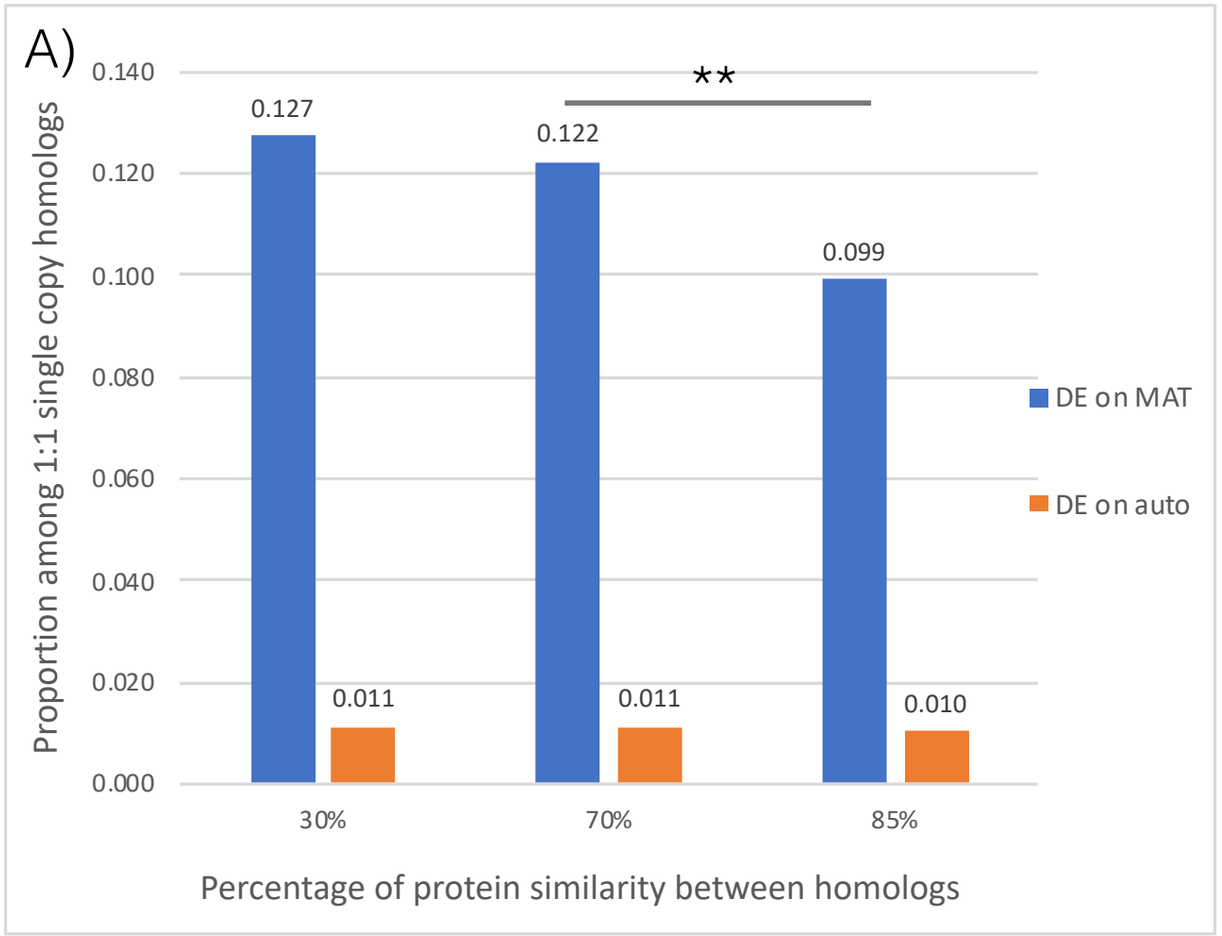

A)
Proportion among 1:1 single copy homologs
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0.127
0.122
0.099
**
0.011
0.011
0.010
30%
70%
85%
Percentage of protein similarity between homologs
DE on MAT
DE on auto

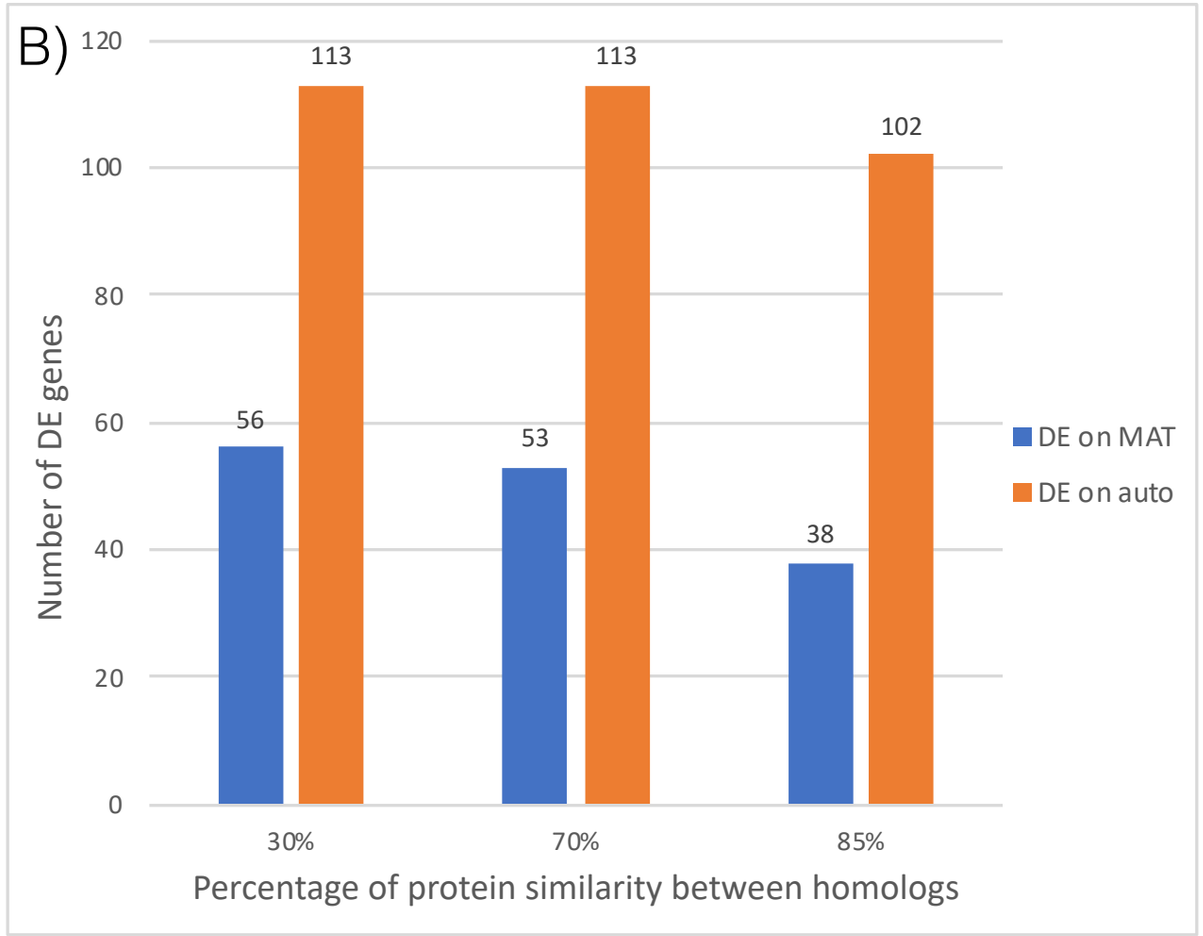

B)
Number of DE genes
120
100
80
60
40
20
0
56
113
53
113
38
102
30%
70%
85%
Percentage of protein similarity between homologs
DE on MAT
DE on auto

Fig. S1

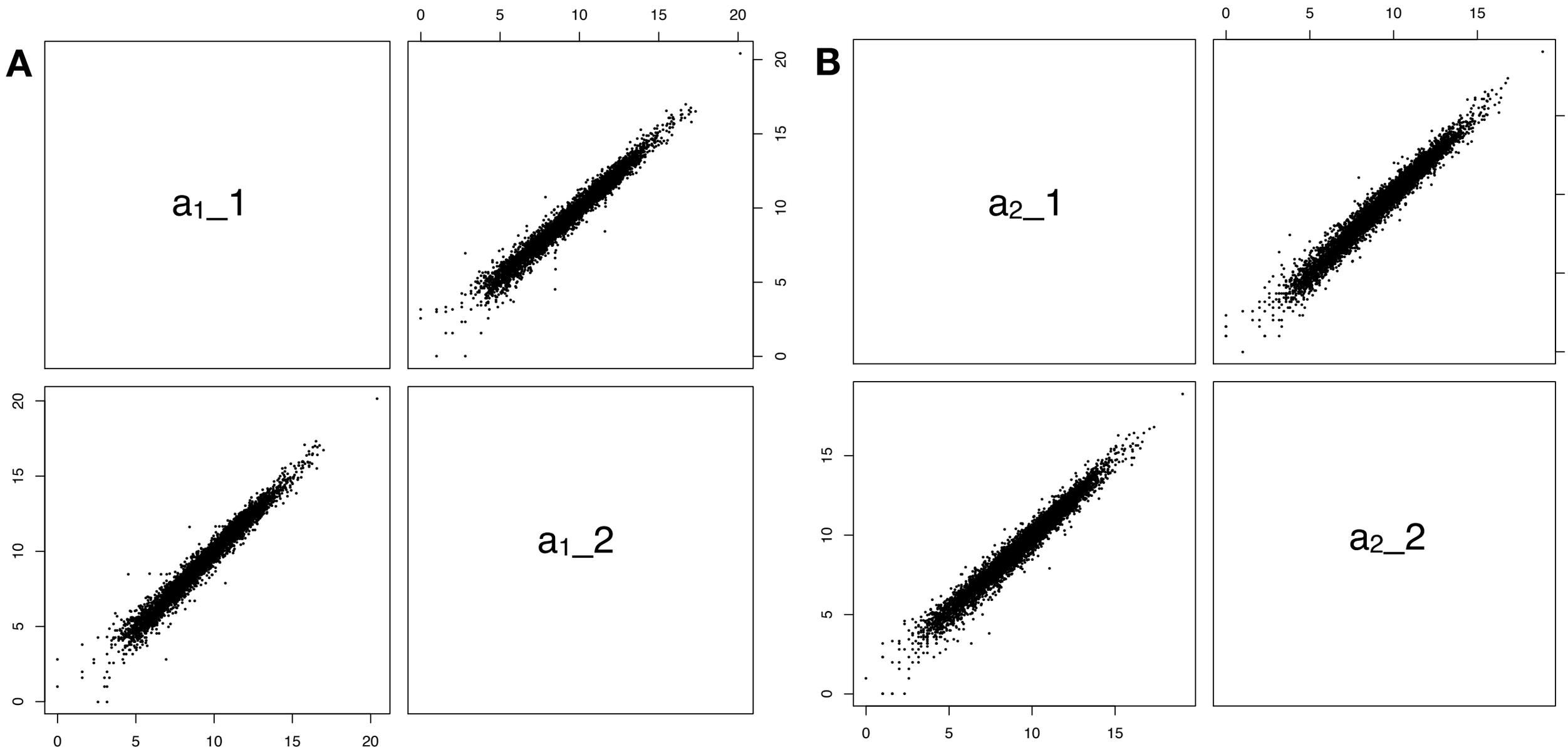


Fig. S2

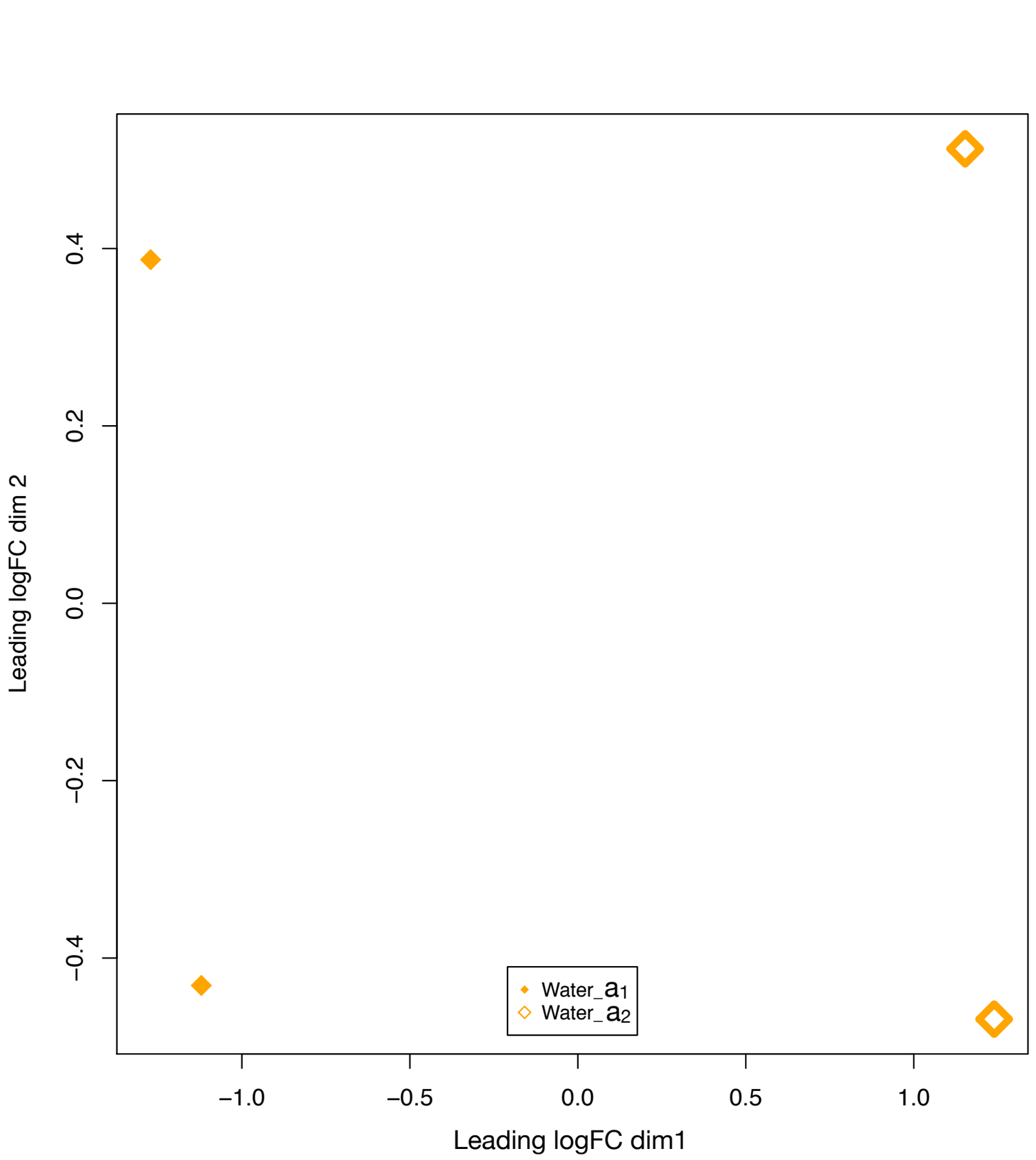

Leading logFC dim 2
0.4
0.2
0.0
−0.2
−0.4
Water_a1
Water_a2
−1.0
−0.5
0.0
0.5
1.0
Leading logFC dim1

Fig. S3

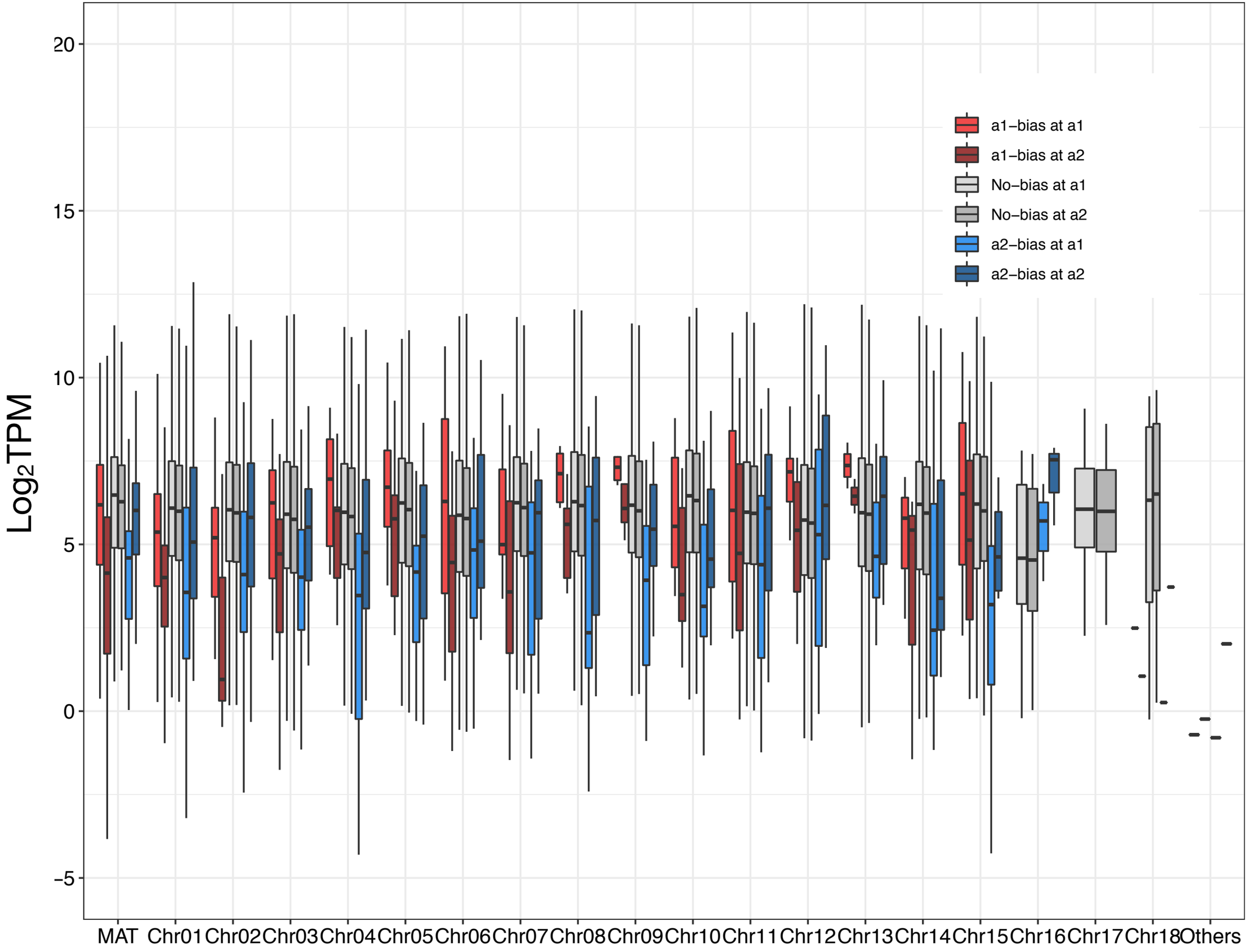

a1−bias at a1
a1−bias at a2
No−bias at a1
No−bias at a2
a2−bias at a1
a2−bias at a2
Log2TPM
20
15
10
5
0
−5
MAT
Chr01
Chr02
Chr03
Chr04
Chr05
Chr06
Chr07
Chr08
Chr09
Chr10
Chr11
Chr12
Chr13
Chr14
Chr15
Chr16
Chr17
Chr18
Others
Chromosomes

Fig. S4

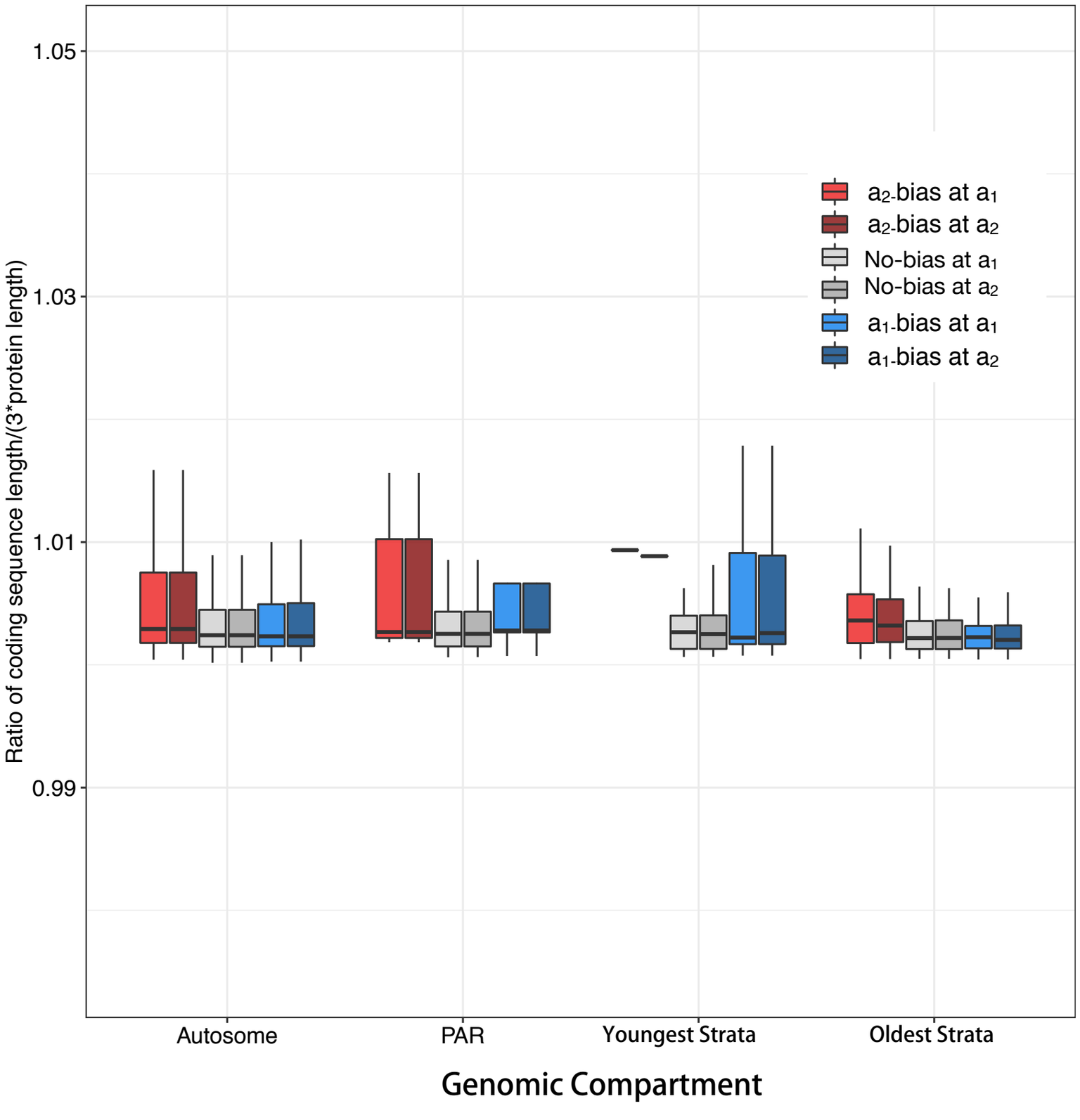

1.05
1.03
1.01
0.99
Ratio of coding sequence length/(3*protein length)
a2-bias at a1
a2-bias at a2
No-bias at a1
No-bias at a2
a1-bias at a1
a1-bias at a2
Autosome
PAR
Youngest Strata
Oldest Strata
Genomic Compartment

Fig. S5

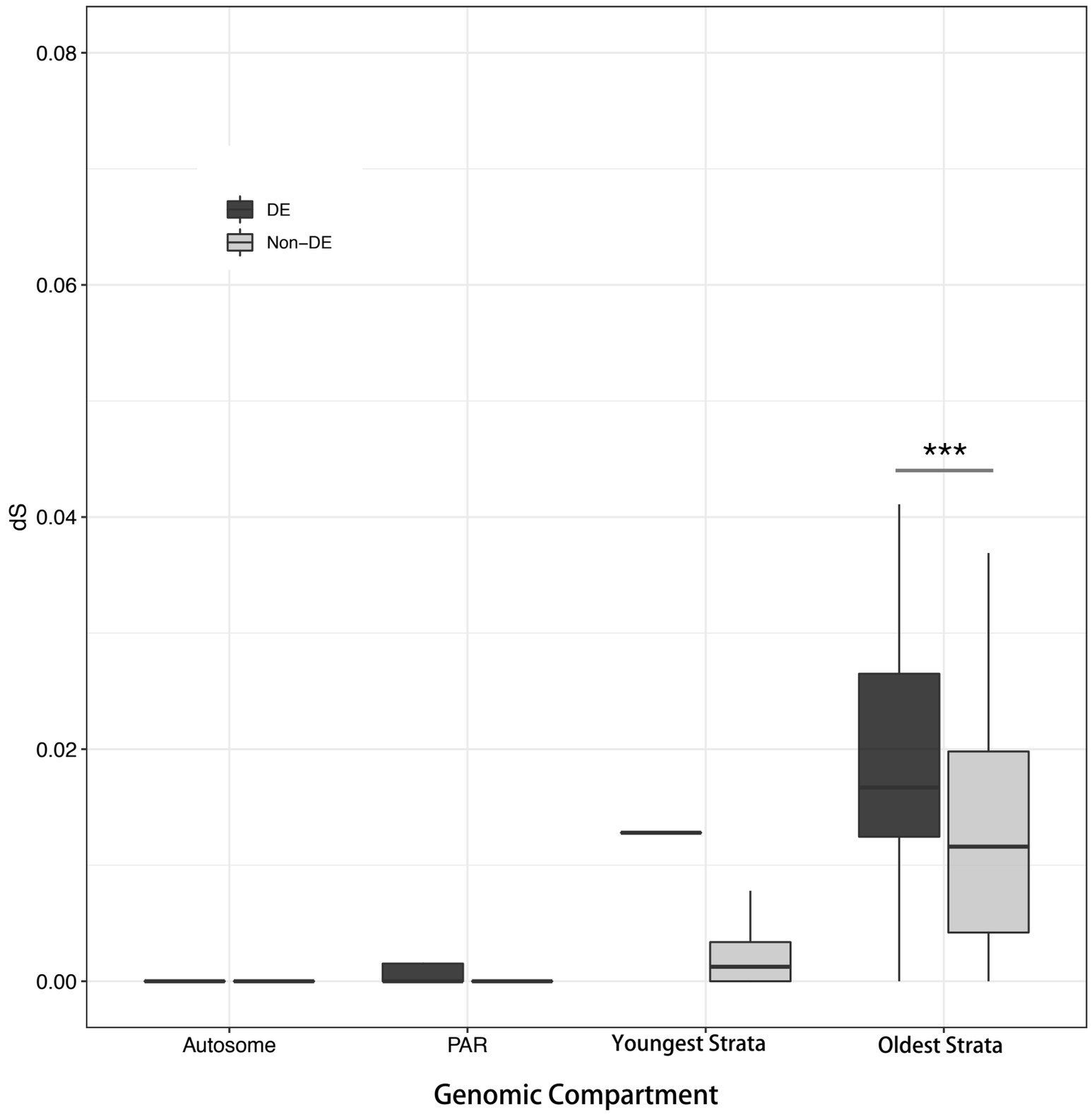

0.08
0.06
0.04
0.02
0.00
dS
DE
Non−DE
***
Autosome
PAR
Youngest Strata
Oldest Strata
Genomic Compartment

Fig. S6

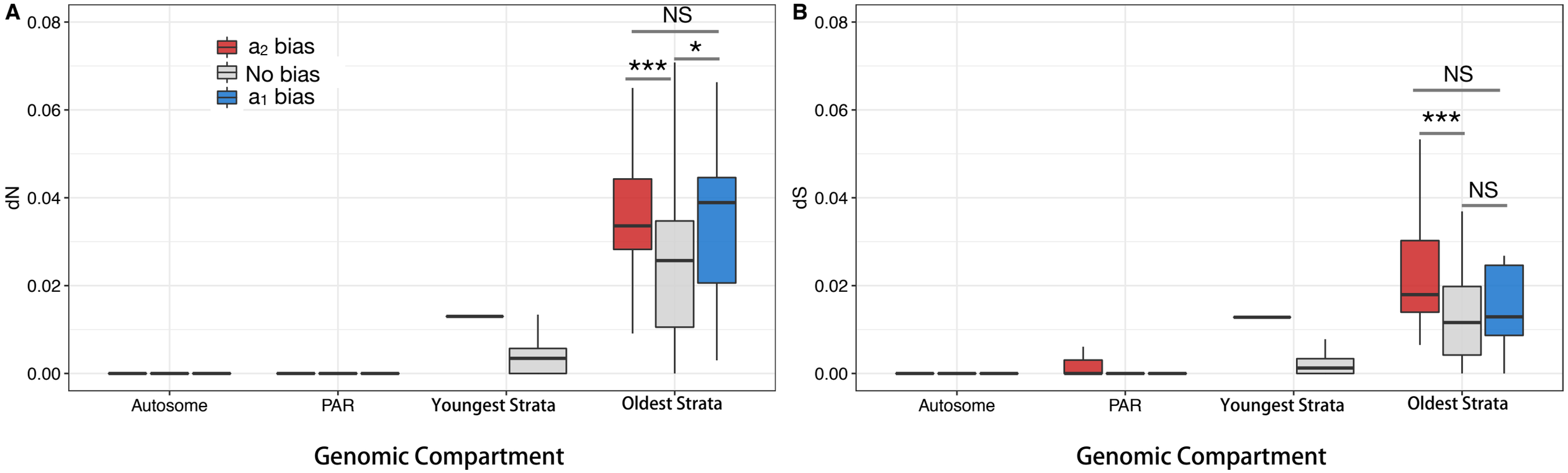

A
B
0.08
0.06
0.04
0.02
0.00
dN
dS
a2 bias
No bias
a1 bias
NS
*
***
NS
***
NS
Autosome
PAR
Youngest Strata
Oldest Strata
Genomic Compartment

Fig. S7

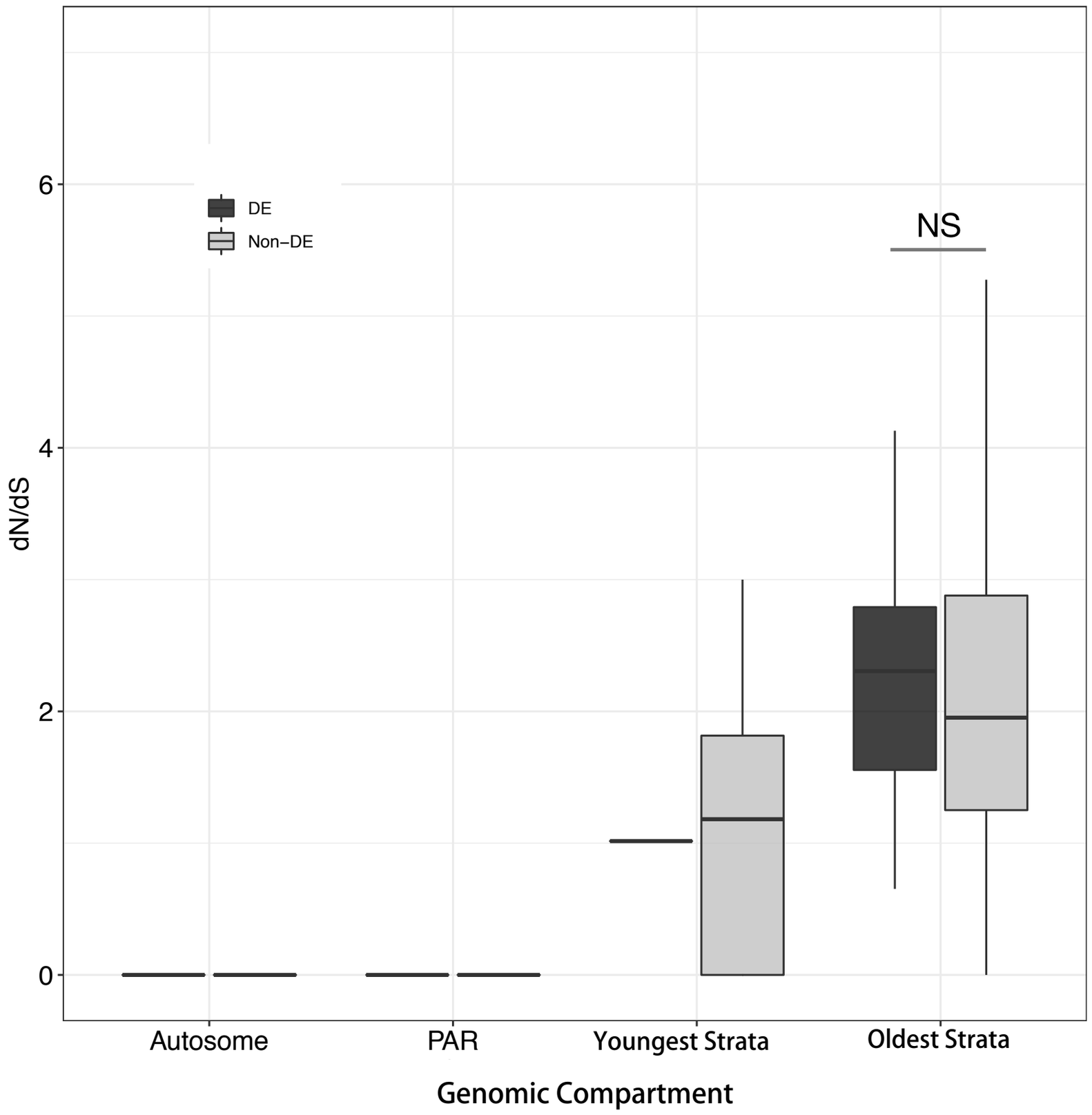

DE
Non−DE
NS
dN/dS
6
4
2
0
Autosome
PAR
Youngest Strata
Oldest Strata
Genomic Compartment

Fig. S8

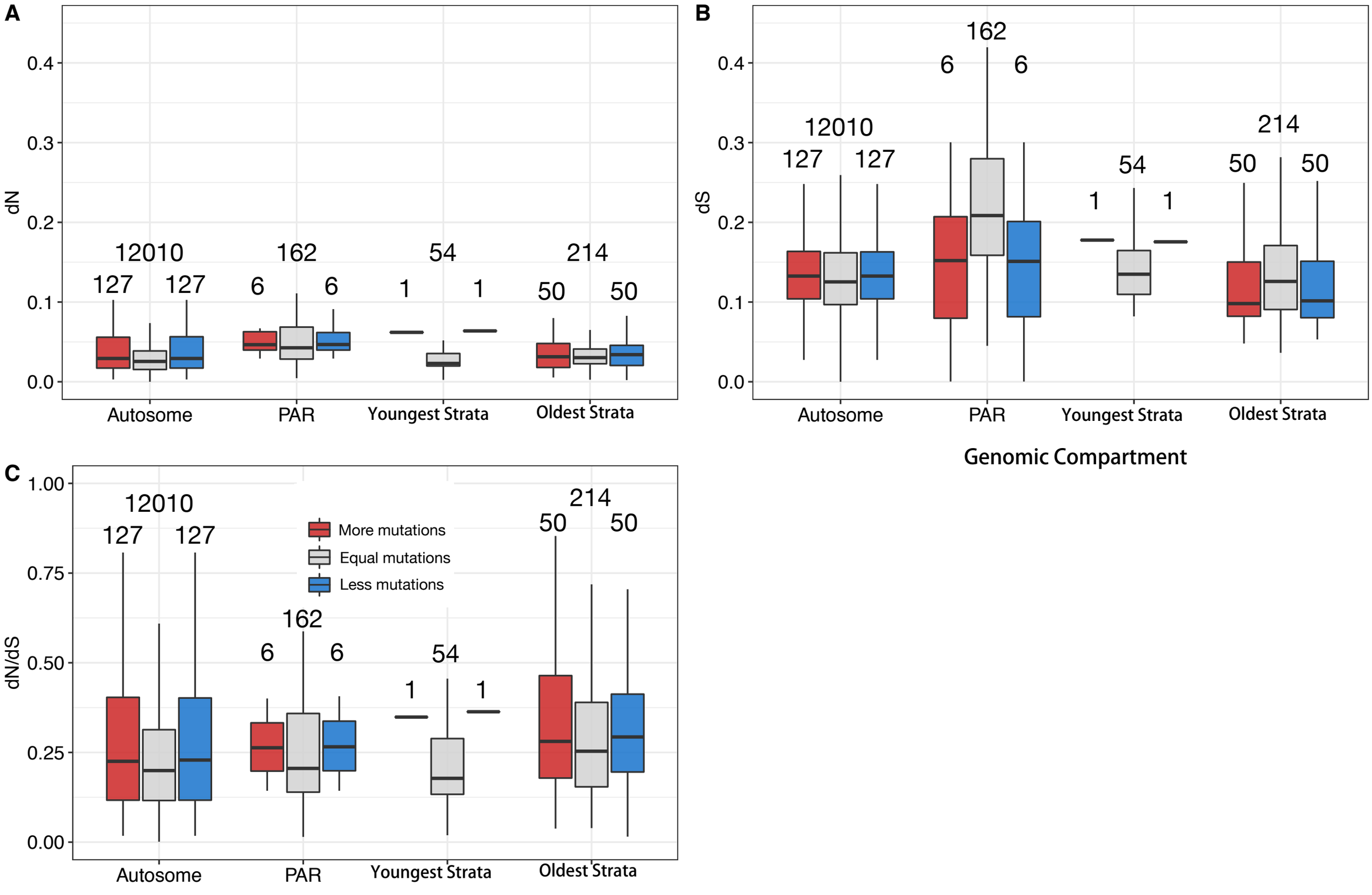
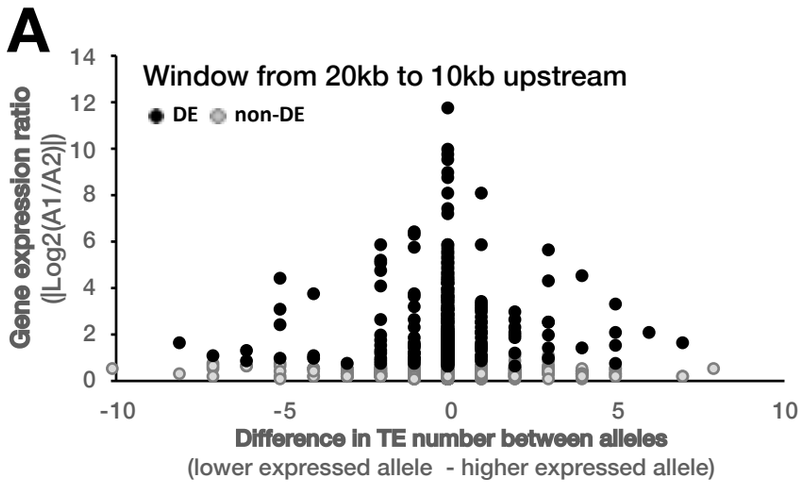
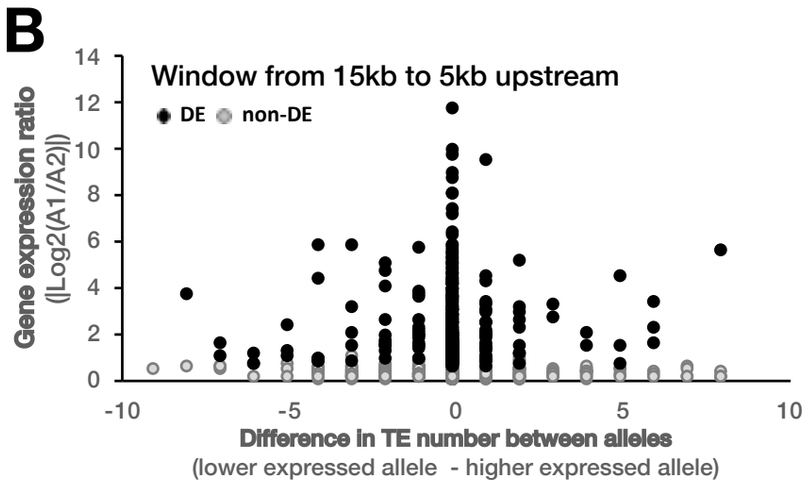
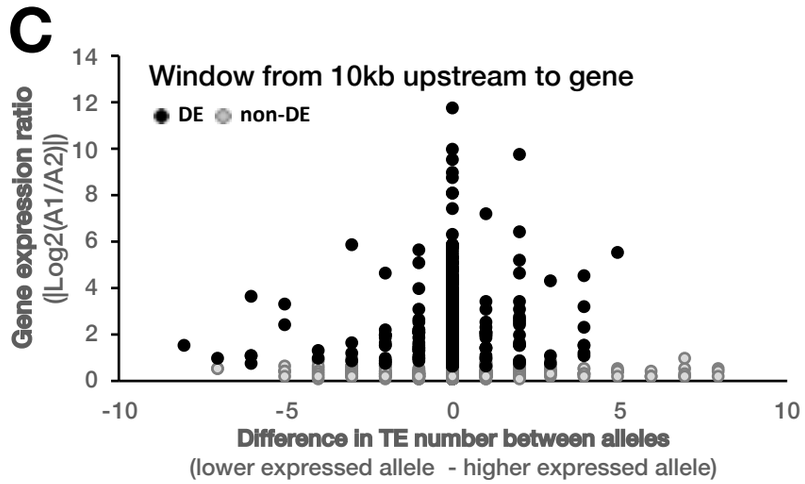
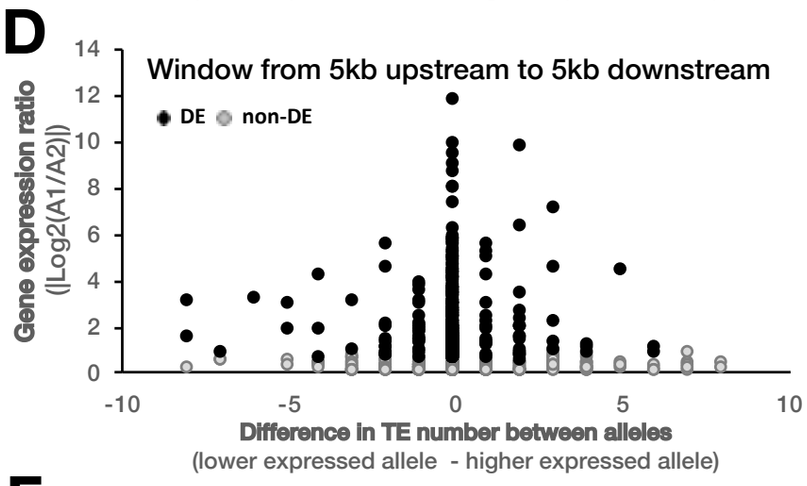

A
dN
0.4
0.3
0.2
0.1
0.0
12010
127
127
162
6
6
54
1
1
214
50
50
Autosome
PAR
Youngest Strata
Oldest Strata
B
dS
0.4
0.3
0.2
0.1
0.0
12010
127
127
162
6
6
54
1
1
214
50
50
Autosome
PAR
Youngest Strata
Oldest Strata
Genomic Compartment
C
dN/dS
1.00
0.75
0.50
0.25
0.00
12010
127
127
162
6
6
54
1
1
214
50
50
More mutations
Equal mutations
Less mutations
Autosome
PAR
Youngest Strata
Oldest Strata

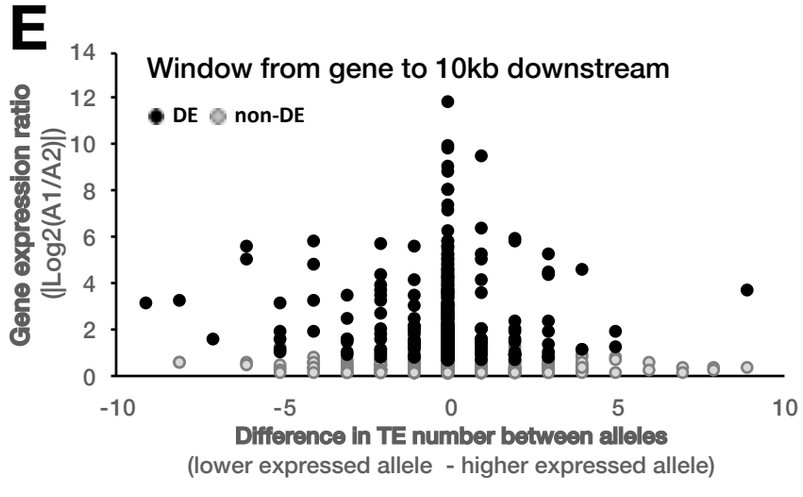
Fig. S9

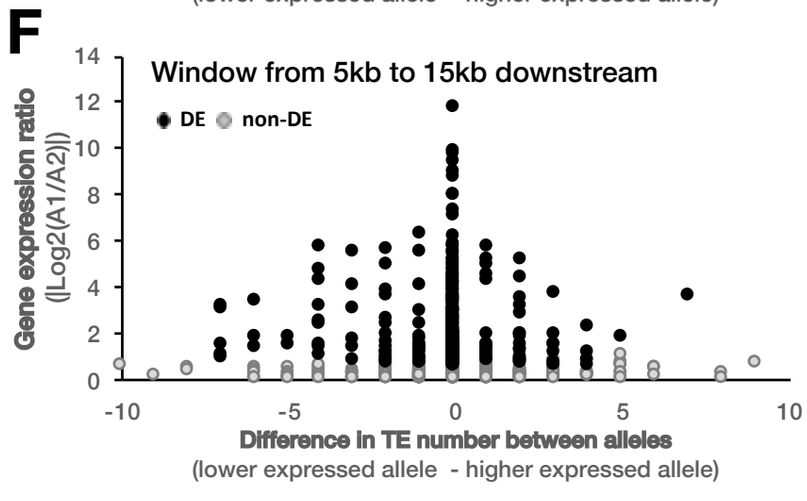

A
Window from 20kb to 10kb upstream
DE non-DE
Gene expression ratio
(|Log2(A1/A2)|)
Difference in TE number between alleles
(lower expressed allele - higher expressed allele)


B
Window from 15kb to 5kb upstream
DE non-DE
Gene expression ratio
(|Log2(A1/A2)|)
Difference in TE number between alleles
(lower expressed allele - higher expressed allele)


C
Window from 10kb upstream to gene
DE non-DE
Gene expression ratio
(|Log2(A1/A2)|)
Difference in TE number between alleles
(lower expressed allele - higher expressed allele)


D
Window from 5kb upstream to 5kb downstream
DE non-DE
Gene expression ratio
(|Log2(A1/A2)|)
Difference in TE number between alleles
(lower expressed allele - higher expressed allele)


E
Window from gene to 10kb downstream
DE non-DE
Gene expression ratio
(|Log2(A1/A2)|)
Difference in TE number between alleles
(lower expressed allele - higher expressed allele)


F
Window from 5kb to 15kb downstream
DE non-DE
Gene expression ratio
(|Log2(A1/A2)|)
Difference in TE number between alleles
(lower expressed allele - higher expressed allele)

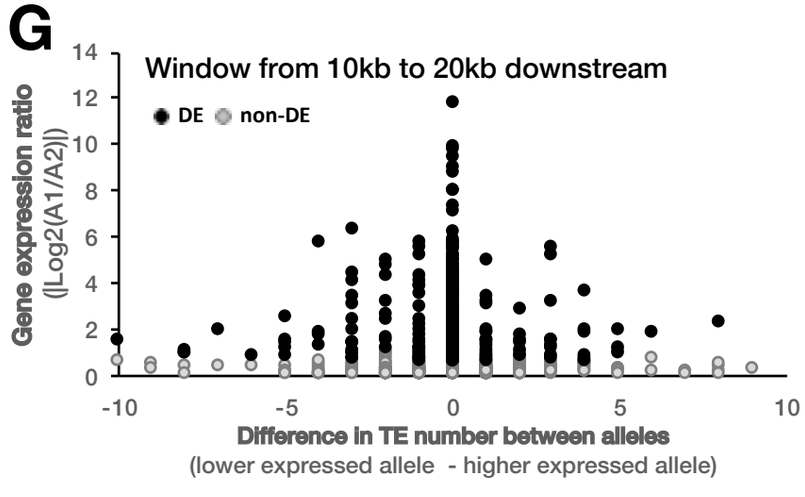
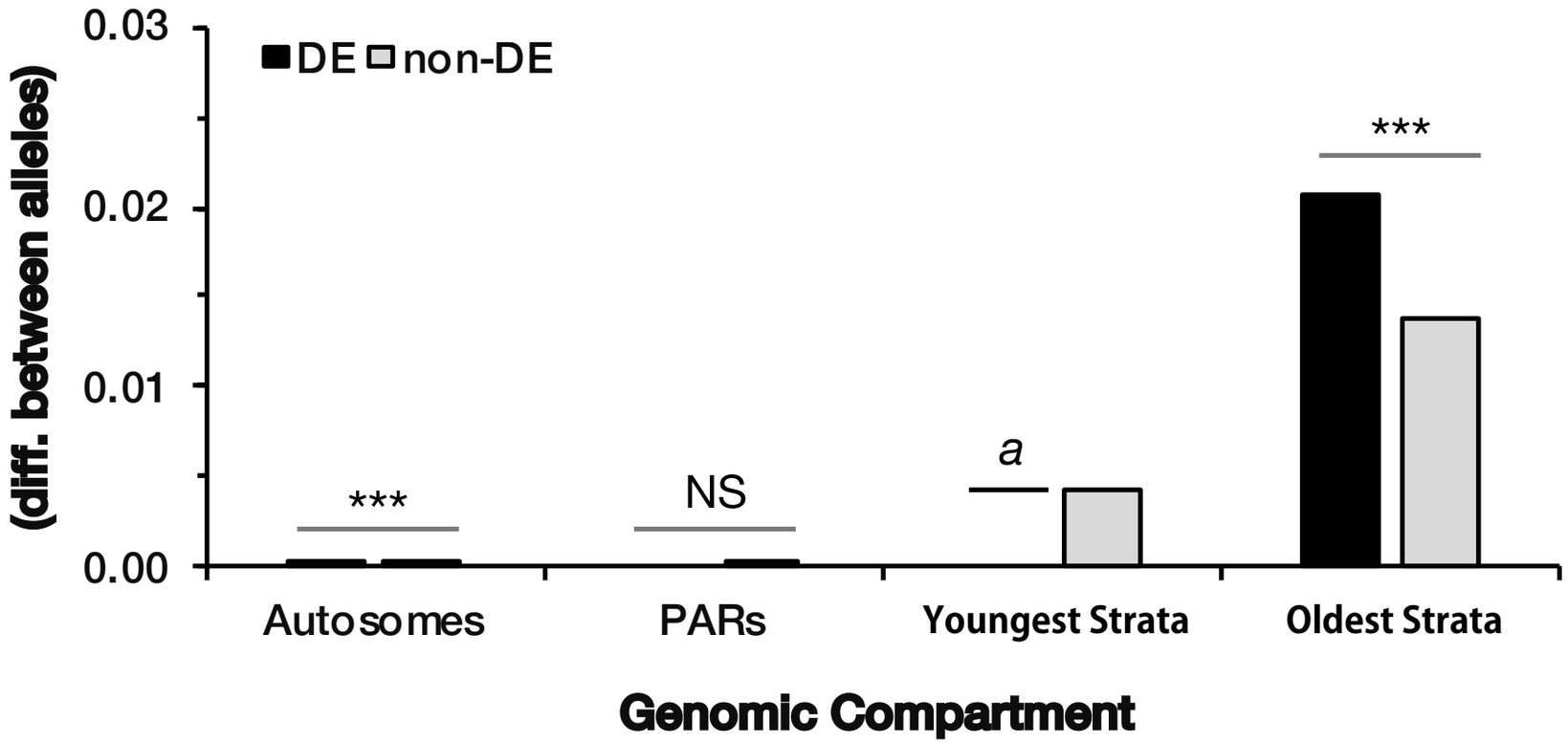

G
Window from 10kb to 20kb downstream
DE non-DE
Gene expression ratio
(|Log2(A1/A2)|)
Difference in TE number between alleles
(lower expressed allele - higher expressed allele)

Fig. S10



0.03
0.02
0.01
0.00
GC3
(diff. between alleles)
DE
non-DE
***
NS
a
***
Autosomes
PARs
Youngest Strata
Oldest Strata
Genomic Compartment

Fig. S11

Table S1. Number of single-copy genes with alleles in both a_1 and a_2 haploid genomes, with the 70% protein sequence identity detection threshold using reciprocal best BLASTp hits, before and after filtering out genes with transposable element (TE)-related functions. Filtering removed 192 paralogous genes in each haploid genome and genes with TE-related functions, including 1,750 and 1,819 from a_1 and a_2 haploid genome, respectively.

Number of single-copy genes	**Mating-type chromosome**	**Autosomes**
Before filtering out genes of TE-related functions	434	10,018
After filtering out genes of TE-related functions	371	9,025

Table S2. Identification of differentially-expressed (DE) genes with either a_1-biased or a_2-biased expression (i.e., higher expression in a_1 or a_2, respectively) under various $Log_2(a_1/a_2)$ criteria.

DE criteria	**a_1 bias**	**a_2 bias**
$Log2(a_1/a_2) > 0$	392	203
$Log2(a_1/a_2) > 1$	286	139
$Log2(a_1/a_2) > 2$	112	48
$Log2(a_1/a_2) > 3$	65	29

Table S3. Numbers and percentages of genes with differential expression (DE) within genomic compartments, including autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. Chi-squared test was used to assess whether DE genes were non-randomly distributed between autosomes and the genomic compartments on mating type chromosomes (MAT). *P* values <0.05 are in bold. NA: not applicable. The youngest strata only had one DE gene, so no statistical comparison could be performed for these strata.

	Higher expression in a_1				**Higher expression in a_2**			
	Autosomes	MAT			Autosomes	MAT		
		PARs	Youngest strata	Oldest Strata		PARs	Youngest strata	Oldest Strata
DE gene number	351	4	0	36	156	8	1	38
Total number	8207	114	29	198	8207	114	29	198
Percentage	4.28%	3.51%	0.00%	18.18%	1.90%	7.02%	3.45%	19.19%
X^2 test (X^2)	NA	0.02	1.24	68.91	NA	11.60	0.04	210.95
X^2 test (*P* value)	NA	0.88	0.41	**9.99E-05**	NA	**6.60E-04**	1	**9.99E-05**

Table S4. Wilcoxon rank sum test statistics for comparisons of mean non-synonymous substitution rate (*dN*, A) and synonymous substitution rate (*dS*, B) of differentially expressed genes (DE) versus non-differentially expressed genes (non-DE) within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. *P* values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, no statistical comparison could be performed for this compartment.

(A)

dN	**Autosome**	**PARs**	**Young strata**	**Old Strata**
Mean of DE genes	0	0	0.013	0.053
Mean of non-DE genes	0	0	0.006	0.033
Number of DE genes	117	4	1	43
Number of non-DE genes	5742	75	26	106
Wilcoxon W	334269	146	NA	1433
P-value	0.449	0.94	NA	**< 0.001**

(B)

dS	**Autosome**	**PARs**	**Young strata**	**Old Strata**
Mean of DE genes	0	0	0.013	0.027
Mean of non-DE genes	0	0	0.003	0.015
Number of DE genes	117	4	1	43
Number of non-DE genes	5742	75	26	106
Wilcoxon W	333918	116	NA	1422
P-value	0.404	0.455	NA	**< 0.001**

Table S5. Wilcoxon rank sum test statistics for comparisons of divergence between each of the two *Microbotryum lychnidis-dioicae* alleles and their *M. lagerheimii* orthologs. This test assessed whether the allele with lower expression in *M. lychnidis-dioicae* was more divergent from orthologs in *M. lagerheimii* than the alleles with higher expression in *M. lychnidis-dioicae*, considering non-synonymous substitution rate (*dN*), synonymous substitution rate (*dS*), and the ratio (*dN/dS*) within genomic compartments, including autosomes pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. We calculated these substitution rates for a_1 and a_2 alleles between these two species separately. NA: not applicable, as the youngest strata only had one DE gene, statistical comparisons could not be performed for this compartment.

Substitution type	Autosome		PARs		Young strata		Old Strata	
	High vs low mutation rates		High vs low mutation rates		High vs low mutation rates		High vs low mutation rates	
	W	*P*-value	W	*P*-value	W	*P*-value	W	*P*-value
dN	8066.0	0.999	18.5	1.000	NA	NA	1262.5	0.934
dS	8060.5	0.995	19.0	0.936	NA	NA	1167.0	0.909
dN/dS	8057.0	0.991	17.0	0.936	NA	NA	1247.5	0.989

Table S6. Wilcoxon rank sum test statistics for comparisons of unoriented transposable element (TE) insertion differences between alleles (within 20kb up and downstream) of differentially expressed (DE) genes versus non-differentially expressed (non-DE) genes within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. P-values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, statistical comparisons could not be performed for this compartment.

Transposable elements (TEs)	**Autosome**	**PARs**	**Young strata**	**Old Strata**
Difference of DE genes	0.312	0.333	1.000	4.567
Differences of non-DE genes	0.107	0.078	2.143	3.903
Number of DE genes	507	12	1	74
Number of non-DE genes	7700	102	28	124
Wilcoxon W	31387793	546	NA	4062
P-value	**<0.001**	0.192	NA	0.173

Table S7. Two-proportion Z test for comparisons of unoriented protein length differences between alleles of differentially expressed (DE) genes versus non-differentially expressed (non-DE) genes within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. *P* values <0.05 are in bold. NA: not applicable, as proportions * sample size was less than five for the PARs and youngest strata, statistical comparisons could not be performed for these compartments.

Protein Length	**Autosomes**	**PARs**	**Young strata**	**Old Strata**
Proportion unequal length DE genes	0.037	0.000	0.000	0.757
Proportion unequal length non-DE genes	0.012	0.020	0.357	0.605
Number of DE genes	507	12	1	74.000
Number of non-DE genes	7700	102	28	124
Z	4.640	NA	NA	2.186
P-value	**0.0002**	NA	NA	**0.029**

Table S8. Wilcoxon rank sum test statistics for comparisons of unoriented differences in intron content between alleles of differentially expressed (DE) versus non-differentially expressed (non-DE) genes within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. *P* values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, no statistical comparison could be performed for this compartment.

Intron	**Autosomes**	**PARs**	**Young strata**	**Old Strata**
Difference between DE alleles	0.007	0.001	0.005	0.169
Difference between non-DE alleles	0.002	0.006	0.02	0.101
Number of DE genes	507	12	1	74
Number of non-DE genes	7700	102	28	124
Wilcoxon W	1920124	605	NA	3205
P-value	**0.033**	0.888	NA	**0.001**

Table S9. Wilcoxon rank-sum test statistics for comparisons of unoriented differences in overall GC content (GC0), and third codon position (GC3) between alleles of differentially expressed (DE) versus non-differentially expressed (non-DE) genes within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. (A) Overall GC content. (B) Third codon position GC content. *P* values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, no statistical comparison could be performed for this compartment.

(A)

GC0	Autosomes	PAR	Young strata	Old Strata
Difference between DE alleles	0.000124	0.00065	0.001373	0.01027
Difference between non-DE alleles	0.000081	0.000054	0.00171	0.0067
Number of DE genes	507	12	1	74
Number of non-DE genes	7700	102	28	124
Wilcoxon W	1907831	578	NA	3010
P-value	**<0.001**	0.318	NA	**<0.001**

(B)

GC3	Autosomes	PAR	Young strata	Old Strata
Difference between DE alleles	0.000344	0	0.004464	0.02063
Difference between non-DE alleles	0.000081	0.000148	0.00423	0.01382
Number of DE genes	507	12	1	74
Number of non-DE genes	7700	102	28	124
Wilcoxon W	1903874	594	NA	3168
P-value	**<0.001**	0.549	NA	**0.001**

References:

1. Branco S, Badouin H, Rodríguez RC, Vega D, Gouzy J, Carpentier F. Evolutionary strata on young mating-type chromosomes despite the lack of sexual antagonism. Proc Natl Acad Sci U S A. 2017;114: 7367–7072. doi:10.1073/pnas.1701658114

Supporting Information Legends

Table S1. Number of single-copy genes with alleles in both a_1 and a_2 haploid genomes, with the 70% protein sequence identity detection threshold using reciprocal best BLASTp hits, before and after filtering out genes with transposable element (TE)-related functions.

Filtering removed 192 paralogous genes in each haploid genome and genes with TE-related functions, including 1,750 and 1,819 from a_1 and a_2 haploid genome, respectively.

Table S2. Identification of differentially-expressed (DE) genes with either a_1-biased or a_2-biased expression (i.e., higher expression in a_1 or a_2, respectively) under various $Log_2(a_1/a_2)$ criteria.

Table S3. Numbers and percentages of genes with differential expression (DE) within genomic compartments, including autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata.

Chi-squared test was used to assess whether DE genes were non-randomly distributed between autosomes and the genomic compartments on mating type chromosomes (MAT). *P* values <0.05 are in bold. NA: not applicable. The youngest strata only had one DE gene, so no statistical comparison could be performed for these strata.

Table S4. Wilcoxon rank sum test statistics for comparisons of mean non-synonymous substitution rate (*dN*, A) and synonymous substitution rate (*dS*, B) of differentially expressed genes (DE) versus non-differentially expressed genes (non-DE) within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata.

P values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, no statistical comparison could be performed for this compartment.

Table S5. Wilcoxon rank sum test statistics for comparisons of divergence between each of the two *Microbotryum lychnidis-dioicae* alleles and their *M. lagerheimii* orthologs.

This test assessed whether the allele with lower expression in *M. lychnidis-dioicae* was more divergent from orthologs in *M. lagerheimii* than the alleles with higher expression in *M.*

lychnidis-dioicae, considering non-synonymous substitution rate (*dN*), synonymous substitution rate (*dS*), and the ratio (*dN/dS*) within genomic compartments, including autosomes pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. We calculated these substitution rates for a_1 and a_2 alleles between these two species separately. NA: not applicable, as the youngest strata only had one DE gene, statistical comparisons could not be performed for this compartment.

Table S6. Wilcoxon rank sum test statistics for comparisons of unoriented transposable element (TE) insertion differences between alleles (within 20kb up and downstream) of differentially expressed (DE) genes versus non-differentially expressed (non-DE) genes within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata.

P-values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, statistical comparisons could not be performed for this compartment.

Table S7. Two-proportion Z test for comparisons of unoriented protein length differences between alleles of differentially expressed (DE) genes versus non-differentially expressed (non-DE) genes within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata.

P values <0.05 are in bold. NA: not applicable, as proportions * sample size was less than five for the PARs and youngest strata, statistical comparisons could not be performed for these compartments.

Table S8. Wilcoxon rank sum test statistics for comparisons of unoriented differences in intron content between alleles of differentially expressed (DE) versus non-differentially expressed (non-DE) genes within genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata.

P values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, no statistical comparison could be performed for this compartment.

Table S9. Wilcoxon rank-sum test statistics for comparisons of unoriented differences in overall GC content (GC0), and third codon position (GC3) between alleles of differentially expressed (DE) versus non-differentially expressed (non-DE) genes within

genomic compartments, i.e., autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata.

(A) Overall GC content. (B) Third codon position GC content. *P* values <0.05 are in bold. NA: not applicable, as the youngest strata only had one DE gene, no statistical comparison could be performed for this compartment.

Fig. S1. Comparison of proportion (A) and number (B) of differentially expressed (DE) genes detected between a_1 and a_2 haploid genomes of *Microbotryum lychnidis-dioicae*.

Differentially expressed (DE) genes on mating-type chromosome (MAT) chromosomes and autosomes (auto), at various percentage protein sequence identities used as threshold for identification of alleles for genes in a_1 and a_2 haploid genomes. **: $P < 0.01$ using Chi-square test. All other comparisons of DE genes on mating-type chromosome and autosomes were not significant.

Fig. S2. Pairwise correlation of raw counts from RNAseq data between replicates for haploid a_1 cell culture (A) and haploid a_2 cell culture (B).

Fig. S3. Multidimensional scaling (MDS) plot of RNAseq libraries of *Microbotryum lychnidis-dioicae*.

Water denotes water agar (i.e. low nutrients) culture condition.

Fig. S4. Boxplot of overall gene expression in terms of Log_2TPM (transcripts per millions) for genes with a_1-biased expression (in red), a_2-biased expression (in blue) and non-biased expression (in grey) on mating-type chromosomes (MAT), as well as autosomes (Chr01-Chr18 and other contigs).

Within each color, the slightly brighter color indicates a_1 alleles, the other being a_2 alleles. For boxplot, the horizontal bars (from bottom to top) represent the 25% quartile, median and 75% quartile respectively.

Fig. S5. The ratio index of coding sequence and protein sequence of *Microbotryum lychnidis-dioicae*.

The ratio of predicted coding sequence length divided by the predicted protein sequence length multiplied by three for a_1-bias expression (in blue), a_2-biased expression (in red) and

non-biased expression (in grey), among the four genomic compartments. Within each color, the slightly brighter color indicates a_1 alleles, the other being a_2 alleles. In the boxplot, the horizontal bars (from bottom to top) represent the 25% quartile, median and 75% quartile, respectively.

Fig. S6. Boxplot of synonymous substitution rate (*dS*) for differentially expressed (DE) and non-differentially expressed (Non-DE) genes of *Microbotryum lychnidis-dioicae*.
Wilcoxon rank sum tests for comparisons of mean non-synonymous substitution rate (*dN*) of differentially expressed genes (DE) versus non-differentially expressed genes (non-DE) within genomic compartments: '***': $P < 0.001$, other comparisons were not significant. Genomic compartments correspond to autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. For boxplot, the horizontal bars (from bottom to top) represent the 25% quartile, median and 75% quartile respectively.

Fig. S7. Boxplot of differentially (a_1-biased in red, a_2-biased in blue) and non-differentially expressed genes (not-biased in grey) and the sequence divergence between alleles of *Microbotryum lychnidis-dioicae*.
Wilcoxon rank sum tests for comparisons of genes with higher allelic expression in the a_1 and a_2 haploid mating type genomes separately to non-differentially expressed genes for the mean non-synonymous substitution rate (*dN*) (**A**), synonymous substitution rate (*dS*) (**B**); '***': $P < 0.001$, '**': $P < 0.01$, '*': $P < 0.5$, '.': $P < 0.1$, NS: not significant. As *dN* and *dS* of almost all genes in autosomes and PARs are zero, and there is only one DE gene on the youngest strata, so no statistical test can be performed in these regions. Sample size for each genomic compartment is listed either above or inside the boxplot. Genomic compartments correspond to autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. For boxplot, the horizontal bars (from bottom to top) represent the 25% quartile, median and 75% quartile respectively.

Fig. S8. Boxplot of differentially expressed (DE) and non-differentially expressed genes (non-DE) and gene evolutionary rate *dN/dS* of *Microbotryum lychnidis-dioicae*.
Wilcoxon rank sum tests for comparisons of evolutionary rate *dN*/*dS* of differentially expressed genes (DE) versus non-differentially expressed genes (non-DE) within genomic compartments; NS: not significant. As *dN*/*dS* of almost all genes in autosomes and PARs are

zero, and there is only one DE gene on the youngest strata, no statistical test can be performed in these regions. Genomic compartments correspond to autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. For boxplot, the horizontal bars (from bottom to top) represent the 25% quartile, median and 75% quartile respectively.

Fig. S9. Boxplot of sequence divergence, non-synonymous substitution rate *dN* (A), synonymous substitution rate *dS* (B), between *Microbotryum lychinidis-dioicae* and *M. lagerheimii*.

Alleles of differentially-expressed genes with higher (red - lower expressed allele) and lower (blue - higher expressed allele) substitution rates and equal (grey; non-differentially expressed genes) mutation rates were pooled from a_1 and a_2 genomes and assessed for divergences from orthologs in *M. lagerheimii*. Genomic compartments correspond to autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. For boxplot, the horizontal bars (from bottom to top) represent the 25% quartile, median and 75% quartile respectively.

Fig. S10. Dot plot of oriented differences of transposable element (TE) insertions and differential gene expression between alleles of *Microbotryum lychnidis-dioicae*.

TE insertions are shown for sliding-window intervals from upstream to downstream of genes, where differences between alleles were calculated as the TE number for the allele with lower expression minus the TE number for the higher expressed allele; a positive value thus represented an excess of TEs in the lower expressed allele. Sliding window intervals are shown as **A**: upstream 20kb to 10kb, **B**: upstream 15kb to 5kb, **C**: upstream 10kb to gene, **D**: upstream 5kb to downstream 5kb, **E**: gene to downstream 10kb, **F**: downstream 5kb to 15kb, and **G**: downstream 10kb to 20kb.

Fig. S11. Comparisons of differentially expressed (DE) and non-differentially expressed (non-DE) genes between mating types of *Microbotryum lychnidis-dioicae* for differences between alleles in third codon position GC content (GC3) within genomic compartments.

Wilcoxon rank sum test statistics for comparisons of unoriented differences in GC content of third codon position (GC3) between alleles of differentially expressed (DE) versus non-differentially expressed (non-DE) genes within genomic compartments; ***: $P < 0.001$, NS: non significant. Genomic compartments correspond to autosomes, pseudo-autosomal regions (PARs), youngest and oldest evolutionary strata. The notation "*a*" indicates that the youngest

167 evolutionary strata contained only one DE gene, precluding comparisons to non-DE genes
168 within this compartment.
169