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2

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4

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7

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18

19

20 **Abstract:**

21 XY systems usually show chromosome-wide compensation of X-linked genes, while in many
22 ZW systems, compensation is restricted to a minority of dosage sensitive genes. Why such
23 differences arose is still unclear. Here, we combine comparative genomics, transcriptomics and
24 proteomics to obtain a complete overview of the evolution of gene dosage on the Z-
25 chromosome of *Schistosoma* parasites. We compare the Z-chromosome gene content of
26 African (*Schistosoma mansoni* and *S. haematobium*) and Asian (*S. japonicum*) schistosomes,
27 and describe lineage-specific evolutionary strata. We use these to assess gene expression
28 evolution following sex-linkage. The resulting patterns suggest a reduction in expression of Z-
29 linked genes in females, combined with up-regulation of the Z in both sexes, in line with the first
30 step of Ohno's classic model of dosage compensation evolution. Quantitative proteomics
31 suggest that post-transcriptional mechanisms do not play a major role in balancing the
32 expression of Z-linked genes.

33 **Introduction**

34 In species with separate sexes, genetic sex determination is often present in the form of
35 differentiated sex chromosomes (1). A sex-specific chromosome can be carried by the male
36 (such as the Y of mammals and fruit flies, in male heterogamety) or by the female (such as the
37 W of birds, in female heterogamety). These sex chromosomes originally arise from pairs of
38 autosomes, which stop recombining after they acquire a sex-determining region (2,3). The loss
39 of recombination between X/Z and Y/W chromosomes is likely driven by selective pressures to
40 link the sex-determining gene and alleles with sexually antagonistic effects, and often occurs
41 through inversions on the sex-specific chromosome (4,5). The inverted Y/W-linked region stops
42 recombining entirely, which hampers the efficacy of selection and leads to its genetic
43 degeneration (3,6). The appearance of further sexually antagonistic mutations can restart the
44 process and select for new non-recombining regions, creating sex chromosome "strata" of
45 different ages (7–10). Eventually, this suppression of recombination can extend to most of the
46 chromosome, leading to gene-poor, mostly heterochromatic sex chromosomes such as the Y
47 chromosome of mammals (11).

48 The loss of one gene copy on the Y/W is predicted to result in a two-fold reduction of expression
49 in the heterogametic sex, as gene expression is correlated to gene copy number (12). This can
50 cause imbalances in gene networks composed of both X/Z-linked and autosomal genes (13).
51 Such imbalances can drive the appearance of dosage compensation mechanisms, which target
52 X/Z chromosomes and regulate their expression to restore optimal dosage (14,15). While X/Z
53 upregulation in the heterogametic sex is required to re-establish balanced levels of expression,
54 global downregulation in the homogametic sex is also observed (e.g. X-inactivation in
55 mammals). Ohno suggested a two-step mechanism, in which the initial upregulation of
56 expression is not sex-specific. This leads to an excess of dosage in the homogametic sex, and
57 secondarily selects for further repressing mechanisms ("Ohno's hypothesis" of dosage
58 compensation, (14)). How relevant this model is to the evolution of mammalian dosage

59 compensation is still under debate (e.g. 16,18,19,21,22). Independent of the underlying
60 mechanisms, balanced gene expression between males and females in species with
61 differentiated sex chromosomes was used as diagnostic of a chromosome-wide (also referred to
62 as “global”, or “complete”) mechanism of dosage compensation in many different clades (21–
63 23).

64 In ZW systems, the loss of genes on the sex-specific W chromosome is generally accompanied
65 by unequal expression levels of the Z-chromosome between ZZ males and ZW females, as well
66 as reduced expression of the Z relative to the autosomes in females. This has generally been
67 interpreted as a lack of chromosome-wide dosage compensation (also referred to as “partial”, or
68 “incomplete”), with individual dosage-sensitive genes being independently regulated instead.
69 Incomplete dosage compensation was described in a wide range of species, including birds
70 (24–27), fishes (28) and snakes (9). So far, Lepidoptera are the only exception to this
71 observation (23,29). Why many ZW systems should fail to acquire a global mechanism of
72 dosage compensation is not entirely clear, though several and non-mutually exclusive
73 hypotheses have been put forward (see Discussion, and Gu and Walters (16) for a review).
74 Another possibility is that the male-bias of the Z is instead caused by an accumulation of genes
75 with male functions due to the male-biased transmission of the Z, which may favor the fixation of
76 sexually antagonistic male-beneficial mutations on this chromosome.

77 While the direct comparison of male and female expression of X/Z-linked and autosomal genes
78 has provided an overview of dosage compensation in many clades, it suffers from several
79 drawbacks (23). First, chromosome-wide dosage compensation can lead to strongly sex-biased
80 expression, if only the initial up-regulation of expression in both sexes has occurred (but not the
81 secondary down-regulation of the Ohno hypothesis (14)). This has been suggested for the flour
82 beetle (30) and for the young sex chromosomes of the threespine sticklebacks (31). Biases in
83 expression levels between sexes and/or chromosomes may also have been present ancestrally,
84 before the present sex-chromosomes evolved, and using a proxy for ancestral expression can

85 yield insights into the direct consequences of sex-linkage (17,32,33). Finally, the vast majority of
86 studies relied only on microarray or RNA-seq data and did not consider any post-transcriptional
87 regulation that might affect gene dosage at the protein level, but not at the transcript level
88 (whereas protein dosage is in most cases the functionally relevant measure). For instance, a
89 proteomic analysis in birds found that several genes appeared to be partially equalized at the
90 protein level despite being strongly male-biased at the transcript level (34). In humans, post-
91 transcriptional regulation does not appear to play a major role in dosage compensation (35).

92 Here we combine comparative genomics, transcriptomics and quantitative proteomics to obtain
93 a complete overview of the evolution of gene dosage on the Z-chromosome of parasites of the
94 genus *Schistosoma*. Schistosomes are a group of blood parasites that can cause
95 schistosomiasis in humans (36). Their complex life cycle is characterized by a phase of clonal
96 multiplication in an intermediate mollusk host, and a phase of sexual reproduction in the final
97 warm-blooded host. Unlike the other 20,000 species of hermaphroditic platyhelminths,
98 schistosomes have separate sexes: sexual reproduction occurs immediately after the male and
99 female primary development in their definitive host, and mating is compulsory for the sexual
100 maturation of females (37,38). Sex determination is genetic, and relies on a pair of
101 cytogenetically well-differentiated ZW chromosomes (39). All schistosomes are thought to share
102 the same ancestral pair of ZW sex chromosomes, but differences in their morphology and in the
103 extent of heterochromatization of the W suggest that different strata were acquired
104 independently by different lineages (39,40).

105 The model blood fluke *Schistosoma mansoni* was one of the first ZW clades to be evaluated for
106 the presence of global dosage compensation, through the comparison of male and female
107 microarray data derived from several tissues (41). It showed reduced expression of Z-linked
108 genes in females relative (i) to the autosomes and (ii) to males, consistent with a lack of
109 chromosome-wide dosage compensation. Interestingly, the reduction of Z-expression in females
110 was less than two-fold, and the Z:autosome ratio of expression was slightly, but consistently,

111 greater than one in males. Our combined genomic, transcriptomic, and proteomic approaches
112 allow us to fully probe the evolution of the male-biased expression of the Z, and suggests a
113 more complex scenario than previously proposed. We discuss this in light of the different
114 hypotheses put forward to account for the evolution of gene dosage on Z chromosomes.

115

116 **RESULTS**

117 1. Genomic differentiation of ZW sex chromosomes in Asian and African lineages.

118 The difference in morphology of the ZW pair in African and Asian schistosomes suggests that
119 the two lineages may differ in their gene content (39). We compared the gene content of the Z-
120 chromosomes of three different species: *S. mansoni* and *Schistosoma haematobium*, which
121 belong to African schistosomes, and *Schistosoma japonicum*, an Asian schistosome (Figure 1).
122 We first identified syntenic blocks between the *S. mansoni* genome and the *S. haematobium*
123 and *S. japonicum* scaffolds. To this end, we mapped all *S. mansoni* protein coding sequences to
124 the genome assemblies of the two other species and selected only the hits with the highest
125 scores, yielding 9,504 *S. mansoni* / *S. haematobium* orthologs and 8,555 *S. mansoni* / *S.*
126 *japonicum* orthologs (Table 1). Scaffolds were then assigned to one of the *S. mansoni*
127 chromosomes, based on their ortholog content (Figure 1-source data 1).

128 We further performed a comparative coverage analysis to define the Z-specific regions of the
129 three species. Z-derived sequences are expected to display half the genomic coverage in ZW
130 females as in ZZ males, and as the autosomes. We thus mapped male and female genomic
131 reads (or only female reads in the case of *S. haematobium*) to the reference genome of each
132 species (42–45). Publicly available raw reads were used for *S. mansoni* and *S. haematobium*
133 (Wellcome Trust Sanger Institute Bioprojects PREJB2320 and PREJB2425), while male and
134 female *S. japonicum* were sequenced for this study. We then estimated the per base genomic
135 coverage. Median coverage values were 18.40 and 18.99 for *S. mansoni* male and female
136 libraries; 23.5 and 7.43 for *S. haematobium* female#1 and female#2 libraries; 23.77 and 20.53

137 for *S. japonicum* male and female libraries. Z-specific genomic regions were defined by a
138 maximum value of the female:male ratio of coverage (*S. mansoni*: $\log_2(\text{female:male})=-0.4$; *S.*
139 *japonicum*: $\log_2(\text{female:male})=-0.84$), or a maximum value of female coverage (*S.*
140 *haematobium*: $\log_2(\text{female})=4.41$). Details of how these cutoff values were obtained are
141 provided in the Methods and Appendix 1. This analysis resulted in 285 newly described Z-
142 specific genes in *S. mansoni* that were previously located on 19 unplaced scaffolds longer than
143 50kb, and to a refined pseudoautosomal/Z-specific structure of the published ZW linkage group
144 (42,43) (Figure 1-source data 2). It further allowed us to define 379 Z-specific scaffolds
145 (containing 1,409 annotated genes with orthologs in *S. mansoni*) in *S. haematobium* (Figure 1-
146 source data 3 for exhaustive list) and 461 Z-specific scaffolds (containing 706 annotated
147 orthologs) in *S. japonicum* (Figure 1-source data 4 for exhaustive list).

148 While the content of the Z was largely shared between the African *S. mansoni* and *S.*
149 *haematobium* (Table1, Figure 1), large differences were found between the African and Asian
150 lineages: only 476 Z-specific genes were shared by *S. mansoni* and *S. japonicum*, while 306
151 were only Z-specific in *S. mansoni* and 137 only in *S. japonicum* (Table1). Of all these Z-specific
152 genes, 613 were already mapped to the *S. mansoni* ZW linkage group (42,43) and, when
153 plotted along the Z-chromosome, outlined three different evolutionary strata: one shared
154 ancestral stratum (S0: 367 genes) and two lineage-specific strata (S1mans, specific to the
155 African schistosomes, with 180 genes; and S1jap, specific to *S. japonicum*, with 66 genes)
156 (Table 1, Figure 1, and Figure 1-source data 1). The presence of pseudoautosomal regions
157 throughout the S0 (Figure 1) is likely due to errors in the genome assembly. All further analyses
158 were run using all newly identified Z-specific genes, but hold when only Z-specific genes that
159 were previously mapped to the ZW linkage group are considered (Appendix 1).

160

161 **Table 1. Number of orthologs assigned as Z-linked and autosomal in *S. mansoni*, *S.***
162 ***haematobium* and *S. japonicum***, based on the female:male (or female for *S. haematobium*)
163 coverage patterns.

164

		<i>SCHISTOSOMA JAPONICUM</i>				<i>SCHISTOSOMA HAEMATOBIIUM</i>			
Categories		Z-specific	Autosomal	Ambiguous	Not covered	Z-specific	Autosomal	Ambiguous	Not covered
<i>SCHISTOSOMA MANSONI</i>	Z-specific	476 (<i>S0</i>)	306 (<i>S1mans</i>)	20	3	847	36	23	10
	Autosomal	137 (<i>S1jap</i>)	7,062	91	13	216	7,462	262	105
	Excluded	57	383	4	3	105	411	20	7
	Orthologs total	8,555				9,504			

165

166

167 2. Consistent patterns of expression in *S. mansoni* and *S. japonicum*.

168 In order to test for dosage compensation, the median expression of Z-specific genes in ZW
169 females can be compared to the median autosomal expression (Z:AA ratio) and/or to the Z-
170 specific gene expression in ZZ males (F:M ratio). In females, a Z:AA or F:M ratio of ~1 supports
171 global dosage compensation, while a ratio between 0.5 and 1 suggests partial or local dosage
172 compensation. We performed this analysis in *S. mansoni* and in *S. japonicum*, using publicly
173 available RNA-seq reads derived from a sexually undifferentiated stage (schistosomula, (46,47))
174 and a sexually mature stage (adults, Wellcome Trust Sanger Institute Bioproject [PRJEB1237](#),
175 (47)). The inclusion of a sexually immature stage is important, as much of the expression
176 obtained from adults will necessarily come from their well-developed gonads. Sex-linked genes
177 are often sex-biased in the germline, even in organisms that have chromosome-wide dosage
178 compensation (e.g. due to sex-chromosome inactivation during gametogenesis), and the
179 inclusion of gonad expression has led to inconsistent assessments of the status of dosage
180 compensation in other clades (16,29). Reads were mapped to their respective genomes, and
181 expression values in Reads Per Kilobase Million (RPKM) were calculated for each gene (Figure

182 2, Figure 2-source data 1 and 2); only genes with a minimum RPKM of 1 in both sexes were
183 considered.

184 We consistently observed a strong male bias in the expression of Z-specific genes in both
185 stages and species (F:M ratio between 0.58 and 0.69, Figure 2 and Supplementary File 1),
186 consistent with local or incomplete dosage compensation. While this was generally supported
187 by the lower expression levels of Z-specific genes in females when compared to the autosomes
188 (Z:AA ratio between 0.73 and 0.85; Figure 2, Supplementary File 1), this difference was only
189 apparent for some filtering procedures (Figure 2-figure supplement 1 to Figure 2-figure
190 supplement 14), and even then was not sufficient to fully account for the strong male-bias of the
191 Z. Instead, the higher expression of the male Z in both stages and species (ZZ:AA ratio between
192 1.25 and 1.46, Supplementary File 1) appeared to also contribute to the male-bias of Z-linked
193 genes. These patterns were qualitatively robust to changes in the methods used to estimate
194 expression (RPKM or TPM [Transcripts Per Kilobase Million]), in the filtering procedure
195 (RPKM>0, RPKM>1, TPM>0 or TPM>1), and when only genes that were previously mapped to
196 the ZW linkage group were considered. These analyses were further performed independently
197 in the S0, S1mans and S1jap strata, which showed no significant difference in the extent of their
198 male bias. All the resulting plots are shown in Figure 2-figure supplement 1 to Figure 2-figure
199 supplement 14. Finally, Z-specific genes were found to be male-biased even when only genes
200 with broad expression were considered (RPKM>1 and RPKM>3 in all samples, and when genes
201 with strong sex-biases in expression were excluded (M:F>2 or F:M>2, Figure 2-figure
202 supplement 15 and Figure 2-figure supplement 16, I-L panels), confirming that this pattern does
203 not appear to be driven simply by the presence of genes with sex-specific functions on the Z-
204 chromosome. No further influence of known protein-protein interactions was detected (Figure 2-
205 figure supplement 17, Appendix 1).

206

207

208 3. Convergent upregulation of the Z in both sexes

209 The previous patterns are consistent with an upregulation of the Z-chromosome in both sexes
210 after the degeneration of the W-specific region, and could represent the intermediate step in the
211 evolution of dosage compensation originally postulated by Ohno. However, they could also be
212 due to high expression of the ancestral proto-Z in both sexes, before sex chromosome
213 divergence. To exclude this, we identified one-to-one orthologs between genes annotated in
214 both species using a reciprocal best hit approach (7,382 orthologs, Figure 3-source data 1). All
215 genes that were classified as Z-specific in one species but as autosomal in the other were
216 considered to be part of the S1 strata (S1jap if they were Z-specific in *S. japonicum* or S1man if
217 they were Z-specific in *S. mansoni*). We then used the pseudoautosomal expression of these
218 lineage-specific strata as a proxy for the ancestral level of expression. For instance, in *S.*
219 *japonicum*, we estimated the S1jap:AA ratio, after normalizing the expression data by their
220 respective (pseudo)autosomal level in *S. mansoni* (Figure 3A and 3B). The reversed analysis
221 was performed for S1mans (Figure 3C and 3D).

222 Figure 3 confirms that the male-biased expression of Z-specific genes is a consequence of their
223 sex-linkage, and that the Z-chromosome has become under-expressed in females relative to the
224 ancestral expression. However, a full two-fold reduction in female expression is not observed,
225 consistent with partial upregulation, and/or full upregulation of a subset of dosage-sensitive
226 genes (Z:AA ranging from 0.68 to 0.83, Supplementary File 1)(48,49). Figure 3 also generally
227 supports an increase in expression in males (ZZ:AA ranging from 0.98 to 1.35, Supplementary
228 File 1). Male adults of *S. japonicum* are the exception, with a ZZ:AA of 0.98. However, given
229 that an excess of expression is observed when (i) we do not take into account the ancestral
230 expression (Figure 2), (ii) we focus on genes previously mapped to the ZW pair (Figure 2-figure
231 supplement 3, 4, 10 and 11), and (iii) we consider the schistosomula stage (with or without the
232 ancestral expression and independent of the classification), this is likely due to noise in the
233 sample and not to a true biological difference (only 58 genes were tested). Figure 3 shows the

234 distributions for all genes with a minimum RPKM value of 1 in males and females of both
235 species. We repeated the analysis using the same filters as before (minimum RPKM of 0, TPM
236 of 0, TPM of 1), and with a publicly available list of 1:1 orthologs (obtained from the Wormbase
237 Biomart, see Methods). The resulting plots are shown in Figure 2-figure supplement 1 to Figure
238 2-figure supplement 14, and Figure 3-figure supplement 1 and 2. Gene expression values for
239 the orthologs of each species are provided in Figure 3-source data 2 and 3.

240

241 4. Male-biased protein dosage of Z-specific genes.

242 We tested for putative post-transcriptional mechanisms by assessing the dosage compensation
243 pattern at the proteomic level in adult *S. mansoni*, using a somatic tissue (head region) as well
244 as the gonads; three replicates were used for each tissue and sex. Heads and gonads were
245 chosen as they allowed us to compare Z-specific gene dosage in tissues with widespread
246 functional sex-specificity (ovary and testis), and in a tissue where most dosage imbalances are
247 likely to be deleterious. We used a label-free quantitative mass spectrometry approach to obtain
248 a relative quantification of the protein levels in each tissue depending of the sex (Figure 4-
249 source data 1 to 5). Post-transcriptional dosage compensation mechanisms would be
250 detectable by (i) an equalization of the Z expression between sexes at the protein level (F:M
251 close to 1 for both the Z and the autosomes); (ii) a different correlation between F:M obtained
252 from mRNA and from proteins for Z-linked and autosomal genes. We used publicly available
253 head and gonad microarray data (50) as the transcriptomic reference (Figure 4-source data 6).
254 A significant and positive correlation was found between the F:M ratio derived from the
255 microarray and from the proteomic data (Figure 4), and between transcript and protein dosage
256 levels in both males and females (Figure 4-figure supplement 1 and 2), confirming the validity of
257 the comparison.

258 Similar to what was observed using RNA-seq, the expression of Z-specific genes was strongly
259 male-biased compared to that of autosomal genes in both heads (F:M of 0.68 for the Z

260 chromosome versus 0.92 for the autosomes; Figure 4A, Supplementary File 1) and gonads
261 (F:M of 0.78 versus 0.99; Figure 4B, Supplementary File 1). These F:M ratios are closer to each
262 other than in our RNA-seq analysis (Figure 2, Supplementary File 1), or than the microarray
263 data (Supplementary File 1), which could suggest a potential contribution of post-transcriptional
264 regulation to dosage equalization. However, Figure 4B shows that Z-linked and autosomal
265 genes show a similar correlation between the F:M ratios found for mRNAs and proteins ($p > 0.05$
266 with a Fisher r-to-z transformation of the correlation coefficients, Figure 4C and 4D), which
267 argues against a major role of post-transcriptional regulation to balance expression. This
268 similarity between Z-linked and autosomal genes holds when only genes with male-biased
269 expression in the microarray data are considered (Figure 4-figure supplement 3), and when the
270 transcript and protein dosage of Z-linked autosomal genes are compared within each sex
271 (Figure 4-figure supplement 1 and 2).

272

273 **DISCUSSION**

274 1. Schistosome sex chromosome evolution in the age of genomics

275 *S. mansoni*, *S. haematobium* and *S. japonicum* are the main species responsible for human
276 schistosomiasis and have been the subject of many molecular and genomic studies. Despite the
277 availability of extensive genomic and transcriptomic resources (e.g. a genome assembly at the
278 near-chromosome level for *S. mansoni* (42,43), or sex- and stage-specific transcriptomes
279 (46,51–53), many basic questions remain regarding their reproduction and biology. For
280 instance, the master sex-determining gene (and whether it is located on the W or Z) is still a
281 mystery (54,55). This is partly due to the inherent challenges of assembling genomes from
282 sequencing data, especially for regions rich in heterochromatin and repetitive sequences, such
283 as sex chromosomes. For instance, 416 scaffolds, including 3,893 genes (29% of the annotated
284 nuclear genes), are still unplaced. By basing our analysis on genomic coverage, we were able
285 to detect a further 285 Z-specific genes in *S. mansoni*; their role in sex determination can be

286 investigated further. Our comparative approach can also reduce the number of candidates, as
287 any gene involved in sex determination should in principle be found in the ancestral Z-specific
288 stratum; similar analyses in other species can in the future refine the candidate region. Another
289 advantage of basing our Z-assignment purely on coverage patterns is that our results should be
290 largely independent of potential biases in the current version of the genome. It should however
291 be noted that many genes are likely still missing from the current assembly (which has a
292 BUSCO score of 76% complete plus fragmented genes,
293 <https://parasite.wormbase.org/index.html> (56,57)), and that repeating these analyses using
294 future improved assemblies will be necessary to obtain the full set of sex-linked genes.

295 A gradient of ZW heteromorphism between schistosome species was revealed by cytogenetic
296 studies (39,58,59); in particular, African schistosomes were found to have much more extensive
297 ZW differentiation and W heterochromatinization than Asian species (60). Our results generally
298 support these cytogenetic data: we confirm the acquisition of independent evolutionary strata in
299 the sex chromosomes of *S. mansoni* and *S. japonicum*, and detect a larger number of Z-specific
300 genes in the African species (8% to 11% of all annotated orthologs, respectively, in *S. mansoni*
301 and *S. haematobium*) than in *S. japonicum* (5.5% of all annotated orthologs). Interestingly,
302 although the sex chromosomes of the African *S. mansoni* and *S. haematobium* differ
303 morphologically, they are largely similar in their gene content (Figure 1), consistent with their
304 much closer phylogenetic relationship (the median synonymous divergence between the two
305 species is around 17%, compared to 65% for *S. mansoni* / *S. japonicum*, Appendix 1). This may
306 be comparable to snakes, where ZW pairs with vastly different morphologies were all equally
307 differentiated at the genomic level (9), and highlights the contribution of other factors, such as
308 differential transposable element accumulation, to the large scale morphology of sex
309 chromosomes.

310

311 2. ZW systems and incomplete dosage compensation: gene-by-gene or partial shift?

312 ZW systems (aside from Lepidoptera) consistently show male-biased expression of the Z
313 chromosome (Gu and Walters, 2017). While female-biased expression of the X occurs in a few
314 young XY systems (16,31,57-61), well established X chromosomes generally show full
315 equalization of gene expression between the sexes. This difference has often been framed as
316 the acquisition of global mechanisms of dosage compensation, which affects the whole X/Z,
317 versus the acquisition of local compensation, in which dosage-sensitive genes become
318 individually regulated (61). Several parameters should influence this, and favor local
319 compensation in ZW systems: (i) The speed of the heterochromosome degeneration: when only
320 a few genes are lost at a time (because the region of suppressed recombination is small, or
321 because degeneration is slow), the establishment of a gene-by-gene dosage compensation may
322 be favored; on the other hand, the loss of many genes at once could favor global mechanisms
323 of dosage compensation (23,62). Since more mutations occur during spermatogenesis than
324 oogenesis, female-specific W chromosomes will generally have lower mutation and
325 degeneration rates than male-specific Ys, favoring local compensation; (ii) The effective
326 population size of Z (N_{eZ}): N_{eZ} is decreased when the variance in reproductive success of ZZ
327 males is larger than that of ZW females (e.g. in the presence of strong sexual selection). This
328 will impair the adaptive potential of the Z (63,64), such that only strongly dosage-sensitive
329 genes can become upregulated in the heterogametic sex, while the others remain
330 uncompensated; (iii) More efficient purging of mutations that are deleterious to males: strong
331 sexual selection can also increase the strength of purifying selection on males, by preventing all
332 but the fittest males from contributing to the next generations. If mutations that compensate for
333 the loss of Y/W-linked genes overexpress the X/Z copy in both sexes, they will be under
334 negative selection in the homogametic sex, and may be more efficiently selected against when
335 males are the homogametic sex (64).

336 Schistosomes are unusual among female-heterogametic clades in that they appear to have a
337 chromosome-wide upregulation of the Z in both sexes; such an increase in males was not

338 detected in birds (17) or snakes (9), even when ancestral expression was taken into account.
339 They therefore likely represent an intermediate between ZW species with true local
340 compensation, and the chromosome-wide compensation of the ZW Lepidoptera. These results
341 further show that, even if mutations that upregulate gene expression in both sexes are more
342 easily fixed on an evolving X-chromosome than on an evolving Z (64), this is not an absolute
343 barrier to the evolution of global dosage compensation. It is however still unclear why the
344 evolutionary dynamics appear to differ between schistosomes and most other ZW clades, as the
345 demographic and population genetics parameters of this group are largely unknown. The
346 observed male biased sex-ratio in adults, combined with a largely monogamous mating system
347 (65,66), may increase the reproductive variance of males and could reduce the effective
348 population size of the Z. This should also lead to stronger sexual selection in males than in
349 females (66,67), suggesting similar evolutionary dynamics as in other ZW systems. A detailed
350 characterization of the population genetics of the Z chromosome and autosomes will therefore
351 be crucial for understanding what may have driven the evolution of this unusual system.

352

353 3. The relevance of the Ohno's hypothesis in the high-throughput sequencing era

354 Ohno's hypothesis predicts that the heterochromosome is initially overexpressed in both sexes,
355 then secondarily downregulated in the homogametic sex (14). This theoretical scenario was
356 first formulated to account for the inactivation of the X in mammals. Since then, similar
357 molecular mechanisms to downregulate the X/Z chromosome have been characterized in
358 nematodes and moths (68,69). If an initial upregulation of the X did occur in both sexes, then
359 inactivation in the homogametic sex should simply restore the ancestral expression levels, a
360 hypothesis that has been tested in many empirical studies in mammals. Most of them assumed
361 that the X and autosomes must have had similar ancestral levels of expression, and simply
362 compared their expression (17,19,20,23,35,70–75). These yielded mixed results, with some (20)
363 finding reduced expression of the X, while others (e.g. (71–73)) found similar levels of

364 expression for X-linked and autosomal genes, in agreement with Ohno's predictions. Taking
365 ancestral gene expression into account, Julien et al. (17) found evidence of an Ohno-like
366 mechanism in the marsupials but not in placental mammals (17). Pessia et al. (49) recently
367 found that while individual dosage-sensitive genes do show evidence of up-regulation, the
368 majority does not. The evolution of X-inactivation may therefore have involved a complex
369 scenario under which a few dosage sensitive genes first became individually upregulated in
370 both sexes (gene-by-gene compensation), followed by the establishment of a chromosome-wide
371 mechanism to downregulate expression in females (global compensation) (48,49).

372 Our results, which consider ancestral expression and do not indicate a major influence of post-
373 transcriptional regulation, suggest a scenario closer to Ohno's original hypothesis, with the male
374 Z showing a consistent increase in expression. A similar pattern was observed in *Tribolium*
375 *castaneum* (Coleoptera, (30)), where the female X was found to be over-expressed relative to
376 the autosomes, and to the male X-chromosome. However, an RNA-seq analysis in the same
377 species did not detect this (76), so it is at this point unclear whether it truly represents an
378 example of Ohno's model in action. The youngest evolutionary stratum of the young XY pair of
379 threespine sticklebacks also shows overexpression in females (31), even when ancestral
380 expression is accounted for (77). However, the interpretation of these patterns is complicated by
381 the fact that such an overexpression is also detected for the pseudoautosomal region, and that
382 the oldest evolutionary stratum appears to lack dosage compensation altogether. Schistosomes
383 may therefore not only represent an ideal system in which to investigate the evolution of dosage
384 compensation in a ZW system, but also an unparalleled system for understanding the relevance
385 of the model and predictions originally made by Ohno.

386

387

388 **MATERIALS AND METHODS**

389 A detailed description of the computational analyses, as well as all the scripts that were used,
390 are provided in Appendix 1.

391

392 **DNA sequencing of *S. japonicum* males and females**

393 Male and female worms preserved in ethanol of *S. japonicum* were provided by Lu Dabing from
394 Soochow University (Suzhou, China). DNA was extracted from 28 pooled males and 33 pooled
395 females. The worms were lysed using the Tissue Lyser II kit (QIAGEN) and DNA was isolated
396 using the DNeasy Blood & Tissue Kit (QIAGEN). DNA was then sheared with a AFA™ with
397 Covaris Focused-ultrasonicator. Library preparation and sequencing (HiSeq 2500 v4 Illumina,
398 125bp paired-end reads) were performed at the Vienna Biocenter Next Generation sequencing
399 facility (Austria). Reads have been deposited at the NCBI Short Reads Archive under accession
400 number SRP135770.

401

402 **Publicly available DNA reads and genome assemblies**

403 *S. mansoni* DNA libraries (100bp paired-end reads) were downloaded from the NCBI Sequence
404 Read Archive, under the accession numbers ERR562989 (~6,000 male pooled cercariae) and
405 ERR562990 (~6,000 female pooled cercariae). Female *S. haematobium* DNA libraries (80bp
406 paired-end reads) were found under accession numbers ERR037800 and ERR036251. No male
407 *S. haematobium* library was available. The reference genome assemblies of *S. mansoni*
408 ([PRJEA36577](#), (78)), *S. haematobium* ([PRJNA78265](#), (44)) and *S. japonicum* ([PRJEA34885](#),
409 (45)) were obtained from the WormBase parasite database
410 (<https://parasite.wormbase.org/index.html>, (56,57)).

411

412

413

414 **Orthology and assignment to the *S. mansoni* chromosomes**

415 *S. mansoni* coding sequences and their respective chromosomal locations were obtained from
416 the WormBase Parasite database (<https://parasite.wormbase.org/index.html>, (56,57)). This
417 gene set was mapped to the *S. haematobium* and *S. japonicum* genome assemblies using Blat
418 (79) with a translated query and dataset (-dnax option), and a minimum mapping score of 50;
419 only the genome location with the best score was kept for each. When more than one gene
420 overlapped by more than 20 base pairs, only the gene that had the highest mapping score was
421 kept. Finally, each scaffold was assigned to one of the *S. mansoni* chromosomes, depending on
422 the majority location of the genes that mapped to it, or on their total mapping scores if the same
423 number of genes mapped to two separate chromosomes. The final chromosomal assignments
424 are provided in Figure 1-source data 1.

425

426 **DNA read mapping and estimation of genomic coverage**

427 For the *S. japonicum* DNA reads, adaptors were removed using Cutadapt (v1.9.1, (80)) and the
428 quality of the reads was assessed using FastQC (v0.11.2,
429 <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>); no further quality trimming was
430 deemed necessary. For the *S. mansoni* and *S. haematobium* reads, potential adaptors were
431 systematically removed, and reads were trimmed and filtered depending on their quality, using
432 Trimmomatic (v0.36, (81)). The resulting read libraries of each species were mapped separately
433 to their reference genomes using Bowtie2 (--end-to-end --sensitive mode, v2.2.9, (82)). The
434 resulting alignments were filtered to keep only uniquely mapped reads, and the male and female
435 coverages were estimated from the filtered SAM files with SOAPcoverage (v2.7.7.,
436 <http://soap.genomics.org.cn/index.html>). Coverage values were calculated for each scaffold in
437 *S. haematobium* and *S. japonicum*, and for each 10kb non-overlapping window in *S. mansoni*.
438 The coverage values for each library are provided in Figure 1-source data 3 and 4.

439

440 **Detection of Z-specific sequences**

441 For each species, we calculated the $\log_2(\text{female}:\text{male})$ coverage of each scaffold or, in the case
442 of *S. mansoni*, of each 10Kb window along the genome. Since only female DNA data was
443 available for *S. haematobium*, the $\log_2(\text{female}_1+\text{female}_2)$ was used instead for this species.
444 In order to determine the 95% and 99% percentile of $\log_2(\text{female}:\text{male})$ of Z-linked sequences,
445 which we use as cut-off values for assignment to Z-specific regions, we first excluded
446 scaffolds/windows that fit an autosomal profile. To do so, the 1st and 5th percentile of
447 $\log_2(\text{female}:\text{male})$ were estimated using all 10kb windows found on the annotated autosomes of
448 *S. mansoni* (78); in *S. haematobium* and *S. japonicum*, all scaffolds that mapped to the *S.*
449 *mansoni* autosomes were used for this purpose.
450 By plotting the distribution of $\log_2(\text{Female}:\text{Male})$ for the scaffolds/windows that map to each
451 chromosome (Appendix 1), we determined that: (i) for *S. mansoni* the 1st percentile
452 ($\log_2(\text{female}:\text{male})=-0.26$) discriminates effectively between autosomal and Z-specific windows;
453 (ii) for *S. haematobium* and *S. japonicum*, which have noisier coverage, the 1st percentile
454 included a significant fraction of Z-derived genes, and the 5st percentile was used instead
455 (respectively $\log_2(\text{Female})=4.57$ and $\log_2(\text{female}:\text{male})=-0.40$). All scaffolds with higher
456 $\log_2(\text{female}:\text{male})$ or $\log_2(\text{female})$ were excluded.
457 The 95th and 99th quantiles of coverage were then calculated for the remaining, putatively Z-
458 linked, sequences. By plotting all the coverage values along the *S. mansoni* Z-chromosome
459 (Appendix 1 and Figure 1), we determined that: (i) for *S. mansoni* and *S. haematobium*, the 95th
460 quantile ($\log_2(\text{female}:\text{male})=-0.4$ and $\log_2(\text{female})=4.41$, respectively) was an effective cut-off
461 for discriminating pseudoautosomal and Z-specific sequences; (ii) for *S. japonicum*, using the
462 95th percentile lead to the exclusion of many genes in Z-specific regions, and the 99st percentile
463 ($\log_2(\text{F}:\text{M})=-0.84$) was used instead. The Z-specific or autosome assignment was finally
464 attributed as follows: (i) in *S. mansoni*, windows were classified as Z-linked if they displayed
465 $\log_2(\text{F}:\text{M})<-0.4$ and as autosomal if $\log_2(\text{F}:\text{M})>-0.4$; (ii) for *S. haematobium*, scaffolds with
466 $\log_2(\text{femalesum})<4.41$ were classified as Z-linked, scaffolds with $\log_2(\text{femalesum})>4.57$ as

467 autosomal, and all others as ambiguous; (iii) for *S. japonicum*, scaffolds with $\log_2(F:M) < -0.84$
468 were classified as Z-linked, scaffolds with $\log_2(F:M) > -0.40$ as autosomal, and all others as
469 ambiguous. For *S. haematobium* and *S. japonicum*, we considered only scaffolds with at least a
470 coverage of 1 in each library (n.a.). In *S. mansoni*, unplaced scaffolds shorter than 50kb were
471 excluded; five consecutive 10kb windows with consistent coverage patterns were required for a
472 region to be classified as either Z-specific or autosomal. Smaller regions, as well as the two
473 10Kb windows surrounding them, were excluded (see Appendix 1). The final classifications for
474 the three species are provided in Figure 1-source data 2, 3, and 4, and summarized for
475 orthologs in Figure 1-source data 1.

476

477 **Definition of Z-specific strata in *S. mansoni* and *S. japonicum***

478 The Z-specific gene content of *S. mansoni* and *S. japonicum* was compared in order to define Z-
479 chromosome strata. Genes that were located on Z-specific scaffolds/windows in both species
480 were assigned to the shared stratum "S0". Genes that were assigned to Z-specific regions in
481 one species but not in the other were assigned to lineage-specific strata: "S1mans" genes were
482 Z-specific in *S. mansoni* and autosomal in *S. japonicum*, while "S1jap" genes were Z-specific in
483 *S. japonicum* and autosomal in *S. mansoni*. While the main figures consider all the genes that
484 were classified as Z-linked or autosomal based on coverage (referred to as the "exhaustive
485 classification" in Appendix 1 and Figures), independent of their original genomic location, we
486 repeated the analyses using only the Z-specific genes that were already assigned to the ZW
487 linkage map of *S. mansoni* (the "stringent classification" in Appendix 1). All genes belonging to
488 the categories "excluded", "ambiguous", "n.a." or that did not have orthologs on *S. japonicum*
489 scaffolds were not further considered (Table 1). "PSA_shared" and "Aut_shared" are common to
490 the two classifications and correspond to genes that were classified as autosomal in both
491 species using coverage, and that were previously mapped to the ZW linkage group or to the
492 autosomes of *S. mansoni*, respectively (78).

493

494 **Publicly available RNA reads and estimation of gene expression**

495 *S. mansoni* and *S. japonicum* RNA-seq libraries were obtained from SRA (NCBI). Accession
496 numbers are: *S. mansoni* adult females: ERR506076, ERR506083, ERR506084; *S. mansoni*
497 adult males: ERR506088, ERR506082, ERR506090; *S. mansoni* schistosomula females:
498 SRR3223443, SRR3223444; *S. mansoni* schistosomula males: SRR3223428, SRR3223429 ;
499 *S.japonicum* adult females: SRR4296944, SRR4296942, SRR4296940; *S.japonicum* adult
500 males: SRR4296945, SRR4296943, SRR4296941; *S. japonicum* schistosomula females:
501 SRR4279833, SRR4279491, SRR4267990; *S. japonicum* schistosomula males: SRR4279840,
502 SRR4279496, SRR4267991. Raw reads were cleaned using trimmomatic (v 0.36,(81)), and the
503 quality of the resulting reads was assessed using FastQC (v0.11.2,
504 <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Reads were mapped to their
505 respective reference genomes used Tophat2 (83). Read counts were obtained with Htseq (84)
506 and expression values (in Reads Per Kilobase of transcript per Millionmapped reads, RPKM)
507 were calculated for each gene in each of the RNA-seq libraries (Figure 2-source data 1). TPM
508 (Transcripts Per Kilobase Million) values were also calculated using Kallisto (85) against set of
509 coding sequences of the respective species. All expression values are provided in Figure 3-
510 source data 2 and 3. A Loess Normalization (R library Affy) was performed on the
511 schistosomulum data and on the adult data separately and all analyses were performed using
512 different thresholds (RPKM>0, RPKM>1, TPM>0 or TPM>1 in all the libraries of the studied
513 stage). The Loess normalization was applied to all conditions at once when we filtered for
514 minimum expression in all stages and sexes (RPKM>1 and RPKM>3, Figure 2-figure
515 supplement 15 -17, Figure 3-figure supplement 1 and 2). Correlation analyses were performed
516 for each developmental stage, considering libraries from both males and females, and both
517 species. As shown in Appendix 1, two *S. mansoni* libraries (ERR506076 and ERR506082) were
518 not well correlated with the other samples, and were excluded from our study. Expression

519 values were averaged for each stage and sex. The significance of differences between medians
520 of expression was tested with Wilcoxon rank sum tests with continuity correction.

521

522 **Detection of *S. mansoni* and *S. japonicum* one-to-one orthologs**

523 *S. japonicum* coding DNA sequences and their respective location on the genome scaffolds
524 were obtained from the WormBase Parasite database
525 (<https://parasite.wormbase.org/index.html>, (56,57)). The *S. mansoni* set of coding sequences
526 (see above) was mapped to the *S. japonicum* gene set using Blat (79) with a translated query
527 and dataset (-dnax option), and a minimum mapping score of 50; only reciprocal best hits were
528 kept. This reciprocal best hit ortholog list is provided in Figure 3-source data 1 and 2. A second
529 list of orthologs was obtained from the Biomart of WormBase Parasite, excluding paralogues,
530 and requiring a gene stable ID for both *S. mansoni* (PRJEA36577) and *S. japonicum*
531 (PRJEA34885) (in Figure 3-source data 1 and 3). In subsequent transcriptomic analyses each
532 list was used independently to ensure that the results were independent of the method used to
533 assign orthology.

534

535 **Microarray analysis**

536 Microarray data for male and female heads and gonads (50) were obtained from the Gene
537 Expression Omnibus (GEO) database (NCBI,
538 <ftp://ftp.ncbi.nlm.nih.gov/geo/series/GSE23nnn/GSE23942/matrix/>). A Loess Normalization (R
539 library Affy) was performed on the head and gonad data separately. When different probes
540 corresponded to one gene, their expression values were averaged. Gene expression was
541 available for a total of 6,925 genes. The normalized data are available in Figure 4-source data
542 6.

543 **Protein extraction and proteomic analysis**

544 Male and female adult *S. mansoni* gonads were sampled using the whole-organ isolation
545 approach described previously (86). Twenty ovaries and 20 testes, as well as five heads of each
546 sex, were sampled, in triplicate, from paired worms. All biological samples were resuspended in
547 Laemmli buffer, denatured and frozen at -20°C until further processing. Subsequent protein
548 treatment and analyses were performed at the “EDyP-service” – proteomic platform (Grenoble,
549 France). The extracted proteins were digested by modified trypsin (Promega, sequencing
550 grade). The resulting peptides were analyzed by nanoLC-MS/MS (Ultimate 3000 RSLCnano
551 system coupled to Q-Exactive Plus, both Thermo Fisher Scientific). Separation was performed
552 on a 75µm x 250mm C18 column (ReproSil-Pur 120 C18-AQ 1.9µm, Dr. Maisch GmbH) after a
553 pre-concentration and desalting step on a 300 µm × 5 mm C18 precolumn (Pepmap, Thermo
554 Fisher Scientific).

555 MS and MS² data were acquired using Xcalibur (Thermo Fisher Scientific). Full-scan (MS)
556 spectra were obtained from 400 to 1600m/z at a 70.000 resolution (200m/z). For each full-scan,
557 the most intense ions (top 10) were fragmented in MS² using high-energy collisional dissociation
558 (HCD). The obtained data were processed in MaxQuant 1.5.8.3 against the database loaded
559 from Uniprot (taxonomy *Schistosoma mansoni*, October 26th, 2017, 13.521 entries) and the
560 MaxQuant embedded database of frequently observed contaminants. The resulted iBAQ values
561 (87) were loaded into ProStaR (88) for statistical analysis. Contaminant and reverse proteins
562 were removed and only the proteins with three quantified values in at least one condition were
563 taken into account.

564 After log₂ transformation, the iBAQ values were normalized by overall-wise median centering
565 followed by imputation using detQuantile algorithm with quantile set to 1 (Figure 4-source data 1
566 and 2). An alternative set of data without imputed values is available in Figure 4-source data 3
567 and 4. 1,988 and 2,750 *Schistosoma mansoni* proteins were identified in heads and in gonads,
568 respectively (See Figure 4-source data 1 and 2 for statistical testing of differential abundance
569 between male and female samples). Among them, 1,741 and 2,516 could be attributed

570 unambiguously to a *Schistosoma mansoni* gene, and were represented by more than one
571 peptide; these were subsequently analyzed (See Figure 4-source data 5).

572

573 **Data availability**

574 DNA reads of male and female *S. japonicum* will be released upon publication on the SRA
575 database under study number SRP135770. Sex and tissue specific *S. mansoni* label-free
576 proteomic data are provided in Figure 4-source data 1 to 4.

577

578 **Code availability**

579 The full bioinformatic pipeline used in this study is provided in Appendix 1.

580

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591

592 **CONFLICT OF INTEREST STATEMENT**

593 The authors declare that they have no conflict of interest.

594

595 **LIST OF FIGURES**

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603

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607

608 **SUPPLEMENTARY FILES**

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610 autosomal genes (Z:A), and comparison of expression between males and females (F:M) in the
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614

615 **SUPPLEMENTARY FIGURES**

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625 **Figure 3 - figure supplement 1.** Z-linked and autosomal female:male ratio of gene expression,
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628 males, normalized by ancestral autosomal expression, and using different filters.

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630 and protein levels in male heads and gonads.

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635 using only genes with male-biased expression in the microarray data.

636

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654

655

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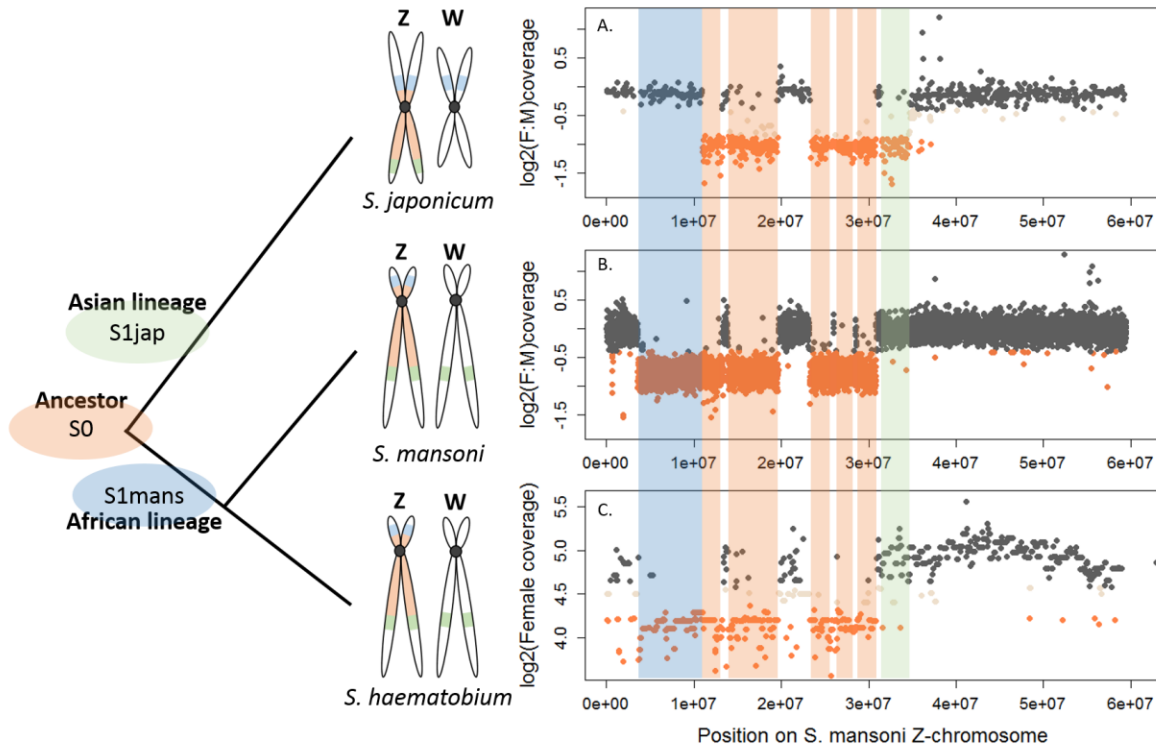
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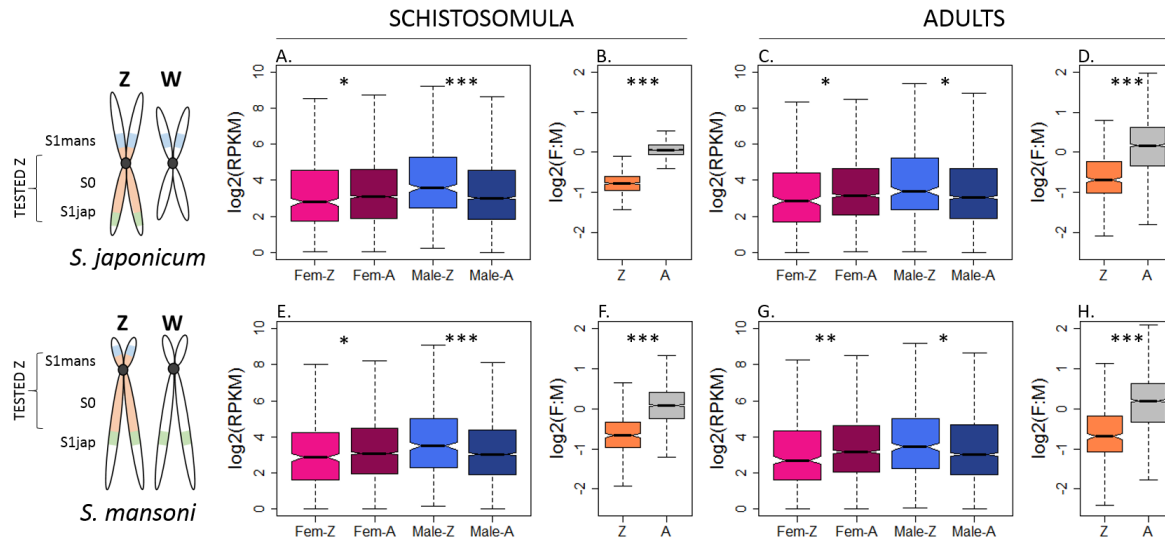
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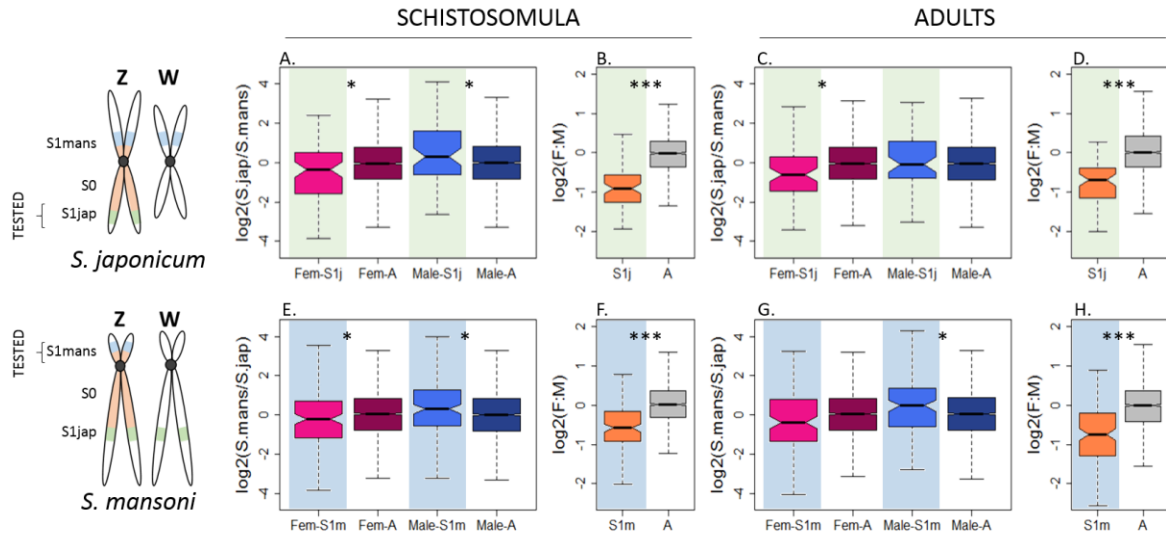
Figure 1. Shared and lineage-specific evolutionary strata on the Z-chromosome.

The phylogeny of the three species is represented on the left. The Female:Male (F:M) ratio of coverage (y-axis) along the Z-chromosome of *S. mansoni* (x-axis) is shown for *S. japonicum* scaffolds (A) and *S. mansoni* 10kb windows (B). Female coverage is shown for *S. haematobium* scaffolds (C). All species share an ancestral Z-linked stratum S0 (marked in orange). The stratum S1jap (in green) is specific to the Asian lineage represented by *S. japonicum*. The stratum S1mans (in blue) is specific to the African lineage, represented by *S. mansoni* and *S. haematobium*. Dot color is attributed depending on the window/scaffold location within each species: Z-specific regions in orange, pseudoautosomal regions in grey, and ambiguous regions in beige.



881
 882 **Figure 2. Patterns of expression on the Z and autosomes of *S. japonicum* and *S. mansoni***
 883 **species.**

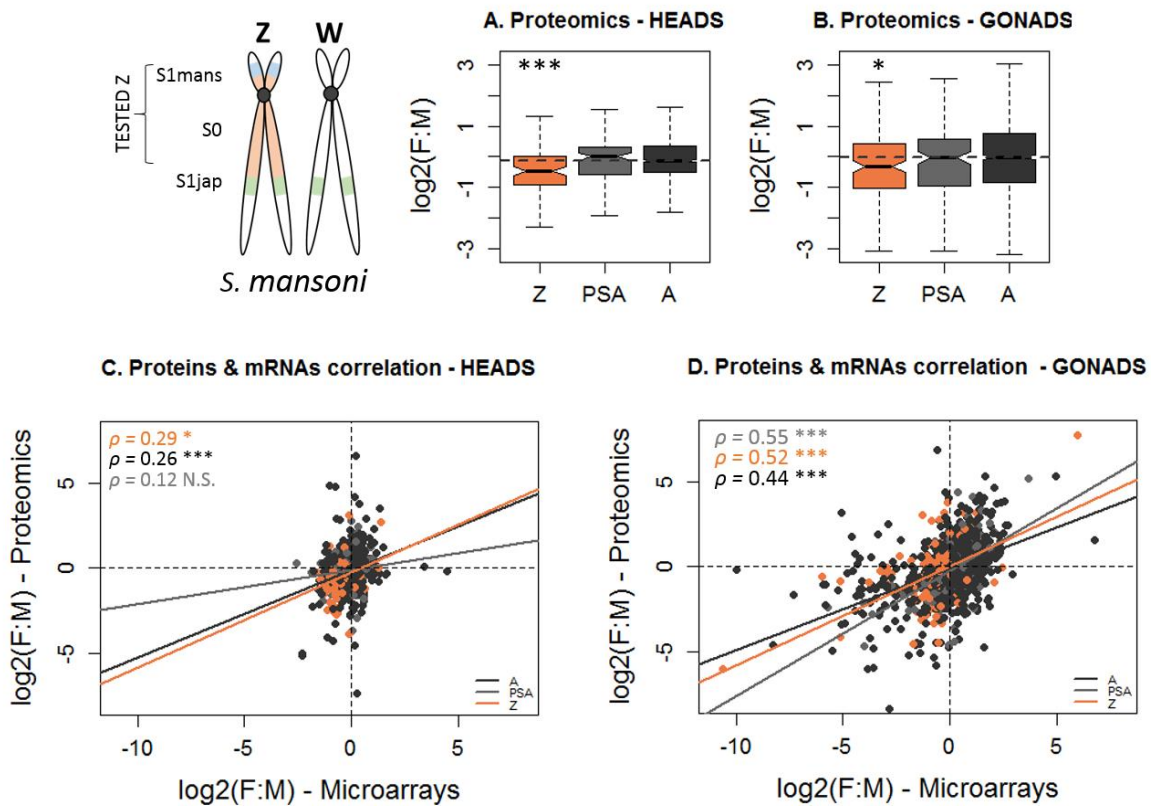
884 Z-linked and autosomal gene expression patterns are shown for *S. japonicum* (A-D) and *S.*
 885 *mansoni* (E-H), in non-differentiated schistosomula and sexually mature adults. In panels A, C,
 886 E, and G, Fem-Z and Male-Z refer to the expression of Z-linked genes in females and males,
 887 respectively, and Fem-A and Male-A to the expression of the autosomal genes in females and
 888 males. In panels B, D, F, and H, Z refers to Z-linked genes and A to autosomal genes. In all
 889 conditions, a strong male bias is observed for the Z-linked genes (B, D, F, H). This male-biased
 890 expression of the Z-linked genes is accompanied by both an under-expression in females and
 891 an over-expression in males, compared to the level of autosomal expression (A, C, E, G). The
 892 level of significance of each comparison (Wilcoxon rank sum test with continuity correction) is
 893 indicated by asterisks: **P-value*<0.05, ***P-value*<0.001, ****P-value*<0.0001.
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Figure 3. Convergent changes in the expression of Z-linked genes in *S. japonicum* and *S. mansoni* after sex chromosome differentiation.

The Z-linked and autosomal gene expression patterns (normalized by ancestral pseudoautosomal or autosomal expression, to show changes since the appearance of the two S1 strata) are shown for S1jap in *S. japonicum* (A-D) and S1mans in *S. mansoni* (E-F), Fem-S1j and Male-S1j refer to the normalized expression levels of genes in the stratum S1jap in females and males, Fem-S1m and Male-S1m refer to the normalized expression levels in S1mans, and Fem-A and Male-A refer to the normalized expression levels of autosomal genes in females and in males, respectively. The level of significance of each comparison (Wilcoxon rank sum test with continuity correction) is denoted with asterisks: **P*-value<0.05, ***P*-value<0.001, ****P*-value<0.0001.



910

911 **Figure 4. Transcript and protein dosage of Z-linked and autosomal genes in *S. mansoni***912 **heads and gonads. (A), (B)** The female:male (F:M) ratio of protein dosage in *S. mansoni*913 heads **(A)** and gonads **(B)**, for Z-specific (Z), pseudoautosomal (PSA) or autosomal (A) genes.

914 The dotted line shows the autosomal median of F:M expression. The level of significance of

915 each comparison (Wilcoxon rank sum test with continuity correction) is denoted with asterisks:

916 **P*-value<0.05, ***P*-value<0.001, ****P*-value<0.0001. **(C), (D)** Pearson correlation between the

917 female:male ratio of expression obtained by proteomics (y-axes) and by microarrays (x-axes) in

918 *S. mansoni* heads **(C)** and gonads **(D)**. A positive correlation (coefficients ρ) is observed for Z-

919 linked (Z, in orange), pseudoautosomal (PSA, in grey) and autosomal genes (A, in darkgrey).

920 The level of significance of each correlation is denoted by asterisks: **P*-value<0.05, ***P*-921 value<0.001, ****P*-value<0.0001, N.S. *P*-value>0.05. No significant difference was found

922 between the correlation obtained for Z-linked and autosomal genes in either tissue (using a

923 Fisher r-to-z transformation).

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925 **Legends of Appendix 1 Figures and Supplementary Figures**

926

927 **Appendix 1 - Figure legends**

928

929 **Appendix 1 - Figure 1.** Determination of Z-specific maximum coverage threshold for *S.*
930 *mansoni* reference species

931 **Appendix 1 - Figure 2.** Determination of Z-specific maximum coverage threshold for *S.*
932 *japonicum* species

933 **Appendix 1 - Figure 3.** Determination of Z-specific maximum coverage threshold for *S.*
934 *haematobium* species

935 **Appendix 1 - Figure 4.** Correlation analysis between RNAseq libraries: heatmaps

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937

938 **Supplementary Figures**

939

940 **Figure 2-figure supplement 1:** Adult expression patterns (in RPKM) of genes located in the
941 different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and autosomal
942 genes. The distribution of expression values is shown for (A) *S. mansoni* females, (B) *S.*
943 *mansoni* males, (C) *S. mansoni* female:male, (D) *S. japonicum* females, (E) *S. japonicum*
944 males, (F) *S. japonicum* female:male, (G) Female expression in *S. japonicum* divided by female
945 expression in *S. mansoni*, (H) Male expression in *S. japonicum* divided by male expression in *S.*
946 *mansoni*, (I) female:male ratio of expression in *S. japonicum* divided by female:male ratio of
947 expression in *S. mansoni*. Asterisks represent levels of significance of comparisons to the
948 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were
949 assigned to each Z-chromosome stratum based only on their coverage patterns; only genes
950 with RPKM>1 were used.

951

952 **Figure 2-figure supplement 2:** Adult expression patterns (in RPKM) of genes located in the
953 different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and autosomal
954 genes. The distribution of expression values is show for (A) *S. mansoni* females, (B) *S. mansoni*
955 males, (C) *S. mansoni* female:male, (D) *S. japonicum* females, (E) *S. japonicum* males, (F) *S.*
956 *japonicum* female:male, (G) Female expression in *S. japonicum* divided by female expression in
957 *S. mansoni*, (H) Male expression in *S. japonicum* divided by male expression in *S. mansoni*, (I)
958 female:male ratio of expression in *S. japonicum* divided by female:male ratio of expression in *S.*
959 *mansoni*. Asterisks represent levels of significance of comparisons to the control autosomal
960 genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were assigned to each Z-
961 chromosome stratum based only on their coverage patterns; only genes with RPKM>0 were
962 used.

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964 **Figure 2-figure supplement 3:** Adult expression patterns (in RPKM) of genes located in the
965 different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and autosomal
966 genes. The distribution of expression values is show for (A) *S. mansoni* females, (B) *S. mansoni*
967 males, (C) *S. mansoni* female:male, (D) *S. japonicum* females, (E) *S. japonicum* males, (F) *S.*
968 *japonicum* female:male, (G) Female expression in *S. japonicum* divided by female expression in

969 S. mansoni, (H) Male expression in S. japonicum divided by male expression in S. mansoni, (I)
970 female:male ratio of expression in S. japonicum divided by female:male ratio of expression in S.
971 mansoni. Asterisks represent levels of significance of comparisons to the control autosomal
972 genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes previously mapped to the
973 ZW linkage group were assigned to each Z-chromosome stratum based on their coverage
974 patterns (stringent classification); only genes with RPKM>1 were used.
975

976 **Figure 2-figure supplement 4:** Adult expression patterns (in RPKM) of genes located in the
977 different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and autosomal
978 genes. The distribution of expression values is show for (A) S. mansoni females, (B) S. mansoni
979 males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum males, (F) S.
980 japonicum female:male, (G) Female expression in S. japonicum divided by female expression in
981 S. mansoni, (H) Male expression in S. japonicum divided by male expression in S. mansoni, (I)
982 female:male ratio of expression in S. japonicum divided by female:male ratio of expression in S.
983 mansoni. Asterisks represent levels of significance of comparisons to the control autosomal
984 genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes previously mapped to the
985 ZW linkage group were assigned to each Z-chromosome stratum based on their coverage
986 patterns (stringent classification); only genes with RPKM>0 were used.
987

988 **Figure 2-figure supplement 5:** Adult expression patterns (in RPKM) of genes located in the
989 different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and autosomal
990 genes. The distribution of expression values is show for (A) S. mansoni females, (B) S. mansoni
991 males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum males, (F) S.
992 japonicum female:male, (G) Female expression in S. japonicum divided by female expression in
993 S. mansoni, (H) Male expression in S. japonicum divided by male expression in S. mansoni, (I)
994 female:male ratio of expression in S. japonicum divided by female:male ratio of expression in S.
995 mansoni. Asterisks represent levels of significance of comparisons to the control autosomal
996 genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were assigned to each Z-
997 chromosome stratum based only on their coverage patterns; only genes with RPKM>0 were
998 used. This figure differs from Supplementary Figure 2 as the Wormbase Parasite list 1:1
999 orthologs was used instead of our own reciprocal best hit results.
1000

1001 **Figure 2-figure supplement 6:** Adult expression patterns (in TPM) of genes located in the
1002 different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and autosomal
1003 genes. The distribution of expression values is show for (A) S. mansoni females, (B) S. mansoni
1004 males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum males, (F) S.
1005 japonicum female:male, (G) Female expression in S. japonicum divided by female expression in
1006 S. mansoni, (H) Male expression in S. japonicum divided by male expression in S. mansoni, (I)
1007 female:male ratio of expression in S. japonicum divided by female:male ratio of expression in S.
1008 mansoni. Asterisks represent levels of significance of comparisons to the control autosomal
1009 genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were assigned to each Z-
1010 chromosome stratum based only on their coverage patterns; only genes with TPM>1 were
1011 used.
1012

1013 **Figure 2-figure supplement 7:** Adult expression patterns (in TPM) of genes located in the
1014 different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and autosomal
1015 genes. The distribution of expression values is show for (A) S. mansoni females, (B) S. mansoni
1016 males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum males, (F) S.
1017 japonicum female:male, (G) Female expression in S. japonicum divided by female expression in
1018 S. mansoni, (H) Male expression in S. japonicum divided by male expression in S. mansoni, (I)
1019 female:male ratio of expression in S. japonicum divided by female:male ratio of expression in S.
1020 mansoni. Asterisks represent levels of significance of comparisons to the control autosomal
1021 genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were assigned to each Z-
1022 chromosome stratum based only on their coverage patterns; only genes with TPM>0 were
1023 used.

1024
1025 **Figure 2-figure supplement 8:** Schistosomula expression patterns (in RPKM) of genes located
1026 in the different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and
1027 autosomal genes. The distribution of expression values is show for (A) S. mansoni females, (B)
1028 S. mansoni males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum
1029 males, (F) S. japonicum female:male, (G) Female expression in S. japonicum divided by female
1030 expression in S. mansoni, (H) Male expression in S. japonicum divided by male expression in S.
1031 mansoni, (I) female:male ratio of expression in S. japonicum divided by female:male ratio of
1032 expression in S. mansoni. Asterisks represent levels of significance of comparisons to the
1033 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were
1034 assigned to each Z-chromosome stratum based only on their coverage patterns; only genes
1035 with RPKM>1 were used.

1036
1037 **Figure 2-figure supplement 9:** Schistosomula expression patterns (in RPKM) of genes located
1038 in the different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and
1039 autosomal genes. The distribution of expression values is show for (A) S. mansoni females, (B)
1040 S. mansoni males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum
1041 males, (F) S. japonicum female:male, (G) Female expression in S. japonicum divided by female
1042 expression in S. mansoni, (H) Male expression in S. japonicum divided by male expression in S.
1043 mansoni, (I) female:male ratio of expression in S. japonicum divided by female:male ratio of
1044 expression in S. mansoni. Asterisks represent levels of significance of comparisons to the
1045 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were
1046 assigned to each Z-chromosome stratum based only on their coverage patterns; only genes
1047 with RPKM>0 were used.

1048
1049 **Figure 2-figure supplement 10:** Schistosomula expression patterns (in RPKM) of genes
1050 located in the different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and
1051 autosomal genes. The distribution of expression values is show for (A) S. mansoni females, (B)
1052 S. mansoni males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum
1053 males, (F) S. japonicum female:male, (G) Female expression in S. japonicum divided by female
1054 expression in S. mansoni, (H) Male expression in S. japonicum divided by male expression in S.
1055 mansoni, (I) female:male ratio of expression in S. japonicum divided by female:male ratio of
1056 expression in S. mansoni. Asterisks represent levels of significance of comparisons to the

1057 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes
1058 previously mapped to the ZW linkage group were assigned to each Z-chromosome stratum
1059 based on their coverage patterns (stringent classification); only genes with RPKM>1 were used.
1060

1061 **Figure 2-figure supplement 11:** Schistosomula expression patterns (in RPKM) of genes
1062 located in the different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and
1063 autosomal genes. The distribution of expression values is show for (A) S. mansoni females, (B)
1064 S. mansoni males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum
1065 males, (F) S. japonicum female:male, (G) Female expression in S. japonicum divided by female
1066 expression in S. mansoni, (H) Male expression in S. japonicum divided by male expression in S.
1067 mansoni, (I) female:male ratio of expression in S. japonicum divided by female:male ratio of
1068 expression in S. mansoni. Asterisks represent levels of significance of comparisons to the
1069 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes
1070 previously mapped to the ZW linkage group were assigned to each Z-chromosome stratum
1071 based on their coverage patterns (stringent classification); only genes with RPKM>0 were used.
1072

1073 **Figure 2-figure supplement 12:** Schistosomula expression patterns (in RPKM) of genes
1074 located in the different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and
1075 autosomal genes. The distribution of expression values is show for (A) S. mansoni females, (B)
1076 S. mansoni males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum
1077 males, (F) S. japonicum female:male, (G) Female expression in S. japonicum divided by female
1078 expression in S. mansoni, (H) Male expression in S. japonicum divided by male expression in S.
1079 mansoni, (I) female:male ratio of expression in S. japonicum divided by female:male ratio of
1080 expression in S. mansoni. Asterisks represent levels of significance of comparisons to the
1081 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were
1082 assigned to each Z-chromosome stratum based only on their coverage patterns; only genes
1083 with RPKM>0 were used. This figure differs from Supplementary Figure 2 as the Wormbase
1084 Parasite list 1:1 orthologs was used instead of our own reciprocal best hit results.
1085

1086 **Figure 2-figure supplement 13:** Schistosomula expression patterns (in TPM) of genes located
1087 in the different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and
1088 autosomal genes. The distribution of expression values is show for (A) S. mansoni females, (B)
1089 S. mansoni males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum
1090 males, (F) S. japonicum female:male, (G) Female expression in S. japonicum divided by female
1091 expression in S. mansoni, (H) Male expression in S. japonicum divided by male expression in S.
1092 mansoni, (I) female:male ratio of expression in S. japonicum divided by female:male ratio of
1093 expression in S. mansoni. Asterisks represent levels of significance of comparisons to the
1094 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were
1095 assigned to each Z-chromosome stratum based only on their coverage patterns; only genes
1096 with TPM>1 were used.
1097

1098 **Figure 2-figure supplement 14:** Schistosomula expression patterns (in TPM) of genes located
1099 in the different strata of the Z (S0, S1man and S1jap), as well as pseudoautosomal and
1100 autosomal genes. The distribution of expression values is show for (A) S. mansoni females, (B)

1101 S. mansoni males, (C) S. mansoni female:male, (D) S. japonicum females, (E) S. japonicum
1102 males, (F) S. japonicum female:male, (G) Female expression in S. japonicum divided by female
1103 expression in S. mansoni, (H) Male expression in S. japonicum divided by male expression in S.
1104 mansoni, (I) female:male ratio of expression in S. japonicum divided by female:male ratio of
1105 expression in S. mansoni. Asterisks represent levels of significance of comparisons to the
1106 control autosomal genes (*P-value<0.05, **P-value<0.001, ***P-value<0.0001). Genes were
1107 assigned to each Z-chromosome stratum based only on their coverage patterns; only genes
1108 with TPM>0 were used.

1109
1110 **Figure 2-figure supplement 15.** Z-linked and autosomal female:male ratio of gene expression
1111 using different filters. The Z-linked and autosomal gene expression patterns are shown in S.
1112 japonicum and S. mansoni. Different filters were applied: a minimum threshold of expression of
1113 1 RPKM in all libraries (A-D), a minimum threshold of expression of 3 RPKM in all libraries (E-
1114 H), or a minimum threshold of expression of 1 RPKM per stage (as in the main manuscript),
1115 together with a filter on fold change to account for strong sex-biases (FC<2 for female:male ratio
1116 and male:female ratio) (I-L). Z refers to the female:male ratio of expression in the Z-specific
1117 region and A refers to the female:male ratio of expression on autosomes. The level of
1118 significance of each comparison is denoted with asterisks: ***P-value<0.0001.

1119
1120 **Figure 2-figure supplement 16.** Z-linked and autosomal gene expression in females and
1121 males using different filters. The Z-linked and autosomal gene expression patterns are shown
1122 for S. japonicum and S. mansoni. Different filters were applied: a minimum threshold of
1123 expression of 1 RPKM in all libraries (A-D), a minimum threshold of expression of 3 RPKM in all
1124 libraries (E-H), or a minimum threshold of expression of 1 RPKM per stage (as in the main
1125 manuscript), together with a filter on fold change to account for strong sex biases (FC<2 for
1126 female:male ratio and male:female ratio) (I-L). Fem-Z and Male-Z refers to the female:male
1127 ratio of expression in the Z-specific region and Fem-A and Male-A refers to the female:male
1128 ratio of expression on autosomes, in female and male respectively The level of significance of
1129 each comparison is denoted with asterisks: *P-value<0.05, **P-value<0.001, ***P-value<0.0001.

1130
1131
1132 **Figure 2-figure supplement 17.** Z-linked and autosomal female:male ratio of gene expression
1133 according to presence/absence of known protein-protein interactions. The Z-linked and
1134 autosomal gene expression patterns are shown for schistosomula and adults, in S. japonicum
1135 (A-B) and S. mansoni (C-D). We considered only genes with minimum level of expression
1136 RPKM>1 in all stages, did not take into account the ancestral state of expression, and used the
1137 “exhaustive strata” set of genes. -PPIs and +PPIs refer to the female:male ratio of expression in
1138 the lineage specific Z-linked strata S1jap and S1mans respectively. The level of significance of
1139 each comparison is denoted with asterisks: ***P-value<0.0001.

1140 **Figure 3-figure supplement 1.** Z-linked and autosomal female:male ratio of gene expression,
1141 normalized by ancestral autosomal expression, and using different filters. The Z-linked and
1142 autosomal gene expression patterns (normalized by ancestral pseudo-autosomal or autosomal
1143 expression, to show changes since the appearance of the two S1 strata) are shown for S1jap in
1144 S. japonicum and S1mans in S. mansoni. Different filters were applied: a minimum threshold of

1145 expression of 1 RPKM in all libraries (A-D), a minimum threshold of expression of 3 RPKM in all
1146 libraries (E-H), or a minimum threshold of expression of 1 RPKM per stage (as in the main
1147 manuscript), together with a filter on fold change to account for strong sex biases (FC<2 for
1148 female:male ratio and male:female ratio) (I-L). S1jap and S1mans refer to the female:male
1149 ratio of expression in the lineage specific Z-linked strata S1jap and S1mans respectively. The
1150 level of significance of each comparison is denoted with asterisks: ***P-value<0.0001.

1151
1152 **Figure 3-figure supplement 2.** Z-linked and autosomal gene expression in females and males,
1153 normalized by ancestral autosomal expression, and using different filters. The Z-linked and
1154 autosomal gene expression patterns (normalized by ancestral pseudo-autosomal or autosomal
1155 expression, to show changes since the appearance of the two S1 strata) are shown for S1jap in
1156 *S. japonicum* and S1mans in *S. mansoni*. Different filters were applied: a minimum threshold of
1157 expression of 1 RPKM in all libraries (A-D), a minimum threshold of expression of 3 RPKM in all
1158 libraries (E-H), or a minimum threshold of expression of 1 RPKM per stage (as in the main
1159 manuscript), together with a filter on fold change to account for strong sex biases (FC<2 for
1160 female:male ratio and male:female ratio) (I-L). Fem-S1j and Male-S1j refer to the normalized
1161 expression levels of genes in the stratum S1jap in females and males, Fem-S1m and Male-S1m
1162 refer to the normalized expression levels in S1mans, and Fem-A and Male-A refer to the
1163 normalized expression levels of autosomal genes in females and in males, respectively. The
1164 level of significance of each comparison is denoted with asterisks: *P-value<0.05, **P-
1165 value<0.001, ***P-value<0.0001.

1166
1167 **Figure 4-figure supplement 1:** Pearson correlations between gene dosage at the transcript
1168 and protein levels in male heads and gonads. A positive correlation (coefficients R) is observed
1169 for Z-linked (Z, in light blue), pseudo-autosomal (PSA, in grey) and autosomal genes (A, in dark
1170 blue). The level of significance of each correlation is denoted by asterisks: *P-value<0.05, **P-
1171 value<0.001, ***P-value<0.0001. No significant difference was found between the correlations
1172 obtained for Z-linked and autosomal genes in either tissue (using a Fisher r-to-z transformation).

1173
1174 **Figure 4-figure supplement 2:** Pearson correlations between gene dosage at the transcript
1175 and protein levels in female heads and gonads. A positive correlation (coefficients R) is
1176 observed for Z-linked (Z, in light pink), pseudo-autosomal (PSA, in grey) and autosomal genes
1177 (A, in dark pink). The level of significance of each correlation is denoted by asterisks: *P-
1178 value<0.05, **P-value<0.001, ***P-value<0.0001. No significant difference was found between
1179 the correlations obtained for Z-linked and autosomal genes in either tissue (using a Fisher r-to-z
1180 transformation).

1181
1182 **Figure 4-figure supplement 3:** Pearson correlations between the female:male ratio of
1183 expression obtained by proteomics (y-axes) and by microarrays (x-axes) in *S. mansoni* heads
1184 and gonads, using only genes with male-biased expression in the microarray data. A positive
1185 correlation (coefficients R) is observed for Z-linked (Z, in orange), pseudo-autosomal (PSA, in
1186 grey) and autosomal genes (A, in darkgrey). The level of significance of each correlation is
1187 denoted by asterisks: *P-value<0.05, **P-value<0.001, ***P-value<0.0001, N.S. P-value>0.05.

1188 No significant difference was found between the correlations obtained for Z-linked and
1189 autosomal genes in either tissue (using a Fisher r-to-z transformation).

1190

1191 **Source data files**

1192 **Figure 1-source data 1:** Comparative genomics: coverage analysis and strata identification

1193 **Figure 1-source data 2:** *S. mansoni* reference species: coverage analysis and Z-Autosome
1194 assignment

1195 **Figure 1-source data 3:** *S. haematobium* autosome vs Z-specific region assignment

1196 **Figure 1-source data 4:** *S. japonicum* autosome vs Z-specific region assignment

1197 **Figure 2-source data 1:** RPKM calculation for *S. mansoni* gene expression

1198 **Figure 2-source data 2:** RPKM calculation for *S. japonicum* gene expression

1199 **Figure 3-source data 1:** One-to-one orthology *S. mansoni* vs *S. japonicum*

1200 **Figure 3-source data 2:** Transcriptomic data, for blat 1-to-1 orthologs

1201 **Figure 3-source data 3:** Transcriptomic data, for WormBase Biomart orthologs

1202 **Figure 4-source data 1:** Proteomic data with imputed values – GONADS

1203 **Figure 4-source data 2:** Proteomic data with imputed values – HEADS

1204 **Figure 4-source data 3:** Proteomic data without imputed values – GONADS

1205 **Figure 4-source data 4:** Proteomic data without imputed values – HEADS

1206 **Figure 4-source data 5:** Correspondance between Gene_Id and Protein_ID

1207 **Figure 4-source data 6:** Microarray data

1208

1209 **Supplementary File 1.** Libraries and captions for source data files.

1210

1211

Appendix 1 – Computational analyses

Supplementary codes and inputs

Additional Perl and R scripts are available at the following link:

https://www.dropbox.com/sh/1jdpd15zokx7z85/AAB4BoRm5Jf6i7qEys8x_ERMa?dl=0

Remarks: Deposited on IST Austria data repository (<http://dx.doi.org/10.15479/AT:ISTA:109>)

Additional input files are available at the following link:

https://www.dropbox.com/sh/r0gz93xbkgsxyq/AABJqAarTNWewqH-Nd_LF1y-a?dl=0

Remarks: Deposited on IST Austria data repository (<http://dx.doi.org/10.15479/AT:ISTA:109>)

Publicly available reference genomes and annotations

All data were obtained at the WormBase Parasite database (<https://parasite.wormbase.org/index.html>)(56,57).

S. mansoni:

Genomic sequences: *schistosoma_mansoni*.PRJEA36577.WBPS9.genomic.fa

Annotation: *schistosoma_mansoni*.PRJEA36577.WBPS9.canonical_geneset.gtf

CDS: *schistosoma_mansoni*.PRJEA36577.WBPS9.CDS_transcripts.fa

S. japonicum:

Genomic sequences: *schistosoma_japonicum*.PRJEA34885.WBPS9.genomic.fa

Annotation: *schistosoma_japonicum*.PRJEA34885.WBPS9.canonical_geneset.gtf

CDS: *schistosoma_japonicum*.PRJEA34885.WBPS9.CDS_transcripts.fa

S. haematobium:

Genomic sequences: *schistosoma_haematobium*.PRJNA78265.WBPS9.genomic.fa

Annotation: *schistosoma_haematobium*.PRJNA78265.WBPS9.canonical_geneset.gtf

Orthology

The same pipeline was applied to *S. japonicum* and *S. haematobium*. Example is shown here for the *S. japonicum* species.

Mapping of *S. mansoni* genes on the *S. japonicum* genome

First, *S. mansoni* gene sequences were mapped to the *S. japonicum* genome using Blat (79) with a translated query and database:

```
CDS=pathwaytosmansonigenome/schistosoma_mansoni.PRJEA36577.WBPS9.CDS_transcripts.fa
```

```
GENOME=~pathwaytojaponicumgenome
```

```
BLATOUTPUT=~pathwaytoblattoutputfile
```

```
~/tools/blat -q=dnax -t=dnax -minScore=50
```

```
${GENOME}/schistosoma_japonicum.PRJEA34885.WBPS9.genomic.fa ${CDS}
```

```
${BLATOUTPUT}/jap_mans.blatt
```

The resulting blat alignment was filtered to keep only the mapping hit with the highest mapping for each *S. mansoni* gene:

```
sort -k 10 ${BLATOUTPUT}/jap_mans.blatt > ${BLATOUTPUT}/jap_mans.blatt.sorted
```

```
perl Script1_besthitblatt.pl ${BLATOUTPUT}/jap_mans.blatt.sorted
```

This produced the first filtered Blat alignment:

```
${BLATOUTPUT}/jap_mans.blatt.sorted.besthit
```

In a second filtering step, when several *S. mansoni* genes overlapped on the *S. japonicum* genome by more than 20bps, we keep only the gene with the highest mapping score:

```
sort -k 14 ${BLATOUTPUT}/jap_mans.blatt.sorted.besthit >
```

```
${BLATOUTPUT}/jap_mans.blatt.sorted.besthit.sorted
```

```
perl Script2_blatreverse.pl ${BLATOUTPUT}/jap_mans.blatt.sorted.besthit.sorted
```

This produced the final filtered Blat alignment:

```
 ${BLATOUTPUT}/jap_mans.blat.sorted.besthit.sorted.nonredundant
```

Assignment of syntenic blocks to *S. mansoni* chromosomal locations

Each *S. japonicum* scaffold was assigned to one of the *S. mansoni* chromosomes based on its gene content, using a majority rule.

(i) As input table, we transformed the output obtained in the previous step into a 3-column file (sorted by column 1): column1=Smansoni_gene_ID, column2=Sjaponicum_contig/scaffold, column3=score.

```
 cat ${BLATOUTPUT}/jap_mans.blat.sorted.besthit.sorted.nonredundant | cut -f 1,10,14 |
 awk '{print $2, $3, $1}' | perl -pi -e 's/-P.//gi' | sort >
 ${BLATOUTPUT}/jap_mans.blat.sorted.besthit.sorted.nonredundant_score.joignabl
```

(ii) A second file containing the chromosomal location of *S. mansoni* genes was also needed for the next step. The file (named here RefChromLocation.joignabl) is a 2-column file resulting from the combination of the *S. mansoni* GFF file, and the newly identified Z-specific genes (*i.e.* the chromosomal location corresponds to the published GTF location, except for 1. the new Z versus pseudoautosomal assignments based on coverage for ZW genes and 2. genes which were newly detected as Z-linked): column1=Smansoni_gene_ID, column2=Smansoni_chromosome. This file should be sorted by column 1. Column2 has 11 possible values: "Chr_1", "Chr_2", "Chr_3", "Chr_4", "Chr_5", "Chr_6", "Chr_7", "Chr_PSA", "Chr_Z", "Chr_newZ", and "Chr_UP" (See Figure 1-source data 1, column #6: "Bestloc_input_mans").

(iii) We joined both files by column1 and sort the joined table by Sjaponicum_contig/scaffold:

```
 join ${BLATOUTPUT}/jap_mans.blat.sorted.besthit_score.joignabl pathwaytosmansonigenome/
 RefChromLocation.joignabl | awk '{print $2, $1, $4, $3}' | sort >
 jap_mans.inputforbestlocation
```

A final script counted how many orthologs from the different *S. mansoni* chromosomes were found in each *S. japonicum* scaffold, and assigned it a chromosome based on the location of the majority of the orthologs. If the same number of genes were located on two different chromosomes, then the chromosome for which the sum of the mapping scores was highest was chosen.

```
 perl Script3_bestlocation.pl jap_mans.inputforbestlocation
```

Identification of 1-to-1 orthologs between *S. mansoni* and *S. japonicum*

S. japonicum genes were first mapped to *S. mansoni* using Blat with a translated database and query:

```
 CDS1=pathwaytosmansonigenome/schistosoma_mansoni.PRJEA36577.WBPS9.CDS_transcripts.fa
 CDS2=pathwaytojaponicumgenome/schistosoma_japonicum.PRJEA34885.WBPS9.CDS_transcripts.fa
 BLATOUTPUT=~pathwaytoblatooutputfile
```

```
 ~/tools/blat -q=dnax -t=dnax -minScore=50 ${CDS2} ${CDS1}
 ${BLATOUTPUT}/jap_mans_onetoone.blat
```

Only best reciprocal hits were kept:

```
 perl Script4_Blat_one2one.pl ${BLATOUTPUT}/jap_mans_onetoone.blat
```

The list of orthologs is provided in Figure 3-source data 1.

Determination of Z-specific regions in each species

Raw read processing

The same pipeline was applied to all the species. Example is shown here for the *S. japonicum* species. The newly sequenced *S. japonicum* reads were provided as BAM files, which we first converted to FASTQ:

```
 READS=~pathwaytoreads
```

```
 for i in `ls ${READS} | grep .bam`; do
 echo ${i}
```

```

127 ~/tools /bedtools.2.25.0/bin/bamToFastq -i ${i} -fq ${i}.fastq1 -fq2 ${i}.fastq2
128 done
129
130 Adapter sequences were removed from the newly sequenced libraries (Only for S. japonicum) using Cutadapt (80):
131
132 LIBRARY_1=Forward reads in fastq format
133 LIBRARY_2=Reverse reads in fastq format
134 READS =~pathwaytoreads
135 CUTADAPTOUTPUT=~pathwaytocutadaptoutput
136
137 ~/tools/cutadapt_1.9.1 --match-read-wildcards -f fastq -a
138 AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC ${READS}/${LIBRARY_1} -o
139 ${CUTADAPTOUTPUT}/${LIBRARY_1}.trimmed
140
141 ~/tools/cutadapt_1.9.1 --match-read-wildcards -f fastq -a
142 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTA ${READS}/${LIBRARY_2} -o
143 ${CUTADAPTOUTPUT}/${LIBRARY_2}.trimmed
144
145 Trimmomatic was used to remove adaptor and low quality sequences from the available libraries (For S. mansoni and S. haematobium, accession numbers are given in the main text):
146
147 READS=~pathwaytoreads
148 LIBRARY=LibraryName
149
150
151 module load java
152 java -jar ~/tools/tools/Trimmomatic-0.36/trimmomatic-0.36.jar PE -phred33
153 ${READS}/${LIBRARY}.fastq1 ${READS}/${LIBRARY}.fastq2
154 ${READS}/${LIBRARY}.fastq1_paired.fq.gz ${READS}/${LIBRARY}.fastq1_unpaired.fq.gz
155 ${READS}/${LIBRARY}.fastq2_paired.fq.gz ${READS}/${LIBRARY}.fastq2_unpaired.fq.gz
156 ILLUMINACLIP:~/tools/Trimmomatic-0.36/adapters/TruSeq2-PE.fa:2:30:10 HEADCROP:12
157 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
158
159 All libraries were checked using FastQC:
160
161 for i in `ls ${CUTADAPTOUTPUT} | grep .trimmed`; do
162 ~/tools/FastQC-v0.11.2/fastqc ${CUTADAPTOUTPUT}/${i} --extract -o ~/fastQOutputs/
163 done
164
165 OR
166
167 for i in `ls ${READS} | grep paired.fq.gz`; do
168 ~/tools/FastQC-v0.11.2/fastqc ${READS}/${i} --extract -o ~/fastQOutputs/
169 done
170
171
172 Read alignment on reference genome
173 The same pipeline was applied to all the species. Example is shown here for the S. japonicum species.
174 Reads were mapped to the respective reference genomes using Bowtie2 (82).
175
176 #Built reference genome
177
178 GENOME=~pathwaytojaponicumgenome
179
180 ~/tools/bowtie2-2.2.9/bowtie2-build
181 ${GENOME}/schistosoma_japonicum.PRJEA34885.WBPS9.genomic.fa ref_genome
182
183 # Mapping
184
185 SPE=Sjaponicum
186 LIBRARY=LibraryName
187 BOWTIE2OUTPUT=~pathwaytobowtie2output
188 THREADS=5
189
190 ~/tools/bowtie2-2.2.9/bowtie2 -x ${GENOME}/refgenome -1
191 ${CUTADAPTOUTPUT}/${LIBRARY_1}.trimmed -2 ${CUTADAPTOUTPUT}/${LIBRARY_2}.trimmed --end-
192 to-end --sensitive -p ${THREADS} -S ${BOWTIE2OUTPUT}/${SPE}_${LIBRARY}.sam

```

Genomic coverage

The same pipeline was applied to all the species. Example is shown here for the *S. japonicum* species. Genomic coverage in the male and female samples was calculated using SOAPcoverage (v2.7.7., <http://soap.genomics.org.cn/index.html>), after filtering for uniquely mapped reads.

```
#Select only uniquely mapped reads

cd ${BOWTIE2OUTPUT}

for i in `ls | grep .sam`; do
echo ${i}
grep -vw "XS:i" ${i} > ${i}_unique.sam
done

#Obtain average genomic coverage for each scaffold

GENOME=~pathwaytojaponicumgenome
SOAPCOVOUTPUT=pathwaytosoapcoverageoutput

cd ${BOWTIE2OUTPUT}

for j in `ls | grep _unique.sam`; do
echo ${j}
~/tools/soapcoverage.2.7.7/soap.coverage -sam -cvg -i ${j} -onlyuniq -refsingle
${GENOME} -o ${SOAPCOVOUTPUT}/${j}.soapcov
done
```

For *S. mansoni*, the genomic coverage was not calculated per scaffold but for 10kb windows:

```
GENOME=~pathwaytomansonigenome
LIBRARY=LibraryName
SOAPCOVOUTPUT=pathwaytosoapcoverageoutput
WIN=10000

cd ${BOWTIE2OUTPUT}

for j in `ls | grep _unique.sam`; do
echo ${j}
~/tools/soapcoverage.2.7.7/soap.coverage -sam -cvg -i ${j} -onlyuniq -refsingle
${GENOME} -window ${WIN} -o ${SOAPCOVOUTPUT}/${j}.soapcov
done
```

The final coverage values are part of the Figure 1-source data 2 (*S. mansoni*), Figure 1-source data 3 (*S. haematobium*) and Figure 1-source data 4 (*S. japonicum*)

Determination of Z-specific maximum coverage threshold for *S. mansoni* reference species

The maximum value of $\log_2(\text{female}:\text{male})$ coverage for Z-specific assignment in *S. mansoni* was determined using the R script Script5_Genomics_mansoni (Part I) with the input file InputFile1_Smansoni_SoapCov_By10kb_ForR, which contains the SoapCov outputs for male (ERR562989_Depth) and female (ERR562990_Depth).

This involved the following steps (shown in Appendix 1-figure 1):

- When plotted, the $\log_2(\text{female}:\text{male})$ ratio of coverage shows a bimodal distribution. The highest peak is distributed around 0 and corresponds to the windows that are localized on the known autosomes (Chromosomes 1 to 7) of the current version of the genome assembly (Protasio et al. 2012 (42), PRJEA36577).
- If we consider only the unimodal distribution of autosomal window coverage, the 5th percentile has a $\log_2(\text{female}:\text{male})=-0.26$.
- We use this value to exclude the pseudoautosomal windows (plotted in grey) of the ZW linkage group and consider only the non-pseudoautosomal windows (plotted in orange) for the next step.
- The non-pseudoautosomal window (InputFile2_Z_10kb_5pr100) coverage displays an unimodal distribution, and the 99th percentile as a value $\log_2(\text{female}:\text{male})=-0.4$.
- When visualizing $\log_2(\text{female}:\text{male})$ along the ZW linkage group, it appears that the $\log_2(\text{female}:\text{male})=-0.4$ threshold allows the discrimination of the three known Z-specific regions (in orange) and pseudoautosomal regions (in grey). To finely define the Z-specific content, we systematically excluded windows for which the

coverage value is not consistent with the adjacent windows: 5 consecutive 10kb windows with consistent coverage were required to be considered either pseudoautosomal or Z-specific. For instance, yellow bands on the graph highlight regions of more than one window but less than five. They are tagged as “Excluded” in Figure 1-source data 2, as are the two adjacent windows.

We then applied this threshold value to all the genome, considering only scaffolds longer than 50kb for classification. The final classification of each scaffold (as “Z” or “Autosome”) is presented in Figure 1-source data 2. Used R script is: Script1_Genomics_mansoni (Part II); and corresponding input file is: InputFile1_Smansoni_SoapCov_By10kb_ForR.

All genes of a window were assigned to “Z” or “Autosome” depending on the window assignment. When a gene overlapped with an excluded window, it was excluded as well. In order to exclude these genes, the following script was used: Script6_GeneSelector, using as input a 3-column file (Chromosome ID, First base of window to exclude, Last base of window to exclude).

Determination of Z-specific maximum coverage threshold for *S. japonicum*.

The maximum value of $\log_2(\text{female}:\text{male})$ coverage for Z-specific assignment in *S. japonicum* was determined using the R script Script7_Genomics_japonicum (Part I) with the input file InputFile3_Sjaponicum_SoapCov_Bestloc_ForR, which contains the SoapCov outputs for male (Depth_40640) and female (ERR562990_Depth), and the assignment to *S. mansoni* chromosomes (See paragraph 1.1).

This involved the following steps (shown in Appendix 1-figure 2):

- A) When plotted, the $\log_2(\text{female}:\text{male})$ ratio of coverage shows a bimodal distribution.
- B) As *S. japonicum* genome is only assembled at the scaffold level (Zhou et al. 2009), we used the $\log_2(\text{female}:\text{male})$ coverage of the scaffold mapping to *S. mansoni* autosomes to get the distribution of autosomal $\log_2(\text{female}:\text{male})$.
- C) If we consider only the unimodal distribution of the autosome-assigned scaffold coverage, the 1st percentile has a $\log_2(\text{female}:\text{male})=-0.4$.
- D) We use this value to classify scaffolds that mapped to the *S. mansoni* ZW linkage group as Z-specific or pseudoautosomal (grey in Appendix 1-figure 2, panel D), and consider only the non-pseudoautosomal scaffolds (plotted in orange) for the next step.
- E) The non-pseudoautosomal scaffold (InputFile4_Sjaponicum_1pr100) coverage displays an unimodal distribution, and the 95th percentile has a value of $\log_2(\text{female}:\text{male})=-0.84$.
- F) When visualizing the $\log_2(\text{female}:\text{male})$ along the ZW linkage group, it appears that no single threshold value can finely discriminate Z-specific and pseudoautosomal scaffolds. So we use the $\log_2(\text{female}:\text{male})=-0.84$ threshold as maximum value for Z-specific regions (in orange), and $\log_2(\text{female}:\text{male})=-0.4$ threshold as minimum value for pseudoautosomal regions (in grey). Scaffolds with $\log_2(\text{female}:\text{male})$ between these two values were classified as “ambiguous”.

We then applied this threshold value to all the genome. Used R script is : Script7_Genomics_japonicum (Part II); and corresponding input file is: InputFile3-2_Sjaponicum_SoapCov_ForR.

The final “Z”, “Autosome” or “Ambiguous” assignment is shown in Figure 1-source data 4.

Determination of Z-specific maximum coverage threshold for *S. haematobium*.

The maximum value of $\log_2(\text{Female})$ coverage for Z-specific assignment in *S. haematobium* was determined using the R script Script8_Genomics_haematobium (Part I) with the input file InputFile5_Shaematobium_SoapCov_Bestloc_ForR, which contains the SoapCov outputs for two female libraries (Depth_ERR037800, Depth_ERR036251), and the assignment to *S. mansoni* chromosomes (See paragraph 1.1).

This involved the following steps (shown in Appendix 1-figure 3):

- A) When plotted, the $\log_2(\text{Female})$ coverage shows a bimodal distribution.
- B) As the *S. haematobium* genome is only assembled at the scaffold level (Young et al. 2012), we used the $\log_2(\text{Female})$ coverage of the scaffold mapping to *S. mansoni* autosomes to get the distribution of autosomal $\log_2(\text{Female})$.
- C) If we consider only the unimodal distribution of the autosome-assigned scaffold coverage, the 5th percentile has a $\log_2(\text{Female})=4.4$
- D) We use this value to classify scaffolds that mapped to the *S. mansoni* ZW linkage group as Z-specific or pseudoautosomal (grey in Appendix 1-figure 3, panel D), and consider only the non-pseudoautosomal scaffolds (plotted in orange) for the next step.
- E) The non-pseudoautosomal scaffold (InputFile6_Shaematobium_5pr100) coverage displays a unimodal distribution, and the 99th percentile as a value $\log_2(\text{Female})=4.6$
- G) When visualizing $\log_2(\text{Female})$ along the ZW linkage group, it appears no single threshold value cannot finely discriminate between Z-specific and pseudoautosomal scaffolds. So we used the $\log_2(\text{Female})=4.6$ threshold as maximum value for Z-specific regions (Appendix 1-figure 3, panel F, in orange), and $\log_2(\text{Female})=4.4$ threshold as minimum value for pseudoautosomal regions (in grey). Scaffolds with $\log_2(\text{Female})$ between these two values were classified as “ambiguous”.

We then applied this threshold value to all the genome. Used R script is: Script8_Genomics_haematobium (Part II); and corresponding input file is: InputFile5-2_Shaematobium_SoapCov_ForR.txt. The final “Z”, “Autosome” or “Ambiguous” assignment is shown in Figure 1-source data 3.

Definition of evolutionary strata

Genes that were Z-specific in both *S. mansoni* and *S. japonicum* were assigned to stratum S0. Genes that were Z-specific only in *S. mansoni* were assigned to stratum S1mans, and genes that were Z-specific in *S. japonicum* were assigned to stratum S1jap.

This assignment was performed separately for two classifications. The exhaustive classification is defined by the comparison of “Z” or “Autosome” assignment, based on coverage (see 2.4 - 2.6), between *S. mansoni* and *S. japonicum* species. The stringent classification is a subset of the exhaustive classification: it only contains genes localized on the ZW linkage map in *S. mansoni* and with a best location on the Z or PSA region in *S. japonicum* (see Figure 1-source data 1).

Transcriptomics

Raw read processing

Reads were first trimmed with Trimmomatic (v0.36, (81)), using the following commands:

(i) *S. mansoni*

```
READS=~pathwaytoreads
```

```
cd ${READS}
```

```
for i in `ls | grep .fastq`; do
```

```
    echo ${i}
```

```
module load java
```

```
java -jar ~/tools/Trimmomatic-0.36/trimmomatic-0.36.jar PE -phred33 ${i}
```

```
${j}.trimmed.fq.gz ILLUMINACLIP:~/tools/Trimmomatic-0.36/adapters/TruSeq3-SE.fa:2:30:10
```

```
HEADCROP:12 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

```
done
```

(ii) *S. japonicum*

```
READS=~pathwaytoreads
```

```
cd ${READS}
```

```
for i in `ls | grep .fastq`; do
```

```
    echo ${i}
```

```
module load java
```

```
java -jar ~/tools/Trimmomatic-0.36/trimmomatic-0.36.jar SE -phred33 ${i}
```

```
${j}.trimmed.fq.gz ILLUMINACLIP:~/tools/Trimmomatic-0.36/adapters/TruSeq3-SE.fa:2:30:10
```

```
HEADCROP:12 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

```
done
```

Read mapping on reference genome

Reads were mapped to their respective reference genomes with TopHat2 (83), an alignment program for RNA-seq reads which takes splice junctions into account.

(i) *S. mansoni*

```
GENOME=~pathwaytogenome/schistosoma_mansoni.PRJEA36577.WBPS9.genomic.fa
```

```
READS=~pathwaytoreads
```

```
OUF=~/.TophatOutput
```

```
# Build the indexed genome for Bowtie2
```

```
~/tools/bowtie2-2.2.9/bowtie2-build ${genome} refgenome
```

```
# Map the RNAseq reads against the reference genome with TopHat2
```

```
cd ${READS}
```

```

387 for i in `ls | grep trimmed.fq`; do
388 echo ${i}
389
390 ~tools/tophat-2.1.1.Linux_x86_64/tophat -p 3 --library-type fr-firststrand --microexon-
391 search -i 10 -I 40000 --min-segment-intron 10 --max-segment-intron 40000 -g 1 -o ${OUF}
392 ~pathwaytogenome/refgenome ${READS}/${i}
393
394 done
395
396 (ii) S. japonicum
397
398 GENOME=~pathwaytogenome/schistosoma_japonicum.PRJEA34885.WBPS9.genomic.fa
399 READS=~pathwaytoreads
400 OUF=~ /TophatOutput
401
402 ~tools/tophat-2.1.1.Linux_x86_64/tophat -p 3 --microexon-search -i 10 -I 40000 --min-
403 segment-intron 10 --max-segment-intron 40000 -g 1 -o ${OUF} ~pathwaytogenome/refgenome
404 ${READS}/${i}
405
406 Read count
407 Reads counts for each gene were obtained from the TopHat2 alignments using HTseq (84).
408
409 (i) S. mansoni
410
411 INF=~ /TopHat_Output
412 GENOME=~pathwaytogenome/schistosoma_mansoni.PRJEA36577.WBPS9.genomic.fa
413 OUF=~ /HTseq_Output
414
415 cd ${INF}
416
417 for i in `ls | grep accepted_hits.bam`; do
418     echo ${i}
419 module load anaconda
420
421 htseq-count -f bam -s reverse -m union --idattr gene_id -o ${OUF}/${i}_Rev.htseq ${i}
422 ${GENOME} > ${OUF}/${i}_Rev.count
423 done
424
425 (ii) S. japonicum
426
427 INF=~ /TopHat_Output
428 GENOME=~ /pathwaytogenome/schistosoma_japonicum.PRJEA34885.WBPS9.genomic.fa
429 OUF=~ /HTseq_Output
430
431 cd ${INF}
432
433 for i in `ls | grep accepted_hits.bam`; do
434     echo ${i}
435 module load anaconda
436
437 htseq-count -f bam -s no -m union --idattr gene_id -o ${OUF}/${i}_Rev.htseq ${i}
438 ${GENOME} > ${OUF}/${i}_Rev.count
439 done
440
441 Alternative pipeline: Kallisto (example shown for S. mansoni)
442 In order to check that our results held independent of the pipeline used to infer expression levels, we further estimated
443 TPM values using Kallisto (85).
444
445 # Create index
446
447 INF=~pathwaytogenome
448 CDS=${inf}/schistosoma_mansoni.PRJEA36577.WBPS9.CDS_transcripts.fa # or input for S.
449 japonicum would be:schistosoma_japonicum.PRJEA34885.WBPS9.CDS_transcripts.fa
450 OUF=~ /Kallistooutput
451
452 cd ${OUF}

```

```

453
454 ~/tools/kallisto_linux-v0.43.1/kallisto index -i transcripts.idx ${CDS}
455
456 # Run Kallisto
457
458 READS=~pathwaytoreads
459 OUF=~Kallistooutput
460 LIST=Input text file containing the library_ID and located in OUF
461
462 cd ${READS}
463
464 while read i; do
465 mkdir ${OUF}/${i}
466
467 ~/tools/kallisto_linux-v0.43.1/kallisto quant -t 16 -i ${OUF}/transcripts.idx -o
468 ${OUF}/${i} -b 100 --fr-stranded ${READS}/${i}_forward ${READS}/${i}_reverse
469
470 done < ${LIST}
471

```

472 **Comparative analysis of gene expression**

473 We perform all the expression analyses in R (Script9_Transcriptomics) with the input files (i) InputFile7_Transcriptomics1
474 corresponding to the newly identified one-to-one orthologs (see 1.2); and (ii) InputFile10_Transcriptomics2
475 corresponding to the list obtained on WormBase parasite.

477 In adults, a correlation analysis revealed an inconsistency between replicates, corresponding to the *S. mansoni* female and
478 male adults: ERR506076 and ERR506082. The corresponding heatmaps are shown in Appendix 1-figure 4. These two
479 libraries were excluded from further analyses.

481 Tables resulting from the Loess normalization are InputFile8_SuperTableNormalized_Transcriptomics1_RPKM,
482 InputFile8-2_SuperTableNormalized_LoessOnAll_Transcriptomics1_RPKM,
483 InputFile9_SuperTableNormalized_Transcriptomics1_TPM, and
484 InputFile11_SuperTableNormalized_Transcriptomics2_RPKM.
485 They can be used directly in the script Script9_Transcriptomics at the step C.

487 **Number of Protein-Protein Interactions (PPIs)**

488 The full set of known PPIs for *S. mansoni* was downloaded from StringDB:
489 <https://stringdb-static.org/download/protein.links.full.v10.5/6183.protein.links.full.v10.5.txt.gz>

491 We kept only interactions supported by experimental evidence or text mining:

```

492
493 cat 6183.protein.links.full.v10.5.txt | awk '($10>0 || $14>0)' | awk '{print $1, $2}' |
494 perl -pi -e 's/6183\.\.//gi' | perl -pi -e 's/___mRNA//gi' | perl -pi -e 's/\.[0-9]*//gi'
495 > 6183.protein.links.full.v10.5_exp.txt
496

```

497 We cleaned the coverage location, so that "Not analysed (Scaffold <40Kb)" was replaced by NA:

```

498
499 cat InputFile14_PPIs_woNA | perl -pi -e 's/Not analysed \(Scaffold \< 40kb\) /NA/gi' |
500 sort > InputFile14_PPIs
501

```

502 Then counted the number of interactions present for each gene:

```

503
504 perl Script11_PPIcounter InputFile14_PPIs.txt 6183.protein.links.full.v10.5_exp.txt
505

```

506 Output: 5 last columns of "InputFile8-2_SuperTableNormalized_LoessOnAll_Transcriptomics1_RPKM" (\$Autocount_exp
507 \$Zcount_exp \$Excludedcount_exp \$NAcount_exp \$TotalPPIs_exp)

510 **Proteomics vs microarrays correlation**

511 We performed all the expression analyses in R (Script10_Proteomics_Microarrays) with the input files (i)
512 InputFile12_Proteomics1_Microarrays for which a low value was imputed to undetected proteins*; and (ii)
513 InputFile13_Proteomics2_Microarrays corresponding to the raw data, without imputation.

515 *such imputation is necessary for the statistical analysis of gene expression differences between males and females (Figure 4-
516 source data 1 and 2).

518

519 **Obtaining Ka & Ks values for *Schistosoma***

520

521 CDS sequences were downloaded from Parasite Wormbase, and 1:1 orthologs detected with a stringent reciprocal best hit
522 approach (Blat with a translated query and database, and a minimum mapping score of 100). These orthologs were
523 aligned with TranslatorX (<http://translatorx.co.uk/>) with the Gblocks option, and Ka and Ks values were obtained with
524 KaKs_calculator (<https://sourceforge.net/projects/kakscalculator2/>, model NG). The specific commands used are
525 provided below. Tables of Ka and Ks values are provided as input files 15 and 16.

526

527 ***S. mansoni* / *S. haematobium***

528

```
529 # Get CDS
```

530

```
531 wget
```

```
532 ftp://ftp.ebi.ac.uk/pub/databases/wormbase/parasite/releases/WBPS10/species/schistosoma  
533 _mansoni/PRJEA36577/schistosoma_mansoni.PRJEA36577.WBPS10.CDS_transcripts.fa.gz
```

534

```
535 gzip -d schistosoma_mansoni.PRJEA36577.WBPS10.CDS_transcripts.fa.gz
```

536

```
537 mv schistosoma_mansoni.PRJEA36577.WBPS10.CDS_transcripts.fa sman_cds.fa
```

538

```
539 wget
```

```
540 ftp://ftp.ebi.ac.uk/pub/databases/wormbase/parasite/releases/WBPS10/species/schistosoma  
541 _haematobium/PRJNA78265/schistosoma_haematobium.PRJNA78265.WBPS10.CDS_transcripts.fa.gz
```

542

```
543 gzip -d schistosoma_haematobium.PRJNA78265.WBPS10.CDS_transcripts.fa.gz
```

544

```
545 mv schistosoma_haematobium.PRJNA78265.WBPS10.CDS_transcripts.fa shaem_cds.fa
```

546

```
547 # Run KaKS pipeline
```

548

```
549 perl Script12_KaKs.pl Sman_Shaem/shaem_cds.fa Sman_Shaem/sman_cds.fa Sman_Shaem/
```

550

551

552 ***S. mansoni* / *S. japonicum***

553

```
554 # Get CDS
```

555

```
556 wget
```

```
557 ftp://ftp.ebi.ac.uk/pub/databases/wormbase/parasite/releases/WBPS10/species/schistosoma  
558 _mansoni/PRJEA36577/schistosoma_mansoni.PRJEA36577.WBPS10.CDS_transcripts.fa.gz
```

559

```
560 gzip -d schistosoma_mansoni.PRJEA36577.WBPS10.CDS_transcripts.fa.gz
```

561

```
562 wget
```

```
563 ftp://ftp.ebi.ac.uk/pub/databases/wormbase/parasite/releases/WBPS10/species/schistosoma  
564 _japonicum/PRJEA34885/schistosoma_japonicum.PRJEA34885.WBPS10.CDS_transcripts.fa.gz
```

565

```
566 gzip -d schistosoma_japonicum.PRJEA34885.WBPS10.CDS_transcripts.fa.gz
```

567

```
568 mv schistosoma_japonicum.PRJEA34885.WBPS10.CDS_transcripts.fa sjap_cds.fa  
569 mv schistosoma_mansoni.PRJEA36577.WBPS10.CDS_transcripts.fa sman_cds.fa
```

570

```
571 # Run KaKS pipeline
```

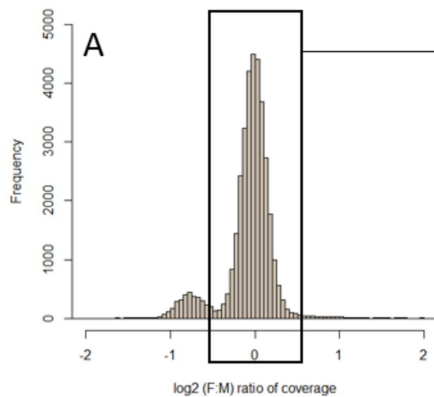
572

```
573 perl Script12_KaKs.pl Sman_Sjap/sjap_cds.fa Sman_Sjap/sman_cds.fa Sman_Sjap/
```

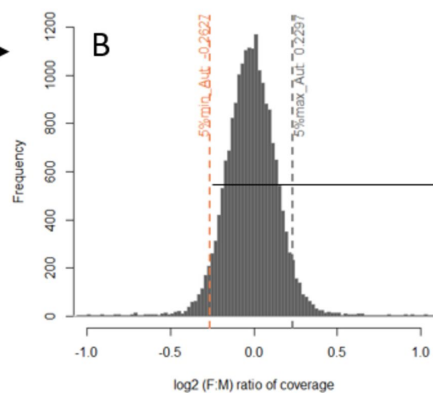
574

575

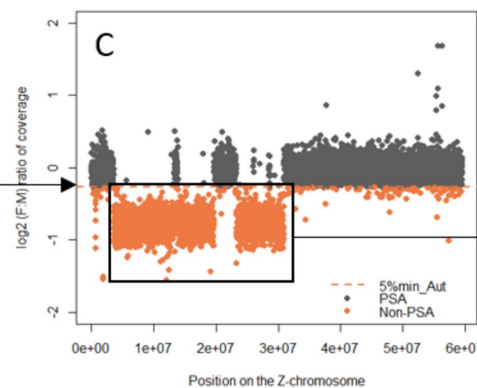
F:M coverage distribution - *S. mansoni*



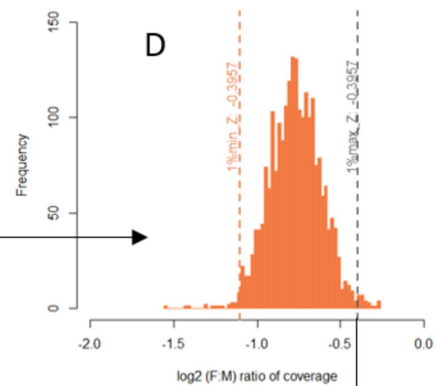
F:M coverage distribution - Autosomes



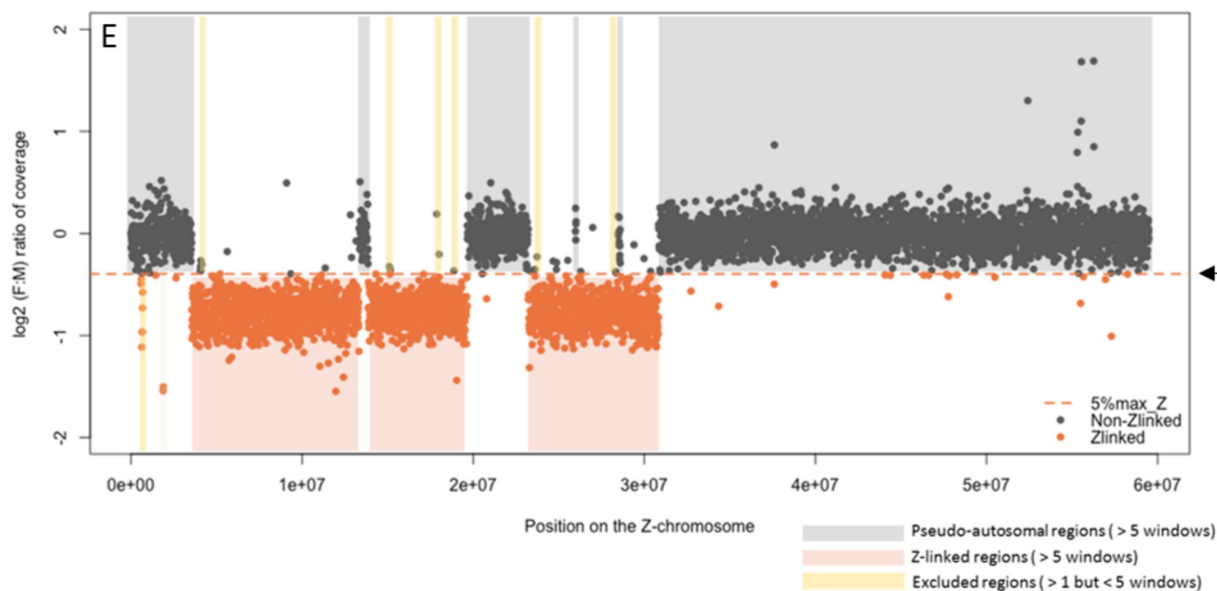
F:M coverage along *S. mansoni* Z-chromosome



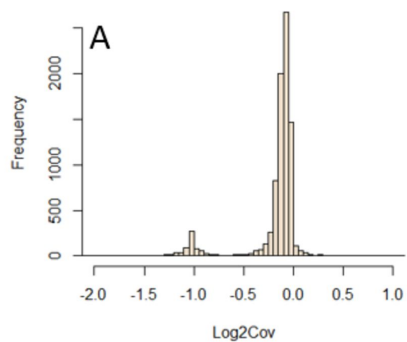
F:M coverage distribution - Non-autosomes



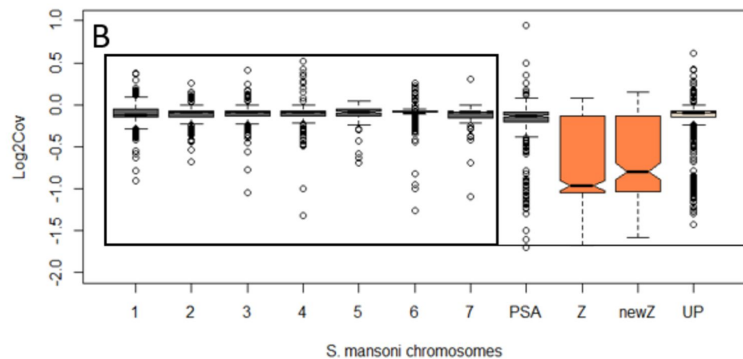
Genomic coverage distribution along *S. mansoni* Z-chromosome



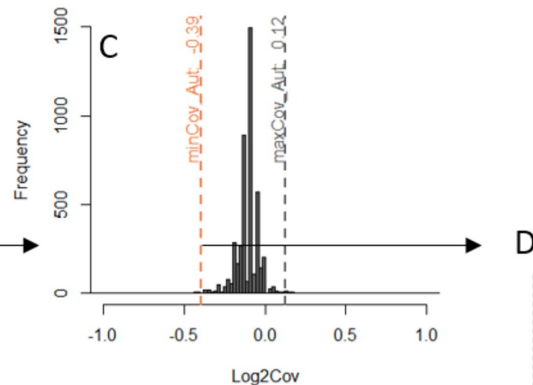
F:M coverage distribution - *S. japonicum*



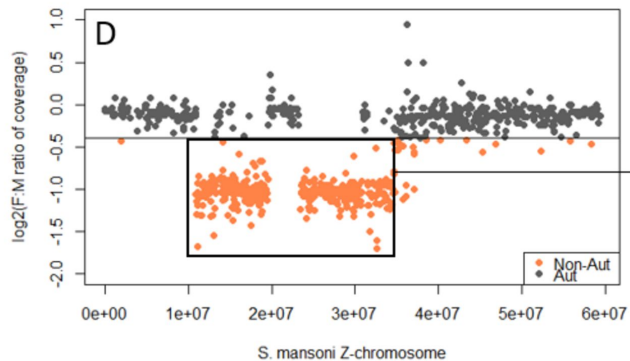
***S. japonicum* (Female:Male) coverage depending on chromosome assignment**



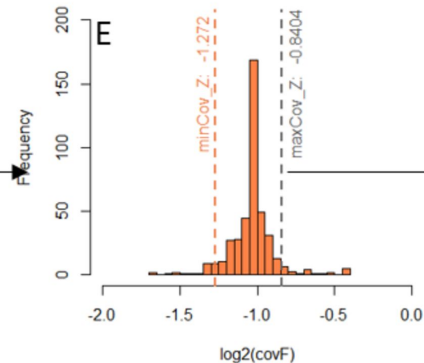
F:M coverage distribution - Autosomes



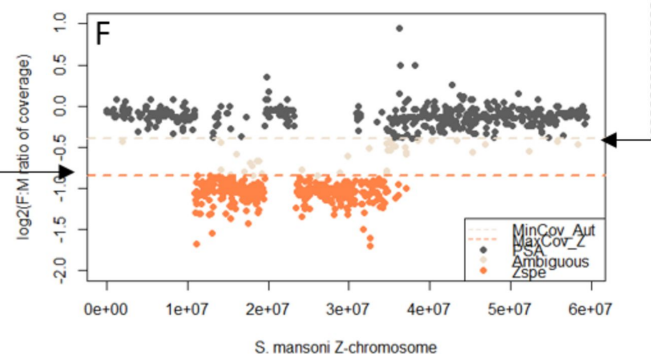
F:M coverage along *S. mansoni* Z-chromosome



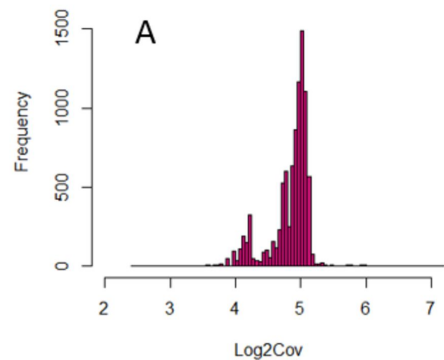
F:M coverage distribution - Non-autosomes



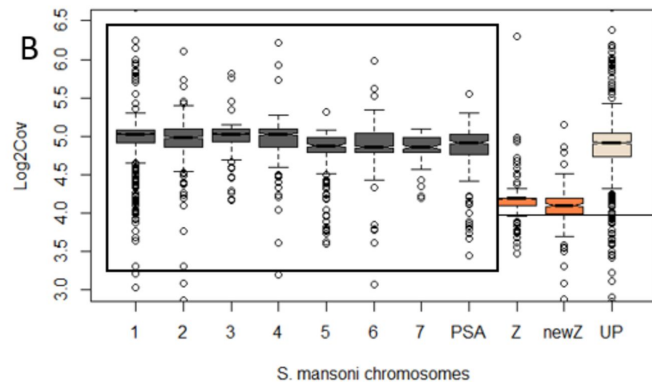
Assignment of *S. japonicum* scaffolds position



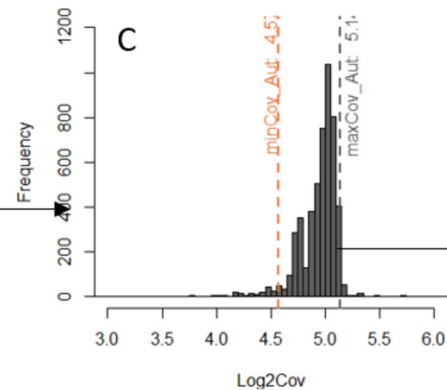
S. haematobium female genomic coverage



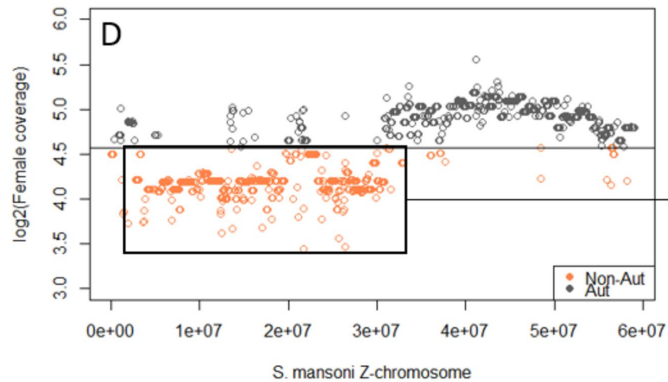
Female coverage depending on chromosome assignment



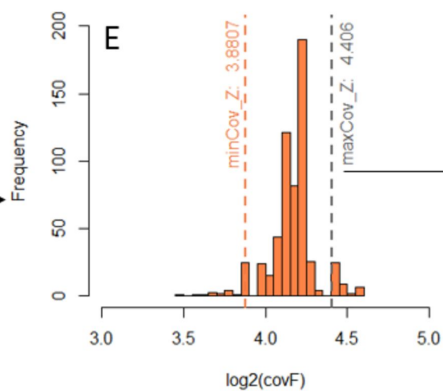
Female coverage - Autosomes



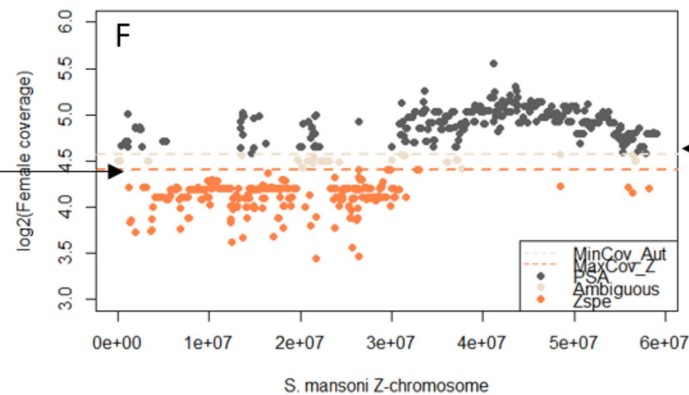
Female coverage along S. mansoni Z-chromosome



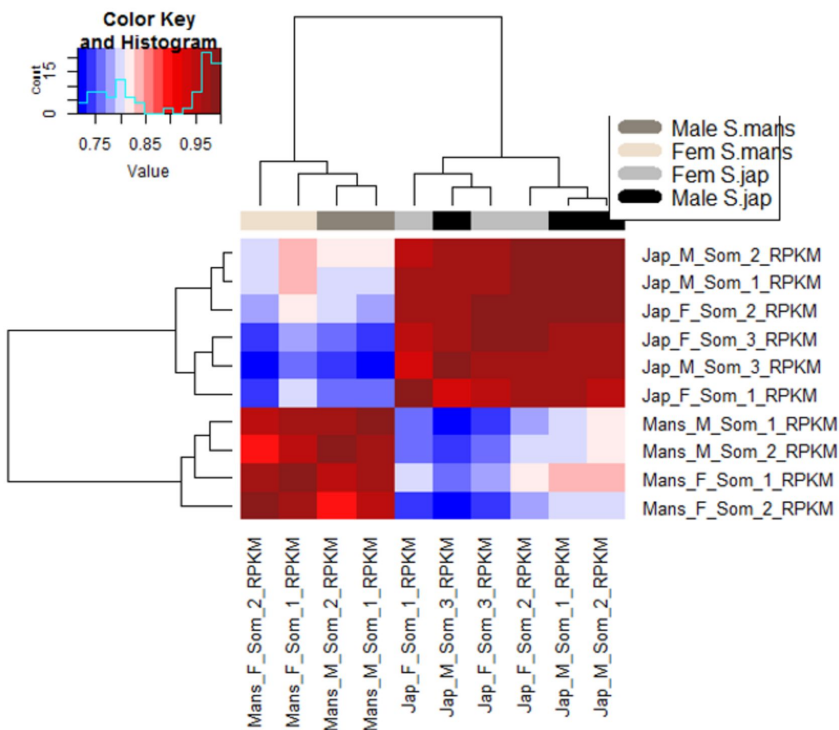
F:M coverage distribution - Non-autosomes



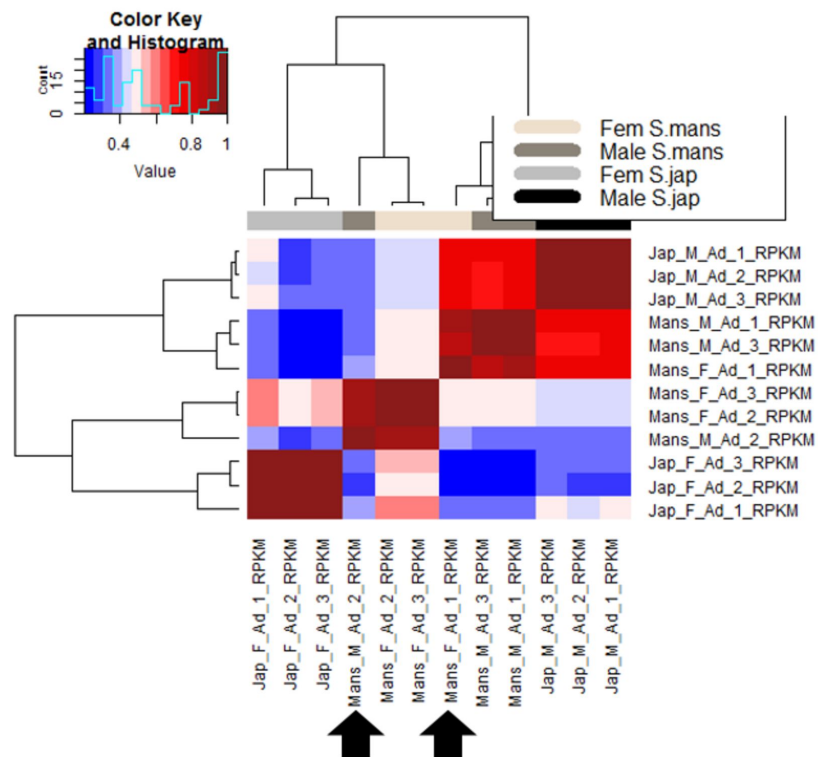
Assignment of S. haematobium scaffolds position



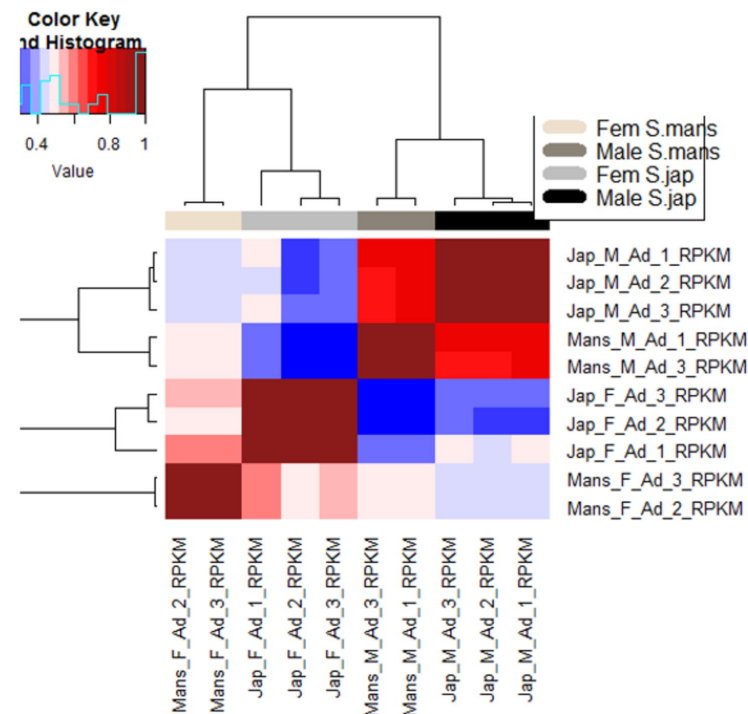
A. Schistosomula (RPKM data)

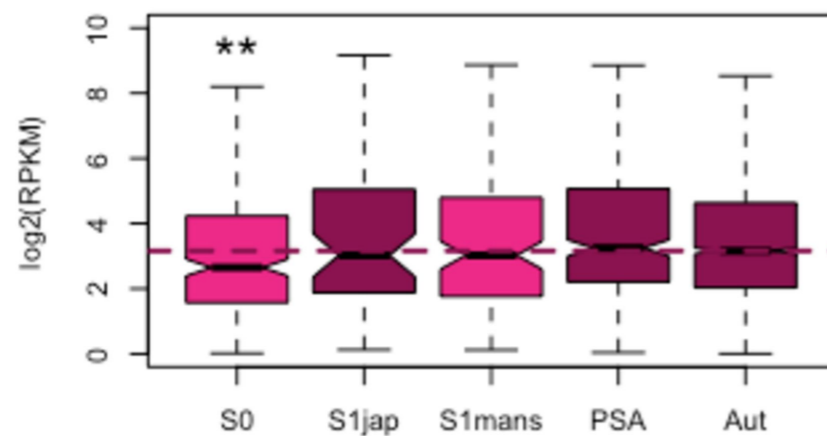
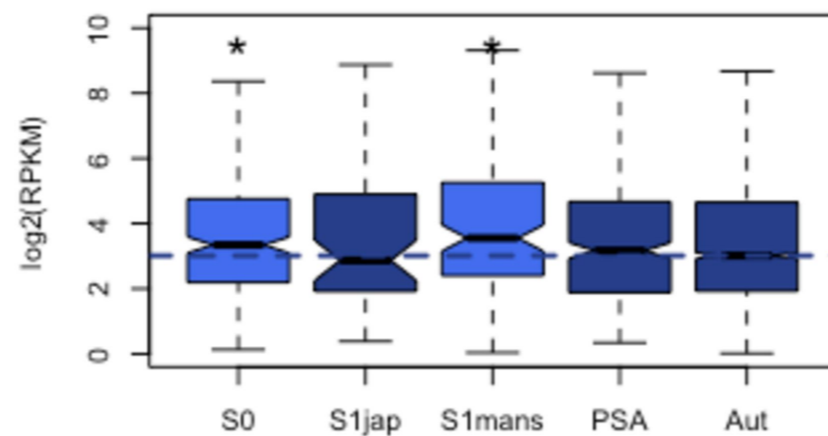
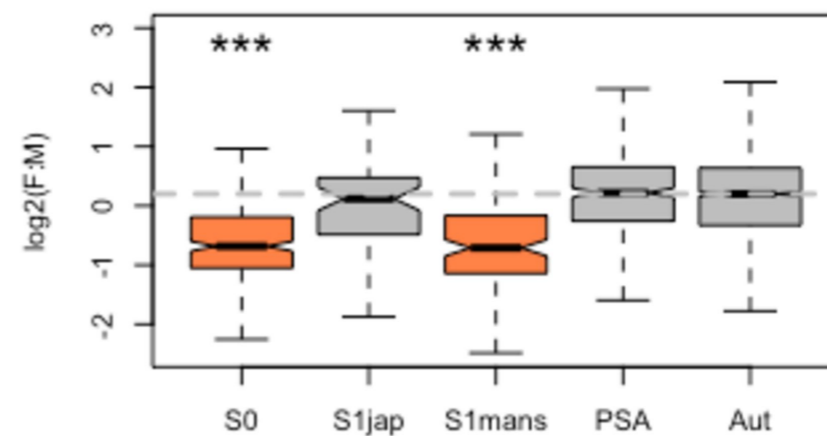
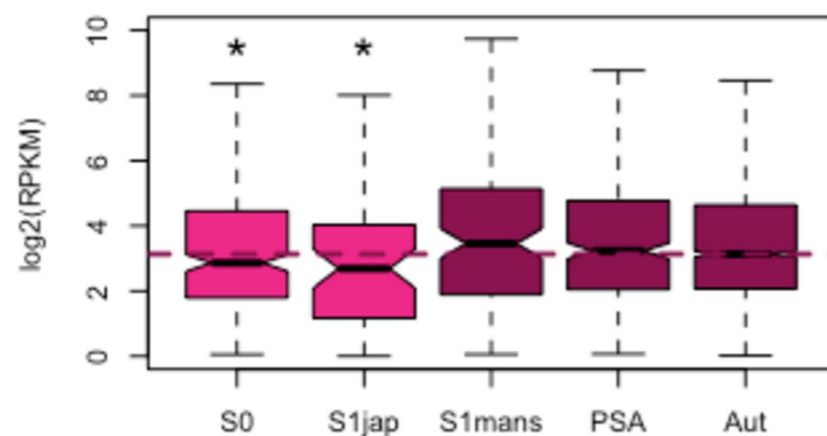
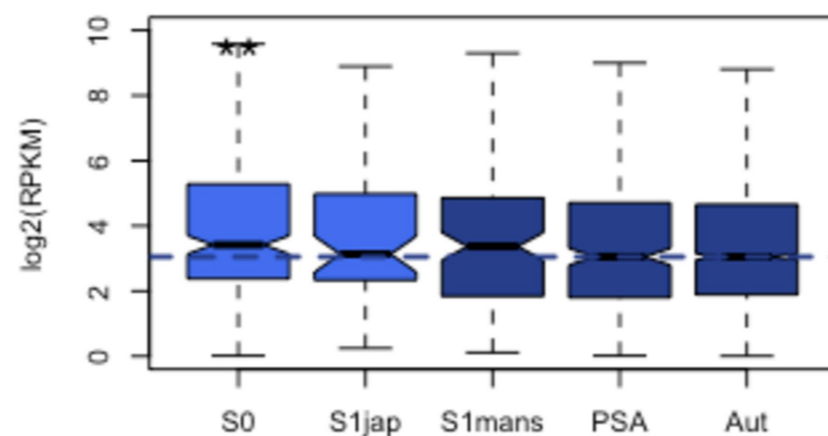
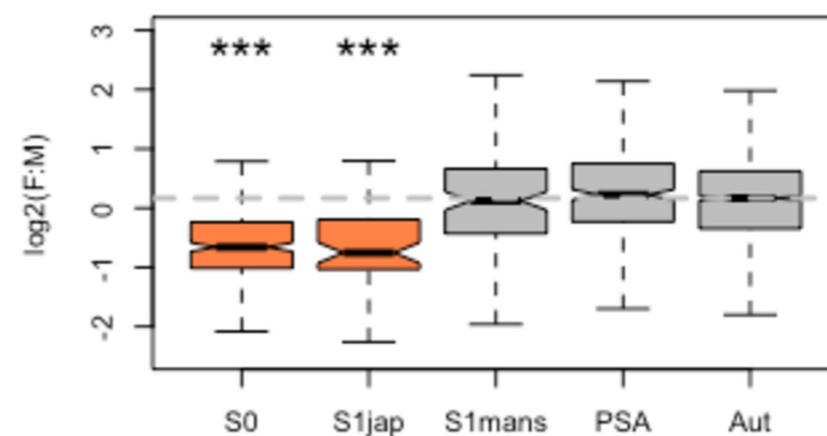
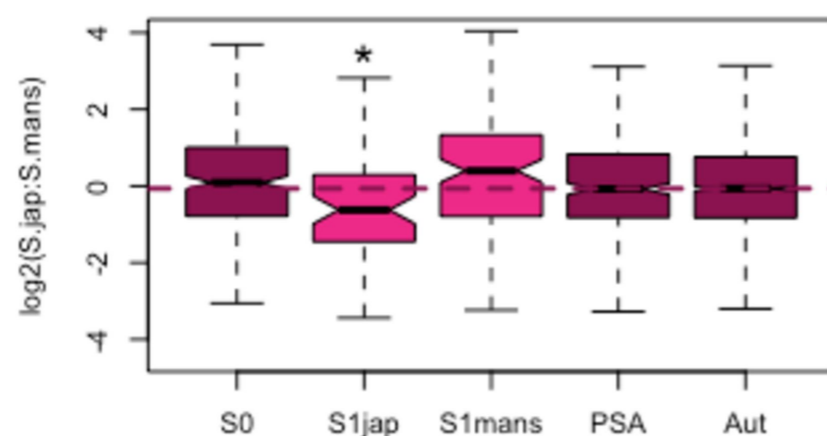
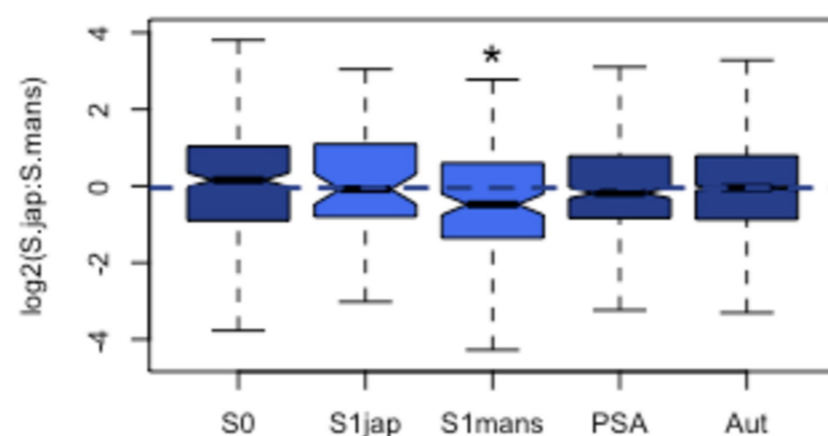
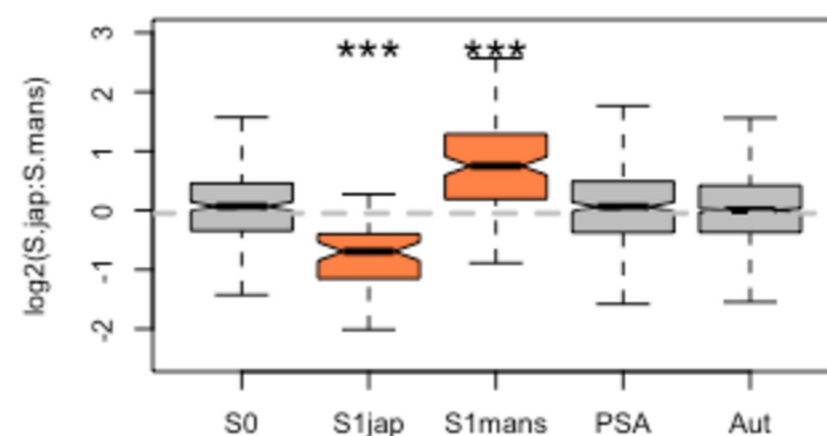


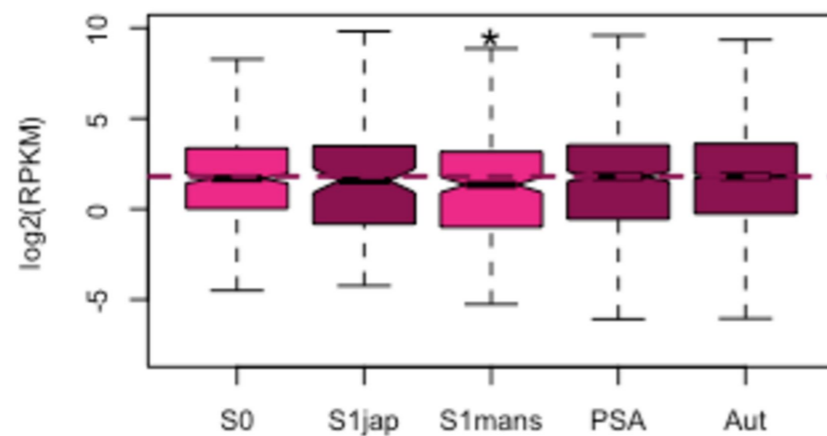
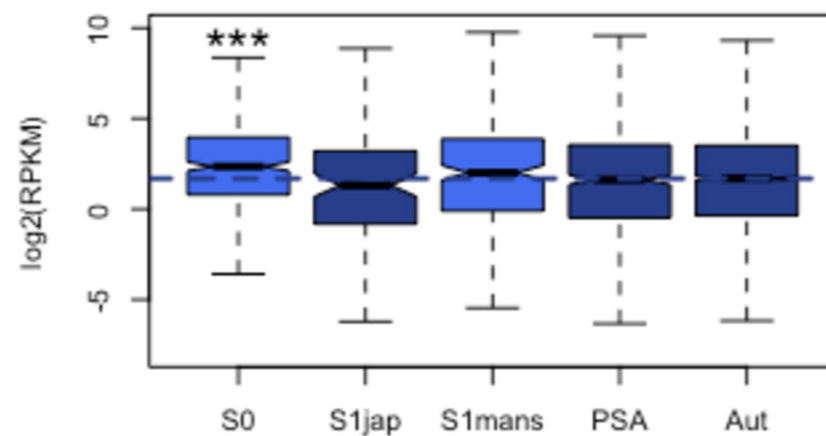
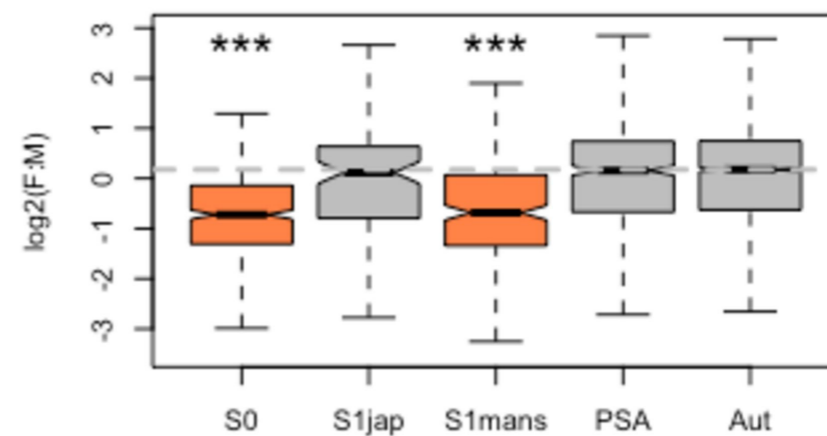
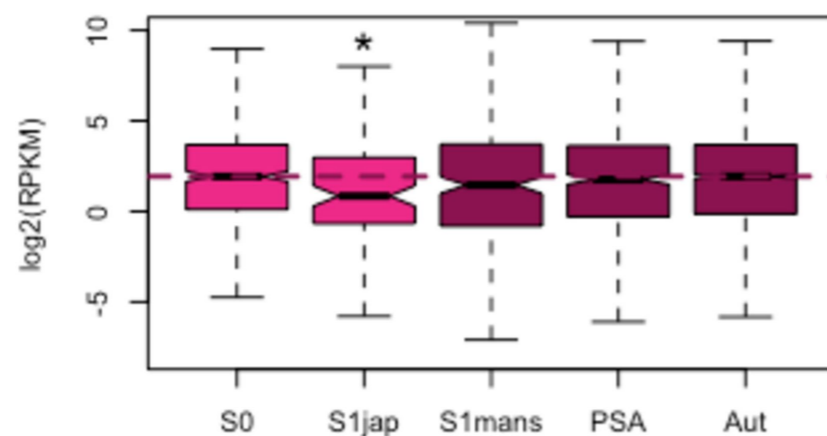
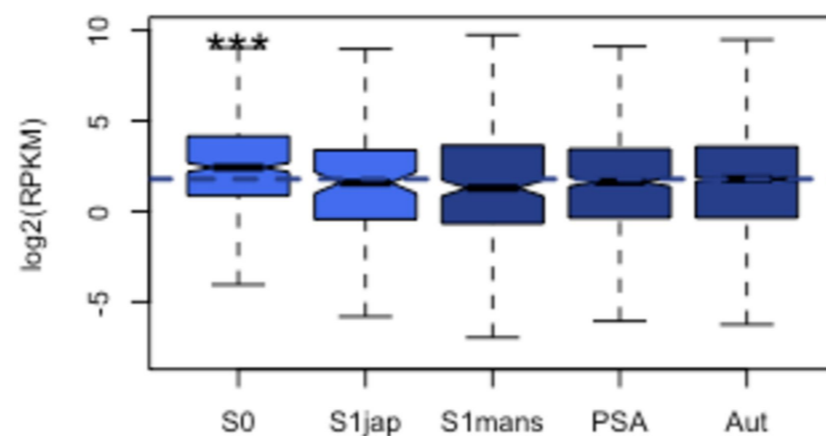
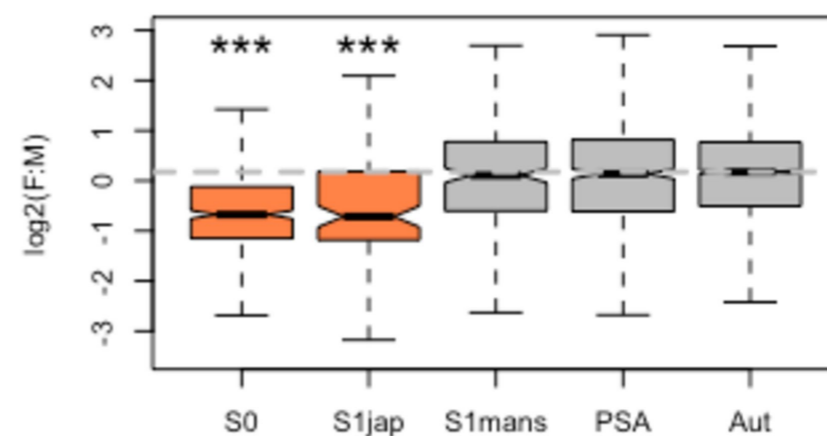
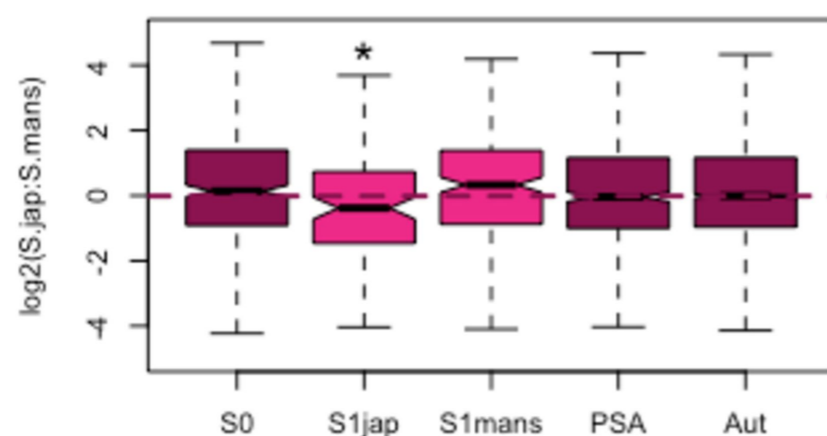
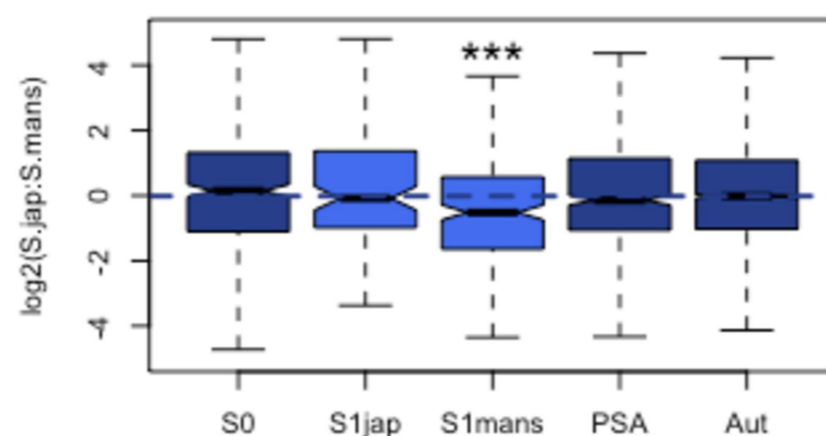
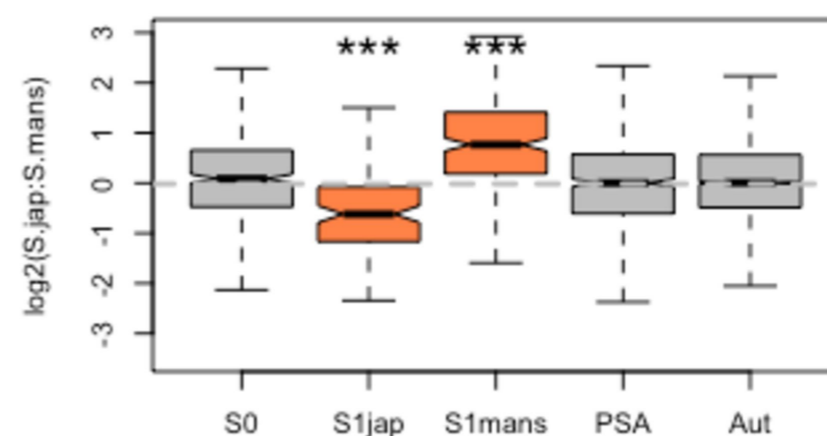
B. Adults (RPKM data)

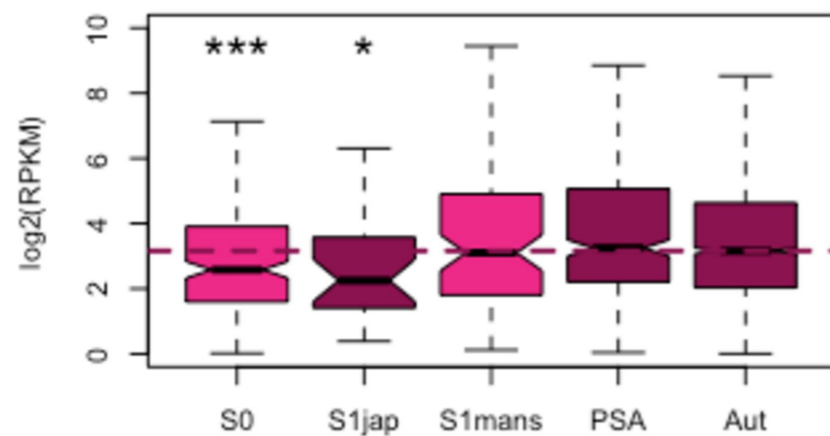
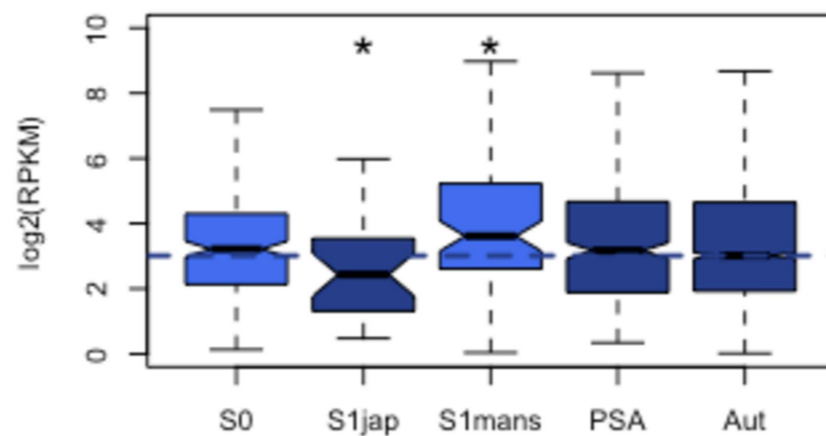
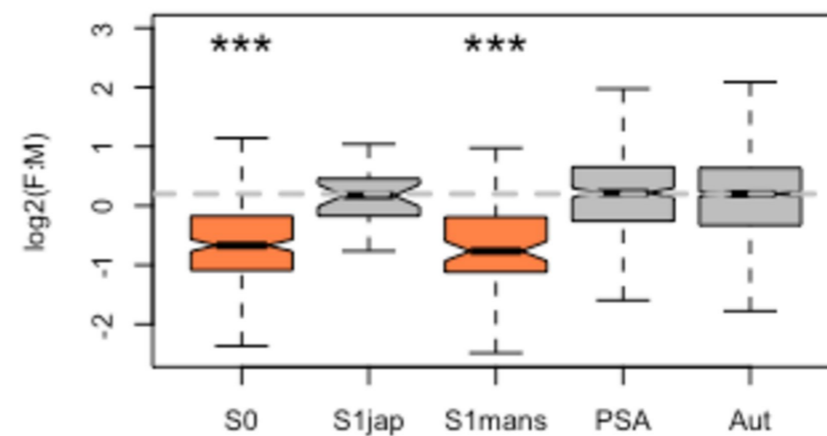
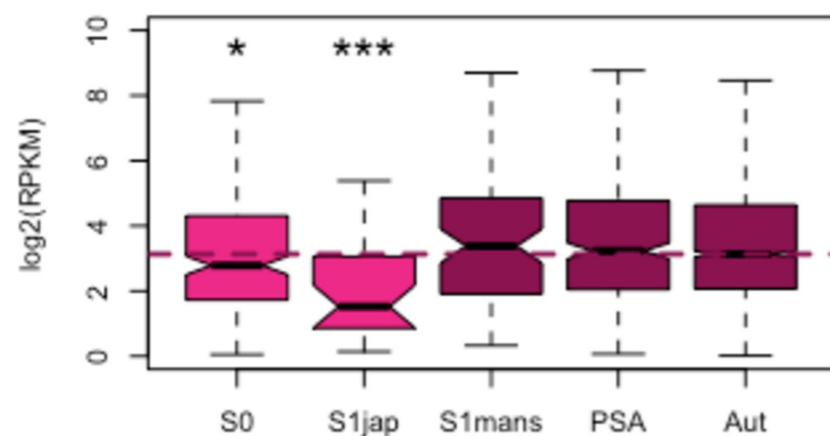
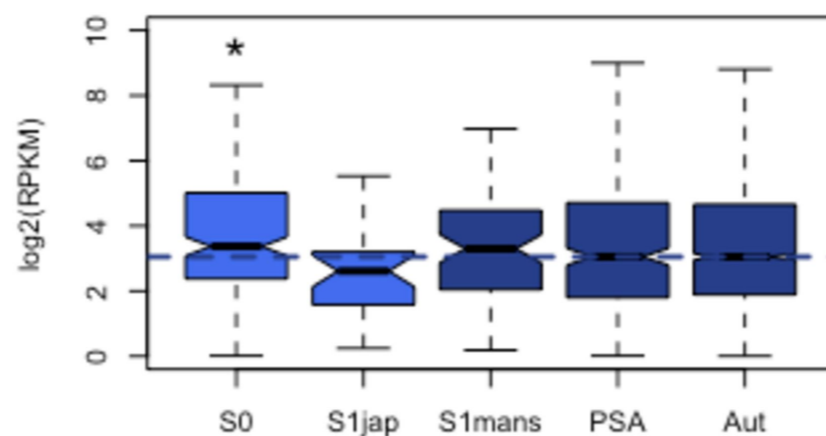
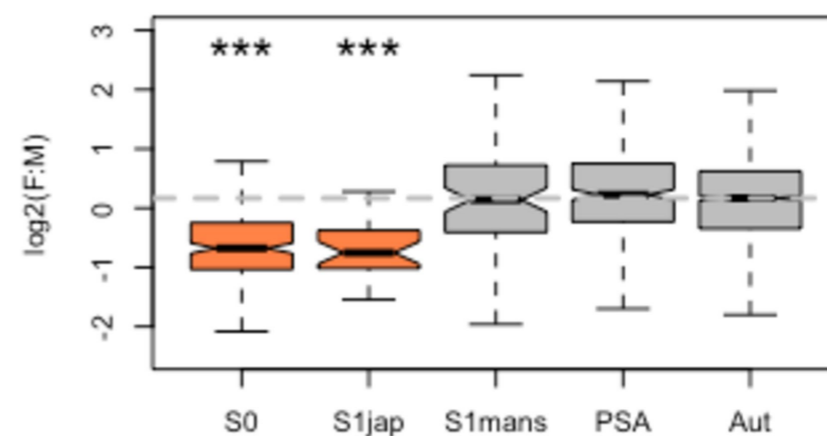
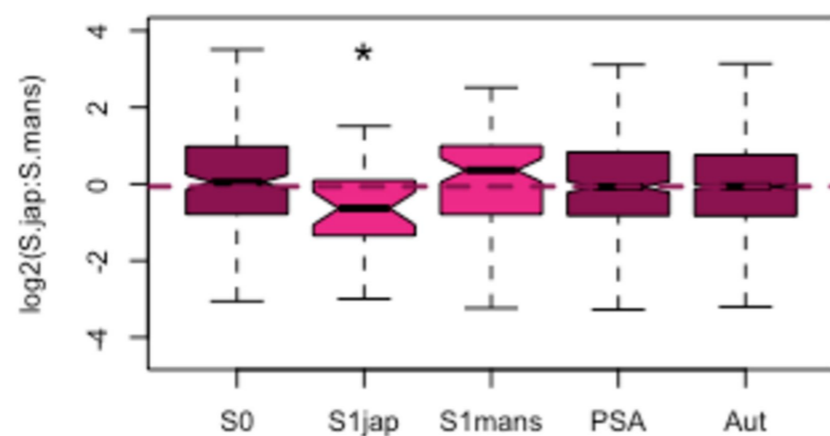
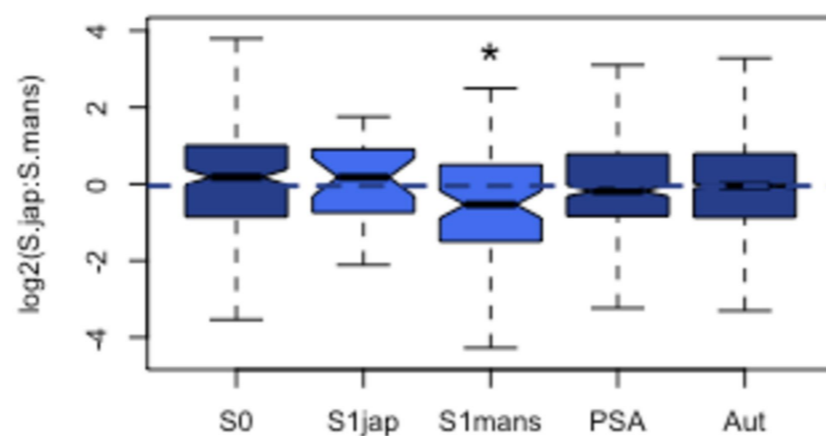
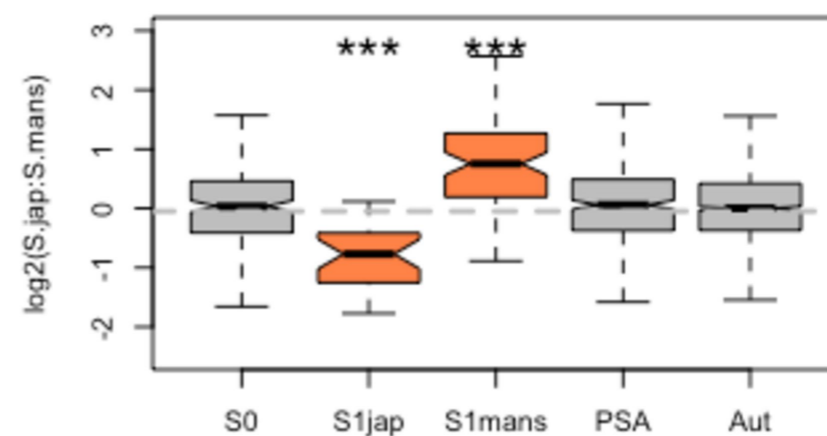


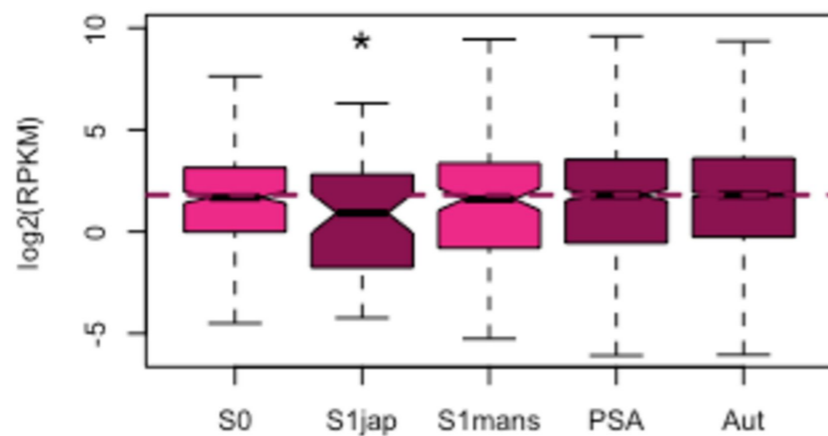
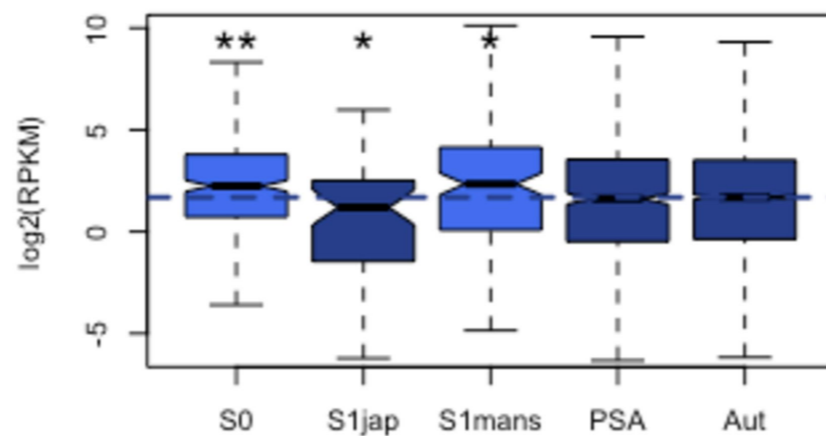
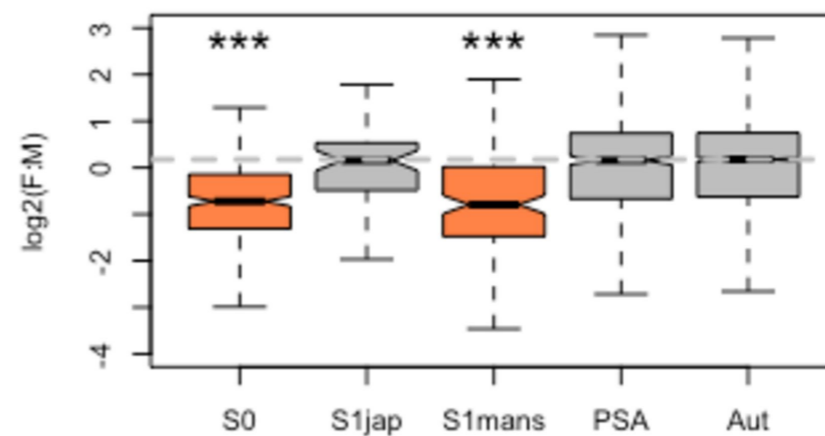
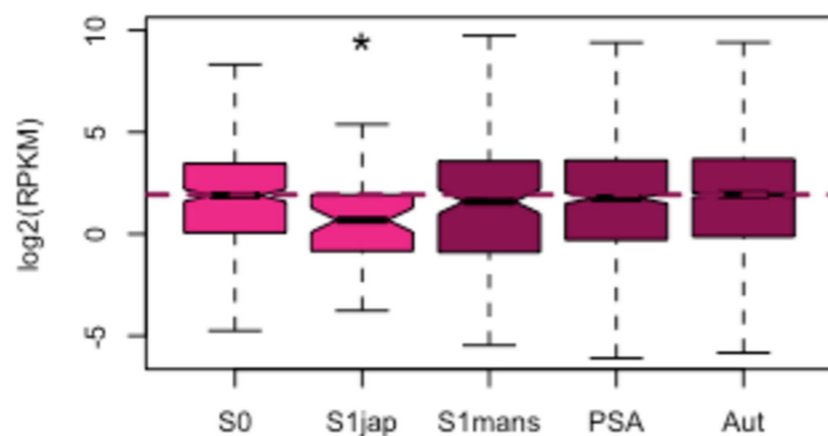
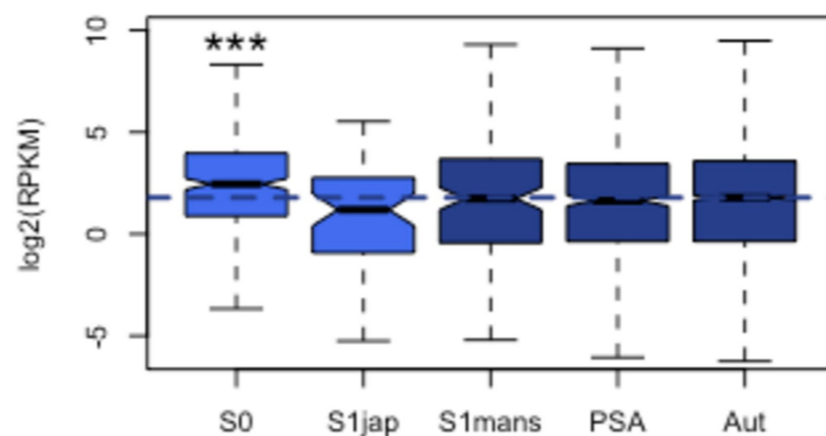
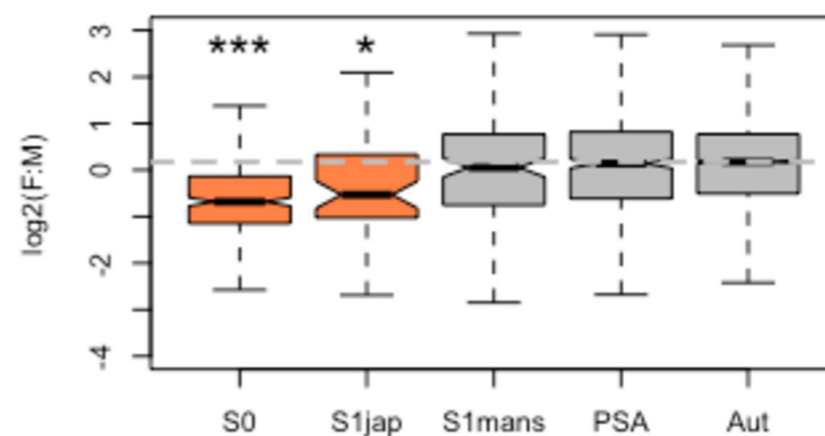
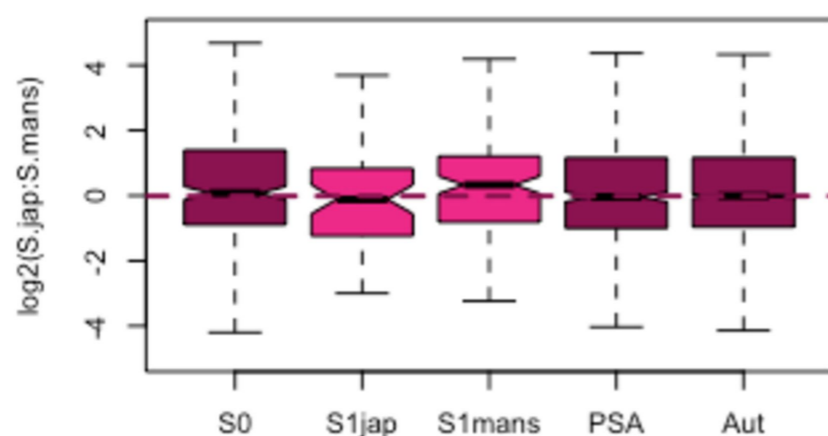
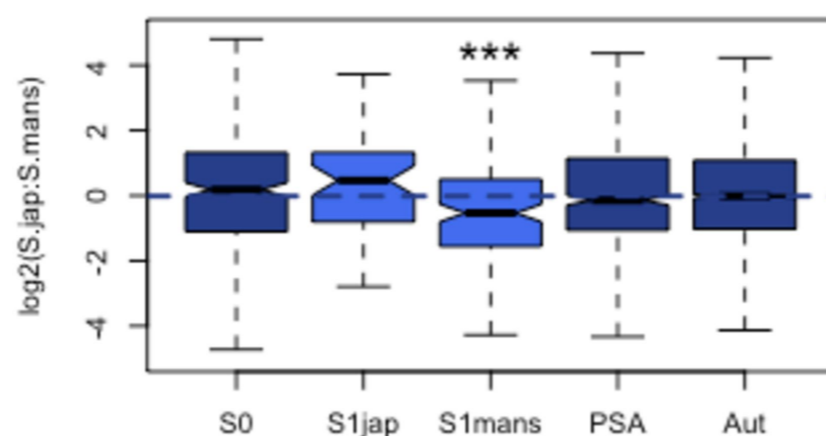
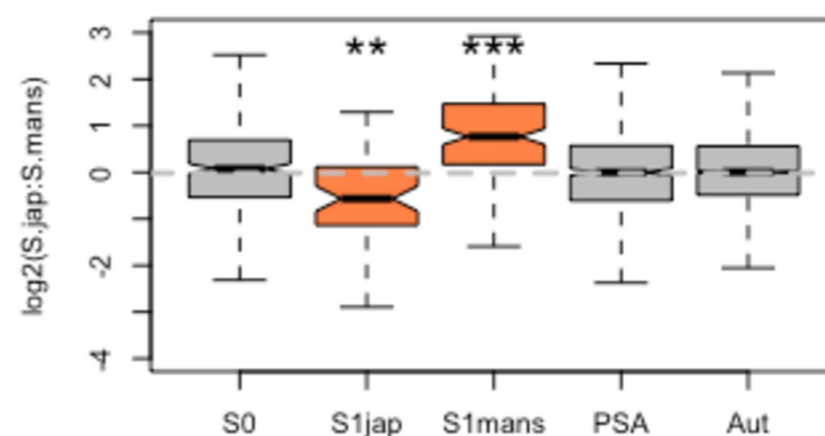
C. Adults (RPKM data) – After exclusion

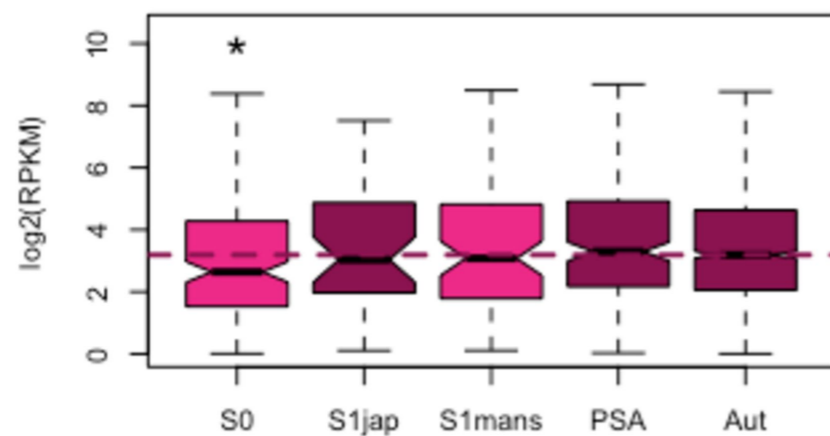
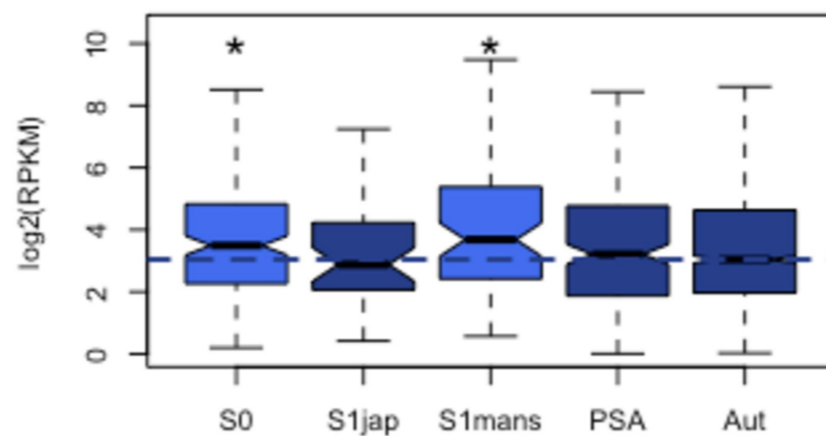
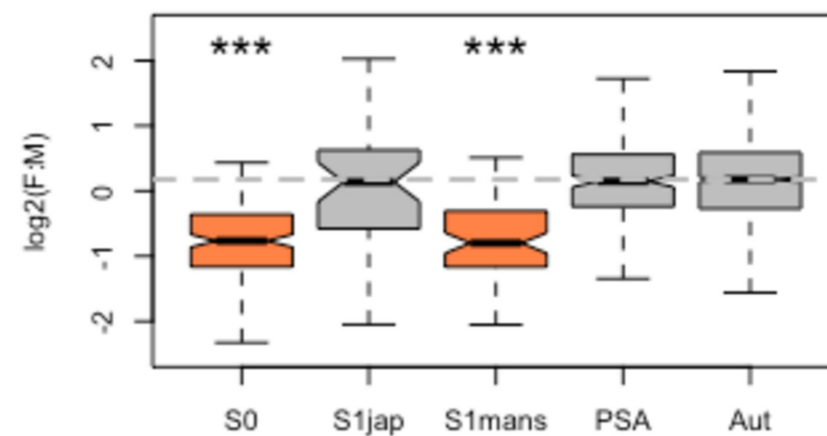
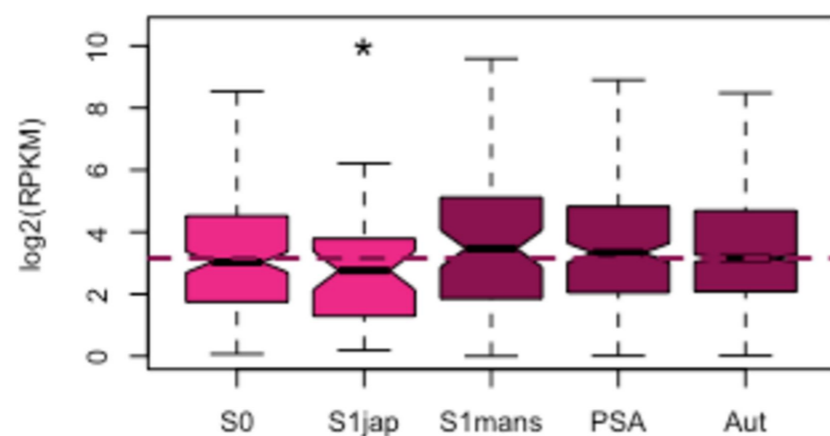
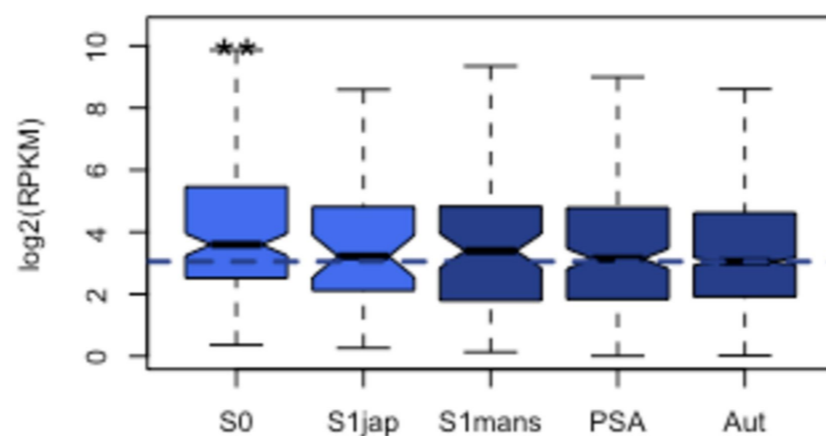
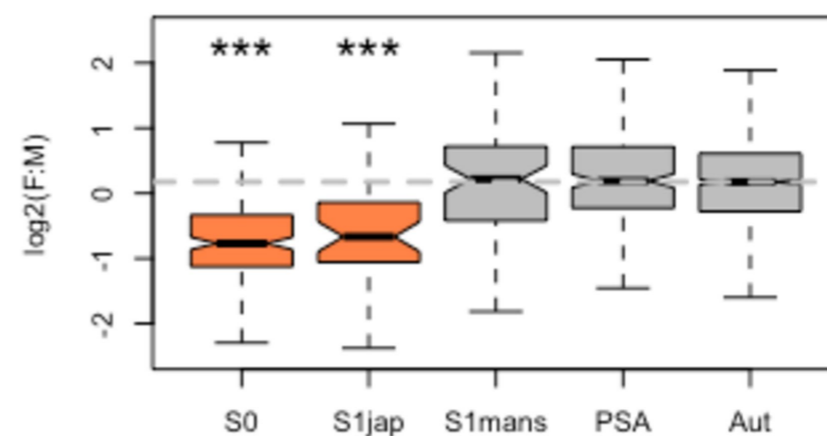
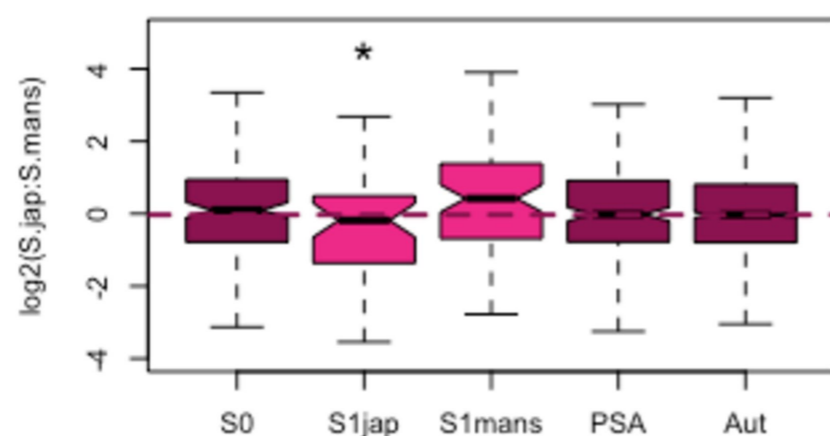
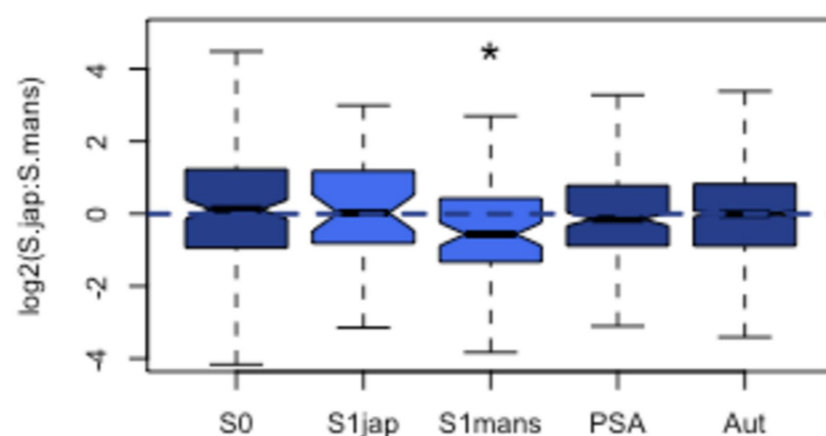
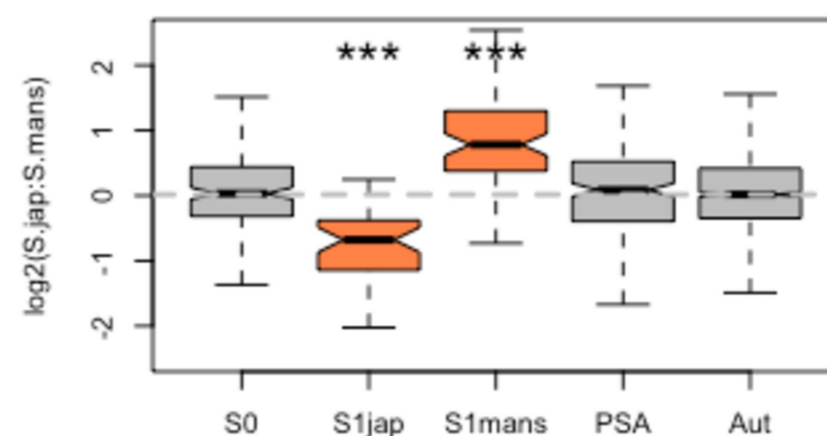


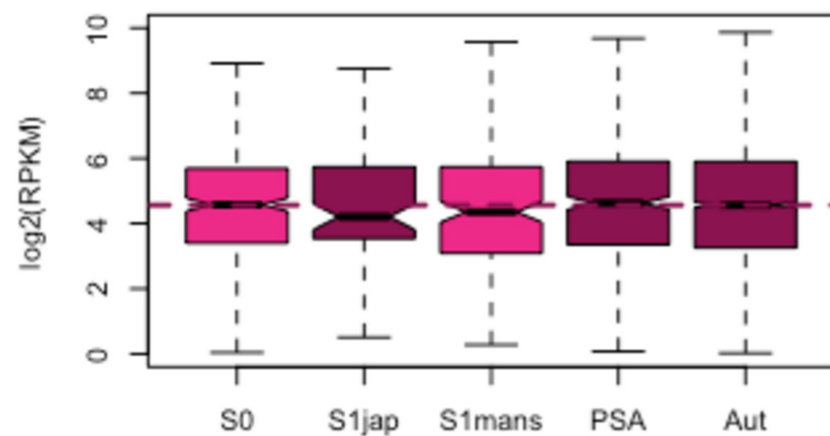
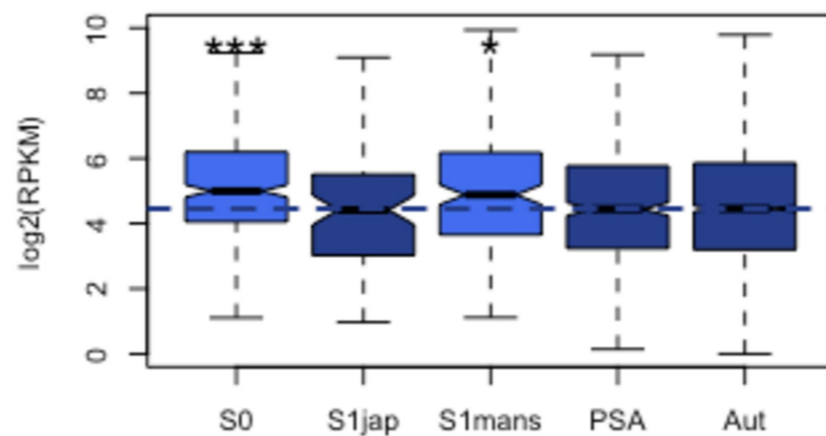
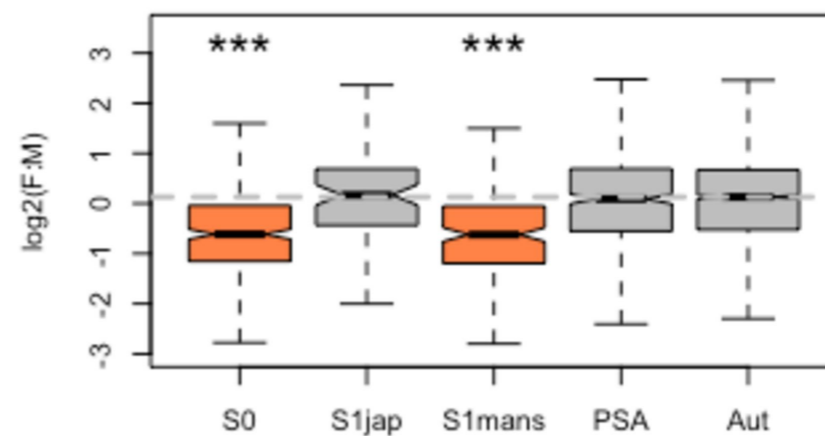
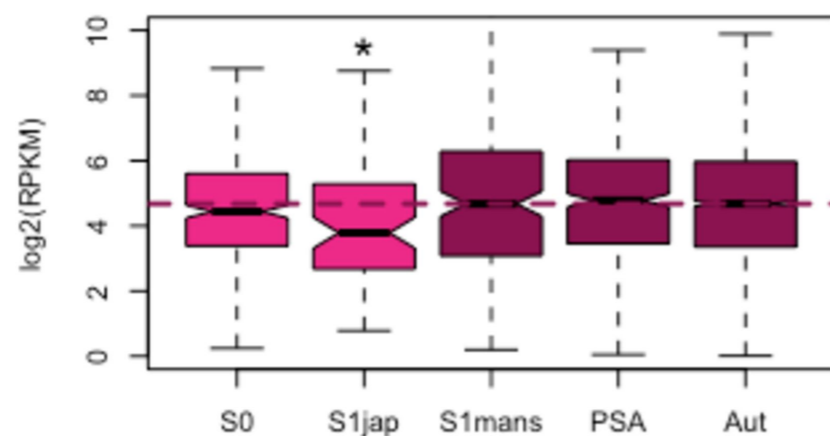
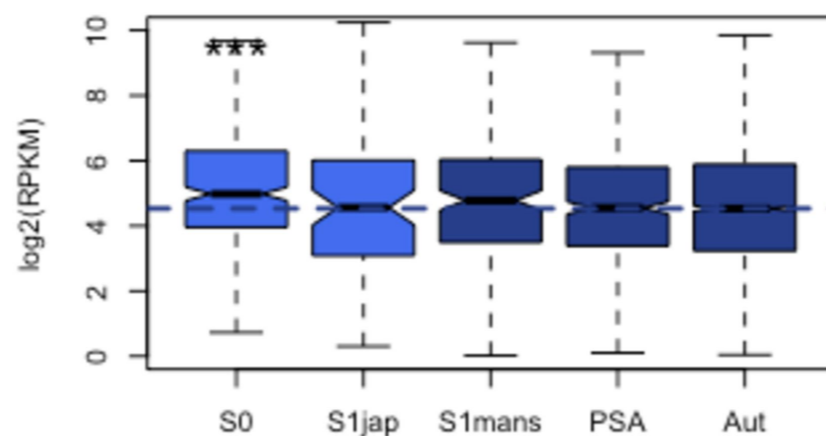
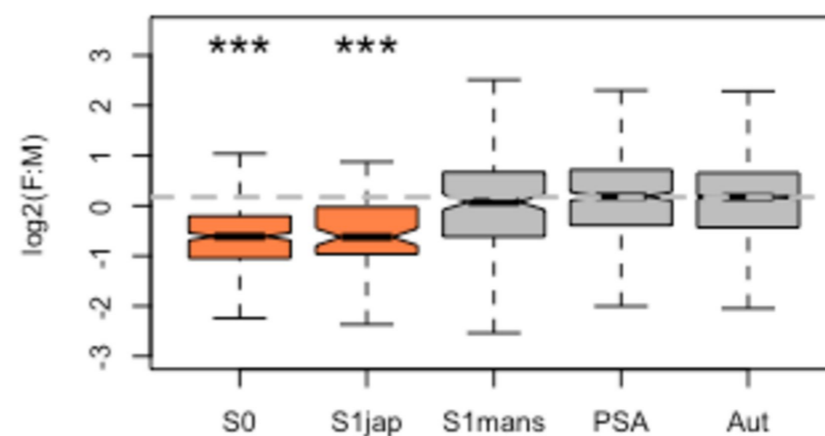
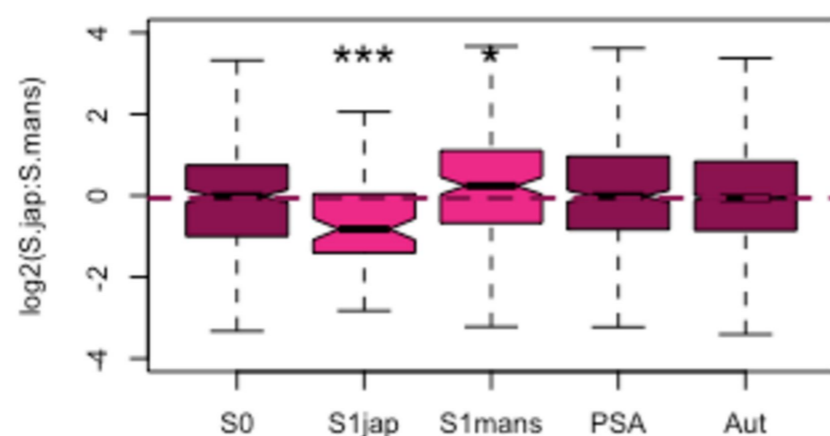
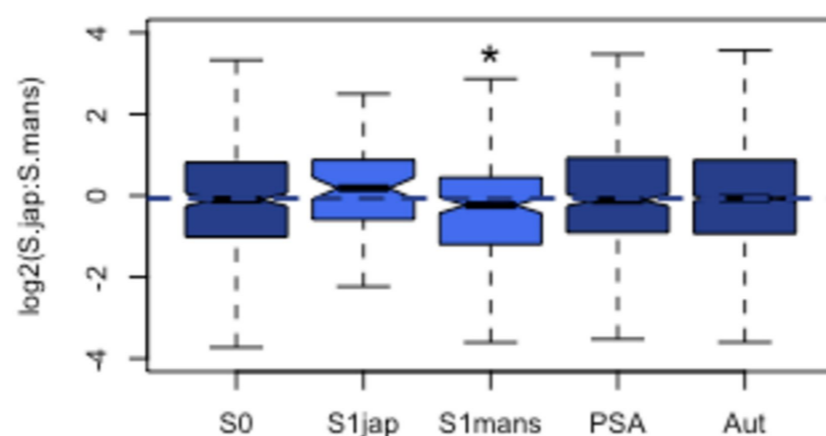
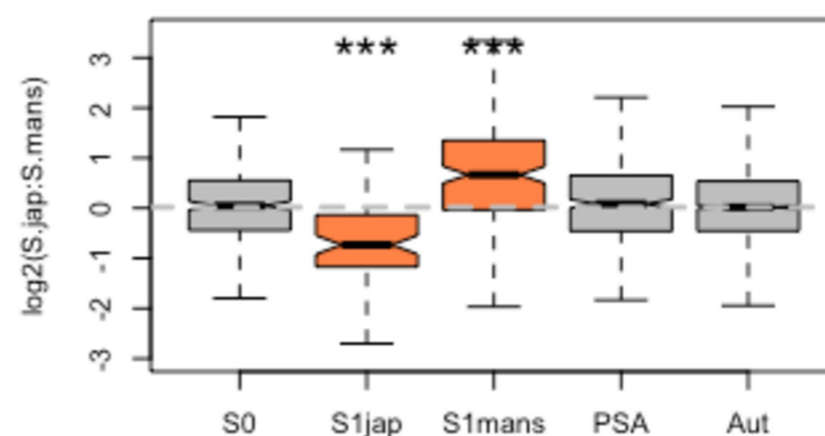
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

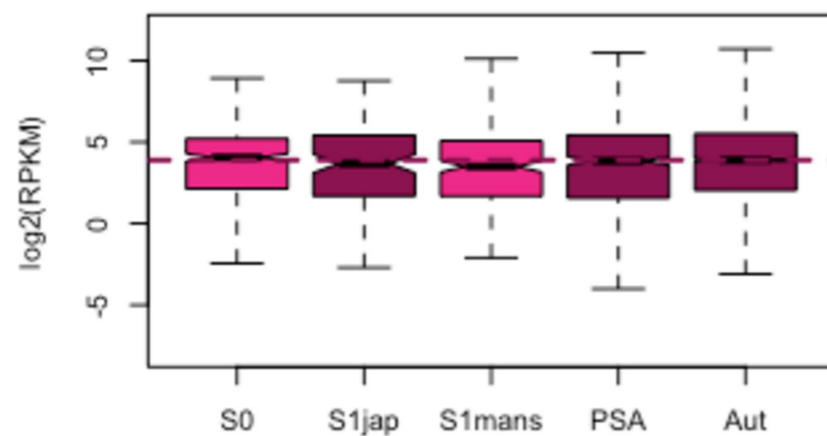
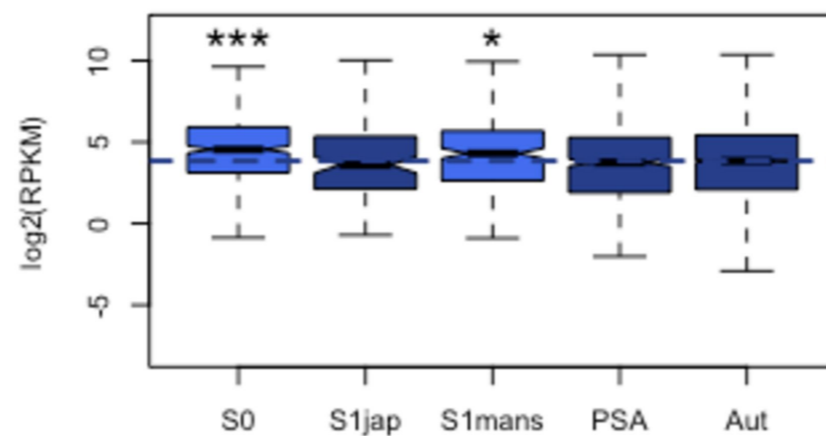
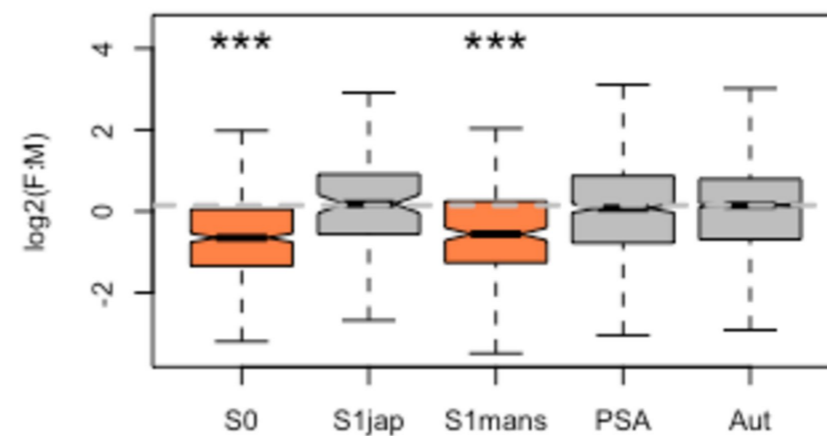
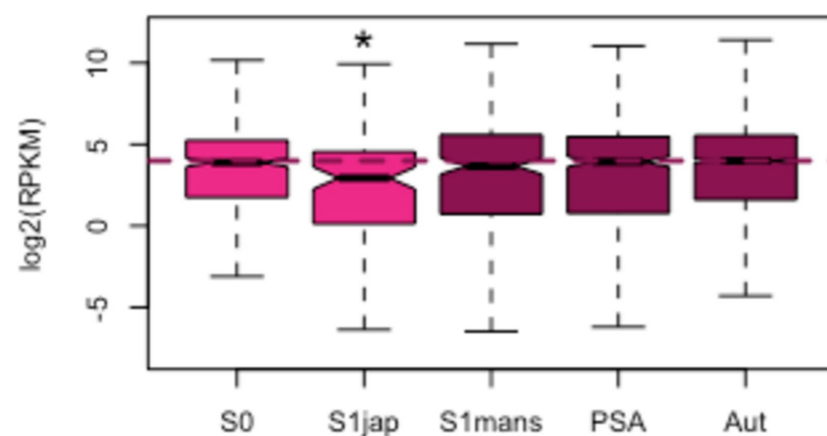
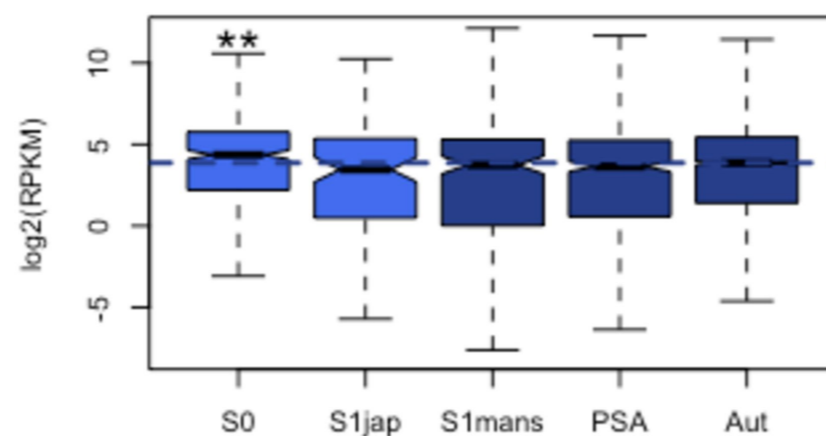
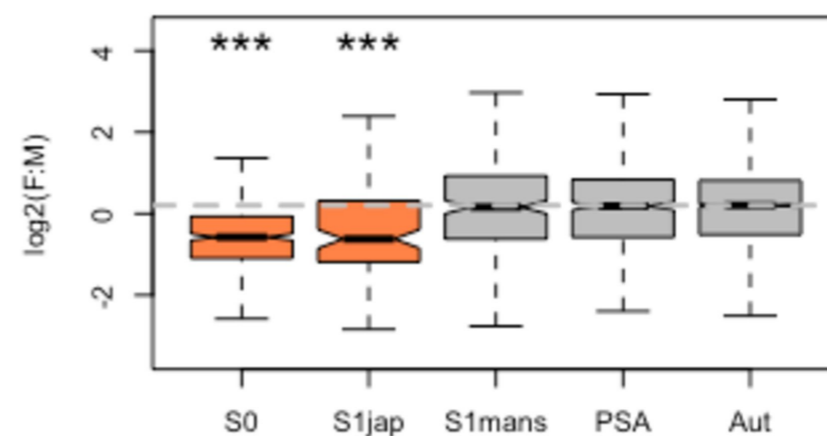
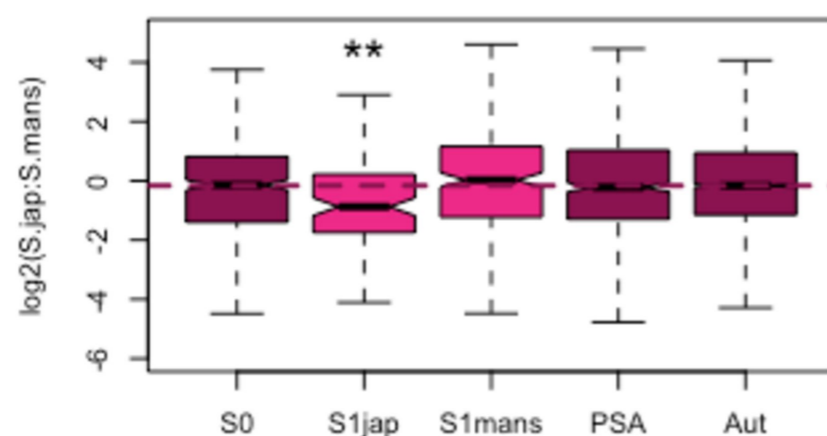
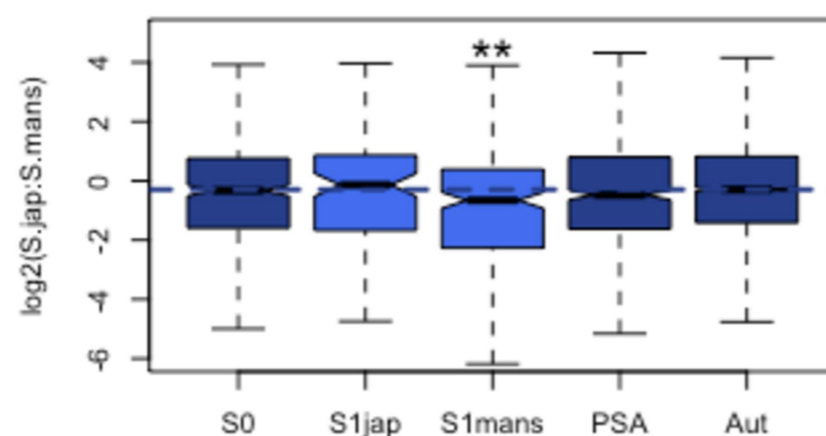
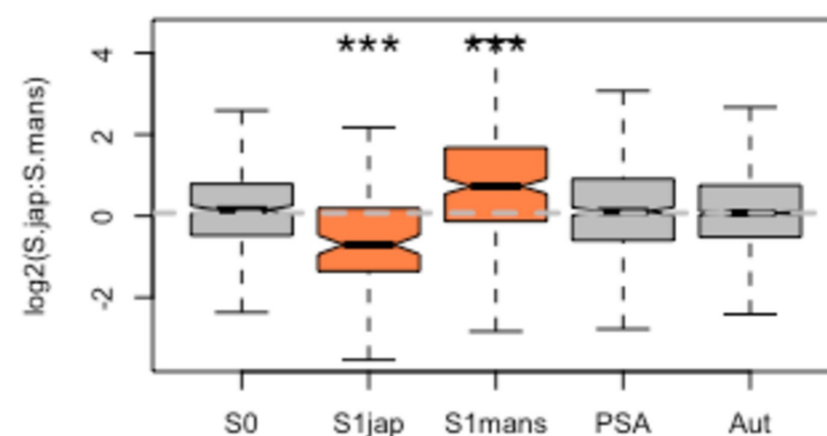
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

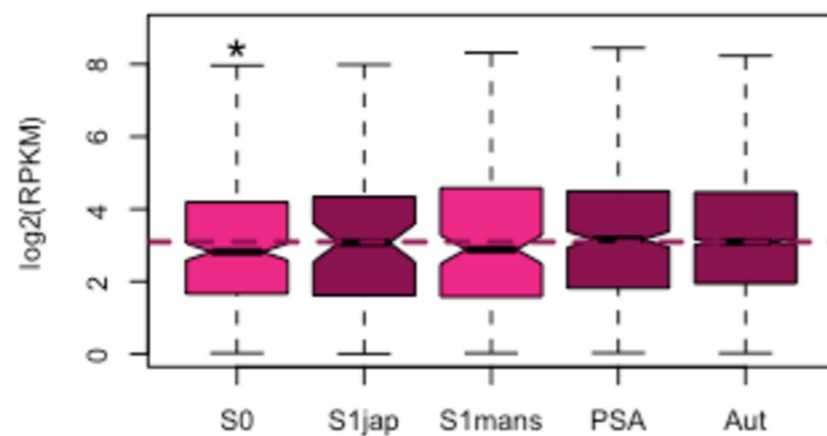
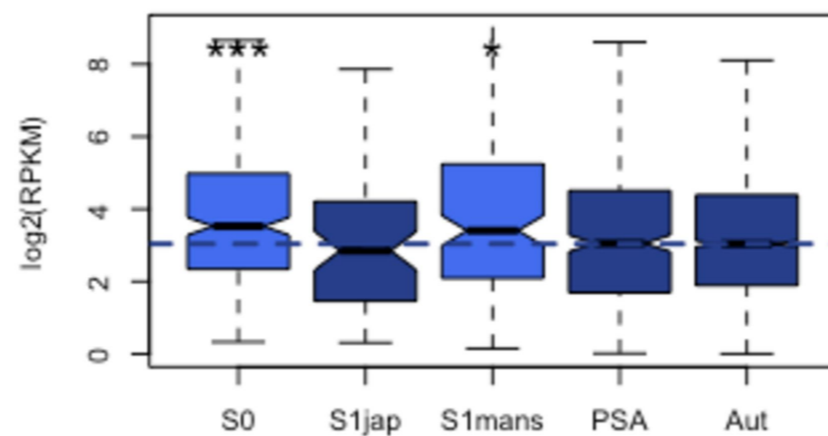
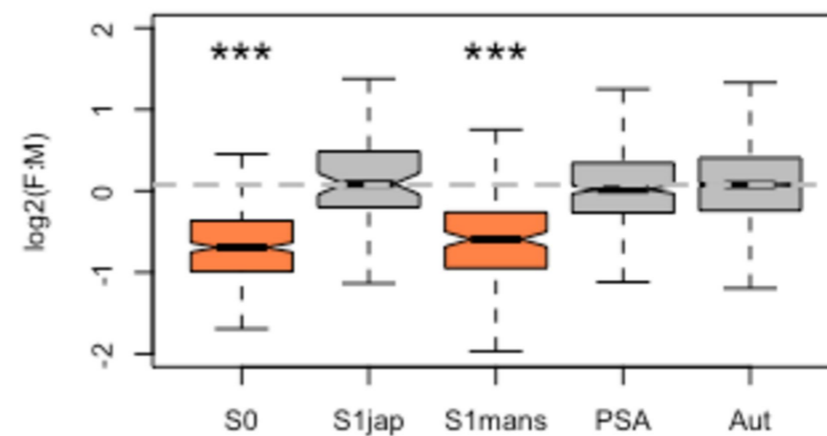
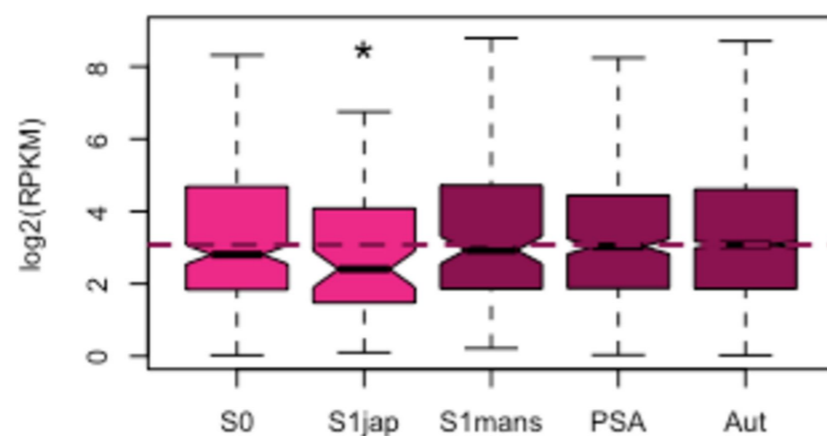
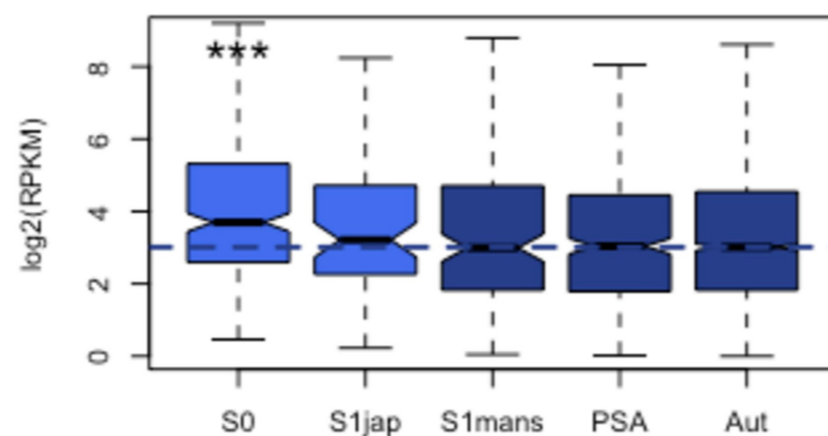
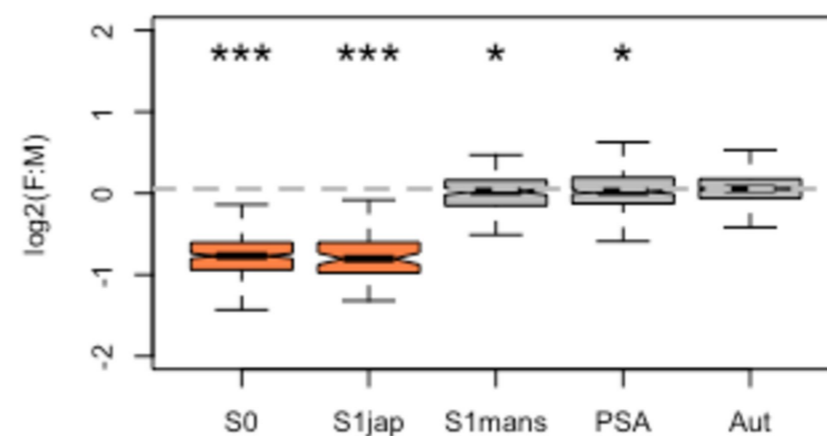
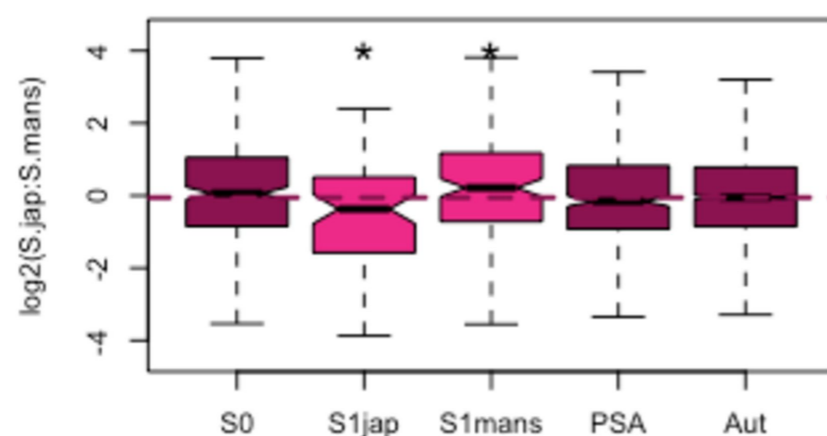
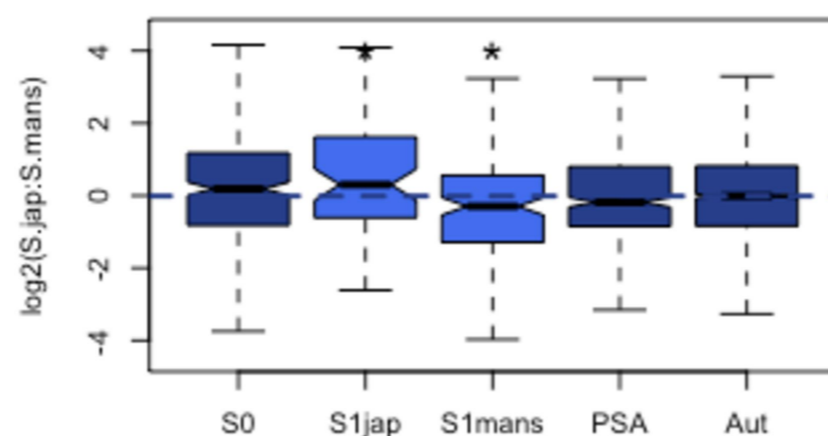
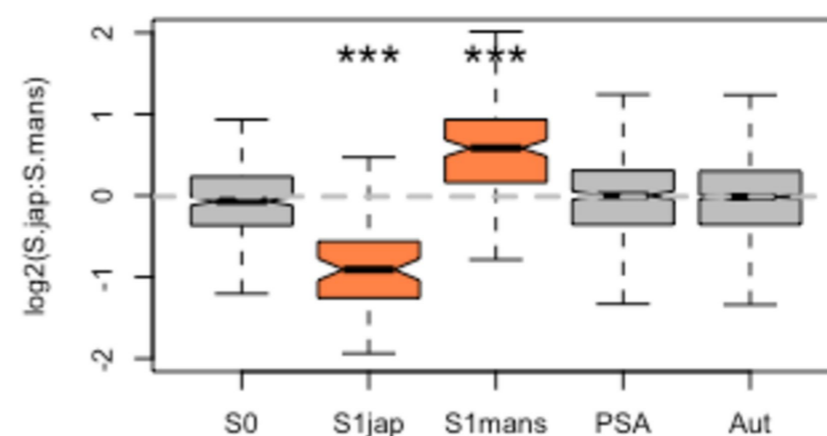
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

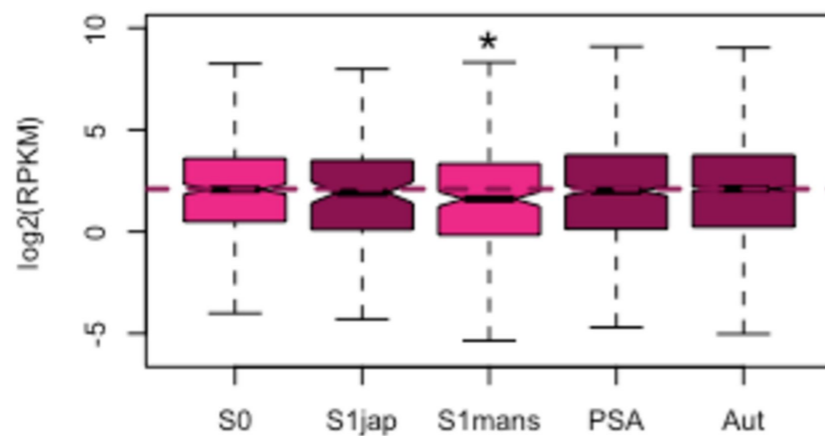
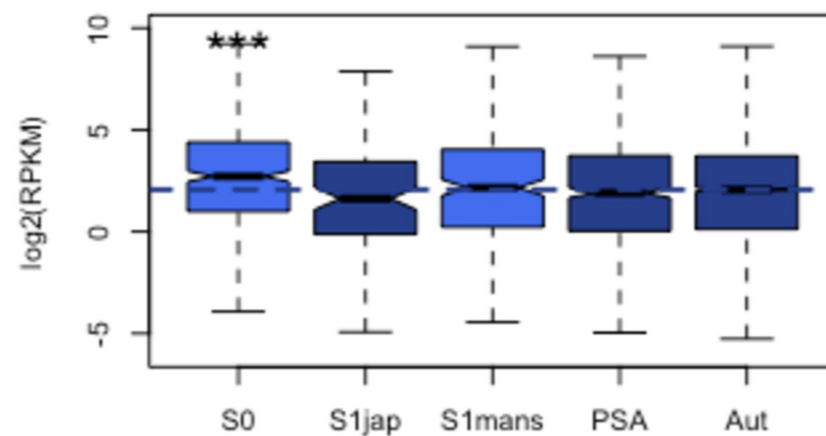
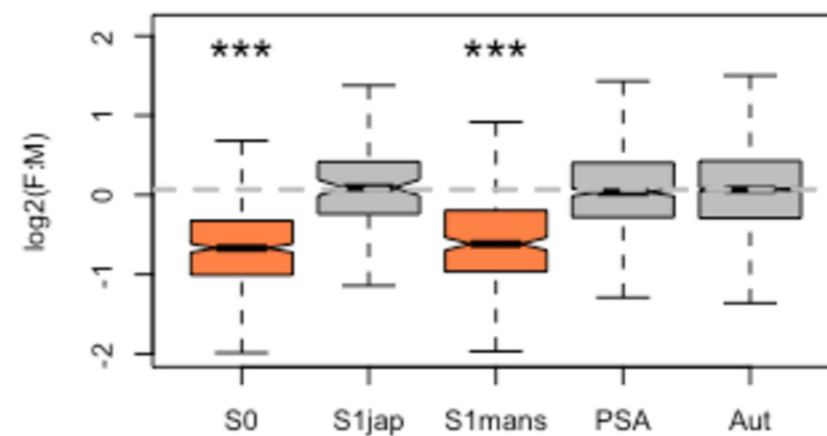
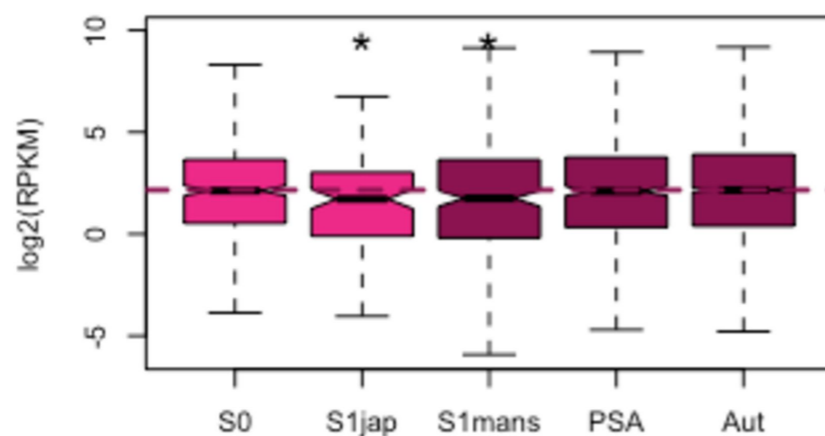
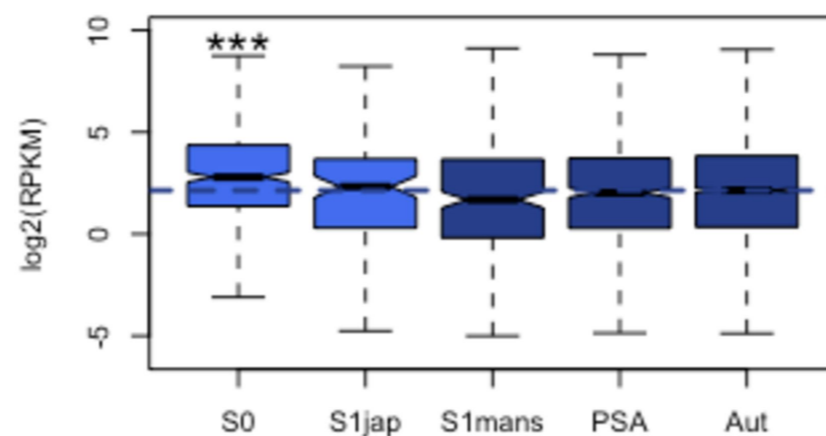
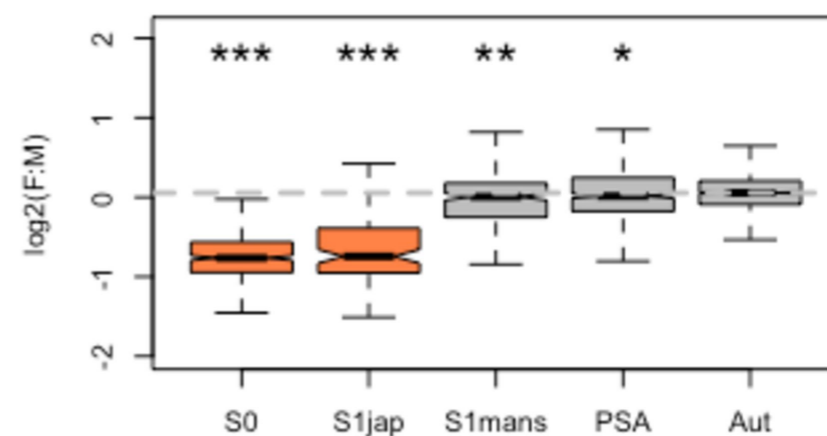
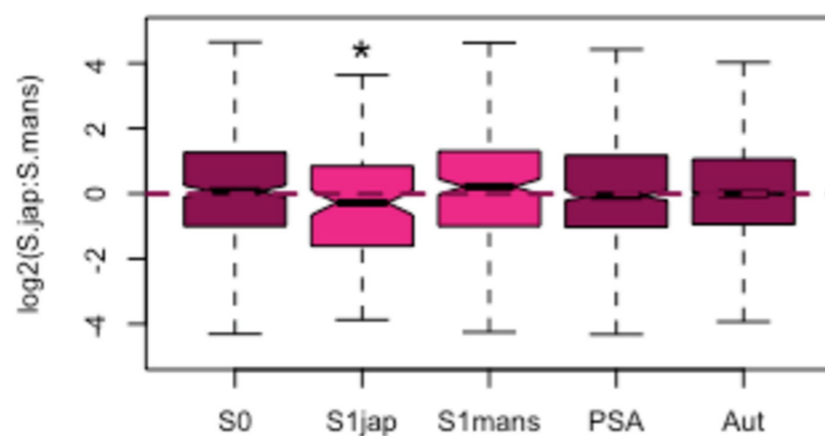
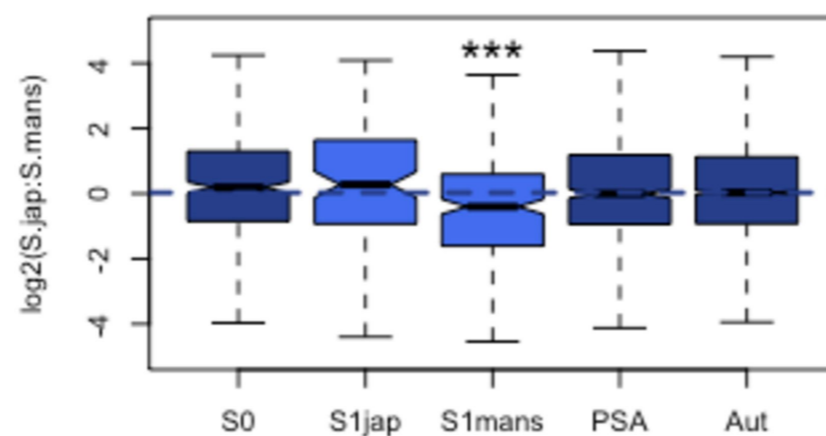
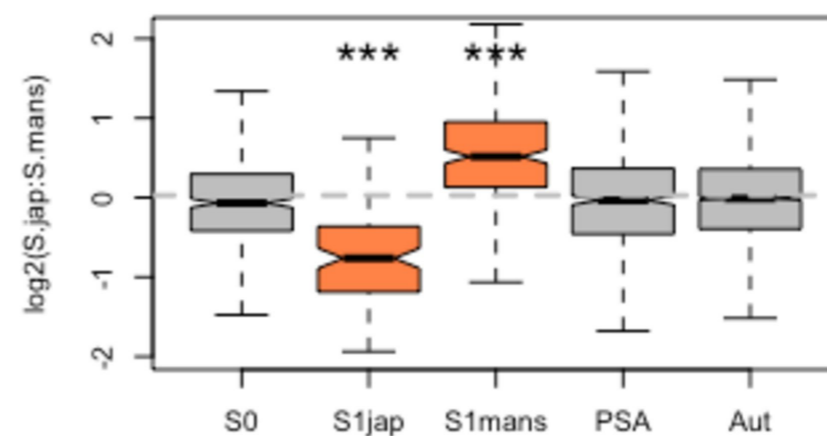
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

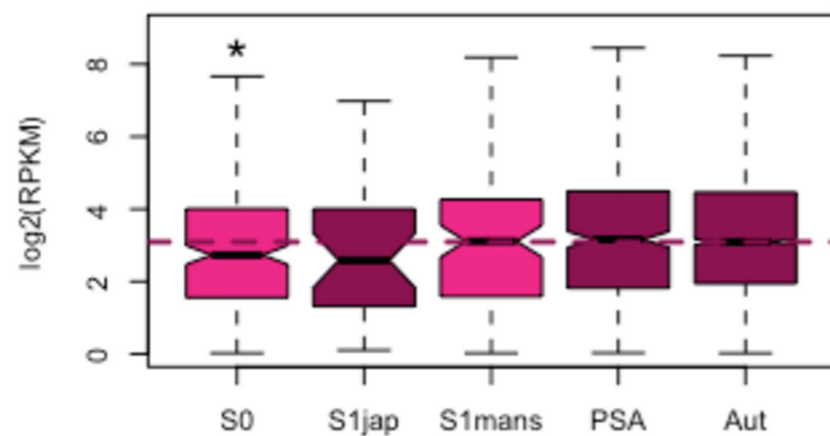
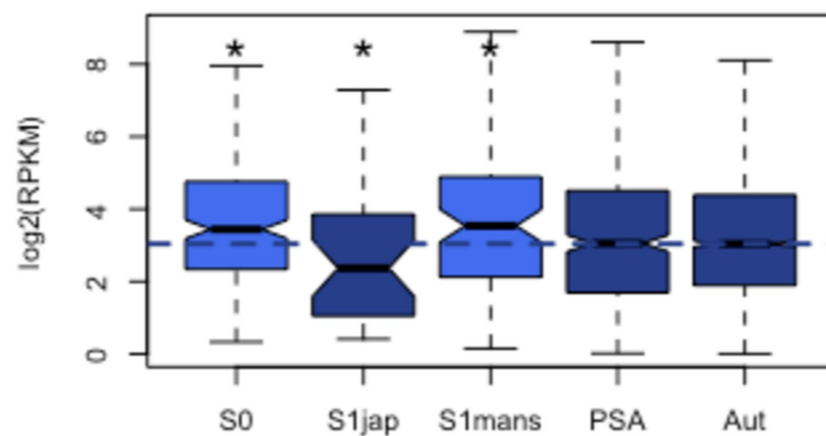
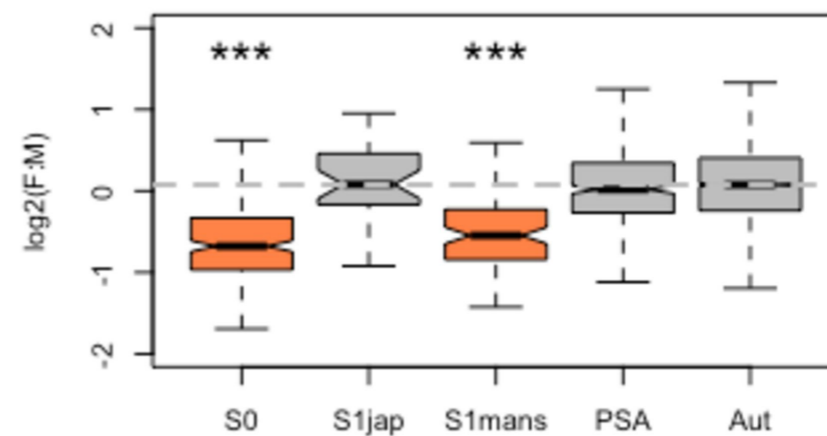
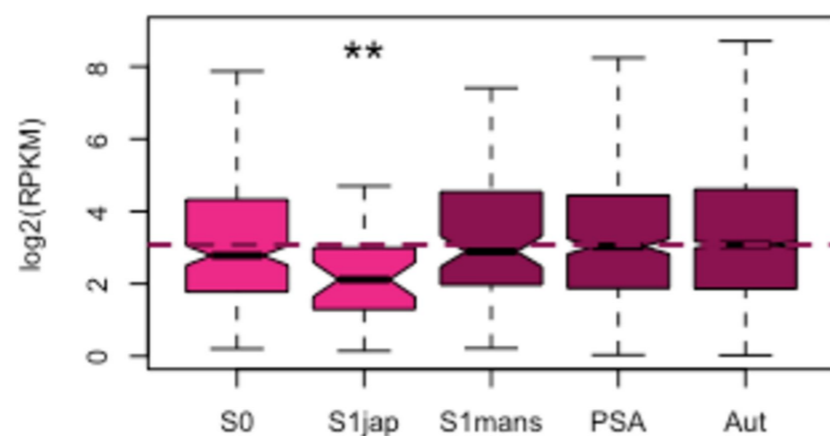
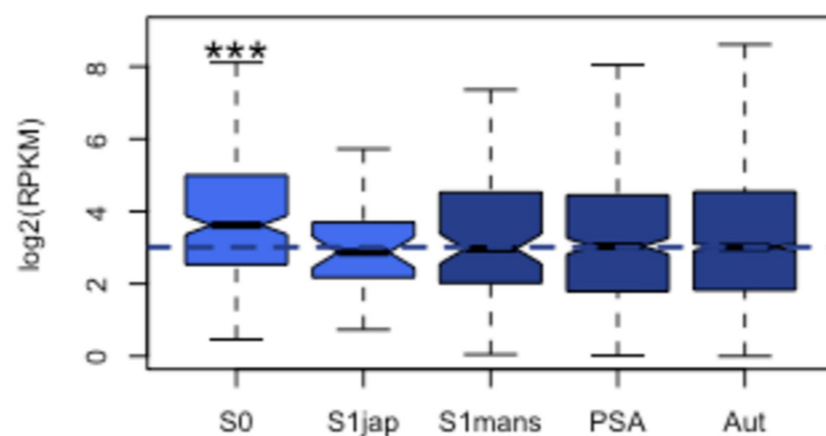
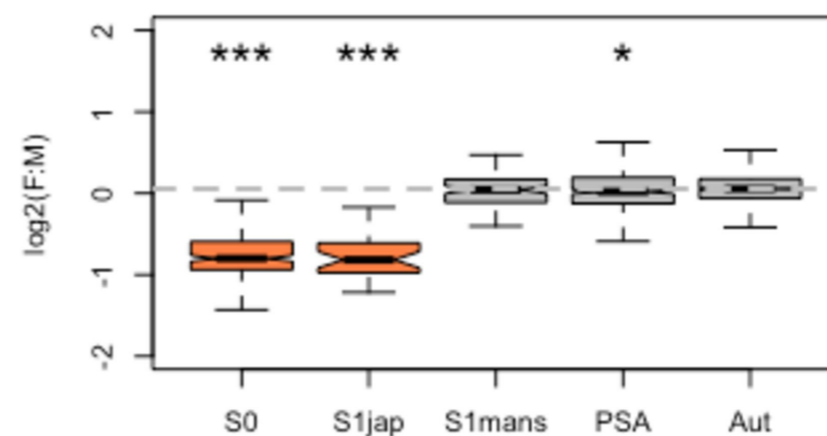
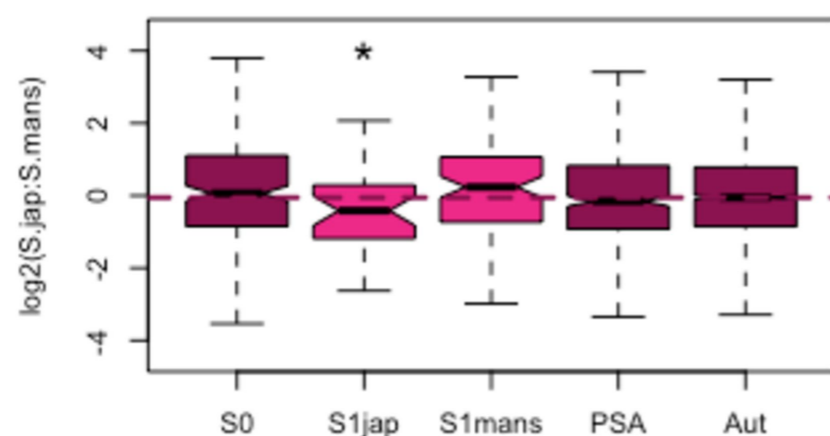
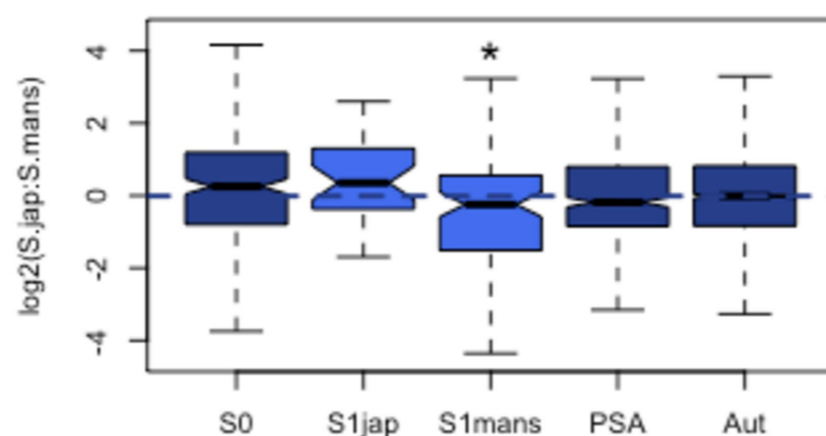
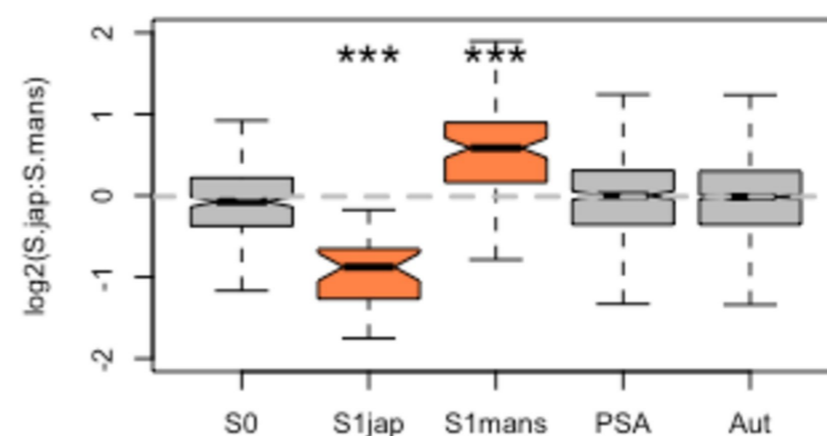
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

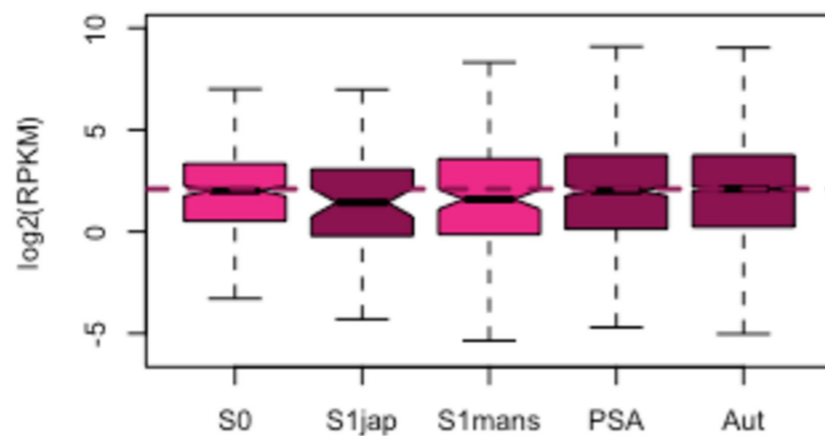
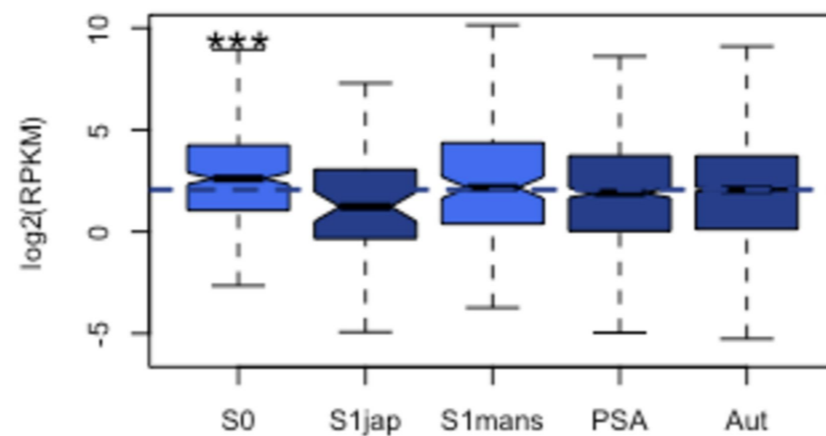
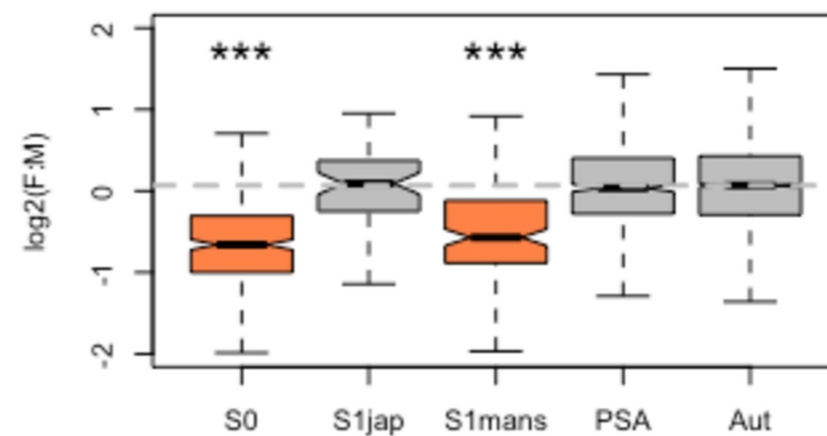
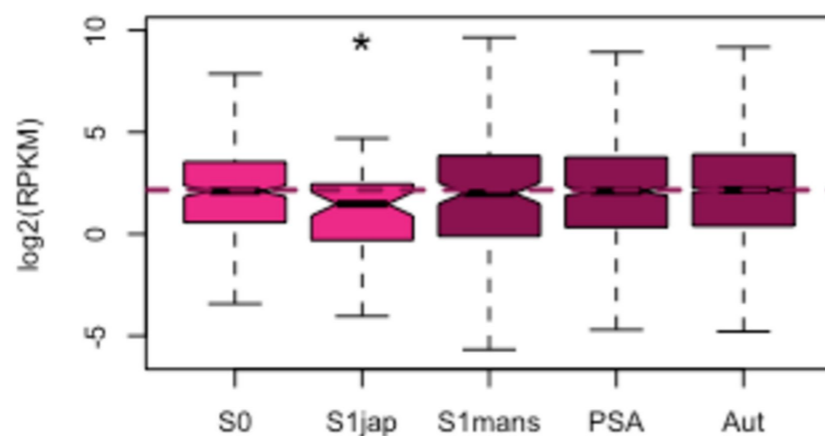
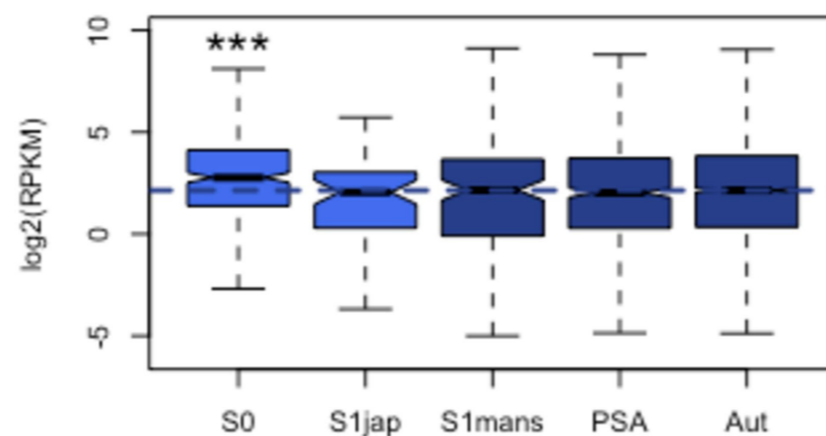
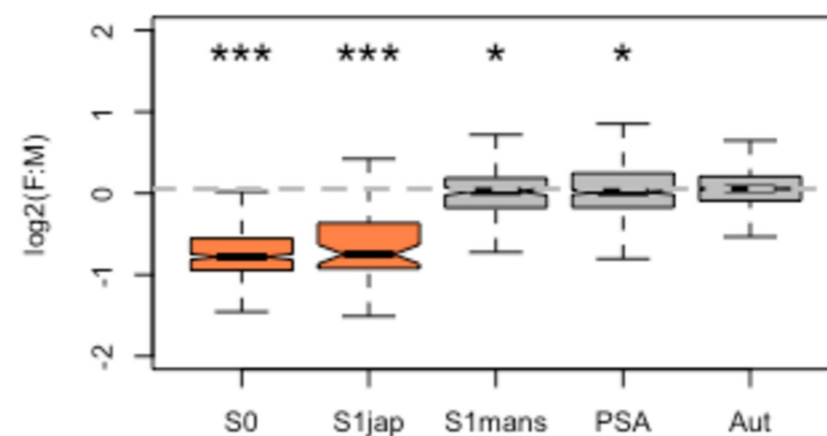
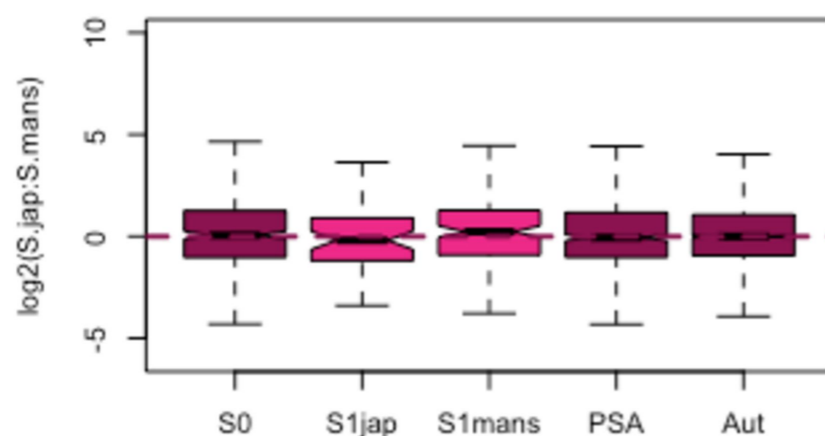
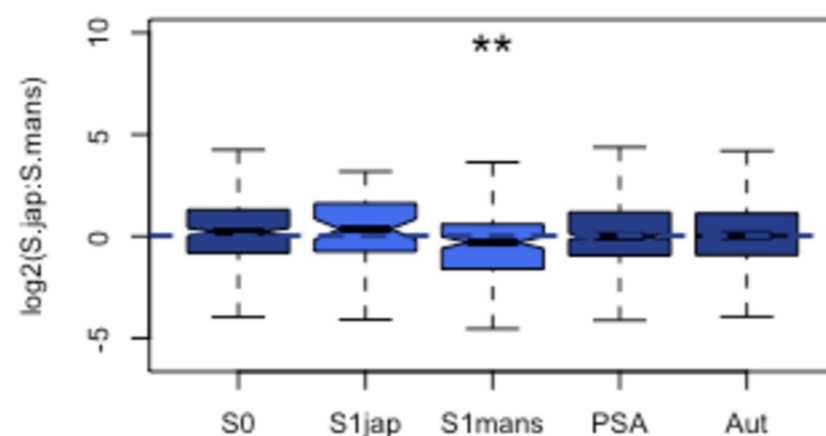
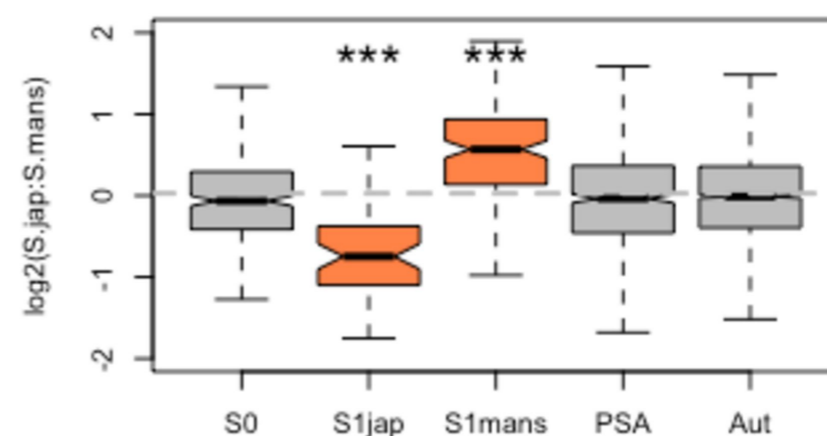
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

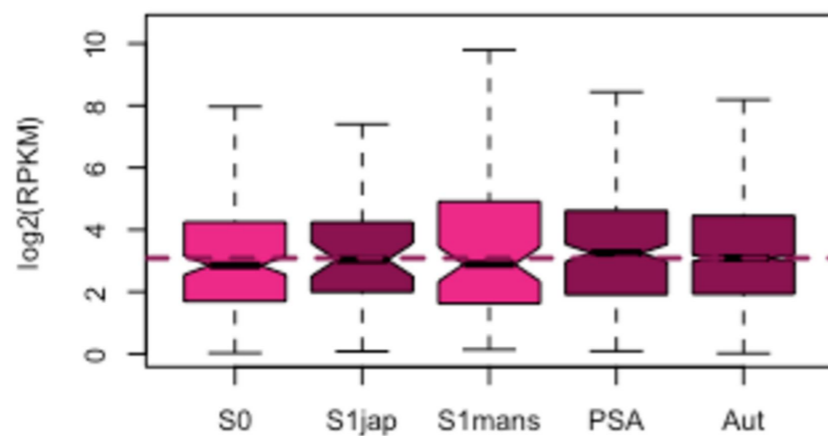
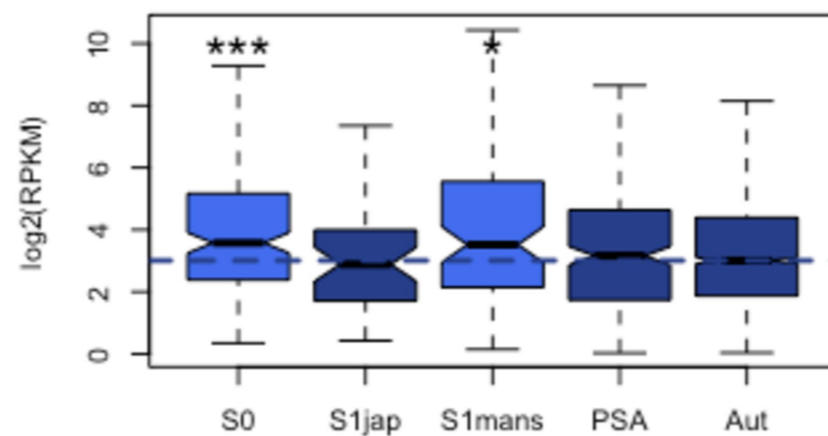
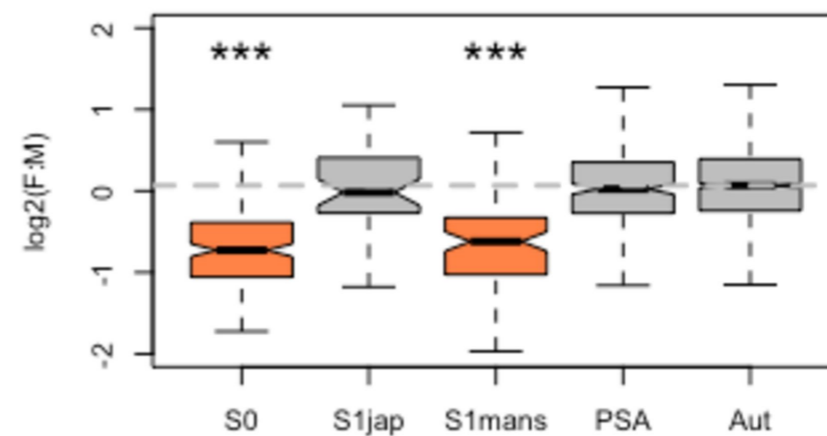
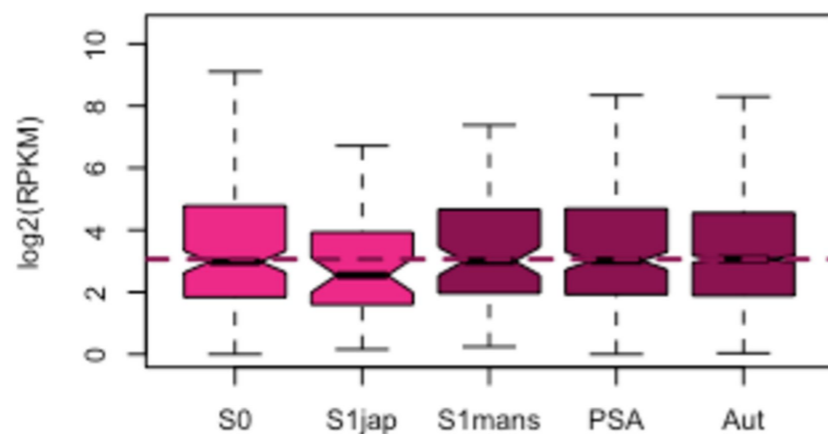
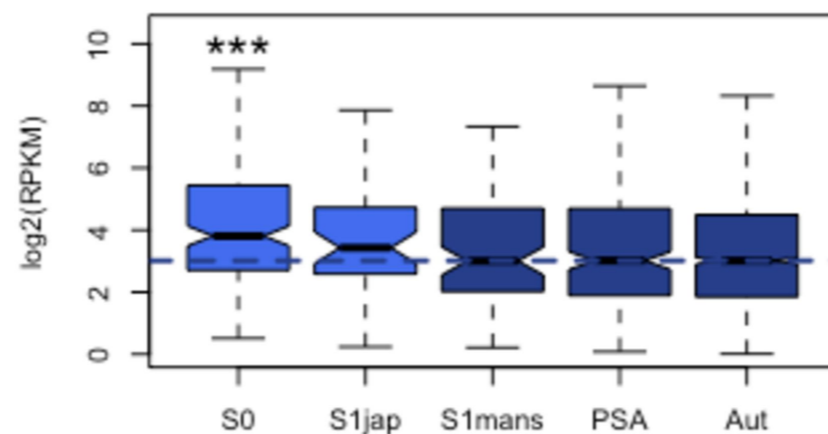
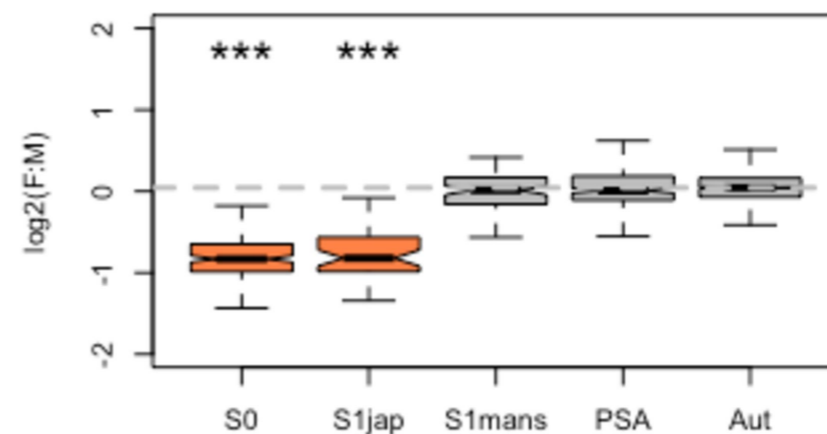
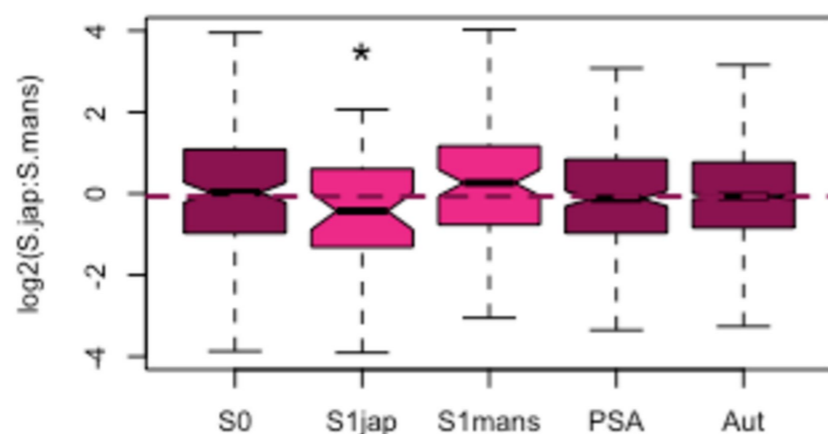
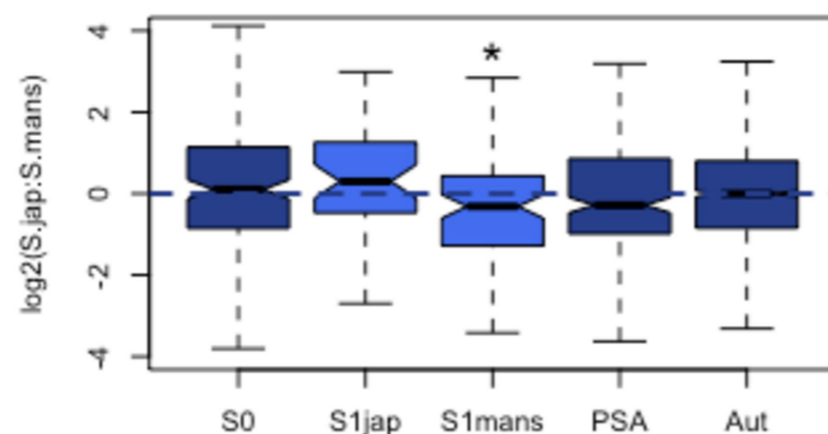
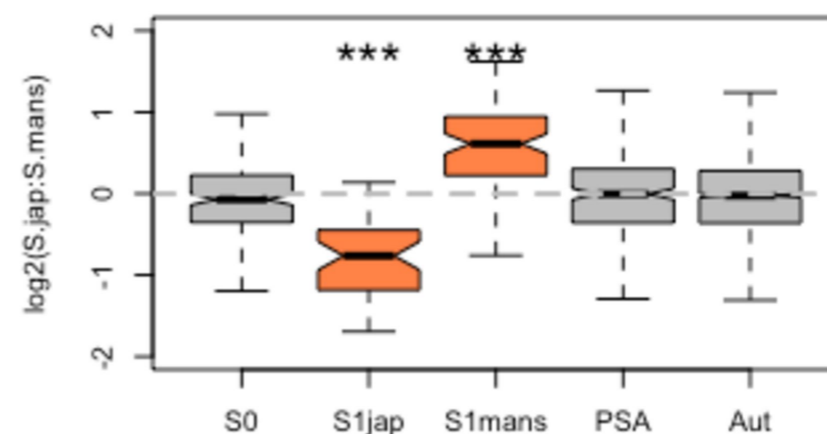
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

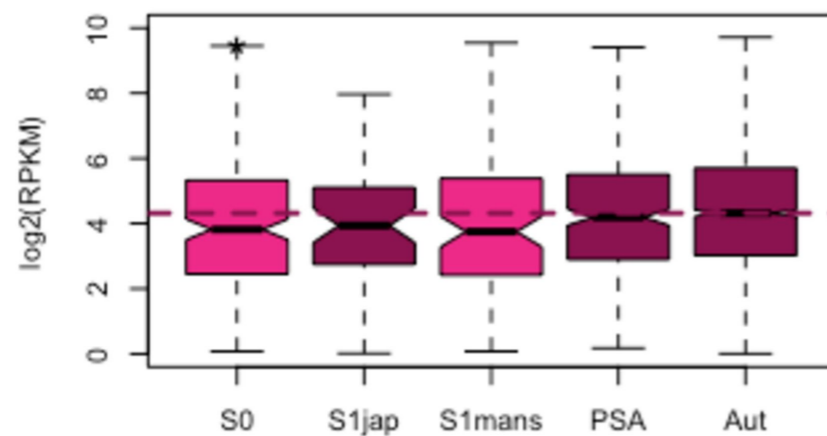
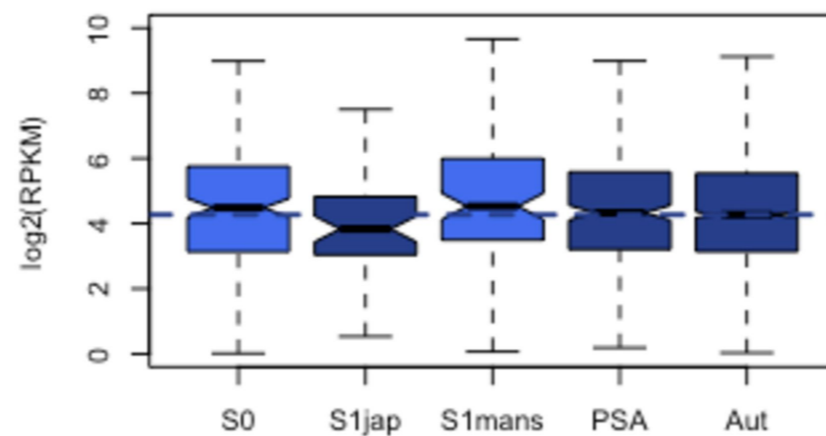
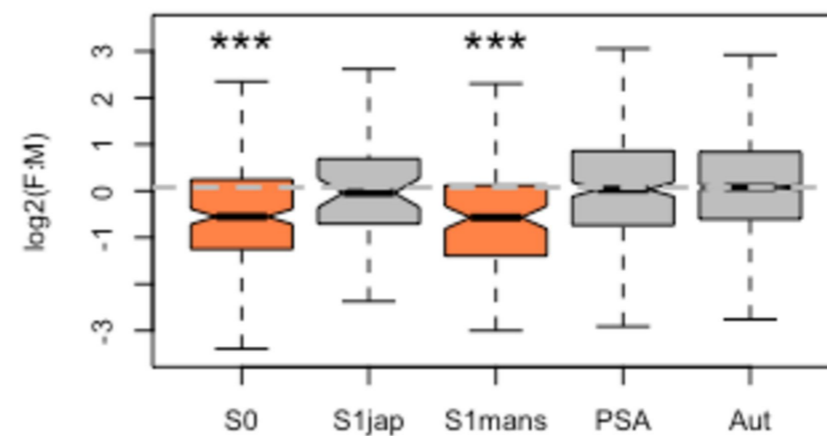
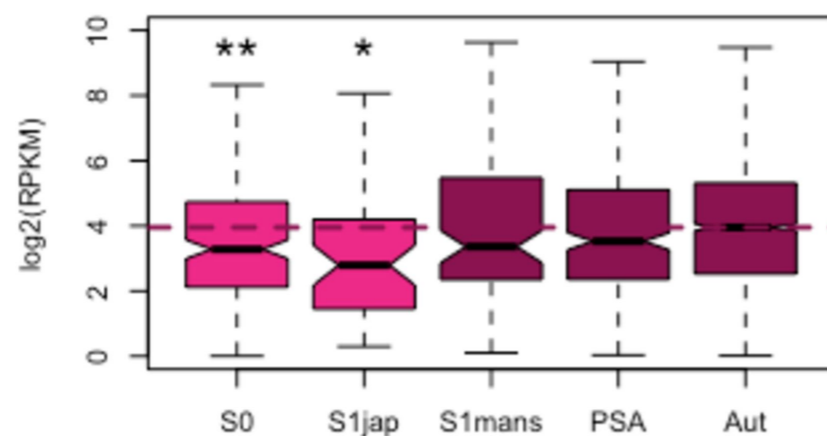
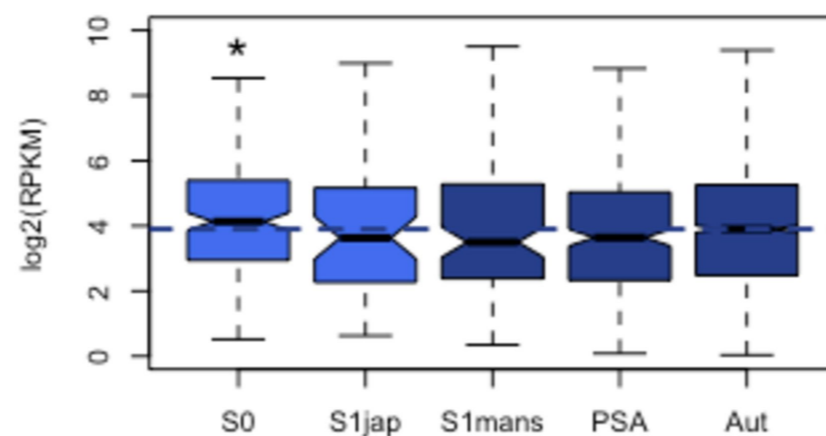
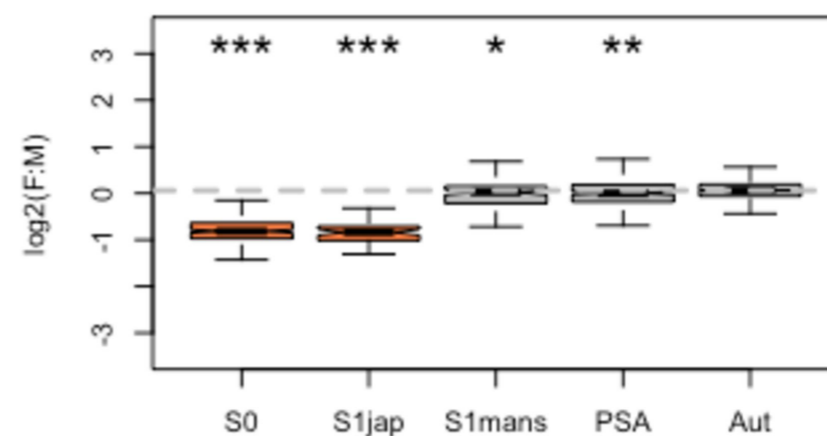
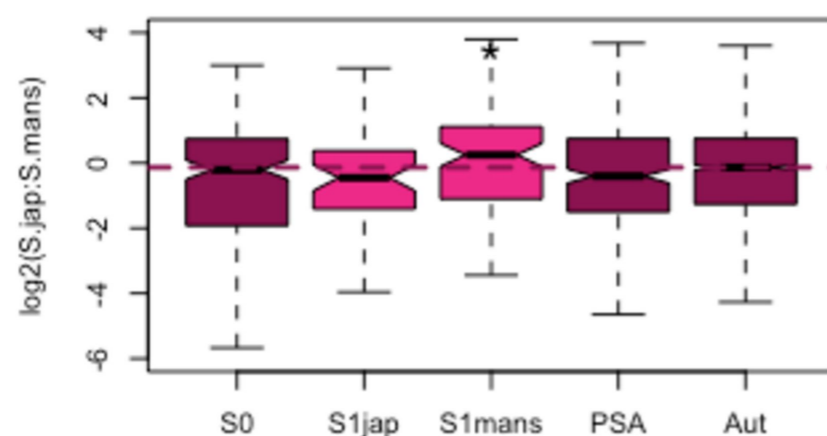
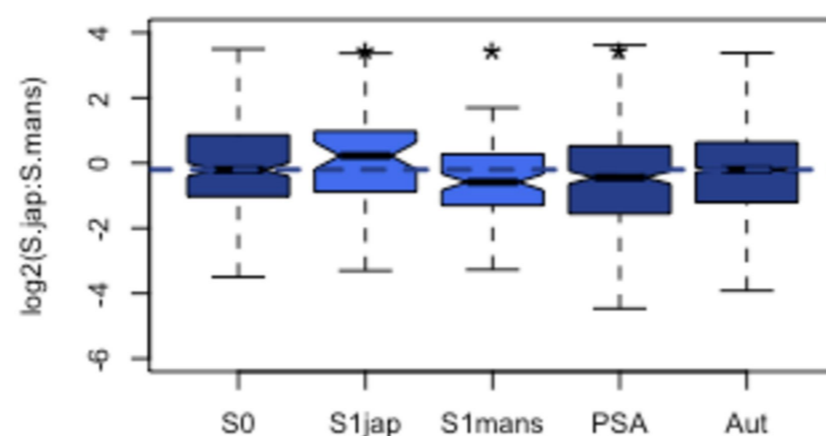
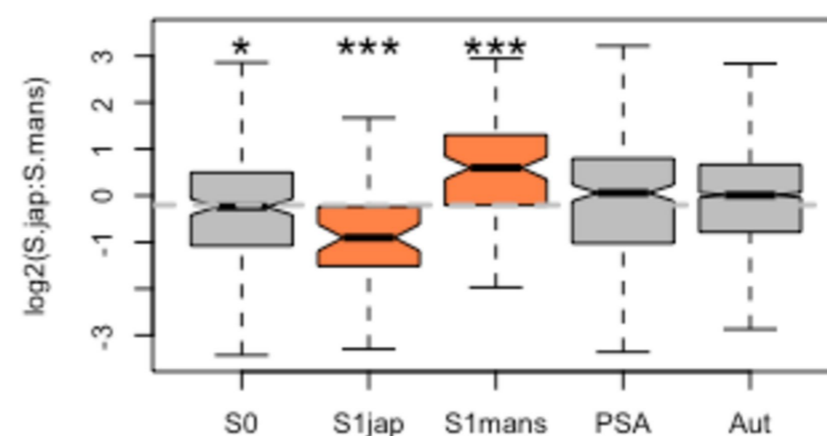
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

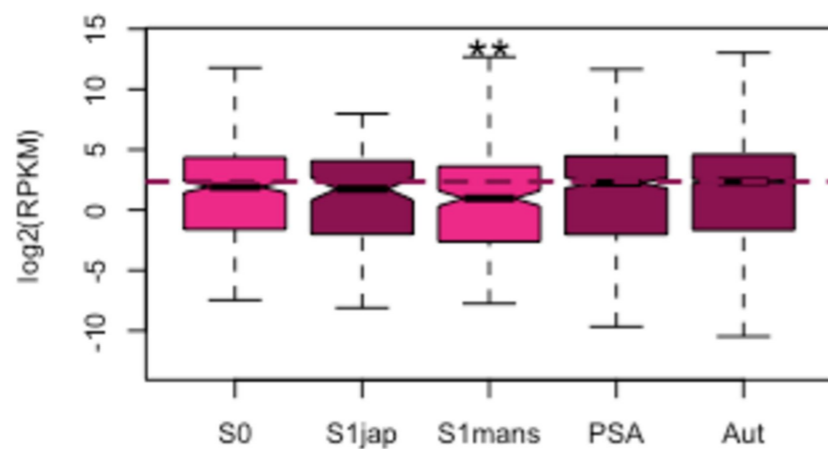
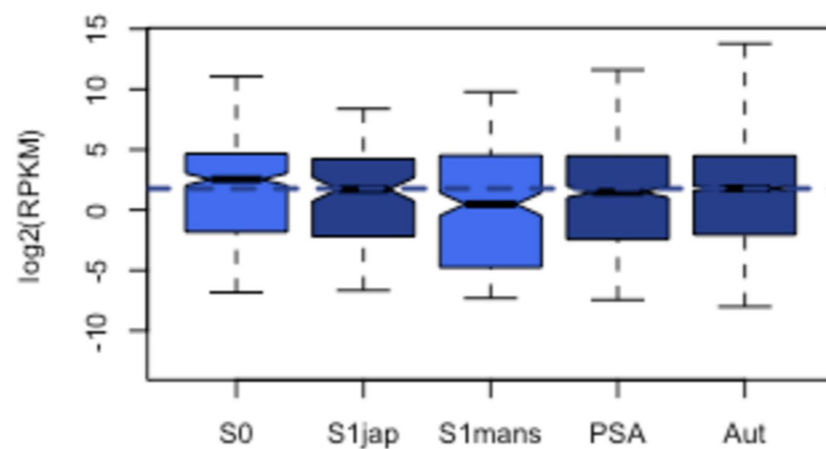
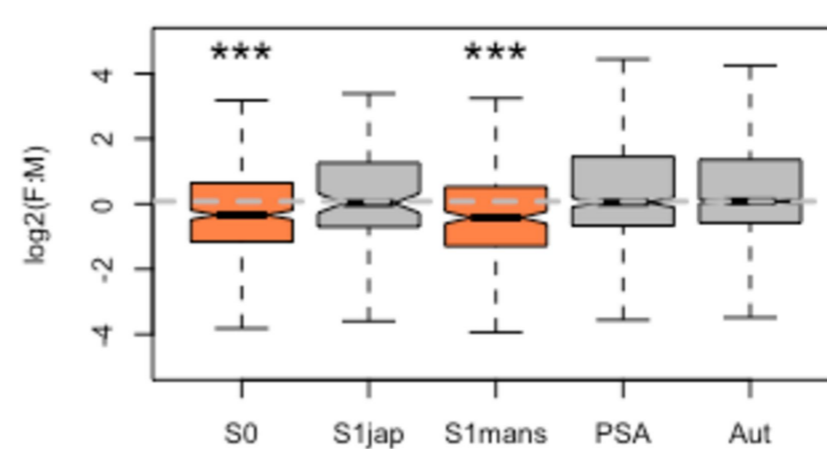
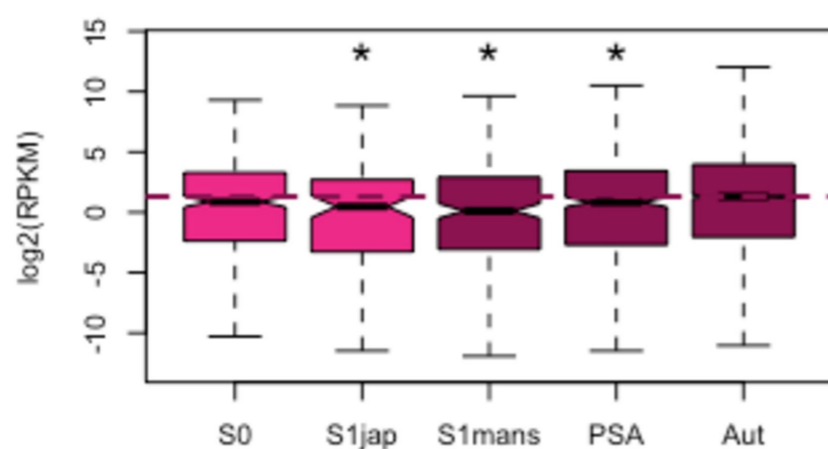
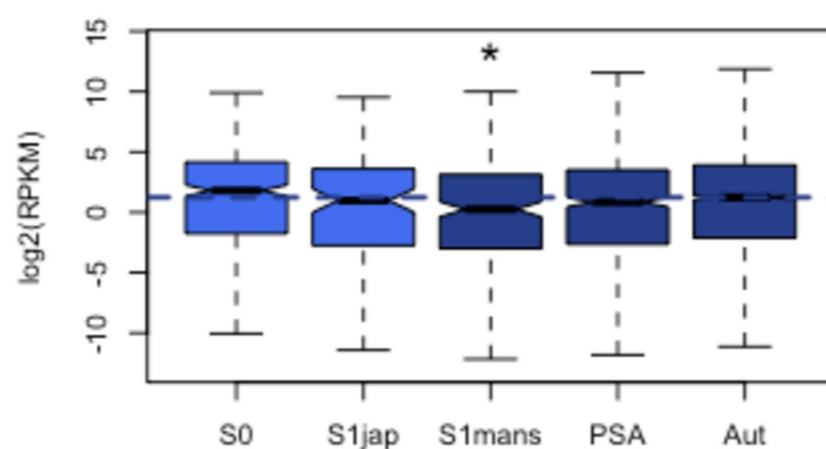
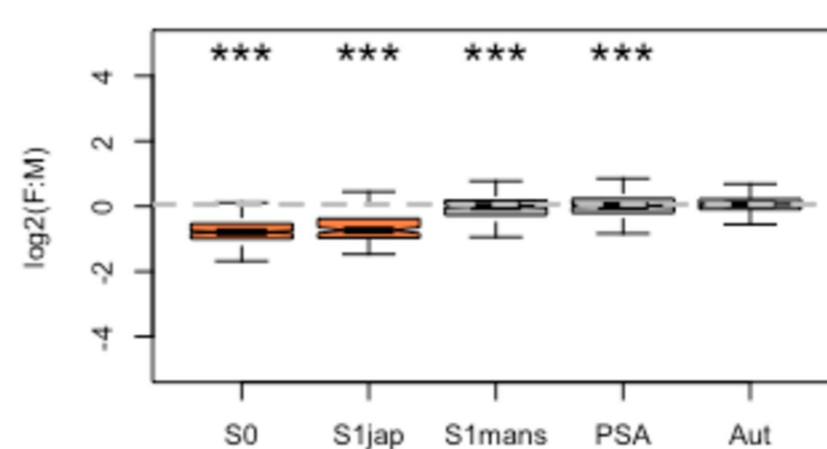
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

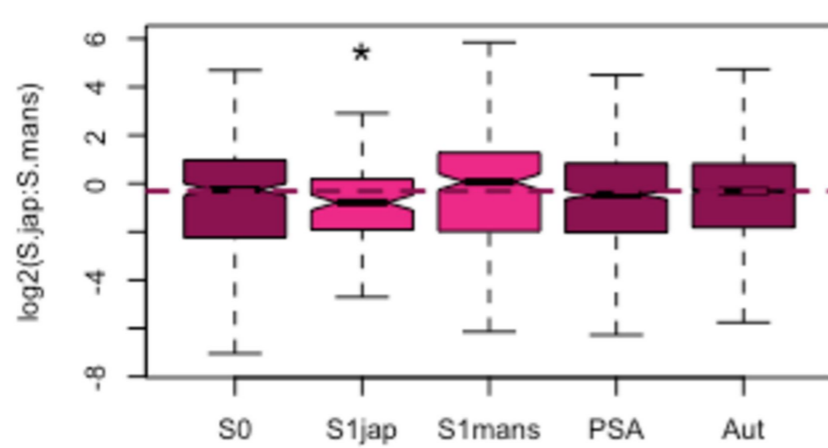
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

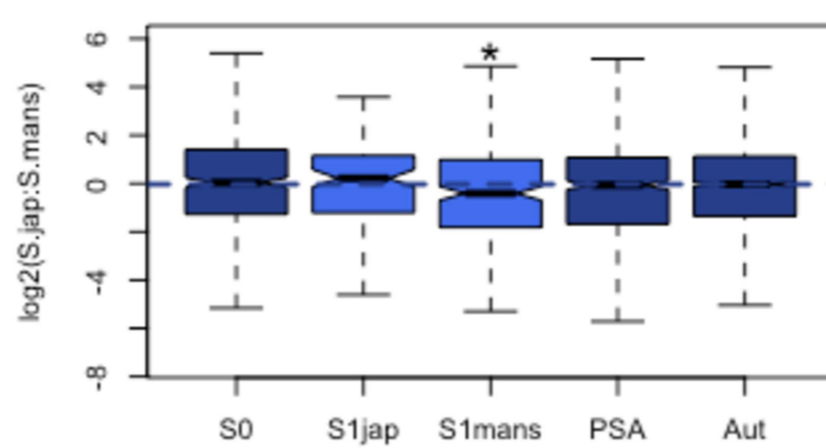
A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*G) Females - *S.jap:S.mans*H) Males - *S.jap:S.mans*I) F:M ratio - *S.jap:S.mans*

A) Females - *S. mansoni*B) Males - *S. mansoni*C) F:M ratio - *S. mansoni*D) Females - *S. japonicum*E) Males - *S. japonicum*F) F:M ratio - *S. japonicum*

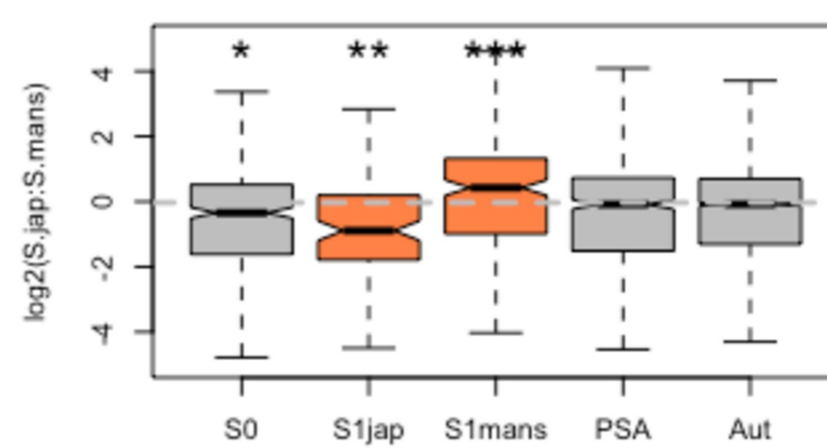
G) Females - S.jap:S.mans



H) Males - S.jap:S.mans

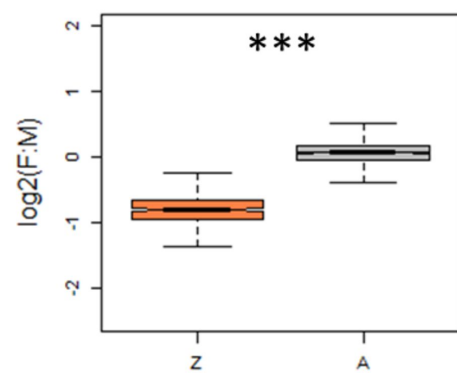


I) F:M ratio - S.jap:S.mans

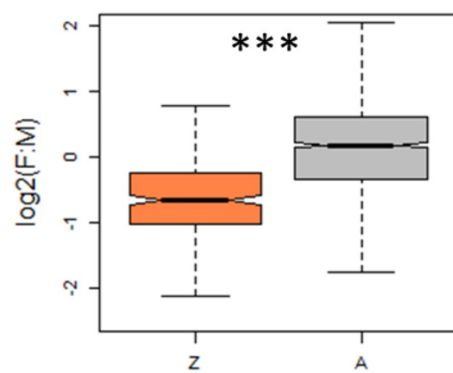


RPKM>1 threshold on all libraries

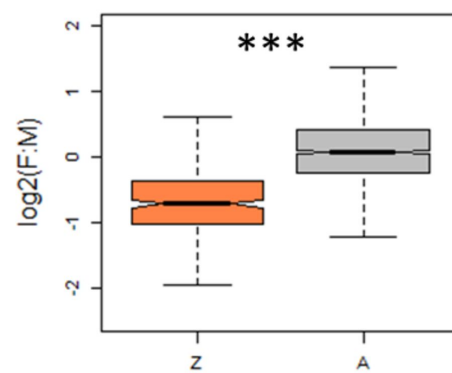
A. Sj SCHISTOSOMULA – F:M



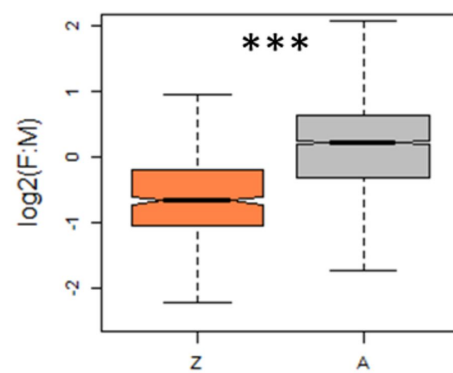
B. Sj ADULTS – F:M



C. Sm SCHISTOSOMULA – F:M

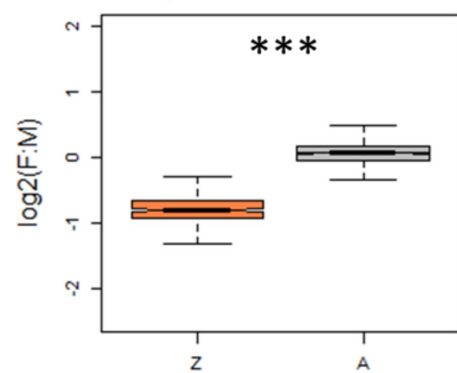


D. Sm ADULTS – F:M

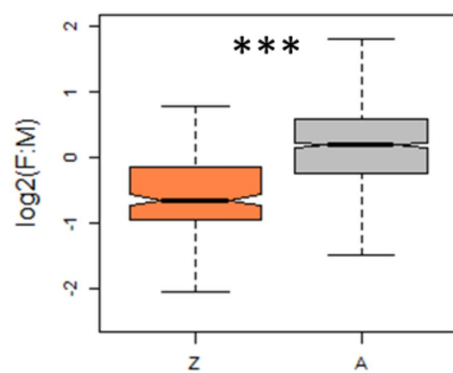


RPKM>3 threshold on all libraries

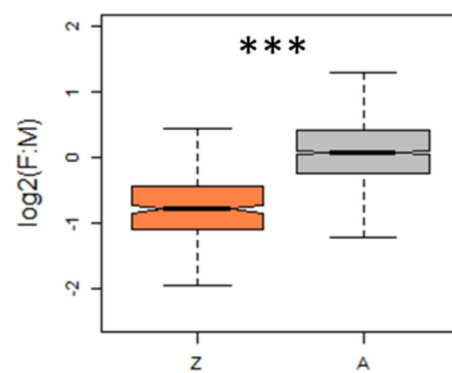
E. Sj SCHISTOSOMULA – F:M



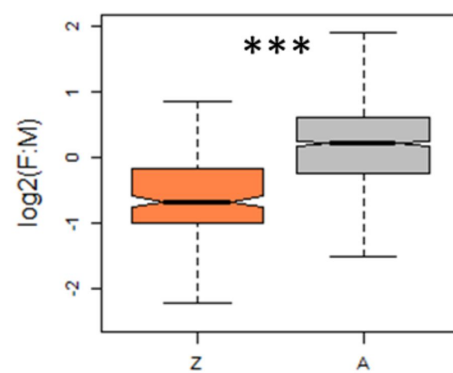
F. Sj ADULTS – F:M



G. Sm SCHISTOSOMULA – F:M

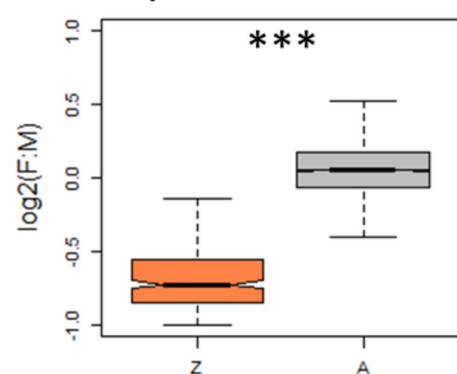


H. Sm ADULTS – F:M

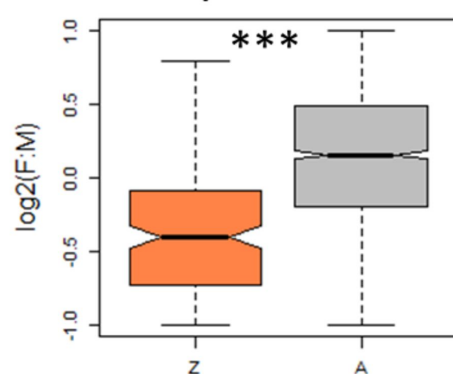


RPKM>1 per stage & FC<2

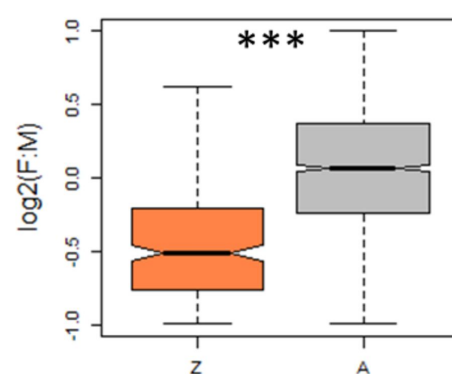
I. Sj SCHISTOSOMULA – F:M



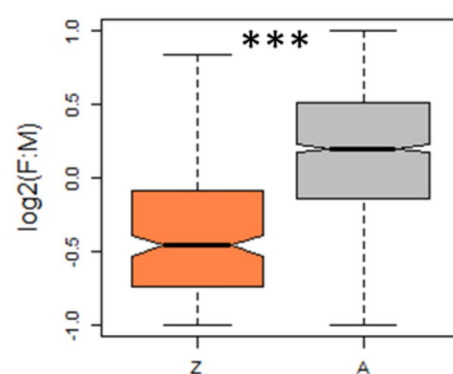
J. Sj ADULTS – F:M



K. Sm SCHISTOSOMULA – F:M

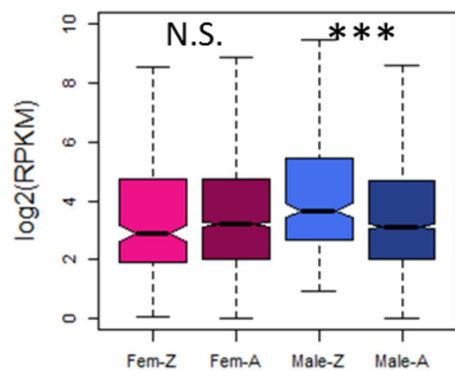


L. Sm ADULTS – F:M

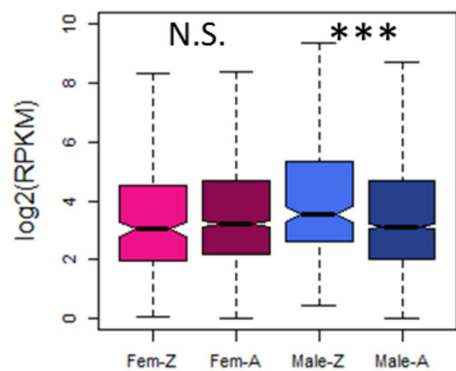


RPKM>1 threshold on all libraries

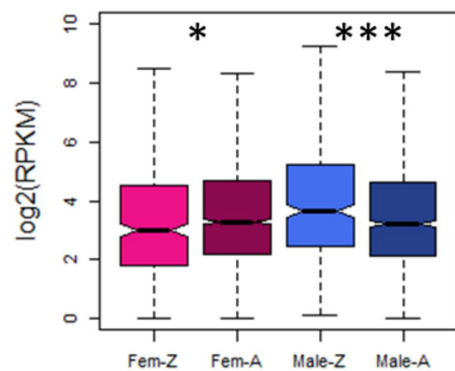
A. Sj SCHISTOSOMULA – Per sex



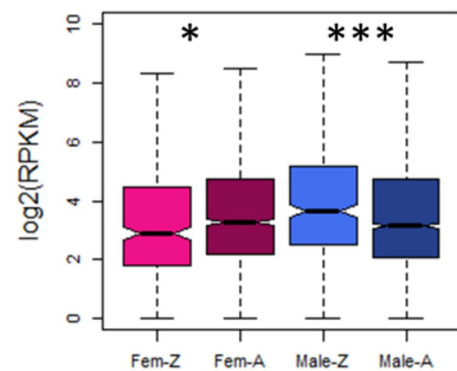
B. Sj ADULTS – Per sex



C. Sm SCHISTOSOMULA – Per sex

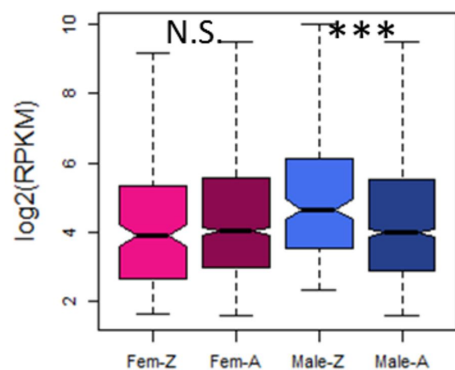


D. Sm ADULTS – Per sex

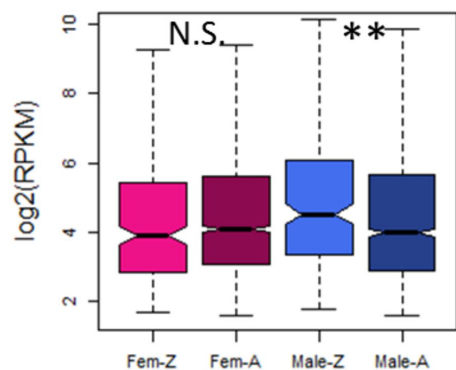


RPKM>3 threshold on all libraries

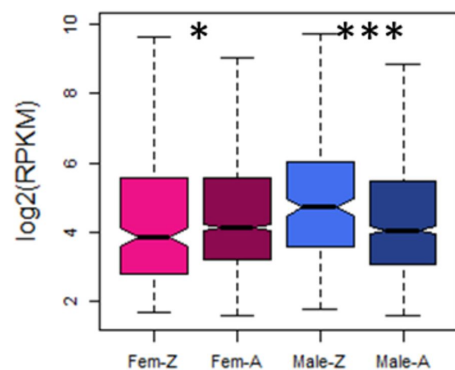
E. Sj SCHISTOSOMULA – Per sex



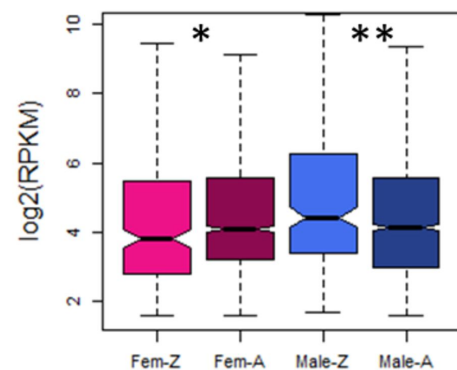
F. Sj ADULTS – Per sex



G. Sm SCHISTOSOMULA – Per sex

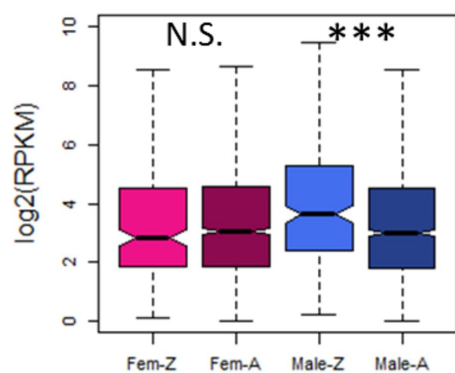


H. Sm ADULTS – Per sex

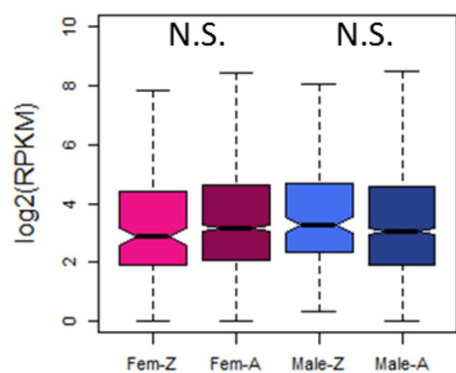


RPKM>1 per stage & FC<2

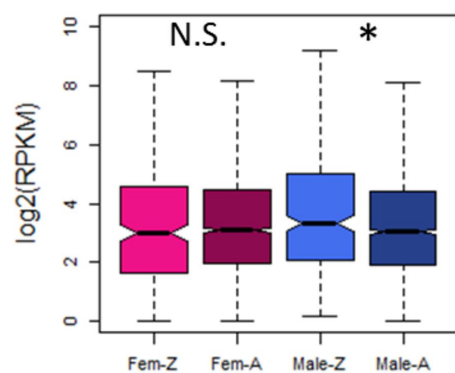
I. Sj SCHISTOSOMULA – Per sex



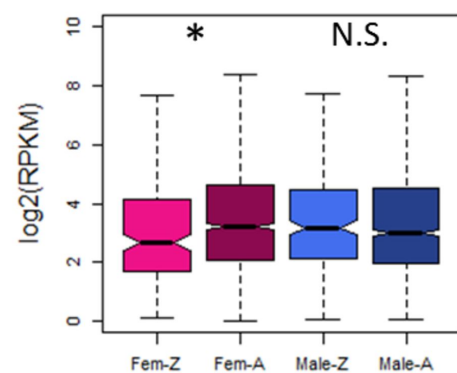
J. Sj ADULTS – Per sex

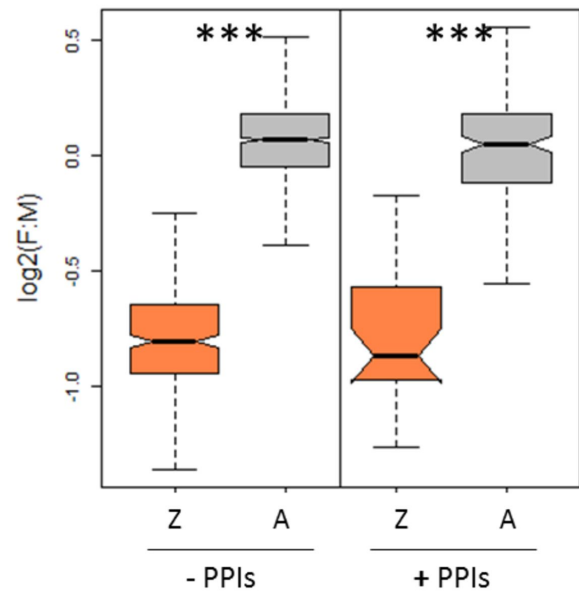
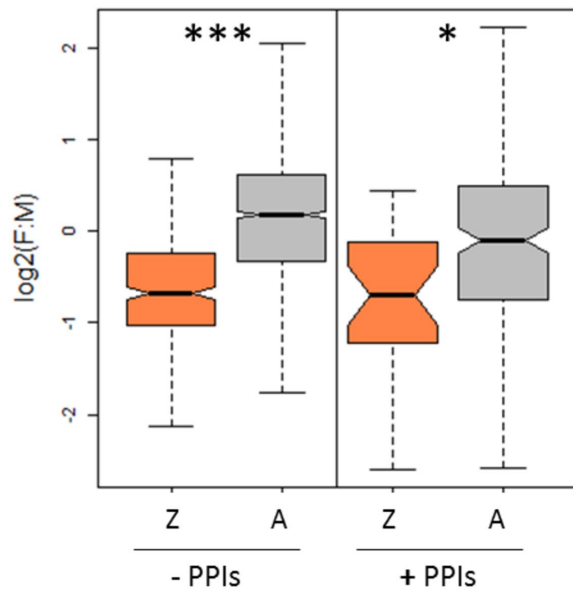
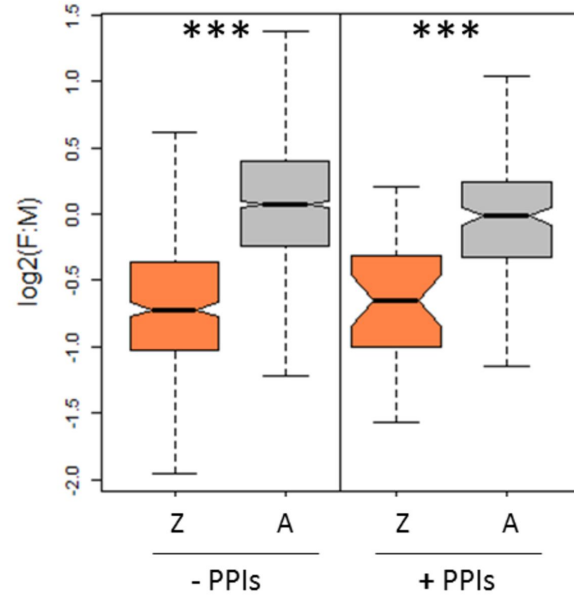
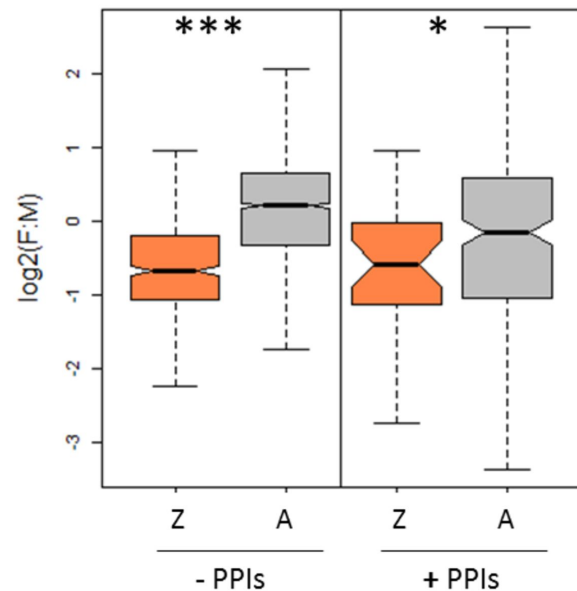


K. Sm SCHISTOSOMULA – Per sex



L. Sm ADULTS – Per sex



A. *S.jap* SCHISTOSOMULAB. *S.jap* ADULTSC. *S.mans* SCHISTOSOMULAD. *S.mans* ADULTS

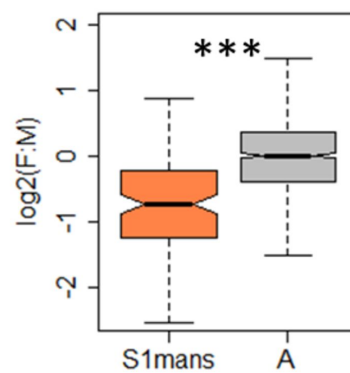
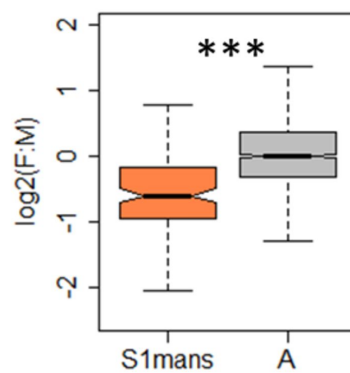
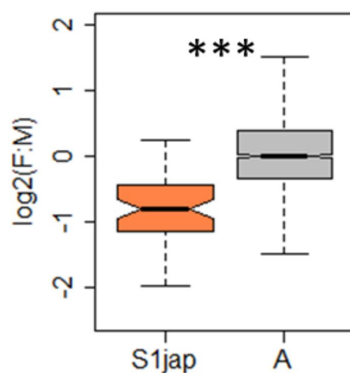
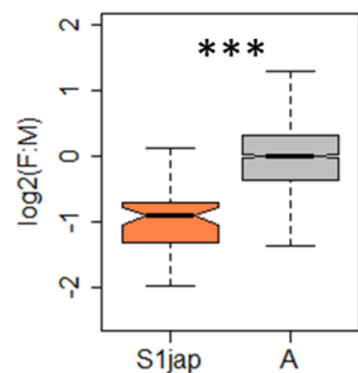
RPKM>1 threshold on all libraries

A. *S.jap* SCHISTOSOMULA

B. *S.jap* ADULTS

C. *S.mans* SCHISTOSOMULA

D. *S.mans* ADULTS



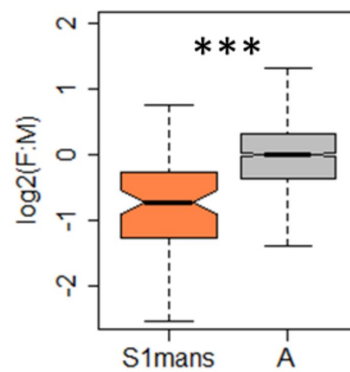
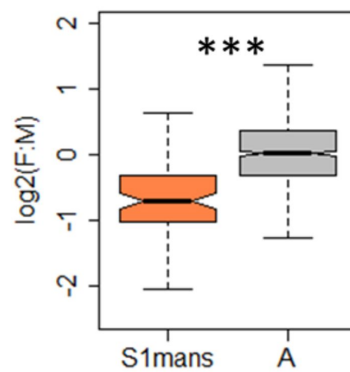
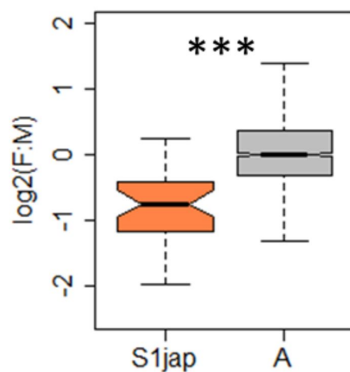
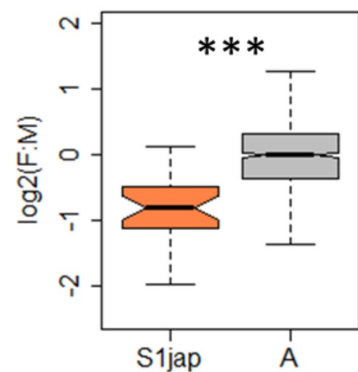
RPKM>3 threshold on all libraries

E. *S.jap* SCHISTOSOMULA

F. *S.jap* ADULTS

G. *S.mans* SCHISTOSOMULA

H. *S.mans* ADULTS



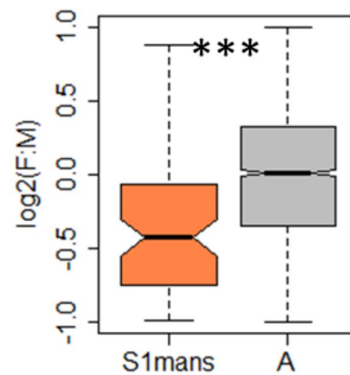
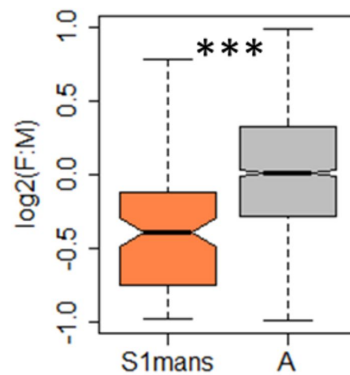
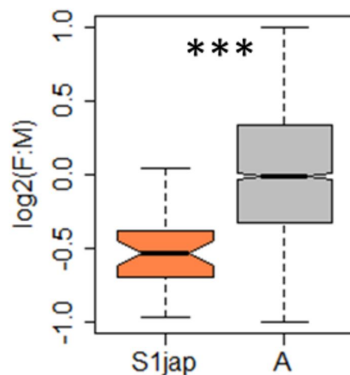
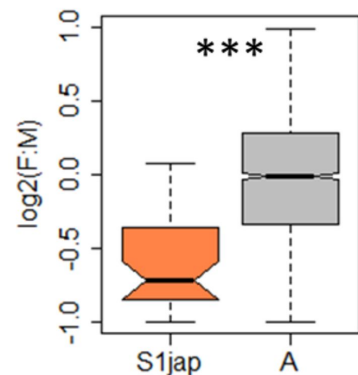
RPKM>1 per stage & FC<2

I. *S.jap* SCHISTOSOMULA

J. *S.jap* ADULTS

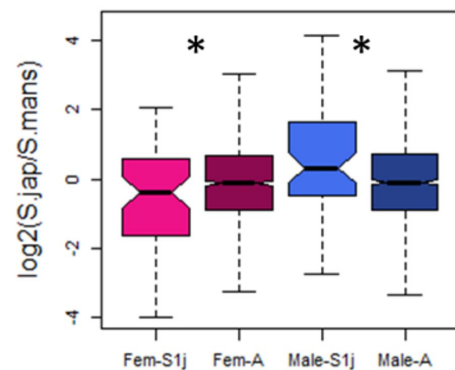
K. *S.mans* SCHISTOSOMULA

L. *S.mans* ADULTS

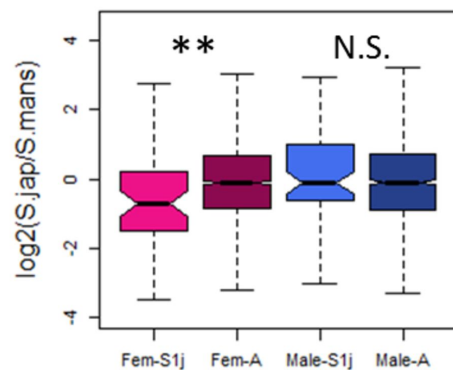


RPKM>1 threshold on all libraries

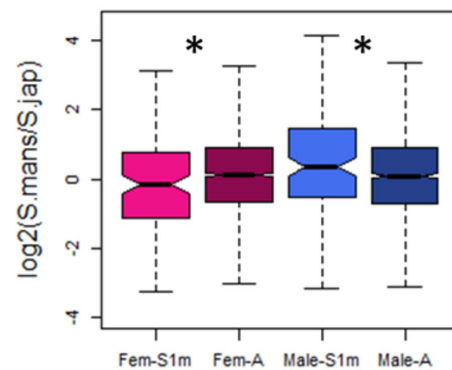
A. Sj SCHISTOSOMULA



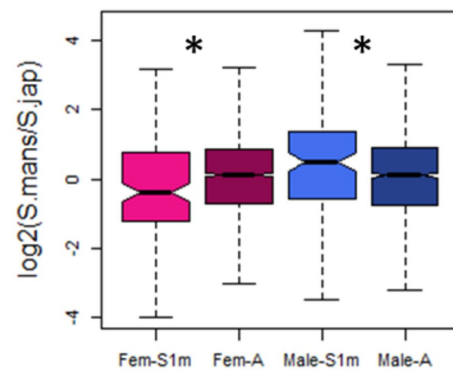
B. Sj ADULTS



C. Sm SCHISTOSOMULA

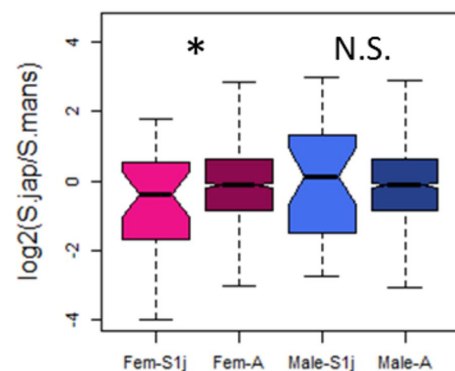


D. Sm ADULTS

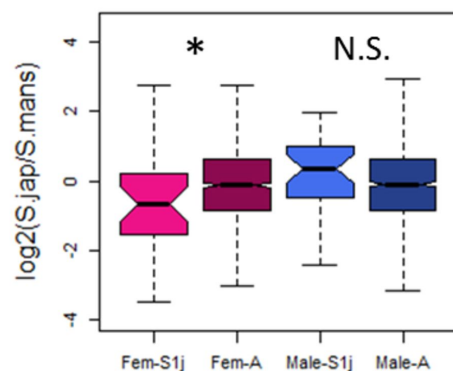


RPKM>3 threshold on all libraries

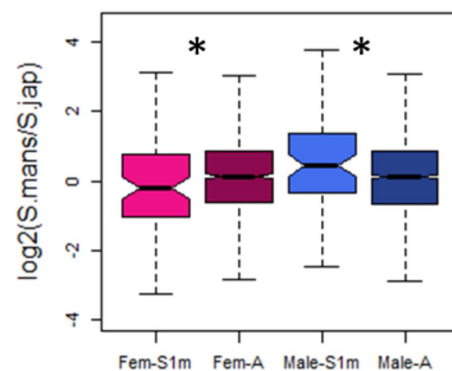
D. Sj SCHISTOSOMULA



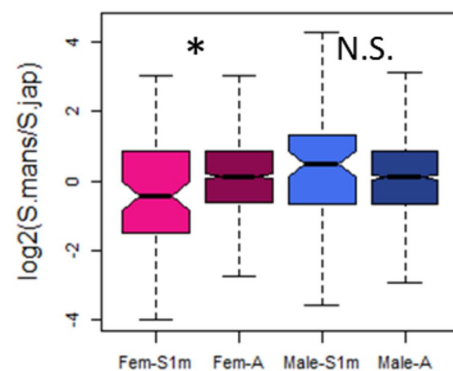
E. Sj ADULTS



F. Sm SCHISTOSOMULA

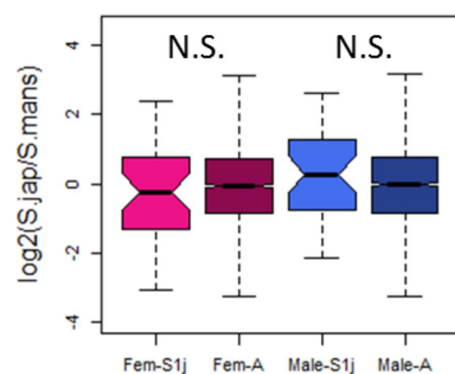


G. Sm ADULTS

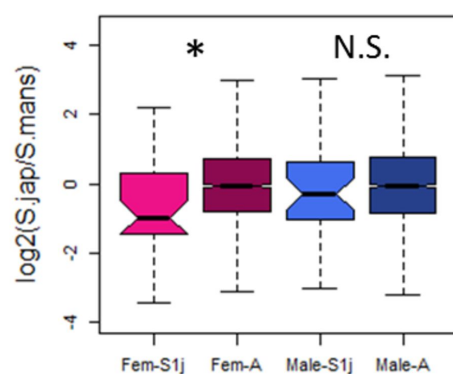


RPKM>1 per stage & FC<2

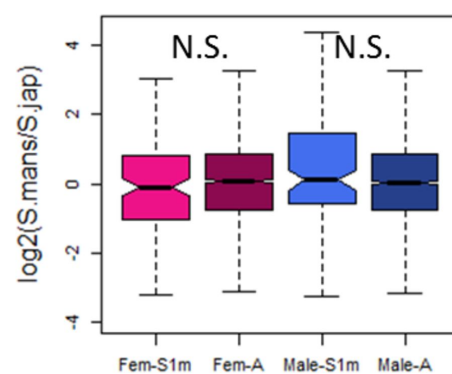
I. Sj SCHISTOSOMULA



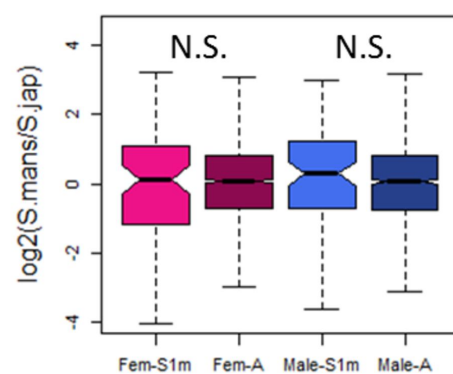
J. Sj ADULTS



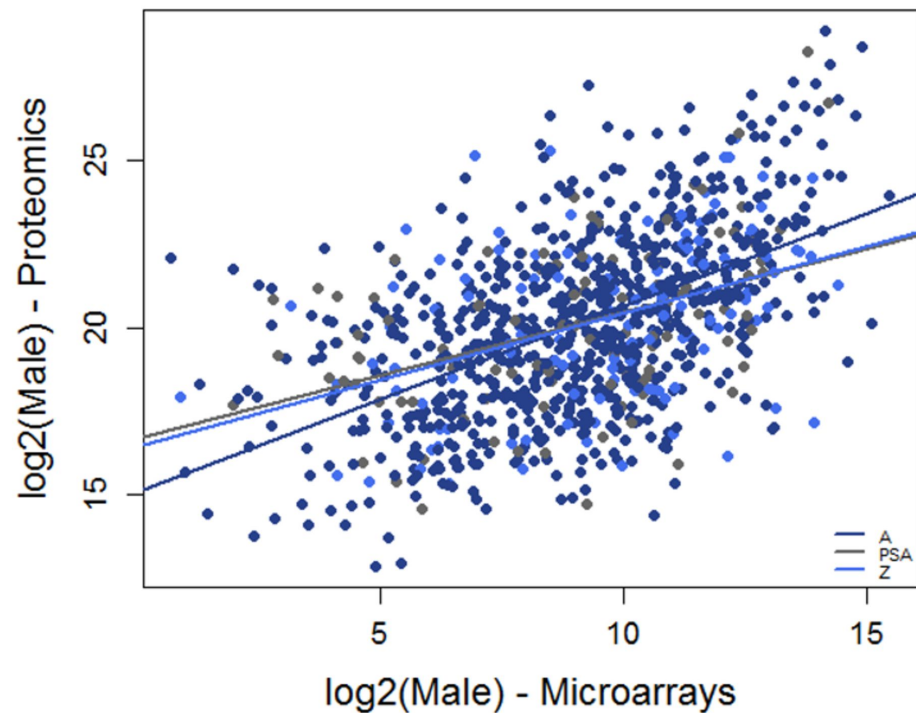
K. Sm SCHISTOSOMULA



L. Sm ADULTS



Male HEADS

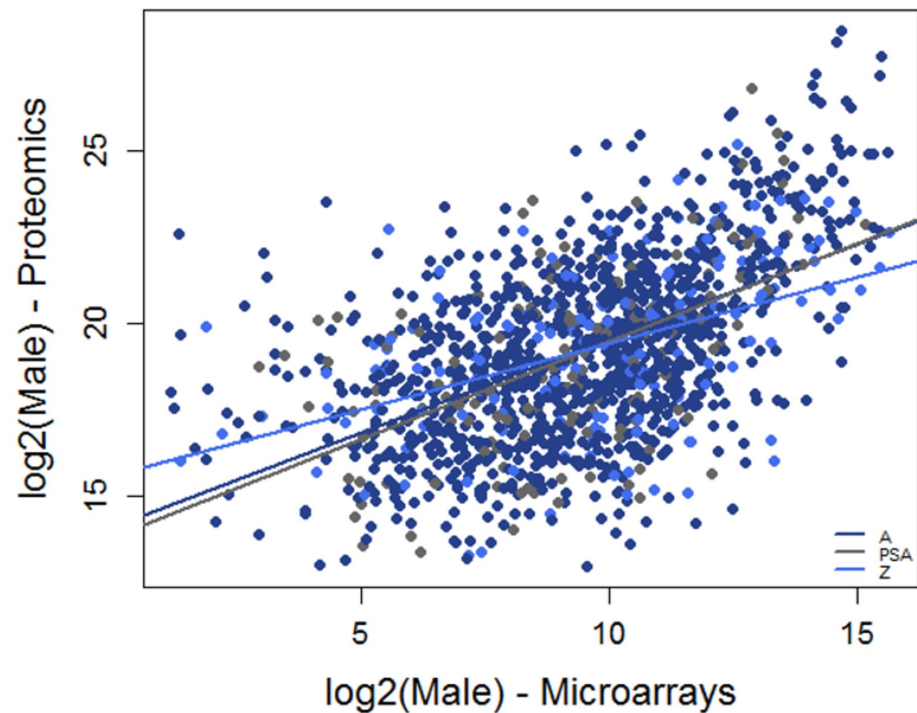


HEADS

A = 738 genes
 PSA = 108 genes
 Z = 123 genes

 R(A) = 0.54***
 R(PSA) = 0.44***
 R(Z) = 0.43***

Male GONADS

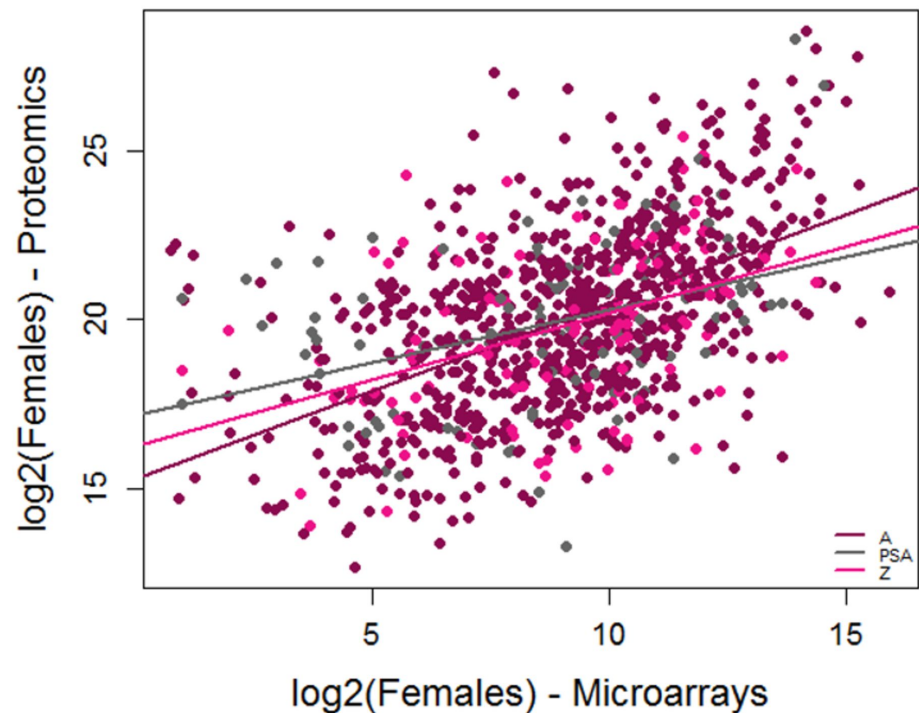


GONADS

A = 1041 genes
 PSA = 159 genes
 Z = 163 genes

 R(A) = 0.54***
 R(PSA) = 0.56***
 R(Z) = 0.47***

Female HEADS

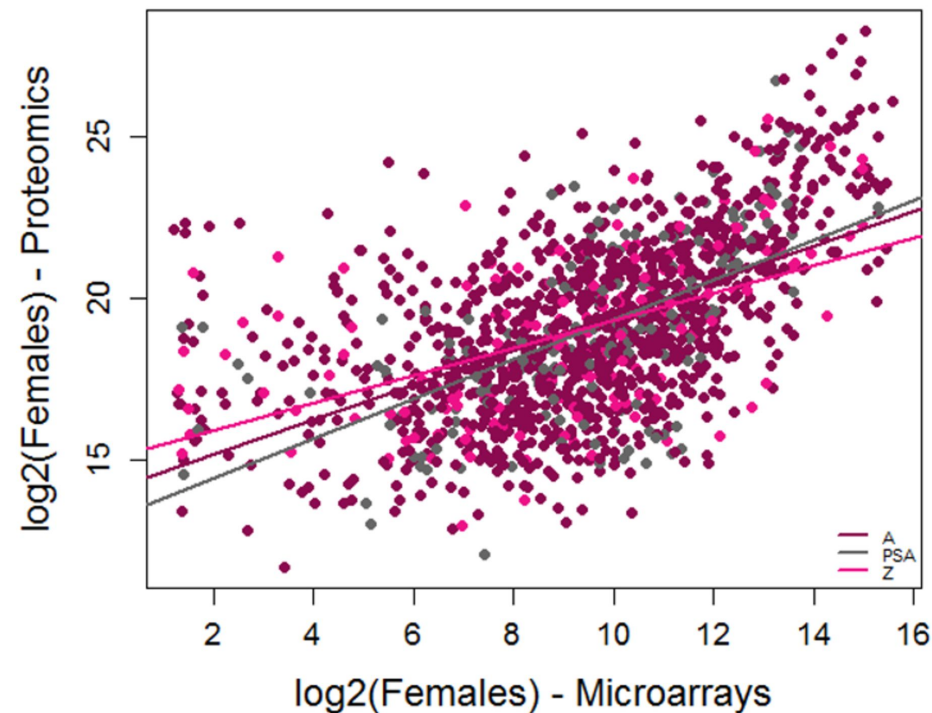


HEADS

A = 738 genes
 PSA = 108 genes
 Z = 123 genes

 R(A) = 0.52***
 R(PSA) = 0.39***
 R(Z) = 0.43***

Female GONADS

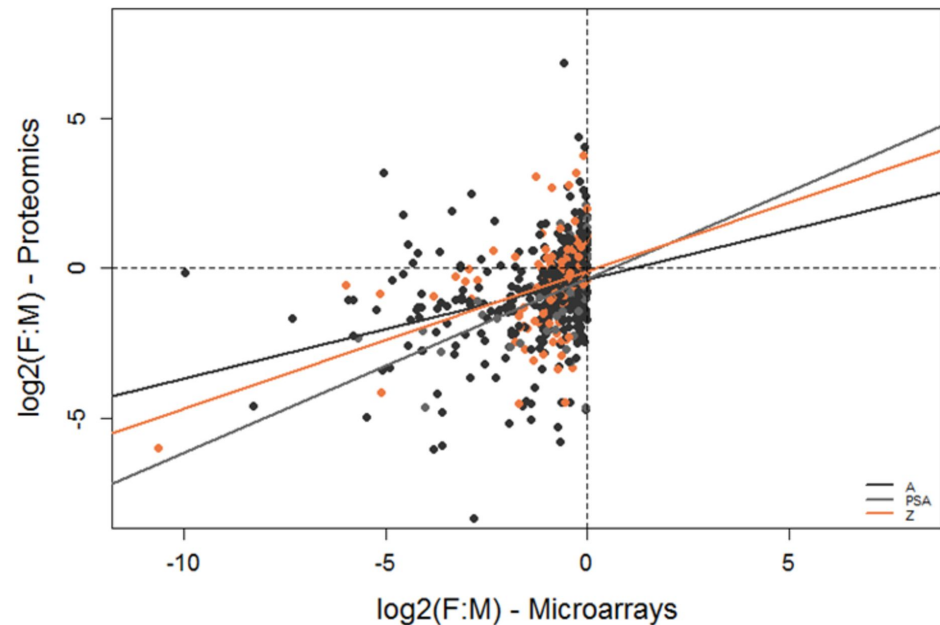


GONADS

A = 1041 genes
 PSA = 159 genes
 Z = 163 genes

 R(A) = 0.55***
 R(PSA) = 0.60***
 R(Z) = 0.53***

Proteins & mRNAs correlation - GONADS

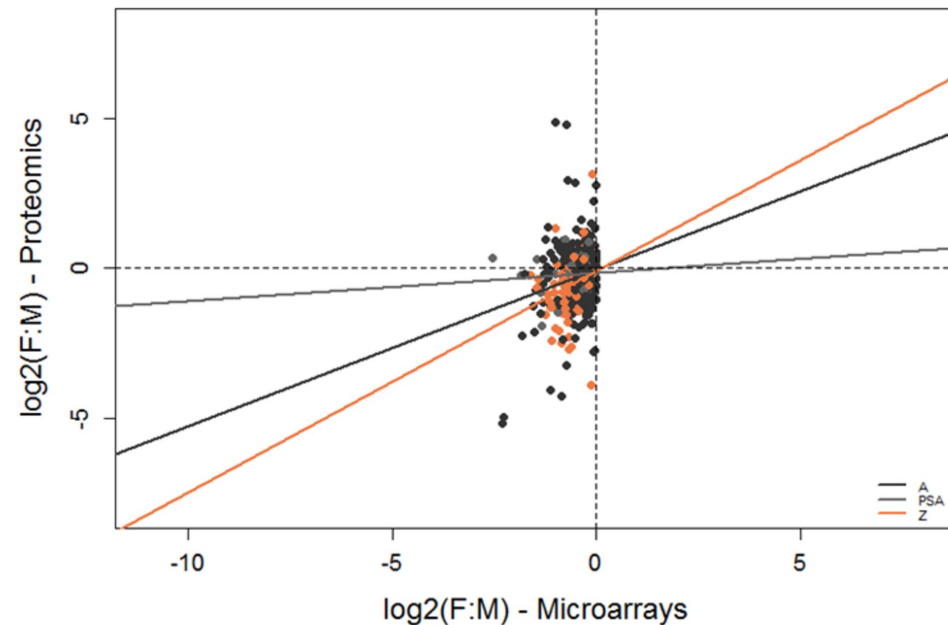


HEADS

A = 410 genes
PSA = 52 genes
Z = 125 genes

R(A) = 0.30***
R(PSA) = 0.48**
R(Z) = 0.41***

Proteins & mRNAs correlation - HEADS



GONADS

A = 339 genes
PSA = 49 genes
Z = 94 genes

R(A) = 0.20**
R(PSA) = 0.08 N.S.
R(Z) = 0.25*