes. FDR = 0.1.

	Number of samples	Number of genes tested	Number of nearby SNPs tested	Number of eGenes	Number of eQTLs	Number of eQTLs affecting multiple genes	Number of secondary eQTLs
Male leaf	69	14,777	1,272,993	2,421	16,674	486	367
Female leaf	74	14,564	1,687,298	3,442	34,238	905	722
Pollen	69	13,785	1,182,102	1,475	9,456	201	159

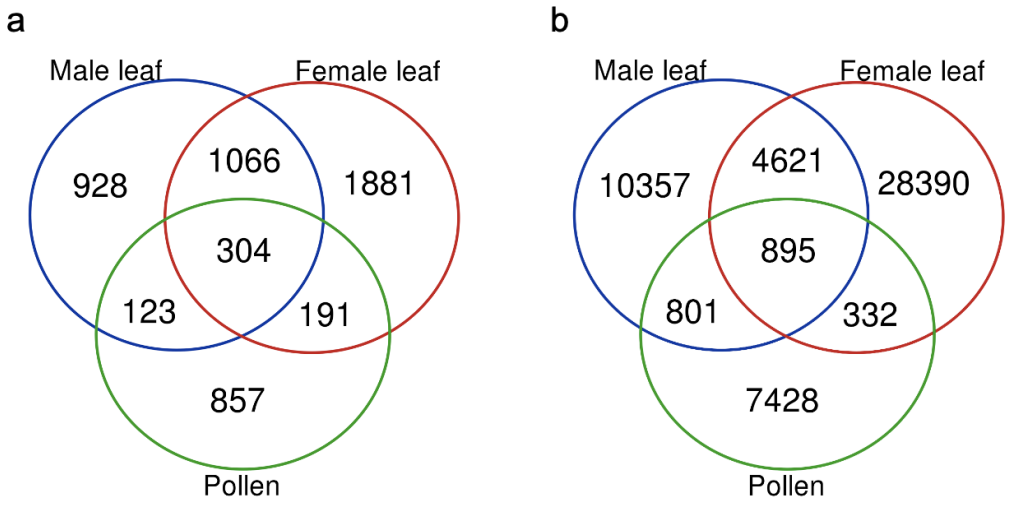

Fig 1. Venn diagrams of eGenes (a) and eQTLs (b) between sexes and life stages on autosomes of *Rumex hastatulus*. FDR = 0.1.

Cis-eQTLs with larger effects end to be closer to the transcription start site (GTEx Consortium et al. 2017). We found a weak but significant negative correlation between effect sizes of eQTLs and their distance to the transcription start site in all tissues when selecting the top eQTL (Spearman correlation, ρ = -0.041, -0.0.049, -0.091 for male leaves, female leaves, and pollen, respectively; $p < 0.0412$) and when selecting a random eQTL (ρ = -0.067, -0.056, -0.113 for male leaves, female leaves, and pollen, respectively; $p < 0.001$). In addition to the primary eQTLs (usually the top eQTLs) in each tissue, for each eGene we examined the independence of its eQTLs and identified secondary eQTLs (Table 1, see Methods). Secondary eQTLs were considered as conditionally independent after accounting for the effect of the primary eQTL of each eGene. We found primary eQTLs were significantly closer to the transcription start site than secondary eQTLs in female leaf and male leaf tissue (*t*-test, p = 0.02, 0.009, 0.32 for female

237 leaves, male leaves, and pollen, respectively). There were 40 - 43% of secondary eQTLs that had
238 opposite effects compared to the primary eQTL of the eGene, consisting of 0.7 - 0.9 % of all
239 eQTLs in each tissue (158 for male leaves, 291 for female leaves, 63 for pollen). The minor
240 allele frequencies of primary and secondary eQTLs were not significantly different (Mann-
241 Whitney *U*-test, $p = 0.12, 0.37, 0.22$ for male leaves, female leaves, and pollen, respectively).
242
243 We performed gene ontology enrichment of eGenes in each tissue and examined whether they
244 showed transcriptional or protein biosynthesis functions. Different tissues showed different
245 patterns of functional enrichment (Table S3). For example, female leaf tissue showed enrichment
246 for protein metabolic process (GO:0019538) whereas pollen showed enrichment for mRNA
247 metabolic process (GO:0016071) and negative regulation of gene expression (GO:0010629).
248 Additionally, protein deubiquitination (GO:0016579) was enriched in both male leaves and
249 pollen, and regulation of RNA metabolic process (GO:0051252) was enriched in female leaves
250 and pollen.
251
252 *Shared genetic architecture between sexes and life stages*
253 To investigate the extent of shared genetic architecture in expression between sexes and life
254 stages, we first estimated the correlation in expression level across genes between sexes and life
255 stages. For comparing sexes, we estimated the correlation in expression between male and
256 female siblings, which is a phenotypic approximation of the genetic correlation between sexes
257 (r_{mf}). For comparing life stages, this represents the phenotypic correlation between traits, which
258 reflects both environmental and genetic variances. We found expression levels were more
259 positively correlated between sexes than life stages based on all genes and eGenes (Fig 2a-b,
260 Mann-Whitney *U*-test, $p < 10^{-16}$ in both comparisons). Even though correlations between life
261 stages involve the same individual (as opposed to siblings of the opposite sex, for comparing the
262 sexes), the stronger correlation in expression between sexes, suggests potentially more shared
263 genetic architecture between sexes than life stages.

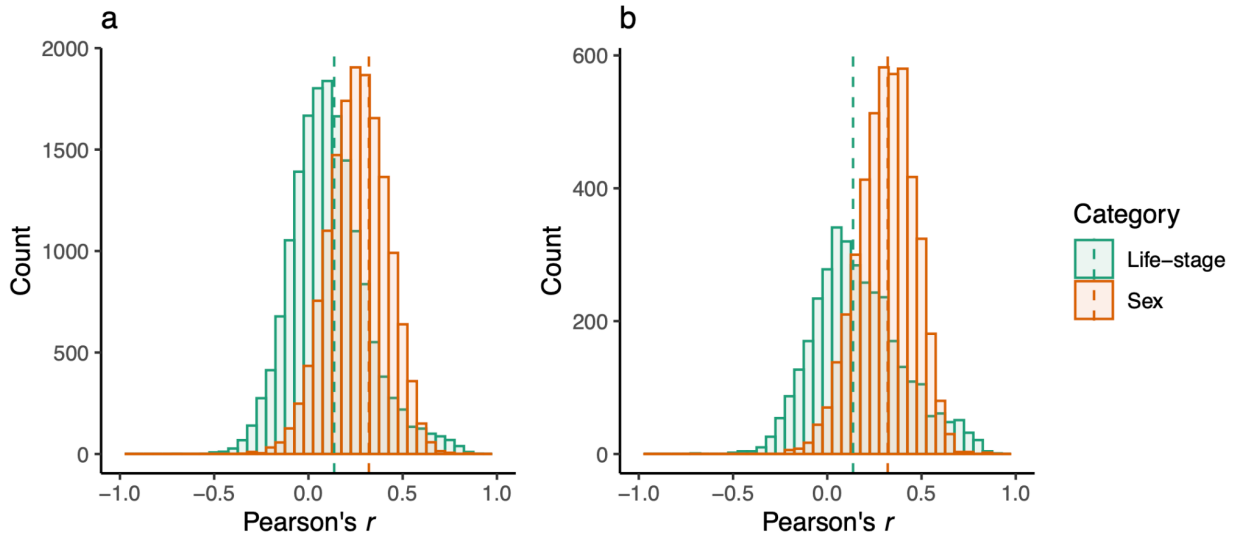

Fig 2. Distribution of Pearson correlation coefficients in gene expression between sexes or life stages in all genes (a) and in eGenes (b) in *Rumex hastatulus*. The dashed line represents the median of each group: 0.093 (Sex) 0.27 (Life-stage) in a, and 0.14 (Sex) 0.32 (Life-stage) in b. Numbers of genes: 14992 (Sex) and 16411 (Life-stage) in a, 4493 (Sex) and 3469 (Life-stage) in b.

Next, we tested whether *cis*-regulatory variation had concordant or discordant effects in gene expression between sexes and life stages. We filtered for nearby SNPs tested in both sexes or life stages that were the top eQTL for the eGene in at least one sex or life stage, resulting in 2,420 eQTLs in the sex comparison and 3,077 eQTLs in the life-stage comparison. The majority of eQTLs showed concordant effects between both sexes and life stages, with their effect sizes positively correlated (Fig 3a-b). The correlation of eQTL effect sizes was much stronger between sexes (Spearman correlation, $\rho = 0.72$, $p < 10^{-16}$) than between life stages ($\rho = 0.35$, $p < 10^{-16}$), suggesting a stronger shared genetic architecture between sexes (Fig 3a-b), as would be predicted from the phenotypic correlations in Fig 2a-b. There were 170 eQTLs with opposite effects between sexes, all of which were the top eQTL in one sex but not an eQTL in the other sex (light orange and light purple dots in Fig 3a). There were 974 eQTLs with opposite effects between life stages, 19 of which were eQTLs in both life stages (Fig S6). Functional enrichment of eGenes, with eQTLs showing opposite effects between sexes or life stages, showed an enrichment in processes relevant to transcription including negative regulation of gene expression (GO:0010629) and regulation of DNA-templated transcription (GO:0006355), and processes

post transcription including regulation of RNA and protein metabolic processes (GO:0051252, GO:0051246) (Table S4).

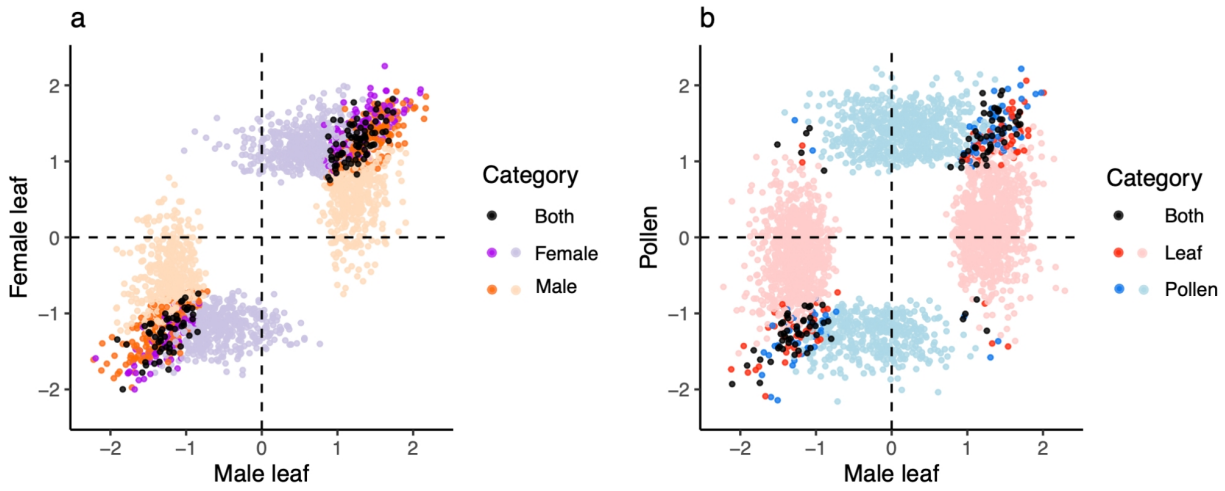

Fig 3. Correlation of effect sizes of autosomal SNPs tested between (a) sexes and (b) life stages in *Rumex hastatulus*. The x and y axes show eQTL effect sizes in different tissues. Black: top eQTL of the eGene in both sexes or life stages; darker colors: top eQTL in the labelled sex/life stage, significant but not the top eQTL in the other sex/life stage; lighter colors: top eQTL in the labelled sex/life stage but not significant in the other sex/life stage. Spearman correlation coefficients ρ: 0.72 (a), 0.35 (b).

In addition to mapping cis-eQTLs in different tissues separately, we tested for Genotype × Sex interaction effects on expression more explicitly in a linear model using both male and female leaf tissues. We obtained 3,684,937 SNPs on autosomes from 143 leaf DNA samples. We found one significant PC in all leaf samples combined that explained 2% of genetic variation (TW statistic = 1.328, $p = 0.0302$) and we included it as a covariate in eQTL mapping. We tested 14,587 genes and 1,083,706 nearby SNPs for the effect of Genotype, Sex, and Genotype × Sex interaction on expression. After correction for multiple testing (Davis et al. 2016), we found 5 eQTLs showing significant Genotype × Sex interaction (adjusted $p < 0.1$, see Methods), all of which changed expression of their eGene in the same direction but with different magnitudes (Fig S7). The small number of eQTLs showing Genotype × Sex is consistent with the previous result that eQTLs showed strongly correlated effects between sexes (Fig 3a).

308 We mapped cis-eQTLs affecting the degree of expression differences between life stages using
309 16,218 genes and 1,404,801 nearby SNPs. Specifically, we aimed to test the effects of the minor
310 allele of each SNP on the differences in expression levels between male leaf tissue and pollen.
311 We found 9,873 eQTLs affecting the expression differences between life stages in 1,564 genes.
312 There were more eQTLs in which the minor allele increased expression differences (6,173) than
313 reduced them (3,700), suggesting new mutations (typically represented by the minor alleles)
314 more often drive the divergence in expression between life stages than constrain it. We examined
315 whether eQTLs for expression differences between life stages were also identified as eQTLs in
316 male leaf tissue or pollen separately. We found 767 eQTLs significantly affected leaf expression,
317 pollen expression, and the differences between them, 735 of which affected leaf and pollen
318 expression in the same direction, consistent with the concordance in eQTL effect sizes shown in
319 Fig 3b. Additionally, we found that 1,464 of 1,564 genes, whose expression differences between
320 life stages were affected by eQTLs, were significantly DE genes between pollen and male leaf
321 tissue (adjust $p < 0.05$, no FC cutoff). Functional analyses showed rRNA processing
322 (GO:0006364) and regulation of RNA metabolic processes (GO:0051252) were enriched in
323 genes with eQTLs affecting life-stage expression differences (Table S5). Lastly, we identified
324 141 conditionally independent eQTLs affecting life-stage expression differences, 54 of which
325 had opposite effects compared to the primary eQTL of the same gene.
326
327 *Selective pressure on eQTLs*
328 We examined the distribution of minor allele frequencies (MAF) of eQTLs to test if selective
329 pressure differs for eQTLs between sexes or life stages. When compared to the neutral
330 expectation, an excess of low-frequency variants indicates purifying selection, whereas an excess
331 of intermediate-frequency variants indicates balancing selection. We kept one eQTL per eGene
332 to have a set of independent eQTLs for MAF comparisons and repeated the analyses when
333 keeping the top eQTL and a random eQTL. In all tissues, there were more rare alleles than
334 common alleles regardless of the eQTL selection method (Fig 4a-f). The median MAFs among
335 all three tissues were similar, with the highest median MAF in female leaves and lowest median
336 MAF in male leaves using both methods of eQTL selection (Fig 4). The differences in mean
337 MAFs between tissues were marginally significant between male and female leaves in top and
338 random eQTLs (Mann-Whitney *U*-test, $p = 0.041$, 0.049, respectively) and between female
339 leaves and pollen in top eQTLs (Mann-Whitney *U*-test, $p = 0.049$) (Fig 4).

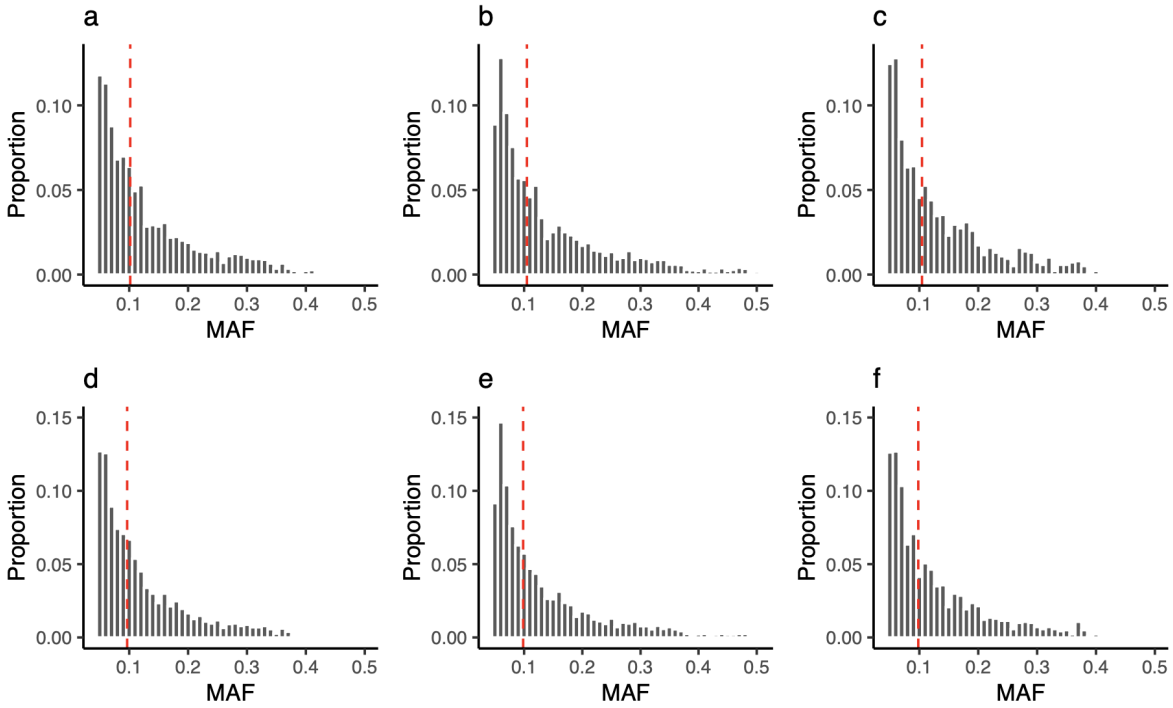

Fig 4. Distribution of MAFs of eQTLs in male leaves (a, d), female leaves (b, e), pollen (c, f) in *Rumex hastatulus*. a-c: top eQTL per eGene, d-e: a randomly selected eQTL per eGene. Binwidth = 0.01. Dashed lines represent the Median MAF in each plot: 0.1017 (a), 0.1048 (b), 0.1045 (c), 0.0965 (d), 0.0984 (e), 0.0982 (f).

To investigate whether the patterns of more rare than common alleles in eQTLs differed from a null expectation accounting for sampling and false positives, we compared the MAFs of true positive eQTLs to a null distribution in each tissue (Fig 5a-c). We generated the null distributions of MAFs using 'false-positive' eQTLs from permuted data by breaking the true assignment of individuals and re-assigning their expression phenotypes. We then used the same procedure to identify false-positive eQTLs from permuted data and true positive eQTLs from observed data (see Methods). In all tissues we found an excess of rare alleles (MAF between 0.05 and 0.1) in the permuted data compared to our observed data, with a small overlap in the proportions of rare alleles in pollen (Fig 5a-c), suggesting that eQTLs do not appear to be less common than would be expected under a null model.

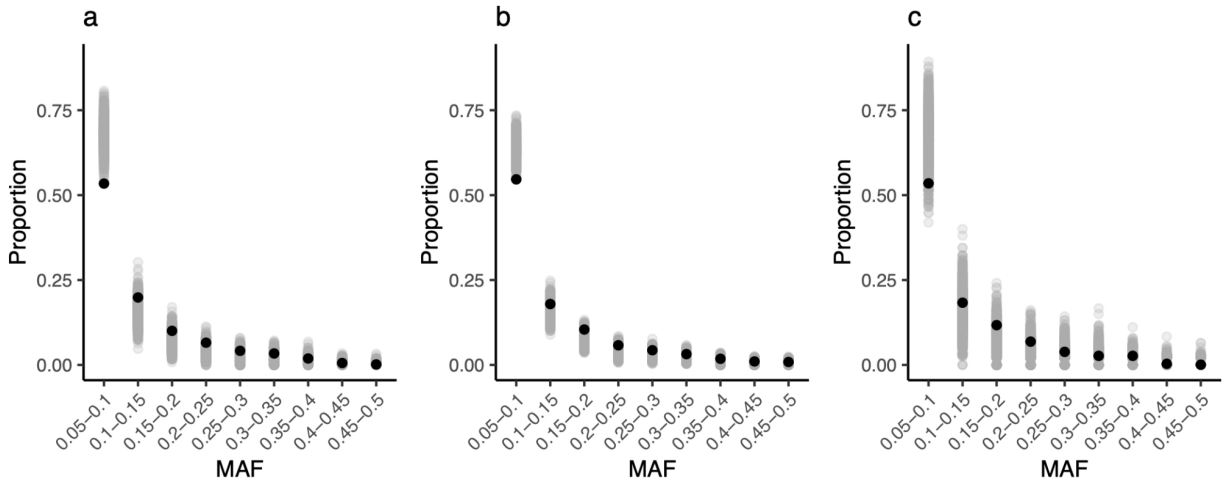

Fig 5. Distribution of MAFs of eQTLs compared to a null distribution in male leaves (a), female leaves (b) and pollen (c) in *Rumex hastatulus.* Black dots: true positive eQTLs (observed data), grey dots: false positive eQTLs (permuted data).

Studies have found more *cis*-regulatory variation in unbiased and moderately biased than strongly biased genes between the sexes of *Drosophila melanogaster*, suggesting potential sexual conflict in regulatory variation (Puixeu et al. 2023; but see Mishra et al. 2024). We tested signals of potential antagonistic selection between sexes or life stages. We first compared MAFs of eQTLs shared between life stages with those that were life-stage specific. We found shared eQTLs had significantly higher MAFs than specific eQTLs (Mann-Whitney *U*-test, $p < 10^{-7}$), with median MAFs of 0.143 and 0.093, respectively. This result was not confirmed by a comparison to a null distribution due to a lack of shared false positive eQTLs between life stages. We also tested signatures of selection on eGenes that were shared between sexes or life stages using neutral diversity statistics. We predicted that shared eGenes would have higher π and Tajima's *D* than specific eGenes, due to the greater potential for conflict in shared eGenes. We found that shared eGenes between sexes had significantly higher Tajima's *D* (Kruskal-Wallis test, $p < 10^{-5}$) but not significantly higher π (Kruskal-Wallis test, $p = 0.5383$) than male- or female-specific eGenes. There were no significant differences in π or Tajima's *D* among shared and specific eGenes in either life stage (ANOVA test, $p = 0.611$, 0.157, respectively). Additionally, we tested the signature of selection on eGenes whose expression differences between life stages were affected by eQTLs and found these eGenes had significantly higher π

378 and Tajima's *D* than eGenes not significant for expression differences (*t*-test, $p = 0.00013$,
379 0.00058, respectively).
380
381 *Burden of rare alleles on gene expression*
382 While our comparisons of allele frequencies of eQTLs to the null distribution suggest a
383 deficiency of rare variants rather than a signal of purifying selection on variants affecting gene
384 expression, rare variants are excluded in eQTL mapping due to the lack of power to detect
385 associations. To address this limitation, we also performed burden of rare allele tests that assess
386 signatures of stabilizing selection in gene expression (Zhao et al. 2016; Kremling et al. 2018;
387 Uzunović et al. 2019). The burden of rare allele test evaluates the shape of the distribution of
388 total counts of rare alleles across ranked bins of varying expression levels. If rare alleles increase
389 or decrease expression level, individuals with highest or lowest expression ranks will show a
390 higher number of rare alleles (a parabolic shape), as would be expected under stabilizing
391 selection against extreme expression. In *Capsella grandiflora*, rare SNPs are equally probable to
392 increase and decrease expression (Uzunović et al. 2019); whereas in maize, rare SNPs upstream
393 of genes are more likely to decrease expression (Kremling et al. 2018). We ranked individuals'
394 expression levels for each gene and counted the number of singletons (i.e., variants that only
395 appear once across all individuals) 5kb upstream of the gene across all genes for each expression
396 rank bin and plotted their relationship (Fig 6). Our results showed that in male and female leaves,
397 rare alleles with both up- and down-regulated expression occurred (Fig 6a-b), similar to the
398 results in *Capsella grandiflora* (Uzunović et al. 2019). There was a slightly stronger effect of
399 rare alleles in increasing than decreasing expression indicated by outliers in the highest rank
400 bins, but overall, the parabolic shape was symmetrical (Fig 6a-b). In pollen we did not see the
401 same effects as in leaf tissues, there was an absence of the parabolic relationship (Fig 6c).
402

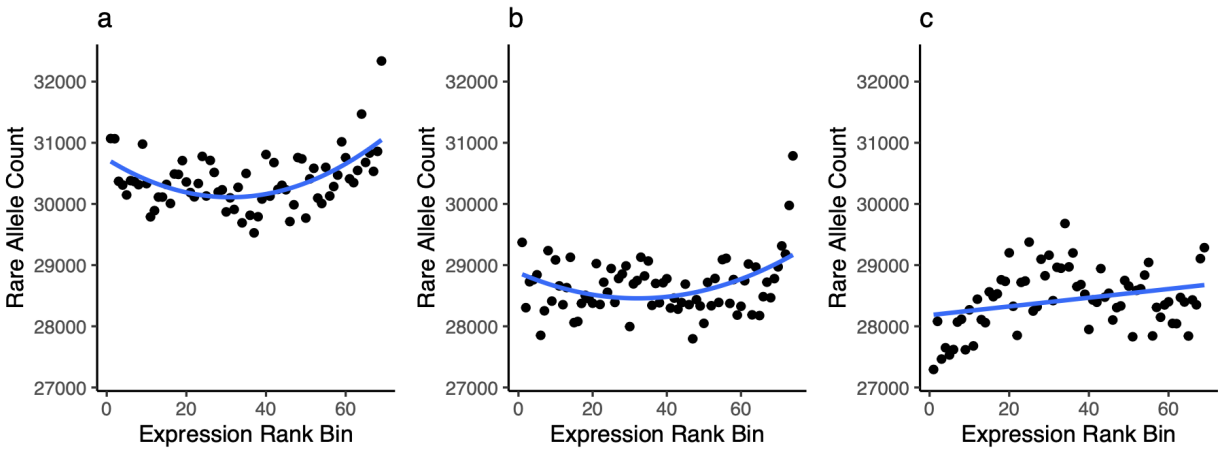

Fig 6. Relationship between rare allele count and gene expression rank bin in male leaves (a), female leaves (b) and pollen (c) in *Rumex hastatulus*. Smooth lines show quadratic regression in a and b and linear regression in c. a. adjusted $R^2 = 0.5288$, $p = 6.149\times10^{-12}$. b. adjusted $R^2 = 0.5484$, $p = 2.067\times10^{-13}$. c. adjusted $R^2 = 0.3481$, $p = 5.756\times10^{-8}$. Lowest rank means lowest expression and highest rank means highest expression.

Discussion

In this study we mapped *cis*-eQTLs in leaf and pollen tissues in the dioecious plant *R. hastatulus* to investigate the potential for conflict between sexes and life stages, based on shared genetic architecture. We investigated the overlap of eQTLs between sexes and life stages and examined their effect sizes and allele frequencies. We discovered a much larger overlap in eGenes and eQTLs between sexes than between life stages. For SNPs tested in both sexes or life stages, there was a stronger correlation of effect sizes between sexes suggesting a more shared genetic architecture between sexes than life stages. There were more minor alleles of eQTLs driving than constraining divergent expression between life stages. Among the eQTLs shared between life stages and affecting their expression differences, most minor alleles changed expression in the same direction with different magnitudes, increasing the degree of divergent expression. MAFs of eQTLs in all tissues exhibited similar distributions with a larger proportion of rare alleles than common alleles, but this pattern did not reflect an excess of rare variants when compared to a null distribution. Investigation of the burden of rare allele on genotypes with more extreme gene expression suggested that rare variants both up- and down-regulated expression. Below we discuss the implications of our results on the extent of a shared genetic architecture between sex

426 and life stages and also the use of eQTL mapping in testing evolutionary forces shaping *cis*-
427 regulatory variation.
428
429 *Potential for conflict between sexes and life stages*
430 With the potential for both sexual and life-stage conflict based on theory (Kidwell et al. 1977;
431 Immler et al. 2012; Peters and Weis 2018), key open questions remain on whether there are more
432 opportunities for conflict between sexes or life stages, and whether sexual and life-stage conflict
433 is still an ongoing process or has already become resolved. We investigated these questions using
434 *cis*-regulatory variation which has a strong effect on gene expression. Our results consistently
435 suggested significantly greater shared genetic architecture between sexes than life stages, thus
436 more potential for ongoing sexual conflict. Furthermore, our results indicated that life-stage
437 conflict is more resolved compared to sexual conflict. These findings are consistent with
438 previous work suggesting considerable scope for ongoing sexual conflict based on intersexual
439 genetic covariances in expression (Allen et al. 20182018; Houle and Cheng 2021; Singh and
440 Agrawal 2023; Grieshop et al. 2025).
441
442 It is also worth mentioning that standing variation in gene regulation might have more sex-
443 differential effects in reproductive tissues such as in flowers compared to leaves. Future studies
444 could benefit from a more diverse sampling of both vegetative and reproductive tissues (e.g.,
445 comparing pollen and ovule or shared floral tissues) than we used in this study. The alternating
446 life cycle of diploid and haploid life stages is shared among all land plants, allowing adaptation
447 to varying environments. While sexual reproduction is an ancient attribute of eukaryotic
448 organisms, dioecy in flowering plants has more recently emerged from hermaphroditic ancestors
449 in most lineages (Bawa 1980), and there probably has not been enough time to break down
450 intersexual genetic correlations. Our conclusion that there is more potential for sexual than life-
451 stage conflict in dioecious flowering plants should be robust in terms of tissue sampling due to
452 the relatively recent evolution of separate sexes compared to the fundamental feature of the
453 haploid-diploid alternating life cycle. In dioecious plants such as *R. hastatulus*, it is likely that
454 there has been a greater amount of time to resolve conflicts between life stages than sexes, where
455 sexual conflict between females and males may still be ongoing. Direct assessments of
456 antagonistic selection between sexes and life stages should provide a more complete picture of
457 the evolution of these two types of conflict.

While our results suggest the potential for unresolved sexual conflict in gene expression on autosomes, sex chromosomes have important roles in the evolution of dioecy (Charlesworth et al. 2005) and can facilitate the evolution of sexual dimorphism (Rice 1984; Zemp et al. 2016). The vast majority of identified sex-biased genes were found to be sex-linked, suggesting a potential role for sex chromosomes in resolving sexual antagonism. Plants are useful systems for comparing opportunities for sexual and life-stage conflict (Moore and Pannell 2011; Beaudry et al. 2020). Whether there is more shared genetic architecture between sexes and life stages in plant lineages with different reproductive systems or sex chromosome systems (presence and absence or age of sex chromosomes) is therefore worth further investigation. Also, whether there are differences in sexual and life-stage conflict in plants with contrasting sexual systems (hermaphroditic vs. dioecious) or different types of life cycles (predominantly diploid vs. haploid) are also promising topics for future research.

With a weak positive correlation of eQTL effect sizes between life stages there is still potential, albeit weaker, for ongoing life-stage conflict. We found that genes on the Y chromosome with higher expression in pollen compared to leaves also tended to be male-biased in leaves, suggesting the possibility of strong selection in pollen, possibly driving up leaf expression in an antagonistic fashion. We examined signatures of conflict on eGenes using diversity statistics. We expected eQTLs with concordant effects between sexes or life stages to experience stronger conflict than those with discordant effects or those exclusive to one sex or life stage. We found that shared eGenes had higher Tajima's *D* than sex-specific eGenes. In contrast to our prediction, eGenes with eQTLs affecting their expression differences between life stages had significantly higher π and Tajima's *D* than eGenes not significant for expression differences; however, this finding might result from an ascertainment bias, as it is more likely to have higher power to detect interactions when diversity is higher. Overall, there was no indication of greater antagonistic selection on eQTLs with shared genetic architecture between the sexes and life stages.

Our previous study tested the genomic signature of intra-locus conflict between life stages in *R. hastatulus* using gene expression but did not find support for a genome-wide signal (Yuan et al. 2025). Conditions for generating balancing selection due to conflict are limited to single large-

490 effect loci (Kidwell et al. 1977). Detecting balancing selection due to conflict using genomic data
491 remains challenging, especially when polygenic traits are involved (Bitarello et al. 2023;
492 Flintham et al. 2025; Ruzicka et al. 2025). While variation in gene expression can underlie
493 important phenotypic variation, it is worth noting that the relationship between expression
494 variation and morphological variation can be complicated and different across tissues. Clearly,
495 there is more to be understood about the relationship among gene expression, phenotypic traits
496 and fitness. For example, the genetic basis of the traits under antagonistic selection needs to be
497 considered when testing for signals of conflict (Rowe et al. 2018). Future studies with more
498 functional information (e.g., on alleles beneficial for pollen competition), combined with
499 previously identified candidate genes under balancing selection (Yuan et al. 2025) should help to
500 uncover the genetic basis of life-stage conflict in *R. hastatulus*.
501
502 *Selective pressure on cis-regulatory variation*
503 Estimating selective pressures in regulatory variation using allele frequencies has resulted in
504 mixed results supporting contrasting hypotheses about the type of selection on *cis*-eQTLs
505 (Josephs et al. 2015; Brown and Kelly 2021). Our results indicated more rare than common
506 alleles among cis-eQTLs in all tissues. However, in contrast to our prediction and previous
507 findings in *Capsella grandiflora* (Josephs et al. 2015), we found a non-significantly higher
508 proportion of rare alleles in the null distribution than our observed data, suggesting a genome-
509 wide signature of purifying selection was unlikely. Assuming the null distribution from genome-
510 wide SNPs reflects neutrality, the slight deficiency of rare alleles could potentially be caused by
511 antagonistic selection or other factors. However, the genome-wide null distribution likely
512 includes a sampling of variants subject to purifying selection, so it is also possible that eQTLs
513 experience relaxed purifying selection. Interestingly, our results are intermediate between those
514 reported in *Capsella grandiflora* and the *cis*-eQTLs in *Mimulus* floral tissues, which showed a
515 higher proportion of common than rare alleles (Brown and Kelly 2021).
516
517 While our *cis*-eQTL mapping removed rare variants and only included variants with large
518 enough effect to be detectable in this study, our burden of rare allele tests provided a more
519 powerful assessment of selective pressure on genetic variation affecting gene expression than
520 comparing genome-wide allele frequency spectra of eQTLs to a null distribution. We found a
521 signal of stabilizing selection on gene expression in leaves using this approach, with an excess of

522 rare allele burdens at both extremes of expression rank, suggesting selection on optimal
523 expression level (Hodgins-Davis et al. 2015). In contrast, we observed no clear signal of
524 stabilizing selection on gene expression in pollen. This may indicate that pollen expression is
525 generally subject to weaker selection in *R. hastatulus*, a conclusion that is supported by our
526 recent results suggesting relaxed purifying selection on pollen-biased genes (Yuan et al. 2025).
527 Future studies should benefit from examining the correlation between effect sizes and MAFs,
528 while controlling for confirmation bias using allele-specific eQTLs (Josephs et al. 2015) and
529 examining *trans*-eQTLs as they have been found in other species to be subject to stronger
530 constraint than *cis*-acting variation (Josephs et al. 2020).
531
532 In conclusion, our results indicate a greater shared genetic architecture in gene expression
533 between the sexes than between the haploid and diploid phase of the lifecycle, and evidence for
534 stabilizing selection on gene expression in leaves but not pollen. While these results imply the
535 potential for a greater constraint in response to antagonistic selection between the sexes because
536 of genetic architecture, the extent of standing variation in sexual antagonism remains difficult to
537 ascertain from our results alone. There are limitations in the application of the site frequency
538 spectrum of eQTLs for testing ongoing antagonistic selection and future work using more direct
539 mapping of fitness effects of genetic variants (e.g., Ruzicka et al. 2019; Delph et al. 2022) will be
540 important to test this further.
541
542 **Methods**
543 *Plant materials*
544 We described the details of plant materials and sequencing in Yuan et al. (2025); here we
545 provide a brief summary and additional relevant information. After generating seedlings using
546 open-pollinated seeds collected from a population in Rosebud, Texas, US (Pickup and Barrett
547 2013), we paired one female and male individual randomly at the time of flowering for crossing
548 and collected seeds to generate F_1 plants. We planted and retained multiple individuals per
549 family for 78 F_1 families to ensure we had one male-female full sibling pair per family for tissue
550 collection. We collected young leaf tissue from each male-female full-sibling pair after flowering
551 for both RNA and DNA sequencing and collected mature pollen grains from males for RNA
552 sequencing. We described the DNA and RNA isolation protocols in Yuan et al. (2025). The
553 DNA samples were sequenced at a depth of 10-15X. Raw sequences of male leaf RNA and

554 pollen RNA were described in Yuan et al. (2025), Initial sample sizes for DNA samples and
555 female leaf RNA samples are presented in Table S1.
556
557 *DNA Seq data and variant calling*
558 We used a phased PacBio genome assembly consisting of four autosomes, X-specific, Y-specific
559 and pseudoautosomal regions for our analyses (Sacchi et al. 2025). We mapped the DNA reads
560 to the genome assembly and added read groups using bwa-mem2 (Vasimuddin et al. 2019). We
561 sorted the BAM, marked PCR duplicates and indexed the final BAM using SAMTools (Danecek
562 et al. 2021). We called variants in parallel on 2 Mb non-overlapping contigs of each chromosome
563 for all samples using BCFtools mpileup (Danecek et al. 2021), then concatenated the VCFs of
564 contigs for each chromosome. We removed one male sample (24fM) due to low genomic
565 coverage and two samples due to incorrect sex labeling (35aM, 40aF), and only kept samples
566 with both DNA and RNA sequences from the same individual. For males, we only kept samples
567 with both leaf and pollen RNA sequences from the same individual. Our sample sizes for VCF
568 filtering were 74 for female leaves, 73 for male leaves, making a total of 147 leaf tissue samples.
569 We performed SNP filtering as follows for female leaf, male leaf and all leaf samples combined
570 separately using VCFtools (Danecek et al. 2011). We kept SNPs with QUAL > 30, a mean depth
571 between 5 and 20, a genotype quality per sample > 30 otherwise marked as missing, and finally a
572 missingness < 20%. For female samples, we used the same filtering criteria for autosomes, PAR
573 and X-specific regions. The numbers of autosomal SNPs were 64,988,762 in male leaf,
574 66,828,708 in female leaf, and 66,341,762 in all leaf samples combined. The numbers of SNPs
575 on X-specific regions and PAR in females were 7,745,941 and 2,164,214, respectively.
576 Additionally, we filtered for invariant sites on autosomes with a mean depth between 5 and 20
577 and a missingness < 20% for all leaf samples combined.
578
579 We removed SNPs with a minor allele frequency lower than 5% or with strong Hardy–Weinberg
580 deviations ($p < 10^{-6}$) on autosomes using Plink (Chang et al. 2015) to test for population structure
581 and for the subsequent eQTL mapping. We removed four male samples (7bM, 27eM, 53bM,
582 5aM) from subsequent analyses for being outliers in principal component analyses in Plink
583 (Chang et al. 2015). Our final sample sizes were 74 for female leaves, 69 for male leaves,
584 making a total of 143 leaf samples. After the filtering using Plink, the numbers of SNPs on
585 autosomes were 3,288,995 in male leaves, 4,509,904 in female leaves, and 3,684,937 in all leaf

586 samples combined; the numbers of SNPs on X-specific regions and PAR in females were
587 769,059 and 96,911, respectively.
588
589 *RNA Seq data and expression analyses*
590 We mapped the RNA reads to our genome assembly in a two-pass mode, sorted the BAM files
591 by coordinates, added read groups using STAR (Dobin et al. 2013), and indexed the output BAM
592 files using SAMTools (Danecek et al. 2021). We generated read counts for each gene using
593 featureCount (Liao et al. 2014). We only kept genes with evidence of expression for our
594 subsequent analyses, i.e., genes with a mean raw read count ≥ 5 in a specific tissue, or in at least
595 one tissue for analyses involving both sexes or life stages. We identified sex-biased genes in leaf
596 tissues and life-stage-biased genes by comparing pollen and male leaf tissues using DESeq2
597 (Love et al. 2014). The cutoffs for significant differentially expressed (DE) genes were
598 Benjamini-Hochberg adjusted $p < 0.05$ and $|\log_2 FC| > 1$ unless stated otherwise.
599
600 We prepared the expression phenotype data for each tissue separately and for all leaf samples
601 combined for eQTL mapping. We normalized raw read counts by sequencing depth using
602 DESeq2 (Love et al. 2014) and performed quantile normalization using the qqnorm function in R
603 (R Core Team 2022). Additionally, we calculated the difference of normalized read counts in
604 pollen and male leaves (male leaf minus pollen) for each gene and performed quantile
605 normalization of its absolute value using the qqnorm function in R (R Core Team 2022).
606
607 *Correlation in gene expression*
608 To characterize the correlation in expression across life stages or sexes, we used Pearson
609 correlations and the final normalized read counts as expression level as described above. For
610 male leaves and pollen, we calculated the correlation of a given gene's expression level in each
611 tissue using 69 male individuals and repeated for 16,411 autosomal genes that had evidence of
612 expression (as described above) to generate a distribution of correlations. These correlations are
613 the same as any phenotypic correlation in behavioral or morphological traits, but here are for
614 gene expression (Stinchcombe and Kelly 2025). To estimate the across-sex correlation in
615 expression, we took advantage of the sib-structure of our design and the fact that we had male
616 and female sibling pairs. We used the expression level of a gene in a male sample and its female

617 sibling as a pair of observations, repeated for 64 sibling pairs in our design, to estimate the
618 correlation in expression across siblings for a given gene. We then repeated this for 14,992 genes
619 with evidence of expression in the samples to generate a distribution of across-sex correlation in
620 expression. Note that because we only had a single male and female sample per family, rather
621 than replicates, this is a phenotypic approximation of the across-sex genetic correlation (r_{mf}),
622 rather than a genetic correlation. We repeated the analyses for genes regulated by eQTLs
623 (eGenes, see below) in at least one life stage or sex following the same steps.
624
625 *eQTL mapping*
626 We performed *cis*-eQTL mapping on autosomes separately for male leaves, female leaves, and
627 pollen tissue. For female leaves, we repeated eQTL mapping on X and PAR using the same
628 approach as the autosomes. For male samples, we performed eQTL mapping using the difference
629 in leaf and pollen expression levels as phenotypes in the same way as in leaves or pollen
630 separately. To control for genetic relatedness, we performed LD pruning in Plink (Chang et al.
631 2015) and identified genetic principal components (PC) using EIGENSOFT smartpca (Patterson
632 et al. 2006; Price et al. 2006). The significance of each PC was determined by the Tracy-Widom
633 test (Patterson et al. 2006; Sztepanacz and Blows 2017). We included the significant PCs ($p <$
634 0.05) as covariates in eQTL mapping.
635
636 We mapped *cis*-eQTL using tensorQTL (Taylor-Weiner et al. 2019) following its guidelines, and
637 tested pre linkage-pruning SNPs within a 20 kb window to the transcription start site for each
638 gene. We performed adaptive permutations to calculate empirical and beta-approximated
639 empirical *p*-values for each gene (Ongen et al. 2016), and generated nominal *p*-values for all
640 SNP-gene pairs. We calculated the Storey *q*-value and the nominal *p*-value threshold for
641 significant associations based on the beta-approximated empirical *p*-value for each gene using a
642 false discovery rate (FDR) of 0.1 (Benjamini and Hochberg 1995; Storey 2002). We filtered for
643 genes that had q-value < 0.1 and at least one eQTL as our eGenes. We filtered for SNPs with a
644 nominal *p*-value smaller than the nominal *p*-value threshold of its eGene as our eQTLs. The
645 minimum *p*-value threshold is 0.0318 for male leaves, 0.0503 for female leaves, and 0.0166 for
646 pollen. We identified conditionally independent eQTLs for eGenes using a forward-backward
647 stepwise regression model in tensorQTL (GTEx Consortium et al. 2017). We defined primary
648 eQTLs as the top ranked eQTL for each eGene, and any additionally ranked eQTLs as secondary

649 or conditionally independent eQTLs. The effect size of an eQTL was defined as the slope in the
650 linear regression model from tensorQTL. In the analyses of eQTLs for differences in expression
651 levels between life stages, effects of the minor allele were tested rather than alternative allele of
652 each SNP.
653
654 We kept one eQTL for every eGene, either the most significant (top) eQTL or a randomly
655 selected eQTL when comparing minor allele frequencies (MAF) and when testing the
656 relationship between effect size and the distance to transcription start site of eQTLs. We
657 removed eQTLs and eGenes when an eQTL affected multiple eGenes when comparing MAFs.
658 We performed gene ontology enrichment of eGenes using topGO (Alexa and Rahnenfuhrer
659 2023), significant GO terms were selected using a *p*-value cutoff of 0.05 based on Fisher's exact
660 tests.
661
662 We ran tensorQTL on all 143 leaf samples to test the linear model: Expression ~ Genotype + Sex
663 + Genotype × Sex. We kept the top association for each gene based on the interaction term (-best
664 only), and calculated multiple-testing corrected (Davis et al. 2016) and Benjamini-Hochberg
665 adjusted *p*-values (pval_adj_bh) following the recommendations from tensorQTL. The cutoff for
666 eQTLs showing significant Genotype × Sex is pval_adj_bh < 0.1.
667
668 *Null distributions of eQTL MAFs*
669 To generate a null distribution for MAFs of eQTLs in each tissue, we performed permutations by
670 randomly re-assigning sample ID's in the expression phenotype file. We performed 1000
671 permutations in both females and males. For female leaves, we performed the same permutations
672 on X and PAR as the autosomes. For male samples, we performed permutations the same way in
673 male leaf and pollen phenotypes and identified the shared and specific eQTLs between male
674 leaves and pollen in each permutation. We identified false-positive eQTLs using the same *p*-
675 value thresholds from the previous analyses on our observed data. We removed eGenes with
676 more than 10 eQTLs, randomly selected an eQTL from each eGene, and removed eQTLs
677 affecting multiple eGenes. We repeated the same procedure of calculating MAF using true
678 positive eQTLs. The proportions of false positive eGenes compared to true positive eGenes were
679 0.059 in male leaves, 0.105 in female leaves, and 0.0318 in pollen, based on the median number
680 of false positive eGenes in 1000 permutations.

681

682 *Diversity statistics*

683 We used the script codingSiteTypes.py

684 (https://github.com/simonhmartin/genomics_general/blob/master/codingSiteTypes.py, accessed

685 in 2020) to extract 4-fold degenerate site for calculating neutral diversity statistics. We generated

686 4-fold VCFs including both variant and invariant sites using all leaf samples combined. We used

687 pixy (Korunes and Samuk 2021; Bailey et al. 2025) to calculate nucleotide diversity (π) and

688 Tajima's *D* for all autosomal genes. We only kept genes with at least 50 sites in the subsequent

689 analyses of π and Tajima's *D.* We removed genes if they were associated with an eQTL that

690 affected multiple eGenes.

691

692 *Burden of rare allele test*

693 We performed the 'burden of rare allele' test separately for male leaves, female leaves and pollen

694 using the methods described in Zhao et al. (2016). We counted the number of singletons in the

695 5kb upstream window from the transcription start site for each gene and each individual. We

696 ranked the individuals for each gene based on their expression level, for each rank we summed

697 the number of local singletons from all genes regardless of their individual identity. Finally, we

698 plotted the sum of rare alleles against rank bins (bin size = 1) and performed quadratic or linear

699 regression to assess relationship between rare allele counts and gene expression ranks.

700

701 **Data availability**

702 Raw sequencing reads are available through the NCBI SRA database under BioProject

703 PRJNA744278. Scripts used in this study are available at

704 https://github.com/SIWLab/Rumex_eQTL.

705

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710

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