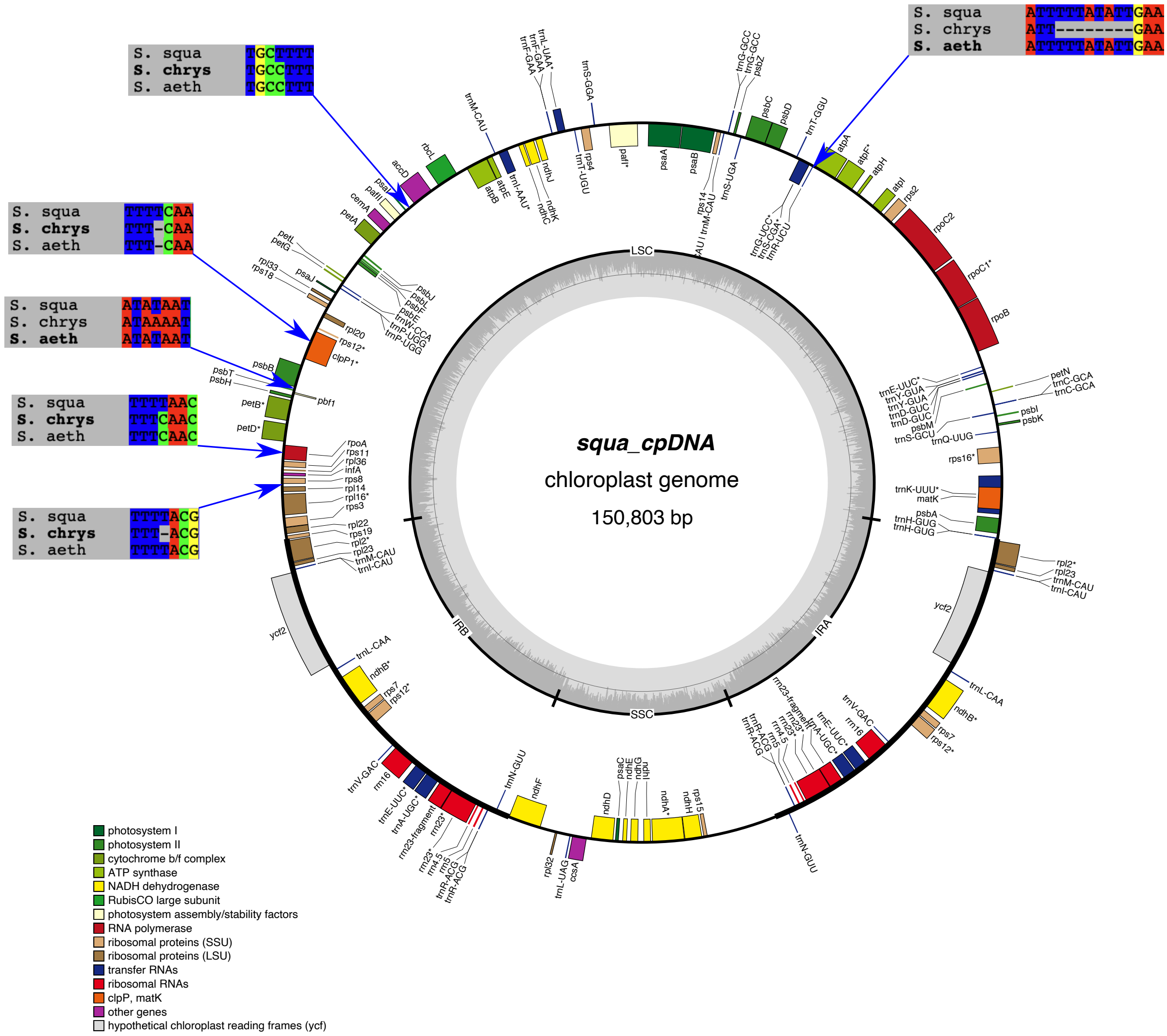

Figure S1: Complete chloroplast genome of *S. squalidus*. Genes annotated in the genome are labelled and color-coded according to function. Genes inside circle are transcribed clockwise, outside circle transcribed counterclockwise. Dark and light grey bars in inner circle denote percentage GC (dark) and AT (light) content. Insets depict the six differences detected across the three species, with the corresponding alleles found in *S. squalidus* (S. squa), *S. chrysanthemifolius* (S. chrys) and *S. aethnensis* (S. aeth). **Related to Figure 2.**

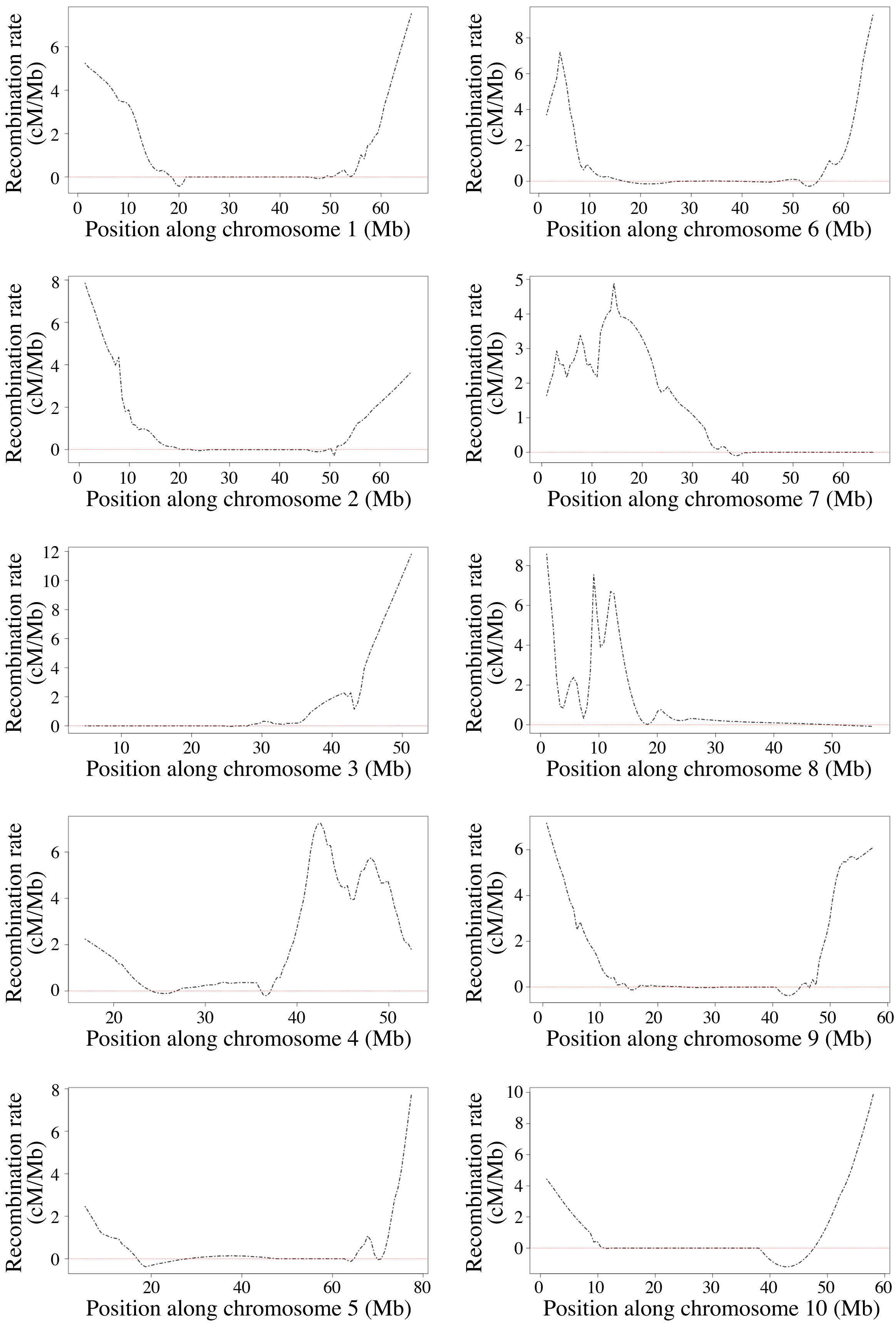

Figure S2. Local recombination rates estimated along each chromosome. Results obtained using the genetic map of reference [1]. Note that axes vary between panels. **Related to Figure 2.**

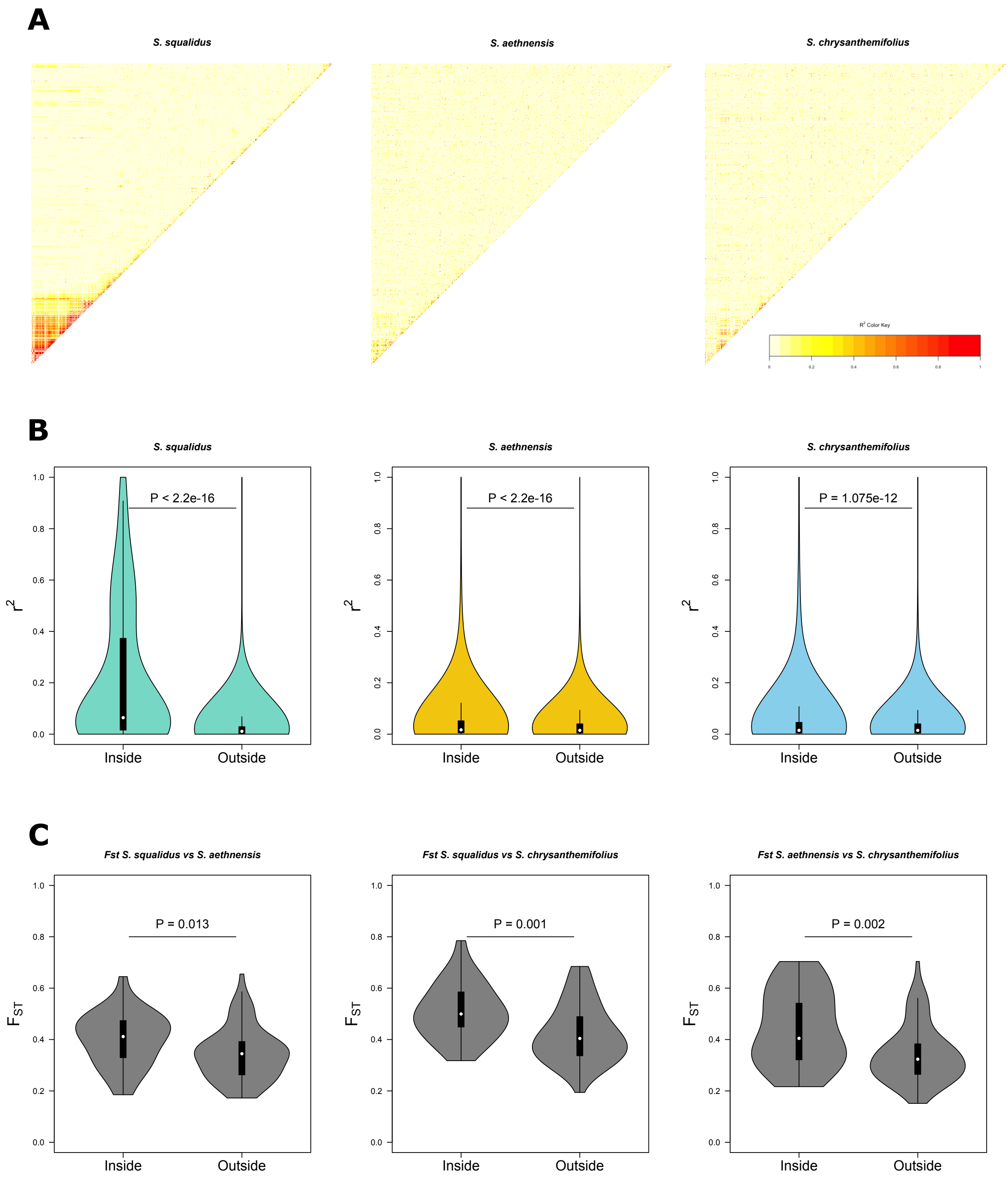

Figure S3: Linkage disequilibrium and pairwise F_{ST} values inside vs outside the first 15 Mb of chromosome 4. (A) Pairwise linkage disequilibrium between pairs of SNPs estimated along chromosome 4 for *S. squalidus* (left), *S. aethnensis* (middle) and *S. chrysanthemifolius* (right). (B) Linkage Disequilibrium values inside and outside the first 15 Mb region of chromosome 4 for *S. squalidus* (left), *S. aethnensis* (centre) and *S. chrysanthemifolius* (right). P-values refer to Welch two sample t-test results, for LD between pairs of SNPs in chromosome 4, inside vs outside the first 15 MB region. (C) F_{ST} values between pairs of species (left to right: *S. squalidus* vs *S. aethnensis*, *S. squalidus* vs *S. chrysanthemifolius*, and *S. aethnensis* vs *S. chrysanthemifolius*) inside and outside this 15 Mb region of chromosome 4. P-values refer to Welch two sample t-test results, for pairwise F_{ST} values estimated in sliding windows of 500 kb, along chromosome 4. For panels B and C, black boxplots indicate the median (white dot), the quartiles (black rectangle), 1.5 times the interquartile range (whiskers), and the kernel probability density (shaded area). **Related to Figure 4.**

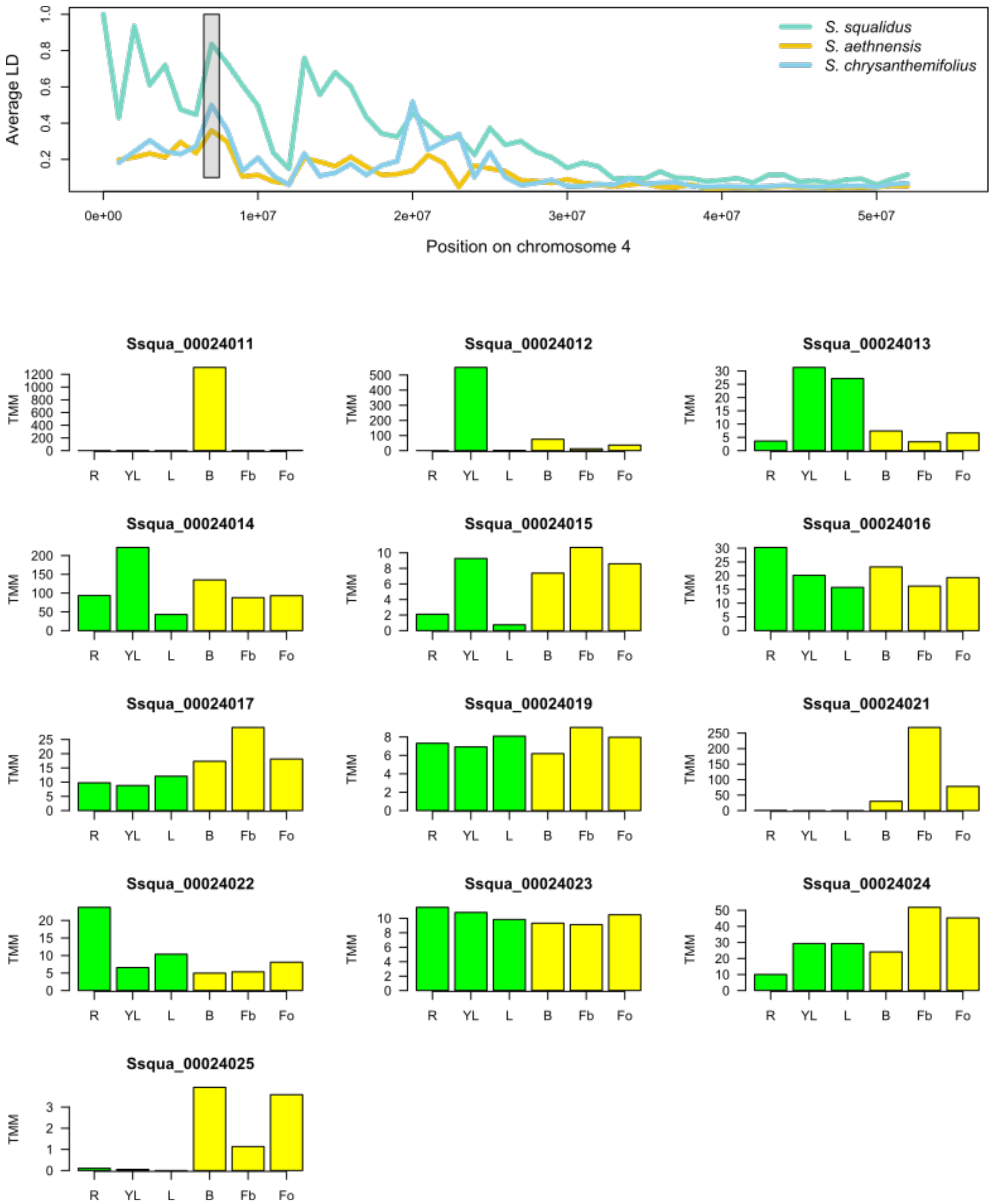

Figure S4: Putative location of the self-incompatibility (*S*)-locus on chromosome 4 of *S. squalidus*. Top panel shows average linkage disequilibrium (LD) between SNPs within 1 Mb windows along chromosome 4, for *S. squalidus* and the two parental species *S. aethnensis* and *S. chrysanthemifolius*. Rectangle highlights the single window (7-8 Mb) with elevated LD in all three species. Bottom inset shows the gene expression estimates of genes annotated in this window, across floral (yellow) and non-floral (green) plant tissues in *S. squalidus* (R: root, YL: young leaf; L: leaf; B: capitulum bud; Fb: flower buds; Fo: whole open flower). **Related to Figure 4.**

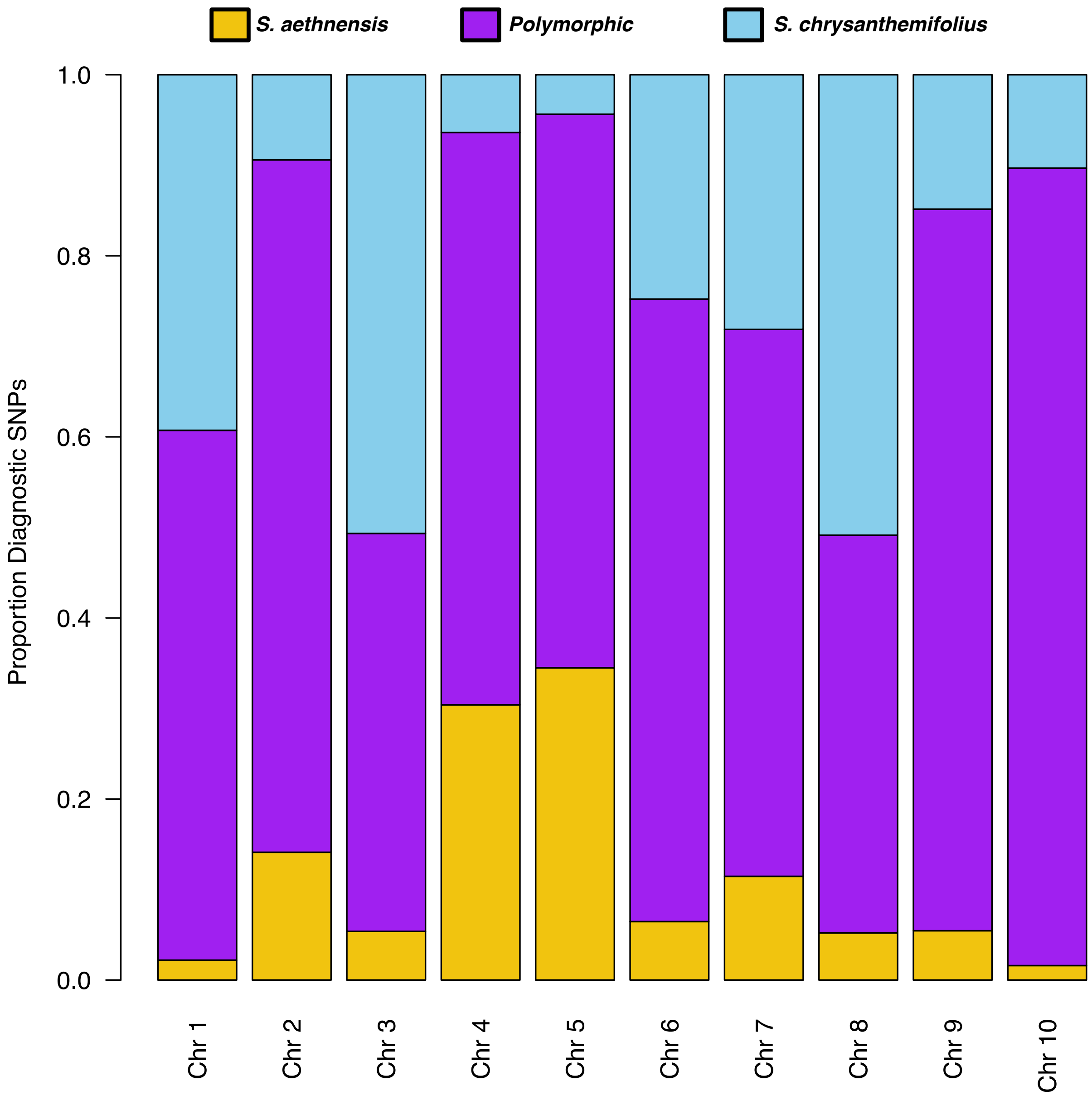

Figure S5. Proportion of diagnostic SNPs (i.e., SNPs that are fixed or nearly fixed for different alleles in the two parental species) sequenced in *S. squalidus* that are fixed or nearly fixed for either parental allele, or polymorphic for the two parental alleles (legend on top). **Related to Figure 4.**

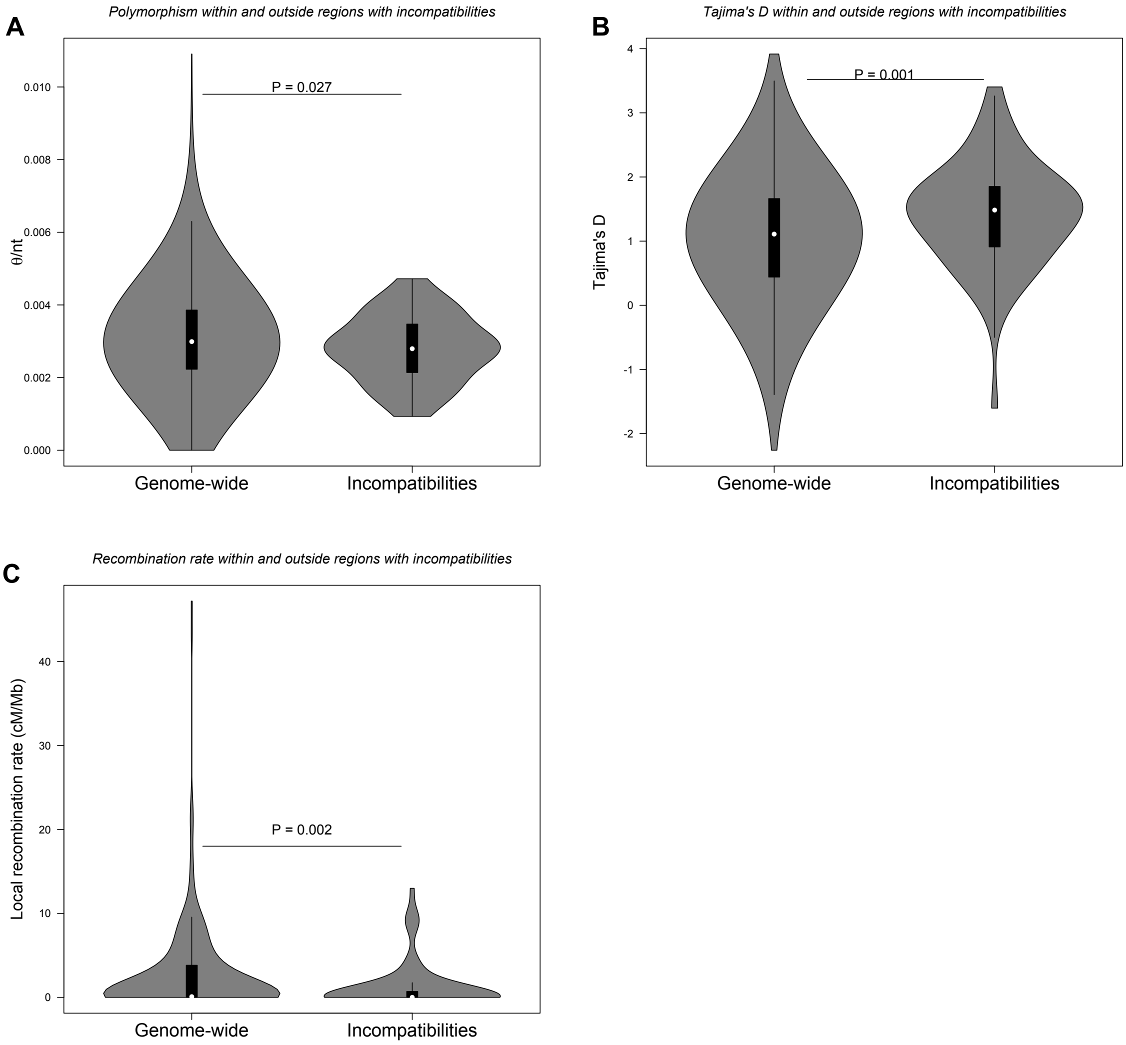

Figure S6: Comparison of summary statistics estimated from windows harbouring genetic incompatibilities vs genome-wide background. Estimates of (A) Watterson's theta, (B) Tajima's D and (C) local recombination rates using non-overlapping windows (500 kb) along the newly assembled genome of *S. squalidus* and based on the population-level RNAseq data. For each panel, left-side distribution refers to values estimated genome-wide (excluding windows harbouring genetic incompatibilities), and right-side distribution values observed in windows harbouring genetic incompatibilities described in [1] between the two parental species of *S. squalidus*. Significance *P* values refer to Welch two sample t-tests in each panel. **Related to Figure 4.**

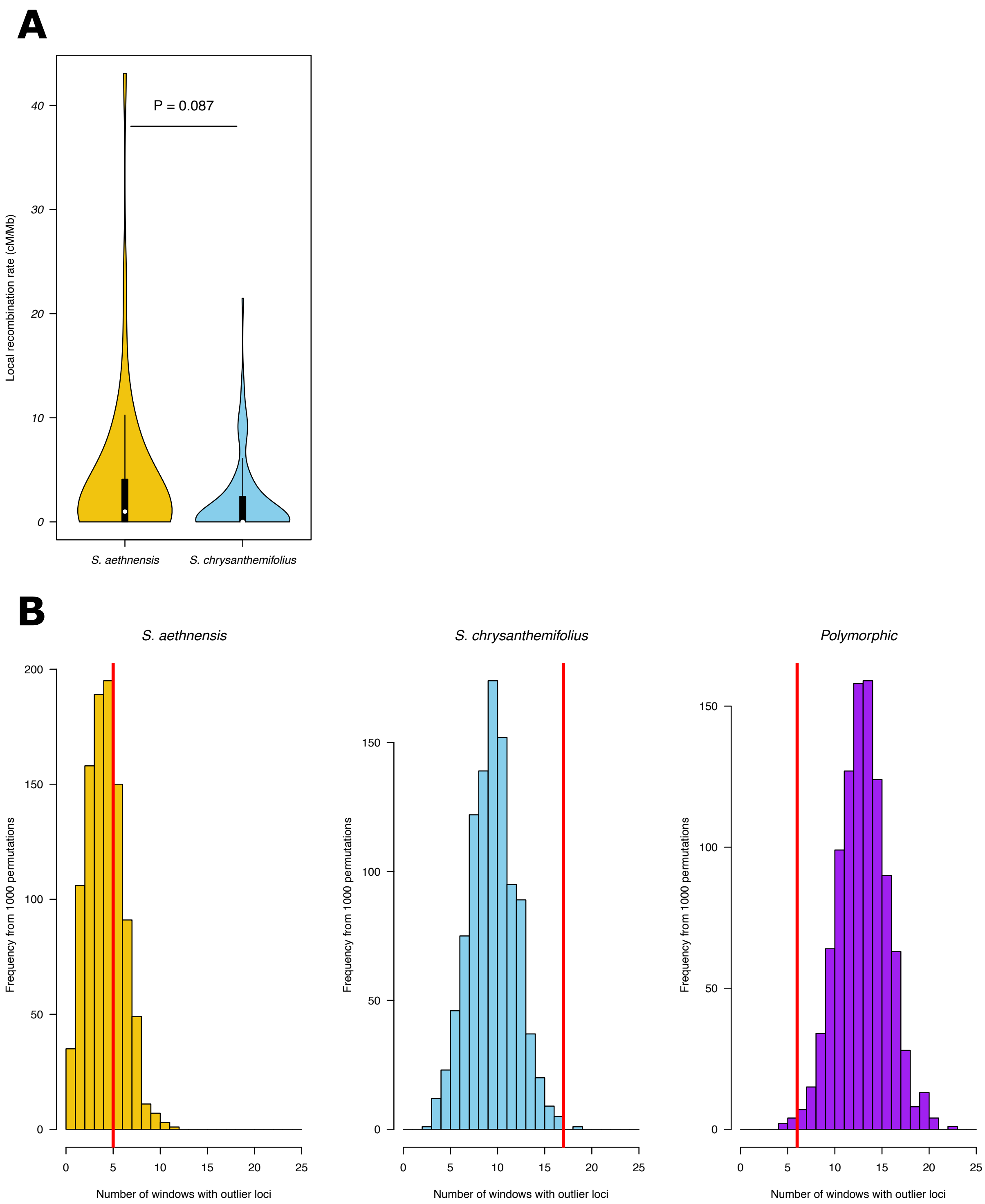

Figure S7: Parental ancestry, recombination rates and natural selection in *S. squalidus*. (A) Local recombination rate in genomic regions with different parental ancestry. Regions with minor parent ancestry (i.e. *S. aethnensis*) exhibit higher recombination rates, but the difference is not significant (*P* values from Welch two sample t-test). (B) The number of genomic windows that have at least 1 outlier locus found to be under divergent selection in parental species on Mount Etna, according to the parental ancestry. Shown are the results for 1000 permutations of outlier loci over all genomic windows with known parental ancestry (i.e., excluding genomic windows without diagnostic SNPs or with mixed ancestry from both parental species). For each graph, red vertical line denotes the number of genomic windows with at least 1 outlier locus observed in the real data. **Related to Figure 4.**

Chromosome	Average (cM/Mb)	Std. Dev. (cM/Mb)
1	1.81	4.44
2	2.28	4.79
3	1.60	4.01
4	4.25	13.77
5	3.99	10.96
6	3.73	6.09
7	3.15	11.42
8	4.40	8.90
9	3.79	8.89
10	1.72	3.23

Table S3: Average recombination rate (and standard deviation) across each chromosome of *S. squalidus* genome assembly. Related to Figure 2.

Gene ID	Start	End	Annotation
00024011	**7230911**	**7234250**	**At5g33370: GDSL esterase/lipase At5g33370**
00024012	7247280	7249670	LTL1: GDSL esterase/lipase LTL1
00024013	7319122	7329476	At1g12620: Pentatricopeptide repeat-containing protein At1g12620
00024014	7571153	7575020	IPI2: Isopentenyl-diphosphate Delta-isomerase II
00024015	7575003	7576002	RIC4: CRIB domain-containing protein RIC4
00024016	7577090	7583128	OTU1: OVARIAN TUMOR DOMAIN-containing deubiquitinating enzyme 1
00024017	7629021	7639557	N/A
00024018*	7631712	7633121	RPS3A: 40S ribosomal protein S3-1
00024019	7644878	7649691	N/A
00024020*	7650632	7653838	At5g48740: Probable LRR receptor-like serine/threonine-protein kinase At5g48740
00024021	**7672536**	**7680339**	**PBL26: Probable serine/threonine-protein kinase PBL26**
00024022	7854038	7855588	NUP205: Nuclear pore complex protein NUP205
00024023	7890574	7891075	P23-1: Co-chaperone protein p23-1
00024024	7891104	7892058	RDM3: Protein RNA-directed DNA methylation 3
00024025	**7893980**	**7896263**	**HD3A: Protein HEADING DATE 3A**

Shown are the gene names, start and end positions and annotation details. Genes in bold show expression primarily in floral tissues (Figure 8). * Not identified in the reference transcriptome.

Table S4: Genes annotated on the window exhibiting high Linkage Disequilibrium on chromosome 4. Related to Figure 4.

PacBio - CLR					
Sample TOLID	Date of sequence generation	Yield (bases)	Read N50	Sample Accession	Run Accession
dSenSqu2	2018-09-04	2,338,629,615	6,472	SAMEA5248730	ERR3313259
dSenSqu2	2018-10-08	2,067,415,936	9,328	SAMEA5248730	ERR3313274
dSenSqu2	2018-12-12	3,928,076,063	5,777	SAMEA5248730	ERR3313389
dSenSqu2	2018-12-13	4,017,274,098	5,787	SAMEA5248730	ERR3313390
dSenSqu2	2018-12-14	3,846,735,368	5,946	SAMEA5248730	ERR3313391
dSenSqu2	2018-12-15	3,904,667,114	6,001	SAMEA5248730	ERR3313394
dSenSqu2	2018-12-15	3,757,120,443	6,033	SAMEA5248730	ERR3313395
dSenSqu2	2018-12-18	3,141,371,637	6,379	SAMEA5248730	ERR3313398
dSenSqu2	2018-12-19	3,721,779,715	6,308	SAMEA5248730	ERR3313399
dSenSqu2	2018-12-19	3,753,597,941	6,196	SAMEA5248730	ERR3313293
dSenSqu2	2018-12-20	3,802,210,460	6,590	SAMEA5248730	ERR3313402
dSenSqu2	2018-12-21	3,404,976,273	6,326	SAMEA5248730	ERR3313403
dSenSqu2	2018-12-22	3,454,174,991	6,280	SAMEA5248730	ERR3313404
dSenSqu2	2018-12-22	3,224,377,864	6,128	SAMEA5248730	ERR3313405
dSenSqu2	2019-06-14	2,386,706,443	10,316	SAMEA5248730	ERR3396649
dSenSqu2	2019-06-21	33,257,705,325	12,306	SAMEA5248730	ERR3421359
All CLR data	**16 runs**	**84,006,819,286**	**7,011**		
Chromium genome					
Sample TOLID	Date of sequence generation	Yield read pairs	Yield (bases)	Sample Accession	Run Accession
dSenSqu2	2018-09-30	199,967,838	30,195,143,538	SAMEA5248730	ERR3316189
dSenSqu2	2018-09-30	181,032,566	27,335,917,466	SAMEA5248730	ERR3316195
dSenSqu2	2018-09-30	179,854,318	27,158,002,018	SAMEA5248730	ERR3316191
dSenSqu2	2018-09-30	201,183,850	30,378,761,350	SAMEA5248730	ERR3316193
dSenSqu2	2018-09-30	220,732,190	33,330,560,690	SAMEA5248730	ERR3316192
dSenSqu2	2018-09-30	208,818,806	31,531,639,706	SAMEA5248730	ERR3316194
dSenSqu2	2018-09-30	221,818,908	33,494,655,108	SAMEA5248730	ERR3316196
dSenSqu2	2018-09-30	207,400,224	31,317,433,824	SAMEA5248730	ERR3316190
All CLR data	**8 part-lanes**	**1,620,808,700**	**244,742,113,700**		
Dovetail Hi-C					
Sample TOLID	Dovetail Library ID	Yield read pairs	Yield (bases)	Sample Accession	Run Accession
dSenSqu2	dSenSqu2_dovetail_hic_lib_001	213,363,960	32,004,594,000	SAMEA5248730	PRJNA1138554
dSenSqu2	dSenSqu2_dovetail_hic_lib_002	224,673,966	33,701,094,900	SAMEA5248730	PRJNA1138554
Dovetail Chicago					
Sample TOLID	Dovetail Library ID	Yield read pairs	Yield (bases)	Sample Accession	Run Accession
dSenSqu2	dSenSqu2_dovetail_chicago_lib_001	191,099,936	28,664,990,400	SAMEA5248730	PRJNA1138554
dSenSqu2	dSenSqu2_dovetail_chicago_lib_002	262,519,028	39,377,854,200	SAMEA5248730	PRJNA1138554

Table S5: Sequence data generated for *Senecio squalidus.* See https://tolqc.cog.sanger.ac.uk/25g/dicots/Senecio_squalidus/ for details. **Related to Table 1.**

Species	Family	Source
Anemone pulsatilla	Ranunculaceae	[2]
Platanus occidentalis	Platanaceae	[2]
Buxus sempervirens	Buxaceae	[2]
Ilex sp.	Aquifoliaceae	[2]
Hydrangea quercifolia	Hydrangeaceae	[2]
Menyanthes trifoliata	Menyanthaceae	[2]
Barnadesia spinosa	Asteraceae	[3]
Centaurea solstitalis	Asteraceae	[3]
Lactuca sativa	Asteraceae	[4]
Helianthus annuus	Asteraceae	[5]
Senecio flavus	Asteraceae	[6]
Senecio madagascariensis	Asteraceae	[6]
Senecio vernalis	Asteraceae	[6]
Senecio vulgaris	Asteraceae	[6]
Senecio glaucus	Asteraceae	[6]
Senecio gallicus	Asteraceae	[6]
Senecio leucanthemifolius	Asteraceae	[6]
Senecio squalidus	Asteraceae	This paper

Table S6. List of species included in the molecular clock analyses. Related to Figure 3.

Fossil	Clade	Minimum age	Soft maximum age
Hyrcantha decussata	Ranunculales	119.6 Ma	128.63 Ma
Aquia brookensis	Proteales	107.59 Ma	128.63 Ma
Spanomera marylandensis	Buxales	100.1 Ma	128.63 Ma
Ilex herycnia	Aquifoliaceae	61.6 Ma	128.63 Ma
Tylerianthus crossmanensis	Cornales	85.8 Ma	128.63 Ma
Tubulifloridites lillei	Asteraceae	72.1 Ma	128.63 Ma
Tubulifloridites antipodica	Asteraceae (Minus *Barnadesia*)	41.5 Ma	128.63 Ma
Mutisiapollis	Asteroideae-Mutisioideae	47.5 Ma	128.63 Ma

Table S7. List of fossil calibrations included in the molecular clock analyses. All calibrations are described and justified in [7]. **Related to Figure 3.**

Supplementary References

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2. Leebens-Mack, J.H., et al., One thousand plant transcriptomes and the phylogenomics of green plants. Nature 574, 679-685.

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4. Reyes-Chin-Wo, S., et al., Genome assembly with in vitro proximity ligation data and whole-genome triplication in lettuce. Nat. Commun. *8,* 14953.

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6. Osborne, O.G., et al., Maintenance of species boundaries despite ongoing gene flow in ragworts. Genome Biol. Evol. *8*, 1038-1047.

7. Barba-Montoya, J., et al., Constraining uncertainty in the timescale of angiosperm evolution and the veracity of a Cretaceous Terrestrial Revolution. New Phytol. *18,* 819-834.