

1 Chromatin dynamics associated with sexual differentiation in a UV sex 2 determination system

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10 Summary

11 In many eukaryotes, such as dioicous mosses and many algae, sex is determined by UV sex
12 chromosomes and is expressed during the haploid phase of the life cycle. In these species, the
13 male and female developmental programs are initiated by the presence of the U- or V-specific
14 regions of the sex chromosomes but, as in XY and ZW systems, phenotypic differentiation is
15 largely driven by autosomal sex-biased gene expression. The mechanisms underlying sex-
16 biased transcription in XY, ZW or UV sexual systems currently remain elusive. Here, we set out
17 to understand the extent and nature of epigenomic changes associated with sexual
18 differentiation in the brown alga *Ectocarpus*, which has a well described UV system. Five
19 histone modifications, H3K4me3, H3K27Ac, H3K9Ac, H3K36me3, H4K20me3, were quantified
20 in near-isogenic male and female lines, leading to the identification of 13 different chromatin
21 states across the *Ectocarpus* genome that showed different patterns of enrichment at
22 transcribed, silent, housekeeping or narrowly-expressed genes. Chromatin states were
23 strongly correlated with levels of gene expression indicating a relationship between the
24 assayed marks and gene transcription. The relative proportion of each chromatin state across
25 the genome remained stable in males and females, but a subset of genes exhibited different
26 chromatin states in the two sexes. In particular, males and females displayed distinct patterns
27 of histone modifications at sex-biased genes, indicating that chromatin state transitions occur
28 preferentially at genes involved in sex-specific pathways. Finally, our results reveal a unique
29 chromatin landscape of the U and V sex chromosomes compared to autosomes. Taken
30 together, our observations reveal a role for histone modifications in sex determination and
31 sexual differentiation in a UV sexual system, and suggest that the mechanisms of epigenetic
32 regulation of genes on the UV sex chromosomes may differ from those operating on
33 autosomal genes.

34 Introduction

35 In species that reproduce sexually, sex is often determined by a pair of sex chromosomes: X
36 and Y chromosomes in male-heterogametic species, Z and W in female-heterogametic species
37 or U and V in haploid sexual systems (Bachtrog et al., 2014). Sex chromosomes originate from
38 pairs of autosomes, but become differentiated after the sex-specific chromosome (Y, W or
39 both the V and U) stops recombining (Bachtrog et al., 2014; Charlesworth, 2017; Umen and
40 Coelho, 2019). Males and females have distinct sex chromosome sets but the extensive
41 phenotypic differences between males and females (sexual dimorphism) are largely caused
42 by differences in autosomal gene expression, so-called sex-biased gene expression. The nature
43 and extent of sex-biased gene expression has been investigated in recent years across a broad
44 range of taxa using genome-wide transcriptional profiling. These studies have revealed that
45 sex-biased gene expression is common in many species, although its extent may vary greatly
46 among tissues or developmental stages (reviewed in Grath and Parsch, 2016).

47 Although many reports have described patterns and evolution of sex-biased genes across
48 several taxa, the molecular mechanisms underlying the regulation of sex-biased expression of
49 hundreds, or even thousands, of genes during sexual differentiation remain poorly
50 understood. One possible mechanism to regulate gene expression is through epigenetic
51 modifications. Epigenetic modifications are defined as reversible changes that affect the
52 genomic structure and regulate gene expression without affecting the DNA sequence itself
53 (Allis and Jenuwein, 2016). Epigenetic modifications may occur through mechanisms such as
54 DNA methylation and histone post-translational modifications (PTMs). DNA methylation
55 regulates transcription in diverse eukaryotes (reviewed in Jones, 2012), and may contribute
56 to transcriptional differences between sexes (Nugent et al., 2015), playing for instance an
57 important role in differentiating female morphs (workers and queens) in the honeybee
58 (Elango et al., 2009). In the liverwort *Marchantia*, male and female gametes have different
59 levels of DNA methylation and this is correlated with differences in the expression of genes
60 involved in DNA methylation (Schmid et al., 2018). Histone PTMs are another important
61 component of transcriptional regulation, and can impact gene expression by altering
62 chromatin structure or recruiting histone modifiers. Combination of histone PTMs (so-called
63 chromatin states) are associated with functionally distinct regions of the genome such as
64 heterochromatic regions and regions of either active transcription or repression (Kouzarides,
65 2007). The role of chromatin states in regulating gene expression patterns during
66 development in animals is well established (Lindeman et al., 2010; Srivastava et al., 2010).
67 However, very few studies have carried out chromatin profiling during sexual differentiation
68 to link profiles with sex-biased expression patterns. The only available study, to our
69 knowledge, described genome-wide maps of histone PTMs coupled with gene expression data

70 to decipher the relationship between the chromatin states and sex-biased gene expression in
71 *Drosophila miranda* (Brown and Bachtrog, 2014). In this study, the genome-wide distribution
72 of both active and repressive chromatin states differed between males and females but sex-
73 specific chromatin states appeared not to explain sex-biased expression of genes.

74 In organisms with XY or ZW sex determination systems, sex chromosomes often exhibit unique
75 patterns of gene expression and unusual patterns of epigenetic marks compared with
76 autosomes (e.g. Brown and Bachtrog, 2014; Schmid et al., 2018). For instance, in *Drosophila*
77 males, where the Y is transcriptionally repressed and the X is hyper-transcribed (Baker et al.,
78 1994), both of these transcriptional modifications are correlated with changes in the
79 chromatin configuration (Gelbart and Kuroda, 2009; Girton and Johansen, 2008; Lemos et al.,
80 2010; Straub and Becker, 2007). Sex chromosomes are derived from autosomes, but they are
81 governed by unique evolutionary and functional pressures (Bachtrog, 2006). The sex-limited
82 chromosome (Y or W) degenerates, i.e., loses most of its ancestral gene content, accumulates
83 repetitive DNA and evolves a heterochromatic appearance (Bachtrog, 2013; Charlesworth and
84 Charlesworth, 2000) whereas the homologous chromosome (X or Z) acquires mechanisms to
85 compensate and evolves hyper-transcription (dosage compensation) (Lucchesi et al., 2005;
86 Picard et al., 2018; Vicoso and Charlesworth, 2009). In *Drosophila* the
87 euchromatin/heterochromatin ratio is different in the two sexes mainly due to the presence
88 of the repeat-rich Y chromosome in males (Brown and Bachtrog, 2014; Yasuhara and
89 Wakimoto, 2008). Similarly, the Z-specific region in schistosomes has a unique chromatin
90 landscape, dominated by gene-activation-associated histone PTMs, that is associated with
91 dosage compensation (Picard et al., 2019).

92 At present, no information is available concerning the regulation of gene expression by
93 chromatin remodelling in organisms with UV sexual systems, such as mosses and algae
94 (Coelho et al., 2018), although recent work has analysed the patterns of histone post
95 translational modifications during the haploid-diploid life cycle of the brown alga *Ectocarpus*
96 (Bourdareau et al., 2020). In UV sexual systems, sex is expressed during the haploid phase of
97 the life cycle. Inheritance of a U or a V sex chromosome after meiosis determines whether the
98 multicellular adult individual will be female or male, respectively (Bachtrog et al., 2014; Coelho
99 et al., 2019). UV systems differ markedly from XY and ZW systems (Bull, 1978; Coelho et al.,
100 2019; Umen and Coelho, 2019). For example, the two sexes are not homozygotic and
101 heterozygotic so mechanisms such as chromosome-scale dosage compensation or meiotic sex
102 chromosome inactivation are not expected. Moreover, whereas Y or W sex chromosomes
103 often undergo genetic degeneration resulting in them being markedly different to their
104 partner X or Z chromosome in terms of size, repeat content and gene density, U and V
105 chromosomes do not tend to exhibit this type of asymmetry because each chromosome

106 functions independently in a haploid context and therefore experiences similar evolutionary
107 pressures (Ahmed et al., 2014).

108 The expression pattern of the genes located on U and V sex chromosomes has been shown to
109 differ from that of the autosomal gene set (Coelho et al., 2019). For example, in the brown
110 alga *Ectocarpus*, most sex-linked genes are upregulated during the haploid, gametophyte
111 phase of the life cycle (Ahmed et al., 2014; Lipinska et al., 2017). The pseudo-autosomal
112 regions (PARs) of the sex chromosomes are enriched in both life cycle-related genes
113 (sporophyte-biased genes) and female-biased genes, compared to the autosomes (Lipinska et
114 al., 2015). Moreover, PAR genes display unusual structural features compared with autosomal
115 genes in terms of their GC content, repeat content and intron sizes (Avia et al., 2018;
116 Luthringer et al., 2015).

117 Here, we investigated the sex-related chromatin landscape of *Ectocarpus*, a model brown alga
118 with a UV sexual system. Comparison of the profiles of five histone PTMs with transcriptomic
119 data showed that chromatin states were predictive of transcript abundance. The chromatin
120 state of genes that exhibited sex-biased expression was markedly different in males and
121 females indicating that histone modifications may play an important role in mediating sexual
122 differentiation. Moreover, an important subset of the PAR genes presented sex-specific
123 chromatin patterns. The U and V sex chromosomes were found to have very different
124 chromatin landscapes to autosomes, despite the absence of a requirement for chromosome-
125 scale dosage compensation in *Ectocarpus* and the fact that the U and V chromosomes do not
126 exhibit strong signs of genetic degeneration.

127 Results

128 Identification of chromatin states in males and females of *Ectocarpus* sp.

129 Near-isogenic male and female gametophyte lines (Table S1, Figure S1) were used to generate
130 sex-specific chromatin immunoprecipitation and sequencing (ChIP-seq) profiles for five
131 different histone PTMs: H3K4me3, H3K9ac, H3K27ac, H3K36me3 and H4K20me3 (Table S2).
132 H3K4me3 is a near-universal chromatin modification that has been found at the transcription
133 start sites (TSS) of expressed genes in a range of eukaryotes, and is associated with gene
134 transcription (Barski et al., 2007; He et al., 2010; Howe et al., 2017). H3K9ac is a chromatin
135 mark that is often associated with ongoing transcription in both animals and land plants
136 (Brusslan et al., 2015; Heintzman et al., 2007). H3K27ac is an important mark that can
137 distinguish between active and poised enhancer elements in animals (Creyghton et al., 2010).
138 H3K36me3 is a gene body mark associated with active gene transcription in animals and plants
139 (Roudier et al., 2011; Shilatifard, 2006). H4K20me3 is a repressive, constitutive

140 heterochromatin mark but also silences repetitive DNA and transposons. H4K20me3 is
141 generally associated with heterochromatin but its presence at gene bodies has been inversely
142 correlated with gene expression in animals (Nelson et al., 2016; Schotta et al., 2004).

143 Given the large phylogenetic distances separating the brown algae from the animal and land
144 plant lineages and the independent evolution of multicellularity in each of these three lineages
145 (Cock et al., 2010), it is possible that the five histone PTMs analysed here are not associated
146 with the same functions in brown algae as they are in animals and land plants. However, a
147 previous analysis of histone PTMs in *Ectocarpus*, which included the marks tested here
148 (Bourdareau et al., 2020), afforded evidence for similar roles. Peaks of H3K9ac, H3K27ac and
149 H3K4me3 were detected within 500 bp of transcription start sites (TSSs). H3K36me3 and
150 H4K20me3 were depleted from TSSs and transcription end sites (TESs), being associated with
151 gene bodies and H4K20me3 was also present in intergenic regions. Together, the five histone
152 PTMs used in our study are therefore expected to provide a broad overview of the *Ectocarpus*
153 sp. chromatin landscape in male and female algae.

154 Thirteen chromatin states (i.e., different combinatorial patterns of histone PTMs) were
155 defined in the *Ectocarpus* genome based on analysis of the genome-wide distribution patterns
156 of the five histone PTMs using MACS2 (Zhang et al., 2008) and SICER (Xu et al., 2014) (Figure
157 1A). States S9-S13 consisted of combinations of histone marks that are usually associated with
158 active transcription (presence of H3K36me3, H3K27ac, H3K9ac, H3K4me3) (Bourdareau,
159 2018). States S2-S8 all included H4K20me3, in most cases in addition to one or more of the
160 above gene activation-associated marks. State S1 corresponded to a ‘background’ state, i.e.,
161 domains that were not enriched for any of the histone PTMs assayed. An example of histone
162 PTM profiles for a 20 kbp region of the *Ectocarpus* genome is shown in Figure 1B.

163 **Chromatin states of different categories of *Ectocarpus* genes**

164 To elucidate the relationship between the observed chromatin states and the expression
165 patterns of *Ectocarpus* sp. genes, RNA-seq data was generated using the same biological
166 samples as were used for the ChIP-seq analysis (see methods) and these data, together with
167 previously published datasets (Lipinska et al., 2015, 2017, 2013), were used to define four
168 categories of genes based on their expression patterns: transcribed genes (TPM \geq 5th
169 percentile), silent genes (TPM $<$ 5th percentile), housekeeping genes (i.e. broadly expressed
170 genes defined as having values of less than 0.25 for the tissue specificity index tau; see
171 methods) and narrowly expressed genes (tau $>$ 0.75; see methods). The housekeeping and
172 narrowly expressed genes (NEGs) were subsets of the transcribed gene set.

173 The most common chromatin state for the transcribed genes (32.9% and 33.9% in males and
174 females respectively) was S13, which corresponds to co-localisation of all four of the histone

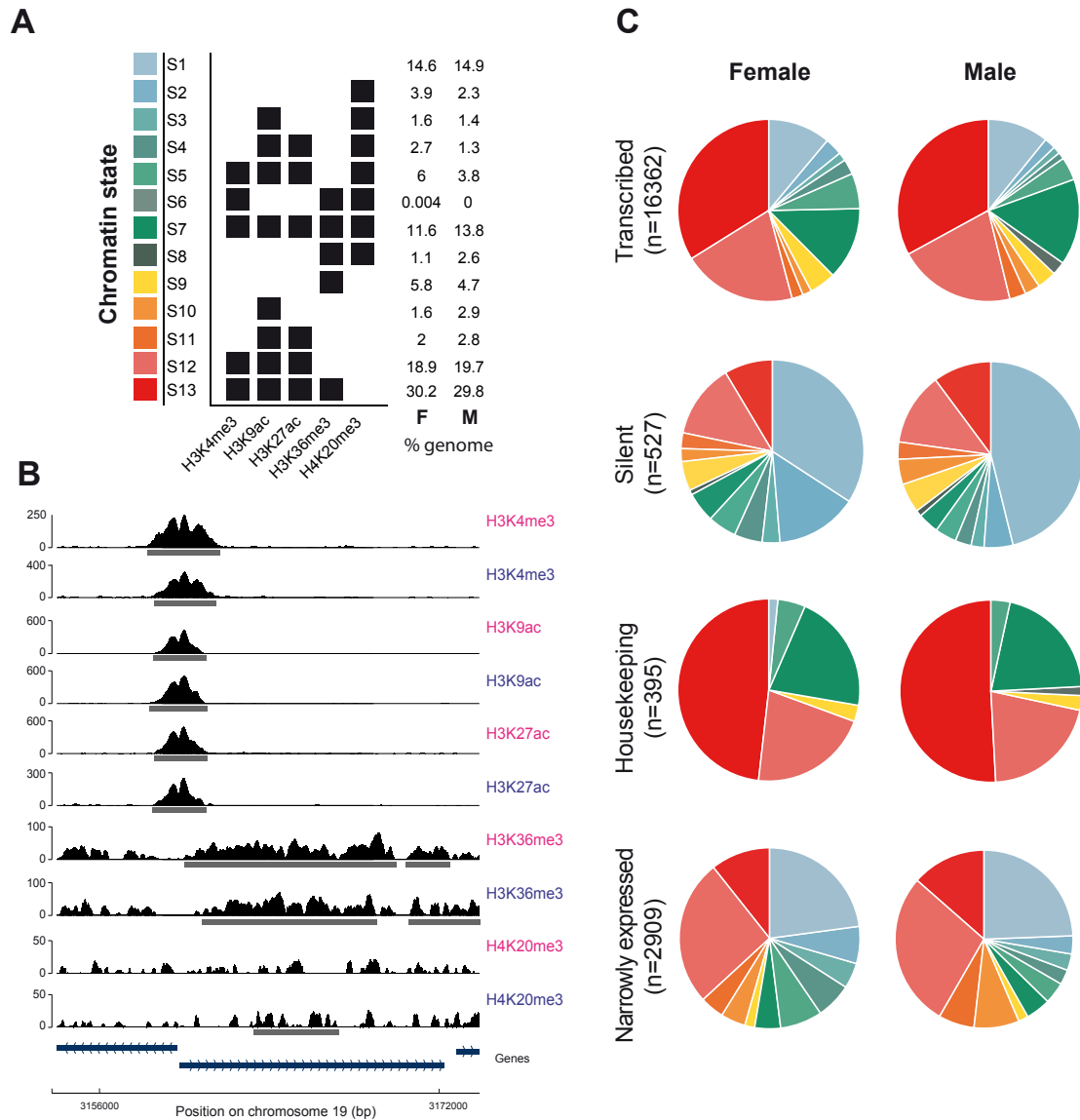


Figure 1. The chromatin landscape of male and female *Ectocarpus* sp. A) Summary of the 13 chromatin states detected in *Ectocarpus* sp. Percentages of the total gene set associated with each chromatin state in males (M) and females (F) are shown to the right. B) Representative region of the chromosome 19 showing profiles of mapped ChIP-seq reads for the five histone PTMs in males and females. Grey bars represent the peaks detected by MACS2 (H3K4me3, H3K9ac and H3K27ac) or SICER (H3K36me3 and H4K20me3). Blue bars represent genes. Pink text, females; blue text, males. C) Proportions of transcribed (TPM \geq 5th percentile), silent (TPM<5th percentile), housekeeping ($\tau < 0.25$) and narrowly expressed genes ($\tau > 0.75$) associated with each chromatin state in males and females.

175 PTMs that are generally associated with gene activation (H3K36me3, H3K27ac, H3K9ac,
 176 H3K4me3; Figure 1C, Table S3). For the ‘silent’ category of genes, S1 (no detectable histone
 177 PTM peak) was the most common state (45.4% and 34.0% in males and females, respectively;
 178 Figure 1C, Table S3). Housekeeping (broadly expressed) genes and NEGs have been shown to
 179 have distinct patterns of chromatin PTMs in *Drosophila* (Brown and Bachtrog, 2014; Filion et
 180 al., 2010). The majority (50.9% and 48.2% in males and females, respectively) of the
 181 housekeeping genes in *Ectocarpus* were associated with state S13 (all four marks associated
 182 with activation) whereas NEGs exhibited no clearly preferred state, the most common state

183 being S12 (H3K4me3, H3K9ac, H3K27ac; 28.2% and 26.2% in males and females, respectively;
184 Figure 1C, Table S3). States that included H4K20me3 were more common at NEGs than at
185 housekeeping genes. Conversely, states associated with H3K36me3 (S6-S9 and S13) were
186 characteristic of housekeeping genes (i.e., 75.9% and 72.3% of the housekeeping genes in
187 males and females respectively had H3K36me3), and this mark was distinctly less prevalent
188 on NEGs (19.8% and 17.1% in males and females respectively; Figure 1C, Table S3, Table S4).
189 Finally, the background state S1 (none of the tested marks associated) was markedly more
190 frequent at NEGs than at housekeeping genes. Together, these data support the association
191 of the tested marks with active or repressed chromatin states in *Ectocarpus*.

192 When the relative proportions of the chromatin states were compared between males and
193 females for each of the four gene categories (transcribed, silent, housekeeping and narrowly
194 expressed), broadly similar patterns were observed in the two sexes, but some small
195 differences were also noticeable (Figure 1C, Table S3). For example, less than 1% of the
196 transcribed genes corresponded to state S8 (i.e., combination of H3K36me3 and H4K20me3)
197 in females, compared to 2.4% in males (Table S3). Also, state S6 (combination of H3K4me3,
198 H3K36me3 and H4K20me3) was exclusively present in a small subset of genes in females.
199 Taken together, these results indicate that overall, the relative proportions of the different
200 chromatin states across the genome remain relatively stable in males versus females.

201 **Identification of histone PTMs associated with gene activation and gene repression**

202 To further investigate the relationship between the observed chromatin states and gene
203 expression, transcript abundances in both males and females were plotted for the sets of
204 genes corresponding to each chromatin state. A clear trend towards increasingly higher levels
205 of transcript abundance was correlated with the gradual acquisition of the histone PTMs
206 H3K9ac, H3K27ac, H3K4me3 and H3K36me3 (in the following order: H3K9ac followed by
207 H3K9ac/H3K27ac, then by H3K9ac/H3K27ac/H3K4me3 and finally by
208 H3K9ac/H3K27ac/H3K4me3/H3K36me3; Figure 2A; Table S5, S6). These observations support
209 the proposed association of these four histone PTMs with gene activation (Bourdareau et al.,
210 2020). These results also suggest that there may be a hierarchy in terms of the deposition of
211 these histone PTMs, with addition of later marks being dependent on the presence of earlier
212 ones in the order H3K9ac, H3K27ac, H3K4me3 and H3K36me3.

213 In pairwise comparisons, sets of genes corresponding to chromatin states that included
214 H4K20me3 consistently exhibited lower transcript abundance than sets of genes with
215 equivalent chromatin states without H4K20me3 (e.g. transcript abundance was significantly
216 lower for S7 than for S13; Wilcox test, p-value= 4.463E-18 Figure 2A; Table S5, S6). These
217 results are consistent with H4K20me3 playing a role in the repression of gene expression in

218 *Ectocarpus*. Note however that because H4K20me3 is frequently associated with transposons
219 (Bourdareau et al., 2020), the observed association with transcriptional repression could also
220 be indirect, via the silencing of intronic transposon sequences.

221 Finally, the background state S1 corresponds to domains that are not associated with any of
222 the assayed histone PTMs, and *Ectocarpus* genes associated with state S1 exhibited very low
223 transcript abundance (Figure 2A, Table S5, S6).

224 Analysis of the RNA-seq data also indicated some differences between the sexes. For example,
225 on average, genes in chromatin state S1, S11 and S12 had significantly higher expression levels
226 in females compared with males (pairwise Wilcoxon, p-value=2.4E-7; p-value=0.02 and p-
227 value=0.001, respectively; Figure 2A). Conversely, on average, genes in chromatin state S2 and
228 S3 had lower expression levels in females than in males (pairwise Wilcoxon, p-value=6.3E-8,
229 p-value=3.4E-8; Figure 2A, Table S5, S6).

230 To further examine the link between chromatin states and transcript abundances in males and
231 females, we classified states S1 and S2 (absence of any of the tested marks or presence of only
232 H4K20me3) as 'repressive' chromatin states, while states S9-S13 were classified as 'active'
233 chromatin states (presence of at least one canonical activation-associated mark H3K9ac,
234 H3K27ac, H3K4me3 and/or H3K36me3). Note that we did not include genes with states S3-S7
235 in this analysis because they exhibited a combination of repression-associated (H4K20me3)
236 and activation-associated marks and because they were expressed at intermediate levels
237 (Figure 2A). As expected, genes marked with states S9-S13 were expressed at higher levels in
238 both sexes than those that were associated with states S1 and S2 (Figure 2B; pair-wise Wilcox
239 test, p-value<2E-16). Interestingly, levels of gene expression in males and females were also
240 significantly different for genes marked with states S9-S13 in one sex but with states S1 and
241 S2 in the other (Figure 2B; pair-wise Wilcox test, p-value<2E-16). Therefore, sex-specific
242 differences in the chromatin states of genes were associated with sex-specific expression
243 patterns.

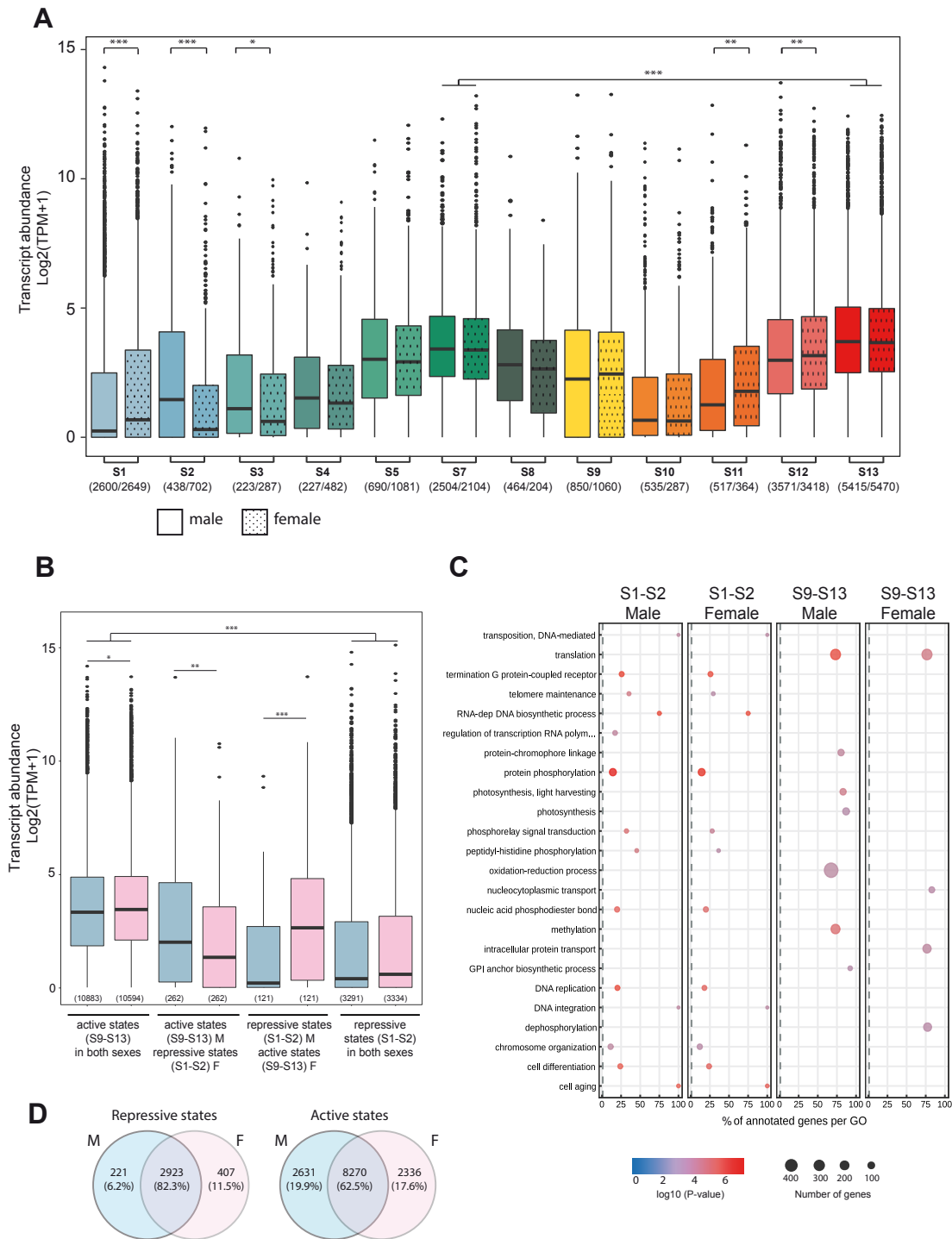


Figure 2. Gene expression and chromatin states. A) Transcript abundances for genes associated with different chromatin states in males and females. The colour code is the same as that used in Figure 1A. The numbers of genes associated with each state are indicated in brackets (males/females). Asterisks above plots indicate significant differences in gene expression (pair-wise Wilcoxon test, * p -value<0.05, ** p -value<0.01, *** p -value<0.001). The full set of statistical tests is presented in Table S6. B) Transcript abundances for genes exhibiting either activation-associated (S9 to S13) or repression-associated (S1 or S2) chromatin states in females (pink) and males (blue). Numbers in brackets indicate the number of genes in each class. Asterisks indicate significant differences (* p -value>0.05, ** p -value<0.01, *** p -value<0.001). C) GO term enrichment for genes marked with activation-associated (S9-S13) or repression-associated (S1-S2) chromatin states in males and females. D) Venn diagrams representing the proportion of genes marked with activation-associated (S9-S13) or repression-associated (S1-S2) chromatin states in males and females.

245 A GO-term enrichment analysis of genes in either activation-associated states (S9-S13) or
246 repression-associated states (S1-S2) showed that the set of genes associated with states S9-
247 S13 was enriched in functions such as translation, oxidation-reduction, methylation and
248 dephosphorylation, whereas the set of genes in S1-S2 states was enriched in functions such
249 as phosphorylation and DNA replication (Figure 2C). GO term enrichment was more stable
250 between sexes for repression-associated chromatin states, whereas sex-specific GO term
251 enrichment was observed for genes in the activation-associated chromatin states S9-S13
252 (Figure 2C, Table S7) but note that a large proportion of the genes S1-S2 exhibited
253 conservation of the repression-associated state in both males and females (82.3% of genes),
254 whereas conservation was less marked for genes in states S9-S13 (62.5%; Figure 2D).

255 **Chromatin states and gene expression of *Ectocarpus* sex-biased genes**

256 To investigate the role of histone PTMs in sexual differentiation, we examined the chromatin
257 states associated with genes that showed sex-biased expression patterns. A comparison of
258 gene expression patterns in the two near-isogenic male and female lines (Figure S1), based on
259 RNA-seq data generated using the same biological samples as were used for the ChIP-seq
260 analysis, identified a total of 268 genes that exhibited sex-biased expression ($p_{adj} < 0.05$, fold
261 change > 2 , TPM > 1 ; Table S5).

262 Presence of the activation-associated chromatin marks H3K36me3, H3K9me3, H3K27ac and
263 H3K4me3 (states S9-S13) was associated with higher transcript abundance for sex-biased
264 genes in both males and females but the difference was only statistically significant for males
265 (Wilcoxon test p-values of 0.012 for males and 0.188 for females; Figure S2, S3). Sex-biased
266 genes therefore display a similar association between the presence of activation-associated
267 marks and increased gene expression levels as observed with the genome-wide gene set
268 (Figure 2A).

269 **Chromatin states of sex-biased genes in males and females**

270 To analyse modifications of chromatin PTMs associated with differential expression of sex-
271 biased genes, transitions between chromatin states in males and females were evaluated on
272 a gene-by-gene basis. This analysis showed that 54.8% of male-biased genes (MBGs) and
273 47.2% of female-biased genes (FBGs) had different chromatin states in males and females
274 (Table S5), underlining the dynamic landscape of histone PTMs on sex-biased genes in males
275 and females.

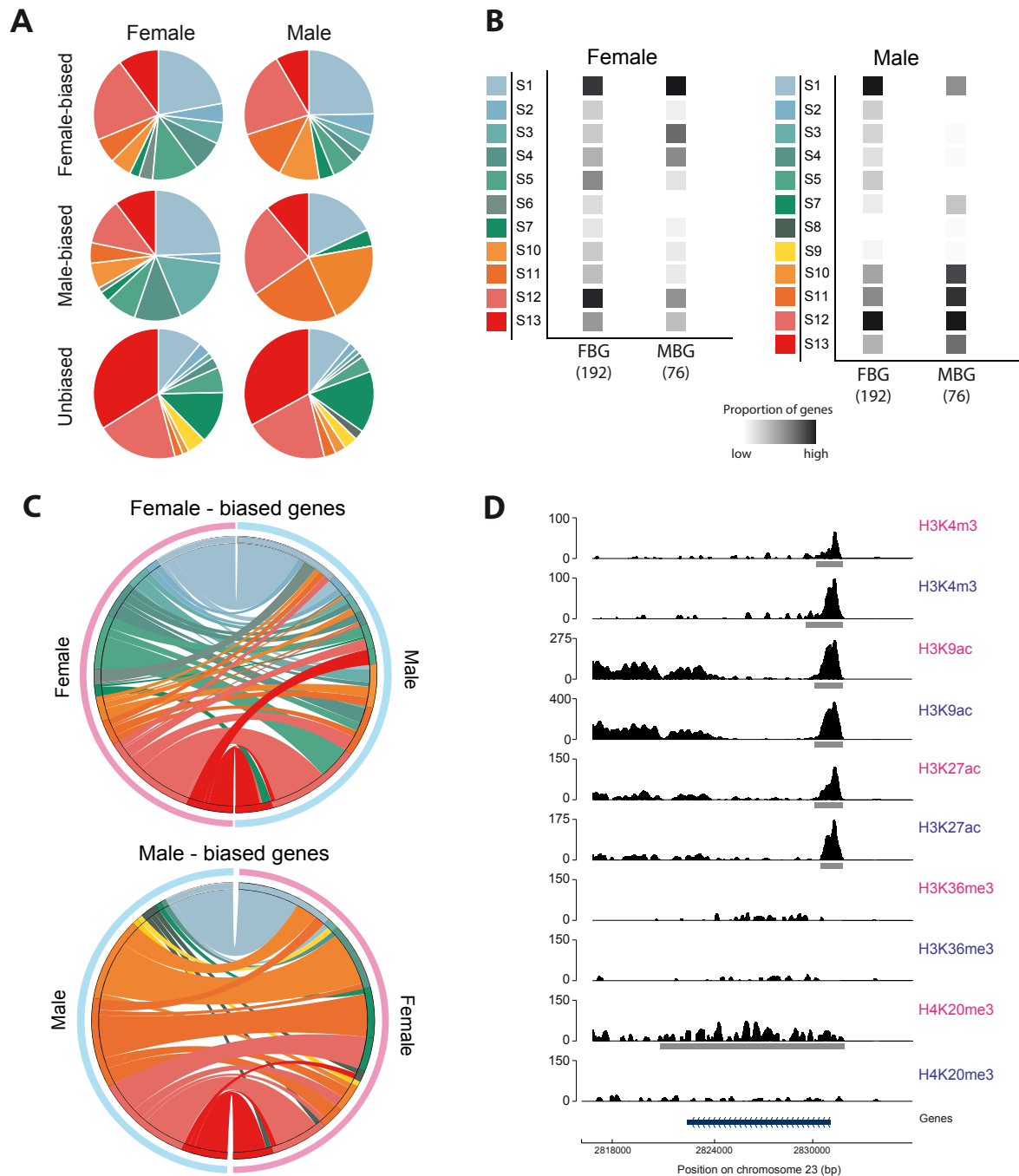
276 Overall, the proportions of the different chromatin states, specifically for MBGs, were
277 significantly different compared with NEGs suggesting that their chromatin landscape is not
278 related to their narrow expression (Chi-square test, p-value = 4.937E-15 and p-value = 0.01608

279 in FBGs vs NEGs in females and males and p-value = 5.627E-4 and p-value = 3.333E-6 for MBGs
280 vs NEGs in females and males respectively; Figure 1C and Figure 3A).

281 For the set of male-biased genes there was a marked difference between the relative
282 proportions of the different chromatin states in males compared to females: in males,
283 chromatin states that included the repression-associated mark H4K20me3 were rare whereas
284 states that included activation-associated marks (H3K9ac, H3K27ac, H3K4me3 and/or
285 H3K36me3, but not H4K20me3) were common (Figure 3A, 3B; Table S3). The proportion
286 represented by chromatin states that contained H4K20me3 (S2 to S8) decreased from 42.1%
287 in females to 3.9% in males whilst the proportion represented by states S9 to S13 (activation-
288 associated states) increased from 34% in females to 73.7% in males (Figure 3A, 3B; Table S3).
289 Almost half (43.4%) of the male-biased genes exhibited a transition from a state that included
290 H3K9ac, H3K27ac, H3K4me3 and/or H3K36me3, but not H4K20me3 (S9-S13) in males to a
291 state that either included H4K20me3 (S2 to S8) or to state S1 (none of the histone PTMs
292 detected) in females (Figure 3C; Table S9). The chromatin state transitions of male-biased
293 genes were consistent with the correlation between the presence and absence of activation-
294 associated and repression-associated histone PTMs and differences in the abundances of the
295 transcripts of sex-biased genes between sexes observed for the complete set of all *Ectocarpus*
296 genes (Figure 2A).

297 Unexpectedly however, female-biased genes exhibited a different pattern of chromatin state
298 transitions when males and females were compared. States that included activation-
299 associated marks (H3K9ac, H3K27ac, H3K4me3 and/or H3K36me3, i.e. states S3-S13) were
300 slightly more frequent in females (76.3%) compared with males (69.2%), but female-biased
301 genes were often associated with H3K20me3 (states S2-S8) in females (37.8%; Figure 3A, B;
302 Table S3). Only 12% of the female-biased genes were associated with chromatin marks S9-S13
303 in females and underwent a transition to a state that included H3K20me3 or to a background
304 state in males (S1-S8) (Figure 3C; Table S9).

305 In conclusion, chromatin state transitions between sexes were concomitant with changes in
306 expression levels of sex-biased genes between males and females. For male-biased genes, the



307

Figure 3. Histone PTM patterns at sex-biased genes in *Ectocarpus* sp. males and females. A) Proportions of the 13 chromatin states for female-biased, male-biased and unbiased genes in females (left) and males (right). B) Proportions of genes associated with each of the 13 chromatin states for female-biased and male-biased genes in females (left) and males (right). The intensity of the grey squares is proportional to the number of genes corresponding to each state. Coloured squares represent the different chromatin states (see Figure 1A). The total numbers of genes analysed for each condition is given in brackets, and the number of genes in each chromatin state are provided in Table S8. FBG: female-biased genes; MBG: male-biased genes. C) Circos plots comparing chromatin states associated with female-biased (above) and male-biased (below) genes in females (pink) and males (blue). The colour code for the chromatin states is the same as that used in Figure 1A. Each link corresponds to the transition from a state in the sex on the left to a state in the sex on the right of the circos plot. D) Representative chromatin profiles for a male-biased gene on chromosome 23 (blue bar). The histone PTMs indicated in blue and pink correspond to those of the male and the female, respectively. The horizontal grey bars under each track correspond to peaks called by either MACS2 (H3K4me3, H3K9ac and H3K27ac) or SICER (H3K36me3 and H4K20me3).

308 patterns of these state transitions were consistent with the tendencies observed for the

309 complete set of all *Ectocarpus* genes, and therefore with the associations between specific
310 histone PTMs and either gene activation or gene repression reported for animals and land
311 plants, as described above. Female-biased genes, however, did not conform to this pattern.

312 **The chromatin landscape of the *Ectocarpus* sex chromosomes**

313 In organisms with diploid sexual systems (XY or ZW), sex chromosomes exhibit different
314 patterns of histone PTMs to autosomes (Brown and Bachtrög, 2014; Picard et al., 2019). In
315 *Drosophila* males for example, the X chromosome is transcribed at a higher level in males than
316 in females, due to dosage compensation of the hemizygous X, and exhibits an enrichment in
317 active chromatin marks (Brown and Bachtrög, 2014). In contrast, in female mammals, the
318 inactivated X chromosome is characterized by DNA methylation and widespread presence of
319 repressive chromatin marks (Brockdorff and Turner, 2015; Lucchesi et al., 2005). In addition,
320 Z-chromosome-localised female-specific hyperacetylation of histone H4 (H4K16Ac) has been
321 described for the chicken (Bisoni et al., 2005) and epigenetic analysis underlined the
322 specialized chromatin landscape of the Z-specific region of *S. mansooni*, which is more
323 permissive than that of the autosomal regions in both male and female *S. mansooni* (Picard et
324 al., 2019).

325 A similar marked difference between sex chromosomes and autosomes was observed in
326 *Ectocarpus* sp. (Figure 4A, Table S5, S10; Figure S4, S5). The relative proportions of each of the
327 13 chromatin states showed some variance between autosomes but the set of genes on the
328 sex chromosomes exhibited strikingly different patterns to those of the autosomes (Figure
329 4A). There was a significant dearth of genes marked with the activation-associated states S12
330 and S13 on the sex chromosomes compared to the autosomes (permutation tests U versus
331 autosomes, $p\text{-value}_{S12}=0.047$ and $p\text{-value}_{S13}=0.039$; permutation tests V versus autosomes,
332 $p\text{-value}_{S12}=0.046$ and $p\text{-value}_{S13}=0.037$; Table S11). Furthermore, in males, the sex
333 chromosome was significantly enriched in states that included the histone PTM H4K20me3
334 compared with autosomes, specifically state S2 ($p\text{-value} = 0.025$), S4 ($p\text{-value} = 0.021$), S5 ($p\text{-}$
335 $\text{value} = 0.008$) and S8 ($p\text{-value} = 0.028$); Figure 4A-C, Table S11).

336 The significantly distinct chromatin patterns between sex chromosome and autosomes were
337 equally manifest when only the pseudoautosomal region (PAR) was taken into account (Chi-
338 square test $p\text{-value} < 2.2E-16$; Figure 4A-C). For example, 67% and 76% of the PAR genes in
339 males and females, respectively, were associated with chromatin states S1-S8 compared with
340 40.1% and 41.5% in males and females, respectively, for autosomal genes (Table S5, Table
341 S12). Although the proportions of chromatin states for the PAR in males and females were not
342 statistically different (chi-square test with continuity correction, $p\text{-value}=0.251$) there were
343 considerably more genes with chromatin state S4 in the PAR in females (12%) than in the PAR

344 in males (4%) (Proportion test, p-value = 6.214e-06) (Table S12). Remarkably, almost half
345 (45%) of the genes located in the PAR were found to be associated with different chromatin
346 states in males and females (Table S5), indicating that a substantial proportion of the PAR
347 genes display sex-dependent chromatin state transitions. Note that only 11 of the 412 PAR
348 genes were classed as sex-biased genes (Table S5), so the sex-related changes in chromatin
349 states of the PAR genes do not appear to be linked with sex-biased PAR gene expression.

350 Analysis of the sex-determining regions of the chromosomes showed that the majority of the
351 genes within the female SDR (i.e., U-specific genes) were in state S1 (i.e., carried none of the
352 assayed marks) whereas the V-specific genes were mostly in state S7 (displayed all of the
353 assayed marks) or state S8 (H3K36me3 and H4K20me3), with some genes in state S13 (all
354 marks except H4K20me3) (Figure 4B-C; Table S13). However, note that, due to the low number
355 of SDR genes, it was not possible to rule out that the difference between chromatin state
356 patterns of the male and female SDRs was due to chance (100 000 permutations tests on
357 Pearson's X^2 statistics).

358 Previous work has shown that the *Ectocarpus* PAR region is enriched in transposons compared
359 with autosomes (Ahmed et al., 2014; Luthringer et al., 2015). Considering that in *Ectocarpus*
360 H4K20me3 co-localizes with transposon sequences (Bourdareau et al., 2020) we asked if the
361 presence of transposons in PAR genes could explain the observed chromatin state distribution
362 patterns. More PAR genes contained a transposon sequence compared to autosomal genes
363 (80% versus 36%, respectively) but there was not a correlated increase in the proportion of
364 PAR genes marked with H4K20me3 (28-29% for the PAR versus 25-27% for autosomes) (Table
365 S14). Moreover, permutation tests using subsets of autosomal genes in which 80% of the
366 genes were selected to contain transposons (i.e., a similar proportion of genes with
367 transposons to that observed for the PAR) indicated that the unusual pattern of chromatin of
368 states in the PAR was not due simply to the presence of additional genes with inserted
369 transposons (Table S14).

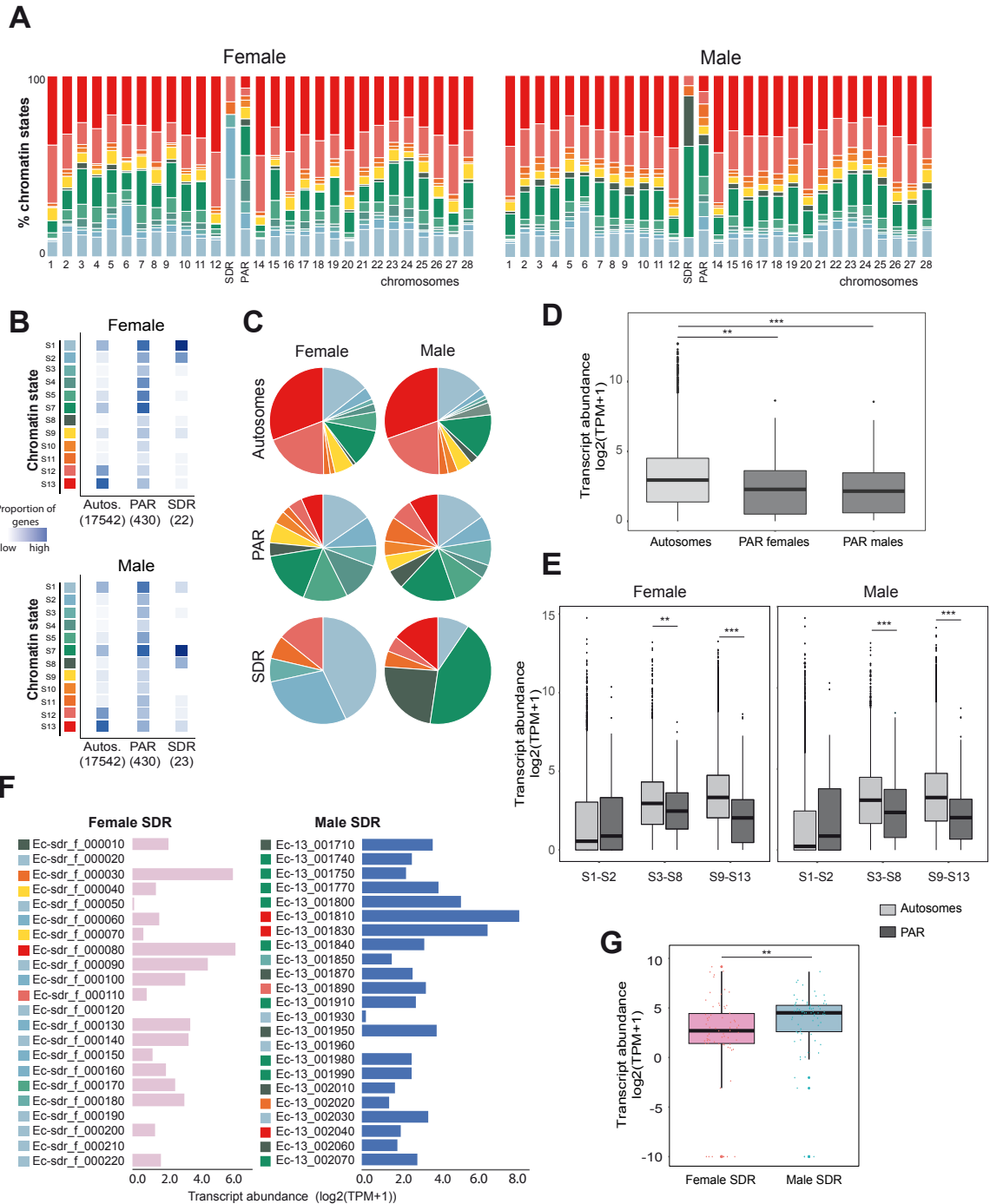


Figure 4. Chromatin landscape of the U and V sex chromosomes compared with the autosomes. A) Chromatin state distribution for each autosome and for the SDR and PAR regions of the sex chromosome in females (left panel) and in males (right panel). B) Proportions of genes associated with each of the 13 chromatin states for all autosomes and for the PAR and SDR regions of the sex chromosome in females (top panel) and in males (bottom panel). The intensity of the blue colour is proportional to the number of genes in each state. The total numbers of genes in each genomic region are represented in brackets. The colour code for the chromatin states is the same as that used in Figure 1A. Autos., autosomes. C) Proportions of chromatin states associated with autosomal, PAR and SDR genes in males and females. The colour code for the chromatin states is the same as in Figure 1A. D) Transcript abundances, measured as $\log_2(\text{TPM}+1)$, for autosomal and for PAR genes in males and females. E) Transcript abundances for autosomal and PAR genes in different chromatin states: repression-associated states S1-S2, states that include canonical activation marks and H4K20me3 (S3-S8) and activation-associated states S9-S13. Significant differences were assessed using pairwise Wilcoxon rank sum test (**p-value < 0.01, ***p-value < 0.0001). F) Transcript abundances, measured as $\log_2(\text{TPM}+1)$, for individual genes located in the female (pink) and male (blue) sex determining regions (SDRs). Coloured squares represent chromatin states corresponding to the colour code indicated in Figure 1A (see also Table S13). G) Transcript abundances of genes located within the sex-specific regions (SDRs) of the U and V sex chromosomes. Asterisks above the plots indicate significant differences (pair-wise Wilcoxon test, **p-value < 0.01).

370 Overall, transcript abundances of genes located in the PAR were significantly lower than those

371 located in autosomes (Wilcoxon p-value=0.003 and p-value=0.0005 for female and male
372 respectively; Table S4, Figure 4D). Potentially, this difference in expression level may also have
373 explained the difference between the chromatin state patterns of the PAR and the autosomes.
374 To test this hypothesis, we selected a subset of autosomal genes that had a similar pattern of
375 transcript abundances to that of the PAR genes (Table S15). The distribution of chromatin
376 states for this set of autosomal genes was different to that of the PAR genes (Figure S6,
377 indicating that gene expression level was not the cause of the difference in chromatin state
378 patterns between the PAR genes and the autosomes.

379 The lower transcript abundance for PAR genes was consistent with the higher proportion of
380 genes in repressive-associated states (S1-S2) compared with autosomal genes (24% for the
381 PAR compared with 13% for the autosomes), but note that even PAR genes in activation-
382 associated states (S9-S13) exhibited significantly lower expression levels than autosomal
383 genes in similar states (pairwise Wilcoxon test, p-value=7.1E-9, p-value=7.1E-9 for female and
384 male respectively; Figure 4E).

385 **Chromatin states and expression levels of sex chromosome genes**

386 Gene expression levels and deposition of chromatin marks were highly correlated for the
387 complete set of *Ectocarpus* genes (see above, Figure 2A). For example, genes in state S13
388 (presence of all four activation-associated marks) had a significantly higher expression level
389 compared with genes in state S7 (presence of all four activation-associated marks plus
390 H4K20me3). In females, when the correlations between chromatin states and levels of gene
391 expression were compared for the autosomes and for the PAR, three chromatin states (S7,
392 S12 and S13) exhibited a significantly weaker correlation with expression for the latter
393 compared with the former. In males, weaker correlation between chromatin state and
394 expression level was also observed for the PAR compared to the autosomes but only for states
395 S7 and S13 (Table S16, Figure S7). In other words, depending on the location (PAR or
396 autosomes) the correlation between chromatin state and gene expression level was not the
397 same.

398 There was no significant correlation between levels of expression of either male or female SDR
399 genes and the presence of particular chromatin marks (likelihood ratio tests, p-value = 0.460
400 and p-value = 0.304 for female and male SDR, respectively; Figure 4F), but the small sample
401 size of SDR genes decreases the power of the statistical test. Note however that H3K36me3,
402 a mark associated with transcript elongation (Huang and Zhu, 2018), was more often present
403 at male SDR genes (in 18/23 genes) than at female SDR genes (1/22 genes)(Figure 4, Figure
404 S4, Table S5) and we also noticed that abundances of transcripts for male SDR genes were

405 significantly higher than for female SDR genes (Figure 4G; pairwise Wilcoxon test with Holm
406 correction, p-value=0.0098).

407 Taken together, these observations suggest that the sex chromosome exhibits exceptional
408 features in terms of its chromatin landscape. The unique features of the PAR are not explained
409 by the preponderance of intragenic transposons nor by the fact that genes in this region have
410 a lower mean level of gene expression. The relationship between chromatin state and gene
411 expression level for the sex chromosomes is different to that observed for the autosomes.

412 Discussion

413 Epigenetic regulation in a haploid UV sexual system

414 Three types of genetic sex determination system exist in nature: XX/XY, ZZ/ZW systems and
415 U/V systems (Bachtrog et al., 2014; Coelho et al., 2018). Studies have focused on
416 understanding sex determination and sex biased gene expression but we know little about
417 chromatin dynamics in males compared to females. The objective of this study was to provide
418 an overview of the sex differences in the chromatin landscape in a haploid UV system, and to
419 investigate the relationship between chromatin states and gene expression differences
420 between sexes and genomic regions, with a particular emphasis on the U and V sex
421 chromosomes.

422 We analysed the genome-wide distribution of five histone PTMs in males and females of an
423 organism with haploid UV sex determination, resulting in the definition of 13 chromatin states
424 corresponding to different combinations of the five histone PTMs. Chromatin states that
425 included different combinations of H3K4me3, H3K9ac, H3K27ac and H3K36me3 were
426 associated with actively transcribed genes, whereas chromatin states that included
427 H4K20me3 were associated with a decrease in gene expression compared to equivalent states
428 that lacked H4K20me3. States that included H3K36me3 were associated with broadly
429 expressed genes, and this mark was less prevalent on genes with narrow expression patterns,
430 a configuration that is compatible with the idea that H3K36me3 is deposited during
431 transcription elongation (Barski et al., 2007). Note that the difference in H3K36me3 levels in
432 NEGs versus housekeeping genes could be related to the lower power to detect H3K36me3
433 binding of tissue-specific genes expressed only in a subset of cells. It was interesting however
434 that the difference between the housekeeping and NEG gene sets was considerably more
435 marked for H3K36me3 than for the TSS-located PTM (Table S4), perhaps indicating a stronger
436 link with gene transcription. A similar association of H3K36me3 with broadly expressed genes
437 has been described for *Drosophila* (Brown and Bachtrog, 2014; Filion et al., 2010), indicating
438 that this correlation has been conserved across distantly related lineages. Overall, the

439 *Ectocarpus* chromatin patterns described here are consistent with H3K4me3, H3K9ac,
440 H3K27ac and H3K36me3 having similar roles in brown algae, land plants and animals (Baroux
441 et al., 2011; Bourdareau et al., 2020; Margueron and Reinberg, 2010; She and Baroux, 2015).
442 The role of H4K20me3, in contrast, appears to be less conserved across eukaryotic
443 supergroups, being associated with low transcriptional levels in both animals and brown algae
444 but with euchromatin and transcriptional activation in land plants (de la Paz Sanchez and
445 Gutierrez, 2009; Fischer et al., 2006).

446 Our analysis has also identified some novel features of the relationship between chromatin
447 marks and gene expression in *Ectocarpus*. For example, we identified a positive correlation
448 between the number of different activation-associated marks (TSS marks and H3K36me3) that
449 were deposited at a gene and transcript abundance. In the absence of canonical repressive
450 marks such as H3K27me3 in *Ectocarpus* (Bourdareau et al., 2020), it is possible that chromatin
451 regulation of gene expression in *Ectocarpus* may be dominated by the synergistic action of
452 activation marks (although it is important to bear in mind the possibility that the activation-
453 associated marks may be deposited a consequence of transcription rather than mediating
454 gene activation). Deposition of H4K20me3 was consistently associated with decreased
455 transcript abundance in *Ectocarpus*, and in that respect this mark can be considered to be
456 'repression-associated'. However, it is currently unclear if H4K20me3 action is direct or
457 indirect through silencing of intronic transposons (Bourdareau et al., 2020).

458 **Relationship between H4K20me3 and gene expression**

459 A complex relationship was observed between H4K20me3 and gene expression. There was
460 clear evidence for a correlation between H4K20me3 and gene expression levels that was
461 independent of the TSS-localised marks and H4K36me3 (Figure 2A). A previous study found
462 that genes marked with H4K20me3 exhibited significantly weaker signals for TSS-localised
463 PTMs (H3K4me2, H3K4me3, H3K9ac, H3K14ac and H3K27ac) (Bourdareau et al., 2020),
464 suggesting a possible effect of H4K20me3 on gene expression via TSS marks. Taken together,
465 these observations suggest that H4K20me3 may act on gene expression via two different
466 pathways, one via an effect on TSS marks and the other by acting directly on gene expression,
467 independently of the TSS marks. However, an alternative hypothesis would be that increased
468 gene expression leads to a decrease in H4K20me3. In other words, activation of a gene might
469 involve (in addition to other processes) suppression of heterochromatin-associated marks
470 such as H4K20me3 leading to a tendency for H4K20me3 to be present at loci that are less
471 marked with TSS-located PTMs.

472 **Chromatin dynamics of *Ectocarpus* sex-biased genes in males and females**

473 Genome-wide, the proportions of genes associated with each chromatin state did not differ
474 substantially when males were compared with females. However, when individual genes were
475 compared, a considerable fraction was associated with different chromatin states in the two
476 sexes, including genes that did not exhibit sex-biased expression patterns. It is possible that
477 the differences correspond to chromatin state 'noise', in which case they would not be
478 expected to be linked with sex-biased gene expression. However, the strong correlation
479 between chromatin states and expression levels argues for a biological role for chromatin
480 state changes. One hypothesis would be that genes display sex-specific chromatin
481 configurations prior to the appearance of significant sex differences in gene expression and
482 phenotypic differentiation. In other words, differences in chromatin state may anticipate sex-
483 biased differences in gene expression at later stages, as has been reported for mammalian
484 fetal germ cells (Lesch and Page, 2013). A more refined study using several stages of
485 development of male and female gametophytes would be needed to gain further insights into
486 this matter.

487 In males, most of the male-biased genes were marked with activation-associated chromatin
488 states (S9-S13), whereas in females, male-biased genes were predominantly marked with
489 repression-associated chromatin states (S1-S2). This observation is consistent with gene
490 expression level modifications reported for sex-biased genes in males compared with females,
491 where male-biased expression is due to a combination of both upregulation in males (i.e.,
492 activation of male-biased genes in males) and decreased expression in females (i.e., repression
493 of male-biased genes in females) (Lipinska et al., 2015). However, more than half of the FBGs
494 were marked with activation-associated chromatin states (S9-S13) in males, whereas in
495 females, FBGs were predominantly marked with chromatin states that included H4K20me3. It
496 appears therefore that female-biased genes do not follow the same trends that were observed
497 genome-wide and for MBGs, where TSS marks were clearly associated with gene activation
498 and H4H20me3 associated with lower transcript abundances.

499 **Unique chromatin organisation features in the U and V sex chromosomes**

500 In organisms with UV sexual systems, the U and V sex-specific regions are both non-
501 recombining, exhibit relatively similar structural features and appear to have been subjected
502 to similar evolutionary pressures (Ahmed et al., 2014; Mignerot and Coelho, 2016). Despite
503 these similarities, the genes in the male SDR exhibited a different pattern of chromatin states
504 to the genes in the female SDR. In particular, H3K36me3, a mark that is often involved in
505 dosage compensation and is usually enriched on X chromosomes (Bell et al., 2008), was
506 detected on 18/23 (78%) of the male SDR genes but only in 6% (1/16) female SDR genes, but
507 note that statistical analysis showed no significant differences between U and V SDRs due to

508 the low number of genes in this region. Deposition of H3K36me3 is associated with increased
509 transcript abundances in plants and animals (Roudier et al., 2011; Shilatifard, 2006), and we
510 found that genes on the *Ectocarpus* male SDR exhibited significantly higher expression levels
511 than female SDR genes (Figure 4G).

512 The *Ectocarpus* PAR has been shown to have unusual structural and gene expression features
513 compared to the autosomes (Avia et al., 2018; Luthringer et al., 2015) and this study found
514 unusual patterns of chromatin states in this genomic region. However, the analysis also
515 showed that neither the levels of gene expression, which are lower, on average, for the PAR
516 compared with autosomes, nor the greater prevalence of transposons and repeat sequences
517 in PAR genes explained the unusual patterns of chromatin states. Moreover, sex-specific
518 differences in chromatin states were prominent on the PAR of the U and V sex chromosomes,
519 where almost half (47%) of the genes displayed different chromatin states between the two
520 sexes. Our observations emphasise the unique features of the PAR of the *Ectocarpus* UV sex
521 chromosomes, and suggest that the effect of chromatin states on transcript abundance may
522 depend on the genomic locations of genes, and that the same chromatin states do not
523 correspond to the same level of transcriptional change in genes located in autosomes and sex
524 chromosomes. It is possible that the expression of genes on the U and V sex chromosomes is
525 regulated by different epigenetic processes to those that regulate the expression of autosomal
526 genes, perhaps involving histone PTMs that have not been assayed in this study. Further
527 investigations employing additional histone PTMs marks will be needed to further understand
528 the extraordinary features of these chromosomes.

529 **Methods**

530 **Biological Material**

531 The near-isogenic male (Ec457) and female (Ec460) *Ectocarpus* sp. lines (Table S1) were
532 generated by crossing brother and sister gametophytes for either four or five generations,
533 respectively (Ahmed et al., 2014). The resulting male and female strains, therefore, had
534 essentially identical genetic backgrounds apart from the non-recombining SDR. Male and
535 female gametophytes were cultured until near-maturity for 13 days as previously described
536 (Coelho et al., 2012) at 13°C in autoclaved natural sea water supplemented with 300 µl/L
537 Provasoli solution (PES), with a light:dark cycle of 12:12 h ($20 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) using
538 daylight-type fluorescent tubes. All manipulations were performed in a laminar flow hood
539 under sterile conditions.

540 **Comparisons of male and female transcriptomes using RNA-seq**

541 RNA for transcriptome analysis was extracted from the same duplicate male and female
542 cultures as were used for the CHIP-seq analysis. For each sex, total RNA was extracted from a
543 mix of 90 gametophytes each, using the Qiagen Mini kit (<http://www.qiagen.com>). RNA
544 quality and quantity were assessed using an Agilent 2100 bioanalyzer, associated with
545 Qubit2.0 Fluorometer using the Qubit RNA BR assay kit (Invitrogen, Life Technologies,
546 Carlsbad, CA, USA), as described previously (Lipinska et al., 2015, 2017).

547 For each replicate sample, cDNA was synthesized using an oligo-dT primer. The cDNA was
548 fragmented, cloned, and sequenced by Fasteris (CH-1228 Plan-les-Ouates, Switzerland) using
549 an Illumina HiSeq 4000 set to generate 150-bp single-end reads. See Table S1 for RNA-seq
550 accession numbers.

551 Data quality was assessed using FastQC
552 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>; accessed May 2019). Reads
553 were trimmed and filtered using Cutadapt (Martin, 2011) with a quality threshold of 33
554 (quality-cutoff) and a minimal size of 30 bp.

555 Filtered reads were mapped to version v2 of the *Ectocarpus* sp. genome (Cormier et al., 2017a)
556 using TopHat2 with the Bowtie2 aligner (Kim et al., 2013). More than 85% of the sequencing
557 reads from each library could be mapped to the genome (Table S1).

558 The mapped sequencing data were then processed with featureCounts (Liao et al., 2014) to
559 obtain counts for sequencing reads mapped to genes. Gene expression levels were
560 represented as transcripts per million (TPMs). Genes with expression values below the fifth
561 percentile of all TPM values calculated per sample were considered not to be expressed and
562 were removed from the analysis. This resulted in a total of 18,462 genes that were considered
563 to be expressed.

564 Differential expression analysis was performed with the DESeq2 package (Bioconductor) (Love
565 et al., 2014). Genes were considered to be male-biased or female-biased if they exhibited at
566 least a twofold difference (fold change; FC) in expression between sexes with a false discovery
567 rate (FDR) < 0.05. A list of the sex-biased genes can be found in Table S5.

568 To calculate breadth of expression we employed the tissue-specificity index tau (Yanai et al.,
569 2005) using published expression data from nine tissues or stages of the life cycle (female and
570 male immature and mature gametophytes, mixed male and female gametophytes, partheno-
571 sporophytes, upright partheno-sporophyte filaments, basal partheno-sporophyte filaments,
572 diploid sporophytes) from *Ectocarpus* sp. (Cormier et al., 2017a; Lipinska et al., 2015, 2019,
573 2017; Luthringer et al., 2015). This allowed us to define broadly expressed (housekeeping)
574 genes (with $\tau < 0.25$) and narrowly expressed genes ($\tau > 0.75$).

575 **Genome-wide detection of histone PTMs**

576 Male versus female *Ectocarpus* sp. gametophyte ChIP-seq experiments were carried for
577 H3K4me3, H3K9ac, H3K27ac, H3K36me3, H4K20me3, and three controls (an input control
578 corresponding to sonicated DNA, histone H3 and immunoglobulin G monoclonal rabbit (IgG))
579 as in (Bourdareau, 2018). RNA-seq data (see above) was generated from the same samples,
580 to ensure that the histone PTM and gene expression data were fully compatible. For ChIP-seq,
581 2.8 g (corresponding to 2800 individual gametophytes) of *Ectocarpus* tissue was fixed for five
582 minutes in seawater containing 1% formaldehyde and the formaldehyde eliminated by rapid
583 filtering followed by incubation in PBS containing 400 mM glycine. Nuclei were isolated by
584 grinding in liquid nitrogen and in a Tenbroeck Potter in nuclei isolation buffer (0.1% triton X-
585 100, 125 mM sorbitol, 20 mM potassium citrate, 30 mM MgCl₂, 5 mM EDTA, 5 mM β-
586 mercaptoethanol, 55 mM HEPES at pH 7.5 with complete ULTRA protease inhibitors), filtering
587 through Miracloth and then washing the precipitated nuclei in nuclei isolation buffer with and
588 then without triton X-100. Chromatin was fragmented by sonicating the purified nuclei in
589 nuclei lysis buffer (10 mM EDTA, 1% SDS, 50 mM Tris-HCl at pH 8 with cComplete ULTRA
590 protease inhibitors) in a Covaris M220 Focused-ultrasonicator (duty 25%, peak power 75,
591 cycles/burst 200, duration 900 seconds at 6°C). The chromatin was incubated with an anti-
592 histone PTM antibody (anti-H4K20me3, anti-H3K4me3, and anti-H3K9ac, Cell Signal
593 Technology; anti-H3K27ac, Millipore; anti-H3K36me3, Abcam) overnight at 4°C and the
594 immunoprecipitation carried out using Dynabeads protein A and Dynabeads protein G.
595 Following immunoprecipitation and washing, a reverse cross-linking step was carried out by
596 incubating for at least six hours at 65°C in 200 mM NaCl and the samples were then digested
597 with Proteinase K and RNase A. Purified DNA was analysed on an Illumina HiSeq 4000 platform
598 with a single-end sequencing primer over 50 cycles. At least 20 million reads were generated
599 for each immunoprecipitation. The ChIP-seq dataset has been deposited in the NCBI Gene
600 Expression Omnibus database under the accession numbers described in Table S2.

601 Quality control of the sequence data was carried out using FastQC (Andrews, 2016). Poor
602 quality sequences were removed and the high quality sequences trimmed with Cutadapt
603 (Hansen et al., 2016; Martin, 2011). Illumina reads were mapped onto the *Ectocarpus* genome
604 (Cormier et al., 2017b) using Bowtie (Langmead et al., 2009). Duplicates were removed using
605 samtools markdup in the Samtools package (v 1.9) (Li et al., 2009).

606 Quality control of ChIP-seq data sets followed the Encode ChIP-seq guidelines and practices
607 (Landt et al., 2012)(Table S2). ChIP-seq analysis was carried out for two biological replicates
608 for each PTM in both the male and female samples. Pearson correlation analysis of replicates
609 was performed with multiBamSummary and then by plotCorrelation (v3.1.2 deepTools)

610 (Ramirez et al., 2014). Replicate samples were strongly correlated (Pearson correlations >0.92 ,
611 Figure S8).

612 To identify peaks and regions of chromatin mark enrichment, each data set, after combining
613 data for biological replicates, was analysed separately for the male and female gametophyte.
614 Peaks corresponding to regions enriched in H3K4me3, H3K9ac and H3K27ac were identified
615 using the MACS2 (version 2.1.1) callpeak module (minimum FDR of 0.01) and refined with the
616 MACS2 bdgpeakcall and bdgbroadcall modules (Zhang et al., 2008). H3K36me3 and
617 H4K20me3 were analysed using SICER (v1.1) (minimum FDR of 0.01) (Xu et al., 2014; Zang et
618 al., 2009) with a window size of 200 bp and a gap size of 400 bp. Note that peaks associated
619 with sex-biased, PAR and SDR genes were manually inspected to validate reproducibility
620 between replicates. The signal was normalized using the Signal Extraction Scaling (SES)
621 method (Diaz et al., 2012).

622 Heatmaps, average tag graphs and coverage tracks were plotted using EaSeq (Lerdrup et al.,
623 2016). Circos graphs were generated using Circos software (Krzywinski et al., 2009).

624 **Detection of chromatin states**

625 To identify the patterns of histone PTM marks associated with each gene (i.e., chromatin
626 states), we used bedtools intersect (intersectBed) from the Bedtools software (v2.26)(Quinlan
627 and Hall, 2010). A total of 13 combinations of histone PTM marks (S1 to S13) were detected.
628 Note that only chromatin states that were present in more than 1% of the genes were taken
629 in consideration for the analysis.

630 **Coverage for each chromatin state**

631 The coverage for each histone PTM per chromosome was calculated using bedtools coverage
632 where the coverage of each PTM was normalized by the size of the chromosome. The
633 pseudoautosomal regions (PAR) and the sex-specific, non-recombining regions (SDR) of the
634 sex chromosome were analysed separately, as in (Brown and Bachtrog, 2014).

635 **Statistical analysis**

636 To test for significant differences in the conservation of chromatin states between sex-biased
637 and unbiased genes, we used mixed generalised linear models with a binomial distribution,
638 modelling conserved vs non-conserved states as a function of bias. We then performed a
639 likelihood ratio test with a null model to assess the significance of bias. Statistical analysis was
640 performed in R 3.6.3. Permutation tests were performed to study the differences of
641 proportions of chromatin states in PAR and SDR genes compared to autosomal genes. We
642 randomly subsampled 100,000 times a number of chromatin states equal to the number of

643 PAR genes, SDR genes or both, from autosomal genes in order to perform proportion tests.
644 We compared observed and simulated Pearson's chi-square statistics to assess whether the
645 observed differences in chromatin state proportions between gene sets (autosomal, SDR, PAR,
646 SDR+PAR) were statistically due to chance. A significant p-value indicates that the observed
647 difference in proportion are not due to chance. In order to eliminate any possible effect of TE
648 prevalence (which is different between PAR, SDR and autosomal genes) we also performed
649 these tests using a randomized set of autosomal genes that displayed exact the same TE
650 prevalence.

651 **GO-term analysis**

652 Gene set enrichment analysis (GSEA) was carried out separately for each sex and each histone
653 state, using Fisher's exact Test implemented in the R package TopGO using the weight01
654 algorithm to account for GO topology (Alexa and Rahnenfuhrer, 2020) We investigated
655 enrichment in terms of molecular function ontology and report significant GO-terms with p-
656 value < 0.01.

657

658 Supplemental Figures

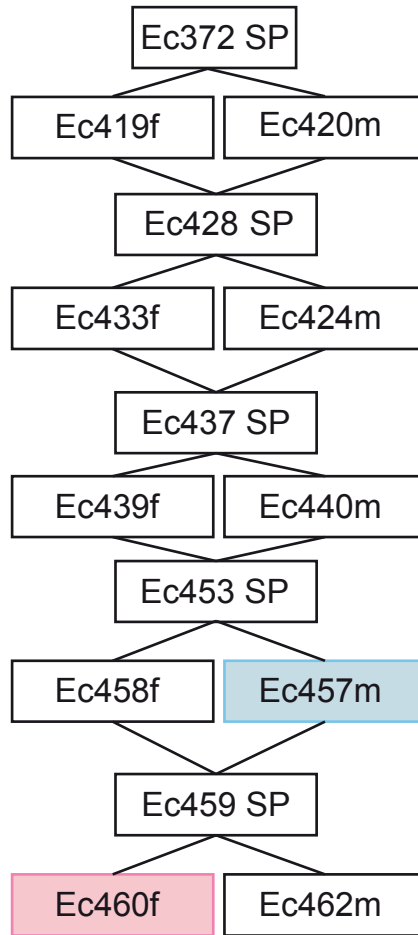


Figure S1. Pedigree of the male and female strains used in this study. SP, sporophyte; m, male gametophyte; f, female gametophyte.

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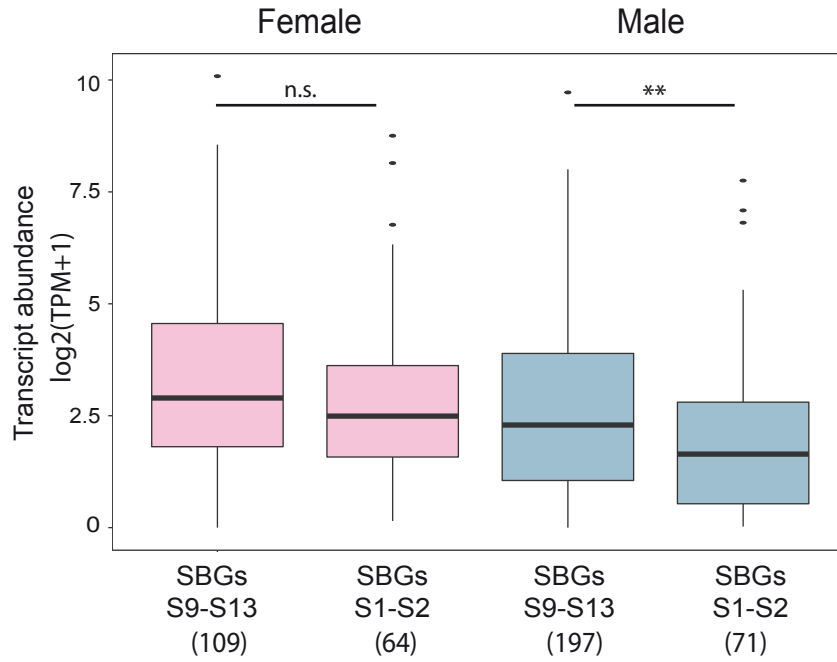


Figure S2. Abundances of the transcripts of sex-biased genes (SBG) marked with different chromatin states in females and males. Abundances of transcripts of SBGs in chromatin states S9-S13 or S1-S2 in females (pink) and males (blue). Values in brackets indicate the number of genes analysed. Asterisks above the plots indicate significant differences (pair-wise Wilcoxon test, **p-value<0.01).

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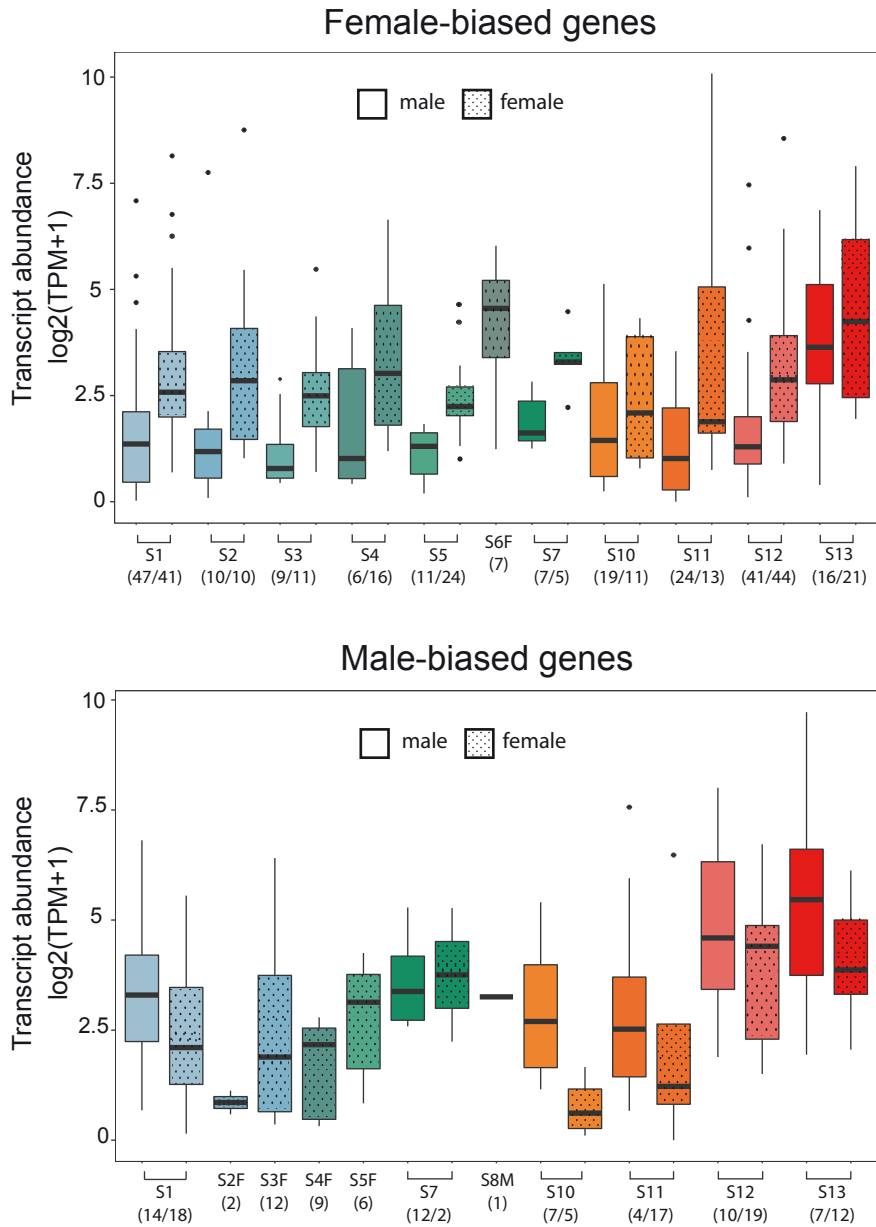


Figure S3. Abundances of transcripts of SBGs associated with each of the different chromatin states in males and females. The colour code is the same as that used in Figure 1A. The total number of SBGs associated with each state are indicated in brackets.

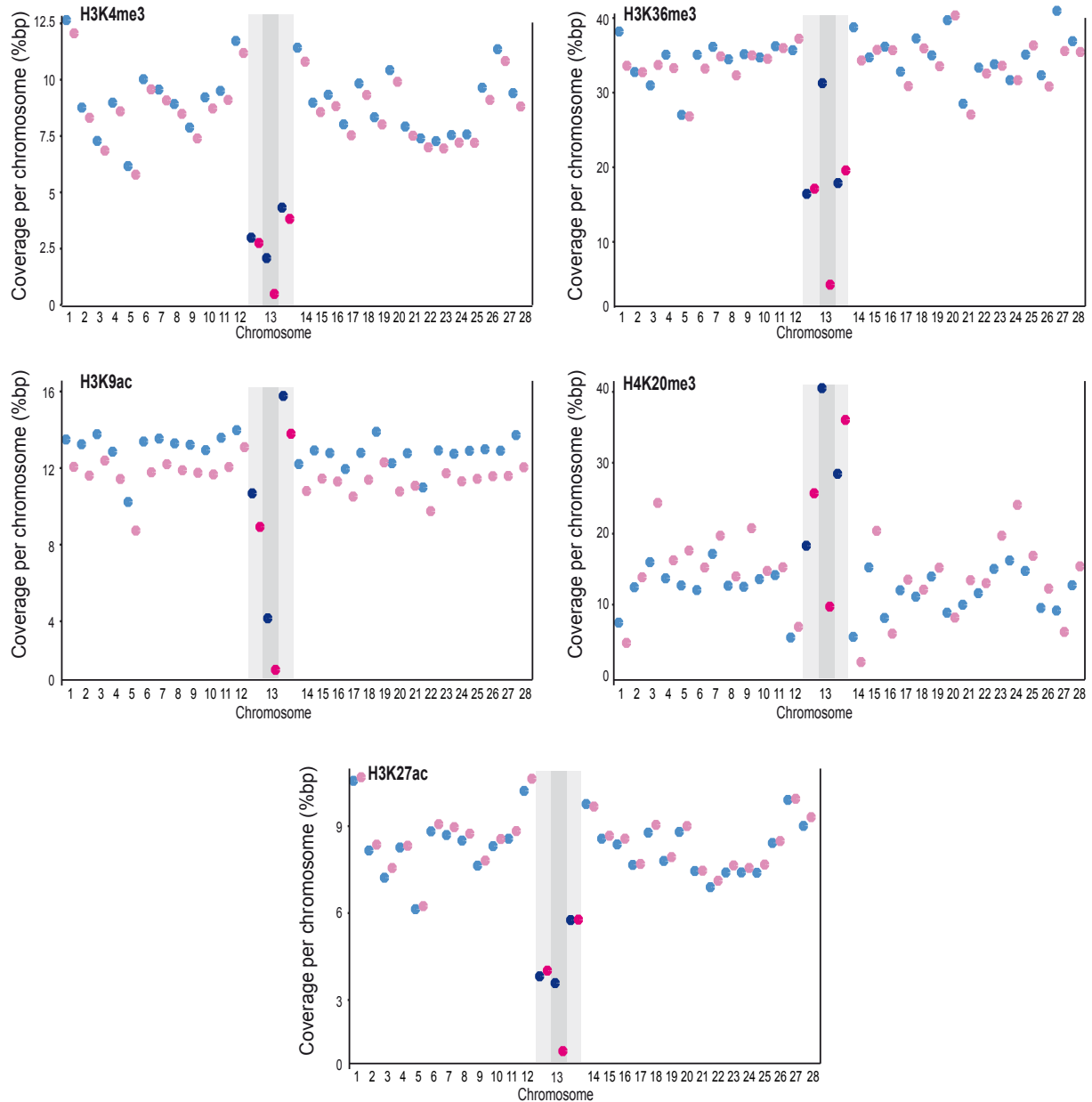


Figure S4. Percentage of coverage for specific histone PTMs for the SDRs, PAR and autosomes in male and females. Scatter plot showing the percent of coverage (in base pairs) for each of the five histone PTMs, H3K4me3, H3K9ac, H3K27ac, H3K36me3 and H4K20me3. Light blue and light pink represent coverage in male and female, respectively. Dark blue and red dots correspond to coverage for the V and U sex chromosomes, respectively. Light shading indicates the two PARs and dark shading the non-recombining, sex specific region (SDR) of the sex chromosome (chromosome 13).

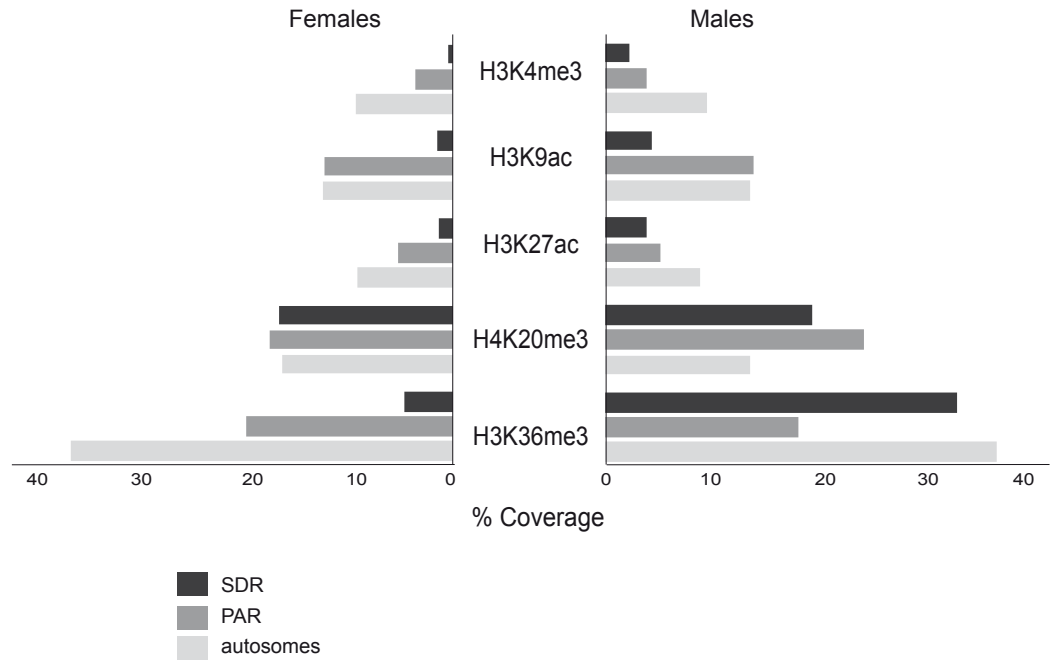


Figure S5. Coverage (represented as percentage of base pairs) in three different genomic regions (PAR, SDR and autosomes) marked with different histone PTMs in females (left) and males (right).

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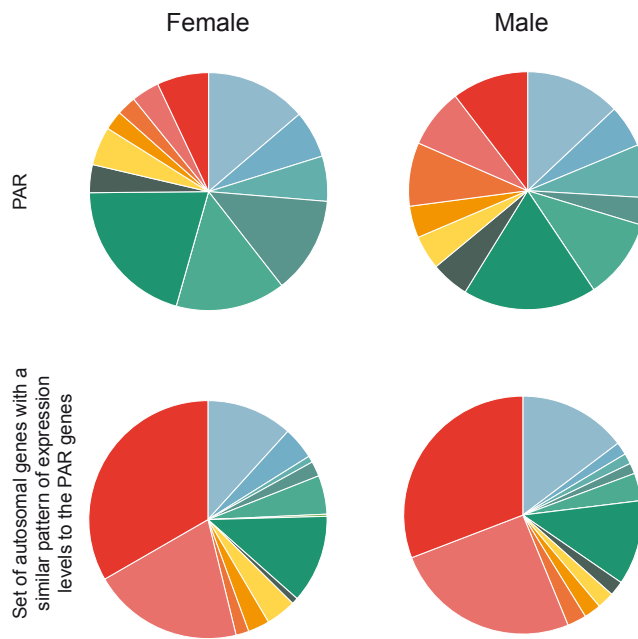


Figure S6. Proportions of chromatin states for PAR genes compared with the proportions of chromatin states for a set of autosomal genes with a similar pattern of expression levels to the PAR genes.

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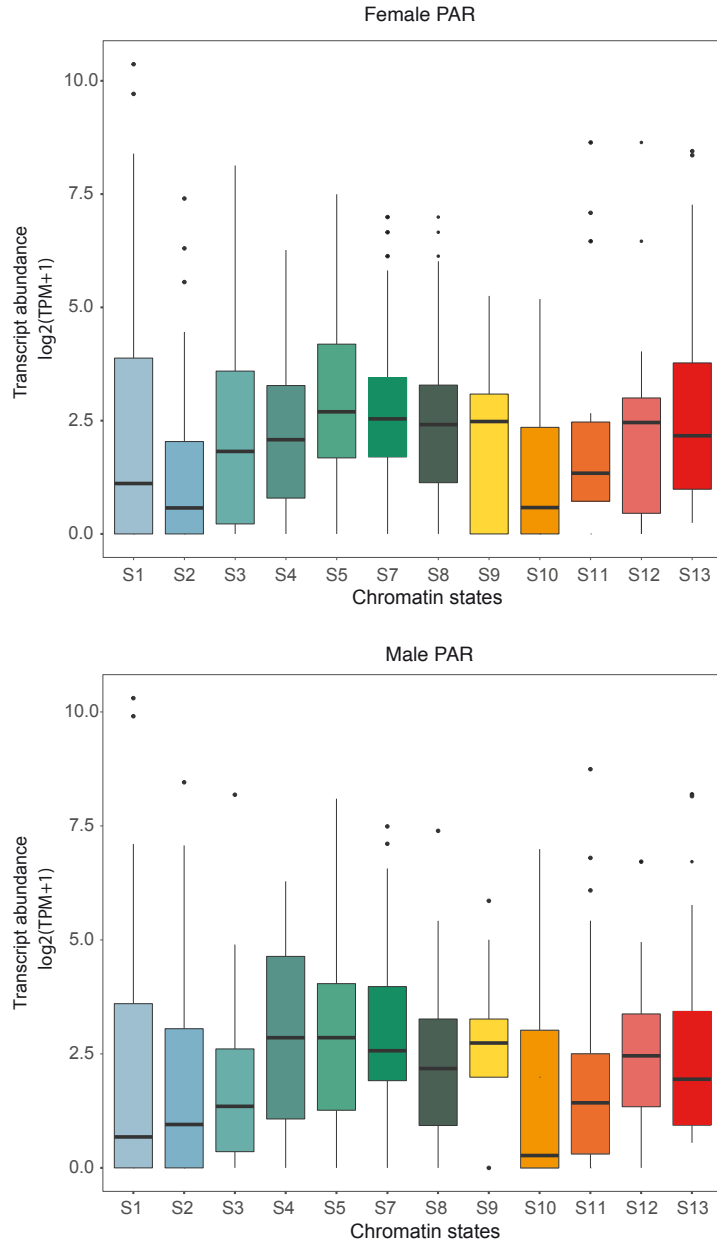


Figure S7. Transcript abundances, measured as $\log_2(\text{TPM}+1)$, for PAR genes associated with different chromatin states in males and females. The colour code is the same as that used in Figure 1A.

668

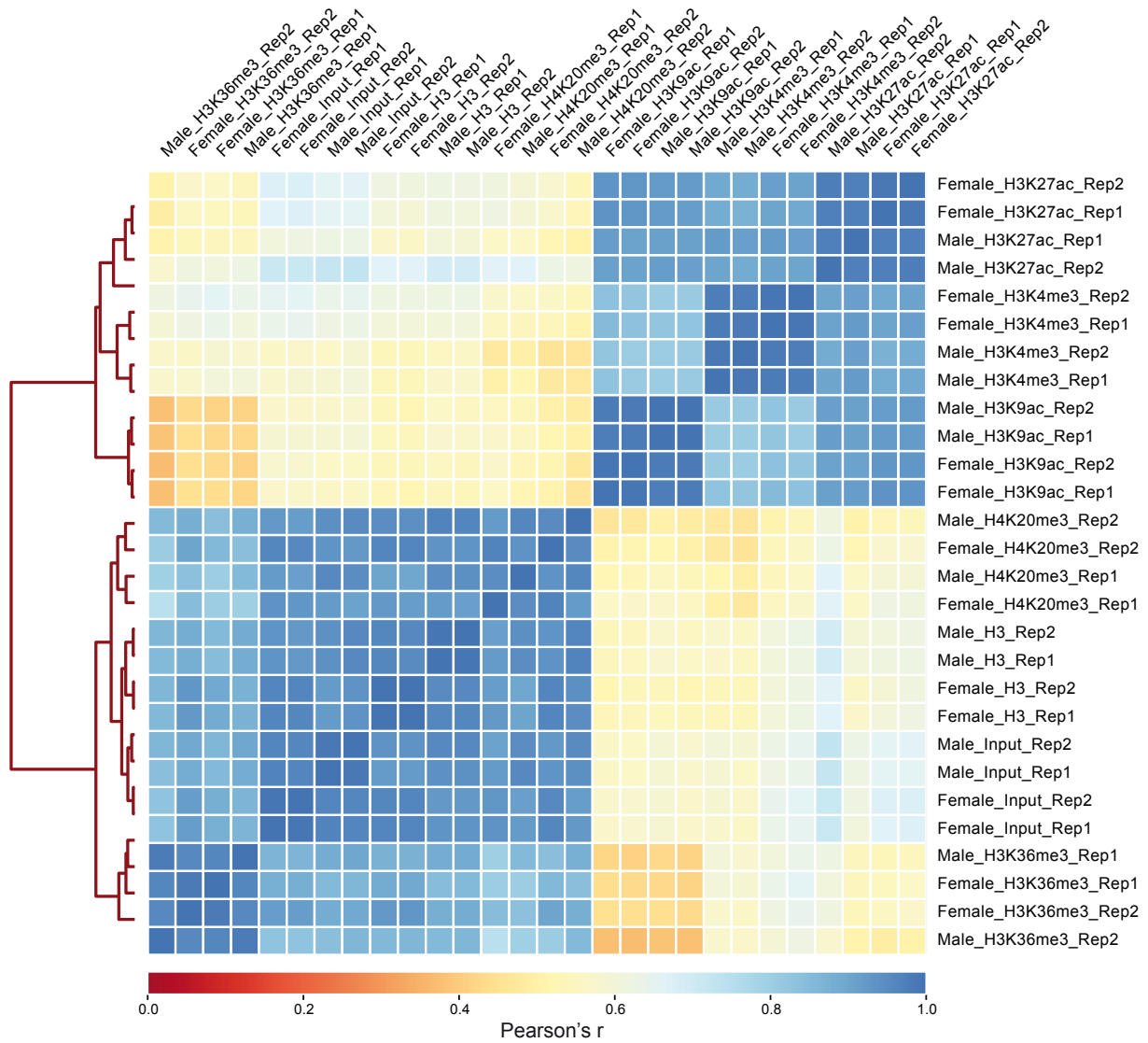


Figure S8. Pearson correlation scores for comparisons of the genomic distributions of ChIP-seq signal peaks for the five histone PTMs.
Rep1, replicate 1; Rep2, replicate 2.

669

670

671 [Supplemental Tables Legends](#)

672 **Table S1. *Ectocarpus* strains used, RNA-seq sequencing statistics and SRA accession**
673 **numbers.**

674 **Table S2. Sequencing statistics for the CHIP-seq analysis and GEO reference for the dataset.**
675 **N. peaks, number of peaks; FRiP, fraction of reads in peaks.**

676 **Table S3. Percentages of genes associated with each of the 13 chromatin states for different**
677 **gene sets in males and females.** Global, all genes in the genome; Transcribed genes, genes
678 with TPM >5th percentile; Silent genes, genes with TPM <5th percentile; Housekeeping and
679 Narrowly-expressed, genes with tau <0.75 and tau >0.75, respectively; Unbiased, no sex-
680 biased expression. For the chromatin states, refer to Figure 1A.

681 **Table S4. Percentages of narrowly expressed genes (NEG) and housekeeping (broadly**
682 **expressed) genes marked with different histone PTMs in males and females.**

683 **Table S5. Chromatin states (S1-S13) and transcript abundances (measured as TPM) for all**
684 ***Ectocarpus* genes in males and females.** FBG, female-biased gene; MBG, male-biased gene.
685 For the chromatin states, refer to Figure 1A. Genes that did not pass the manual inspection
686 (see methods) and were excluded from the analysis of chromatin state transitions are marked
687 in grey.

688 **Table S6. Pairwise Wilcoxon tests for statistical differences between the expression levels**
689 **of genes associated with specific chromatin states (Figure 2A).** S, Chromatin states (S1 to
690 S13); F, females; M, males. The values indicate pairwise Wilcoxon test p-values corrected for
691 multiple comparisons. For the chromatin states, refer to Figure 1A.

692 **Table S7. GO term enrichment for genes associated with each of the chromatin states in**
693 **males and females.** All significantly enriched Biological Process GO terms identified using
694 Blast2GO are presented.

695 **Table S8. Number of sex-biased genes in each of the chromatin states S1-S13 in males and**
696 **females.** FBG, female-biased gene, MBG, male-biased gene

697 **Table S9. Transitions between chromatin states observed for male-biased and female-**
698 **biased genes in males compared with females.** For chromatin states, refer to Figure 1A.

699 **Table S10. Coverage of the five histone PTMs across male and female genomes.** The sex
700 chromosome (chromosome 13) is divided into PAR1 (pseudo-autosomal region 1), SDR (sex-
701 determining region) and PAR2 (pseudo-autosomal region 2).

702 **Table S11. Permutation tests performed to determine whether the relative proportions of**
703 **the different chromatin states were statistically different in different regions of the genome.**

704 We randomized the genomic location of autosomal genes 100,000 times and tested the
705 difference between the observed proportions for the SDR, the PAR or the entire sex
706 chromosome and the permuted gene sets using chi-square statistics. Tests were performed
707 independently for each chromatin state. Significant p-values (<0.05) are highlighted in bold.

708 **Table S12. Chromatin states of PAR genes in males and females.**

709 **Table S13. Chromatin states and transcript abundances ($\log_2\text{TPM}+1$) for SDR genes** (see also
710 Figure 4F).

711 **Table S14. The presence of transposon sequences in the majority (80%) of PAR genes does**
712 **not explain the distinct chromatin landscape of the PAR.** Correlation between the presence
713 of transposable elements within introns and the presence of H4K20me3 in PAR genes and
714 autosomal genes (left table). Permutation tests comparing the proportion of each chromatin
715 state in the PAR with the proportion of that state in 100,000 samples of 430 autosomal genes
716 with transposon sequences in 80% of the genes. For most chromatin states, the proportion on
717 the PAR was significantly different from those of the autosomal gene samples indicating that
718 transposon content does not explain the unusual pattern of chromatin states observed for the
719 PAR. Significant p-values (<0.05) are highlighted in bold (right table).

720 **Table S15. Comparison of chromatin states of the PAR genes with those of a set of autosomal**
721 **genes with a similar pattern of gene expression levels.** To establish the autosomal gene set,
722 for each PAR gene, the full set of autosomal genes was searched for the gene that had the
723 most similar level of expression. When the TPM of the PAR gene was zero, an autosomal gene
724 with a TPM of zero was selected at random. Figure S6 presents the proportions of chromatin
725 states associated with the two gene sets.

726 **Table S16. Linear models to test whether there was a significant correlation between**
727 **expression level ($\log_2(\text{TPM} + 1)$) and chromatin state (upper table) or to test whether**
728 **location of a gene on the PAR or on an autosome significantly influenced the expression**
729 **level associated with each chromatin state (bottom table).** Significant interaction terms, in
730 bold, represent a significantly different effect of the chromatin state on gene expression level
731 in the PAR region compared to autosomal genes. None of the interaction terms between SDR
732 and chromatin state showed a significant effect on gene expression so they are not reported
733 in the table (likelihood ratio test; p-value=0.460 and p-value 0.304 for female and male SDR
734 respectively).

735

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