Title: Virgin Birth: A genetic basis for facultative parthenogenesis

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Abstract

Sexual reproduction evolved 1-2 billion years ago and underlies the biodiversity of our planet. Nevertheless, devolution of sexual into asexual reproduction can occur across all phyla of the animal kingdom. The genetic basis for how parthenogenesis can arise is completely unknown. To understand the mechanism and benefits of parthenogenesis, we have sequenced the genome of the facultative parthenogen, *Drosophila mercatorum*, and compared its organisation and expression pattern during parthenogenetic or sexual reproduction. We identified three genes, *desat2*, *Myc*, and *polo* in parthenogenetic *D. mercatorum* that when mis-regulated in a non-parthenogenetic species, *D. melanogaster*, enable facultative parthenogenetic reproduction. This simple genetic switch leads us to propose that sporadic facultative parthenogenesis could evolve as an 'escape route' preserving the genetic lineage in the face of sexual isolation.

Introduction

Parthenogenesis is a form of reproduction resulting in uniparental offspring having only the maternal genome; it is a virgin birth. There are two types of parthenogenesis: facultative, having the ability to switch back to sexual reproduction; and obligate, in which this is not possible. Sexual reproduction requires a carefully orchestrated program whereby the genome is first duplicated before undergoing two divisions in the absence of DNA synthesis to generate a complement of haploid gametes that can be combined with those of the opposite sex, or mating type in the context of lower eukaryotes, to generate a diploid zygote. Facultative parthenogens retain the key meiotic machinery and yet have a hitherto unknown, but likely heritable, change that enables them to regain diploidy after meiosis and initiate mitotic divisions. By contrast, obligate parthenogens can theoretically have a block anywhere in meiosis and may eliminate it completely. It is therefore likely that different mechanisms underlie parthenogenesis depending upon which stage of sexual reproduction is blocked. Parthenogenesis was first observed in aphids by Charles Bonnet in approximately 1740 and yet, its underlying mechanism has not been identified in any animal. Despite being poorly understood, parthenogenesis is generally regarded as being a deleterious reproductive strategy because it fails to generate genetic diversity. Nevertheless, parthenogenesis has evolved repeatedly across different phyla of animals and plants. One reason for the failure to identify any genetic cause of naturally occurring parthenogenesis in animals is that ancient obligate parthenogenetic lineages are often compared to similar, sexually reproducing counterparts that have sometimes diverged millions of years ago. It then becomes impossible to separate the primary cause from multiple downstream consequences. If we are to understand parthenogenesis, we must look at new species or, better yet, examine those able to switch from sexual to parthenogenetic reproduction. We postulated that a genetic cause likely underlies facultative parthenogenesis because it can undergo selection in *Drosophila*, locuts,

47 and chickens and increase in frequency over several generations [1-4]. We therefore sought
48 to uncover the genetic cause behind facultative parthenogenesis in *Drosophila mercatorum*,
49 by sequencing its genome and comparing gene expression patterns during the oogenesis of
50 females undertaking sexual or parthenogenetic reproduction. We, and now report a genetic
51 cause of sporadic facultative parthenogenesis in *D. mercatorum* and show how these traits
52 can be transferred to a sexually reproducing species, *Drosophila melanogaster.*
53

54 **Results**

55 **The parthenogenetic ability of *D. mercatorum***

56 The facultative parthenogen, *D. mercatorum*, is unique in that some strains can behave as
57 obligate parthenogens upon transitioning to parthenogenetic reproduction and can then be
58 maintained in the lab indefinitely as healthy and easily expandable female only stocks [4-6].
59 *D. mercatorum* belongs to the *repleta* species group of South American cactus feeders which
60 are approximately 47 My diverged from *D. melanogaster* [7]. However, *D. mercatorum*
61 appears invasive and has spread, far beyond the range of most other *repleta*, to Australia and
62 as far north as New York [4, 8]. As nearly all strains of *D. mercatorum* studied to date show
63 some degree of parthenogenetic capability [4], we began by determining the baseline of
64 parthenogenesis in 8 different *D. mercatorum* strains using a classical assay adapted from the
65 first study of *Drosophila* parthenogenesis [3]. Large numbers of virgin females were
66 maintained on fresh food for the duration of their lives and the food examined for offspring at
67 any developmental stage. The numbers of progeny ranged from the generation of a small
68 number of developing embryos that died before hatching to the production of a small number
69 of fertile adult flies (Table S1). We observed that parthenogenetic offspring were produced
70 from middle aged mothers (Table S1). We also confirmed by PCR with general Wolbachia
71 primers that there was no Wolbachia infection (Table S1), since it is known to cause

72 parthenogenesis in other arthropods [9], although Wolbachia is only known to cause
73 cytoplasmic incompatibility in *Drosophila* [10]. We also confirmed that the strains examined
74 were indeed all *D. mercatorum* since they were able to interbreed producing viable and fertile
75 male and female offspring, although the parthenogenetically reproducing strain had slight
76 impediment to breeding and did not consistently produce offspring (Table S2). As a result of
77 these experiments, we selected two *D. mercatorum* stains for further study, a parthenogenetic
78 strain from Hawaii and a sexually reproducing strain with very low parthenogenetic
79 capability from São Paulo, Brazil.
80

81 **The genome of *D. mercatorum***

82 In search for genetic changes permitting parthenogenesis, we chose to sequence and compare
83 the genomes of the chosen sexually reproducing and parthenogenetic strains of *D.*
84 *mercatorum*. We produced polished chromosome-level genome assemblies, using Oxford
85 Nanopore Technology (ONT) and Illumina sequencing technology, that were then annotated
86 (Fig. 1A). Most of the genes were on the 14 largest contigs (Fig. S1A-B). We ensured that the
87 sequencing depth and coverage were uniform by plotting the reads over the assembled
88 genome (Fig. S1C-D). The quality of the assemblies was assessed using standard metrics of
89 N50, coverage, genome size, and gene content (Fig. 1A). When aligned, the sexual and
90 parthenogenetic genomes were highly similar having only 1.2% divergence (Fig. 1B), which
91 is consistent with pairwise heterozygosity, and thus further confirming that they are indeed
92 the same species. We observed inversions on the 2L chromosome arm which had previously
93 been noticed between *D. mercatorum* populations collected from South and North America
94 [8, 11].
95 We found 24.4% divergence between both parthenogenetically and sexually reproducing *D.*
96 *meractorum* genome assemblies and the *D. melanogaster* reference genome (release 6).

97 There was clustering of each contig from both *D. meractorum* genomes to specific
98 chromosome arms in *D. melanogaster* (Fig. S2A-B), indicative of the shuffling of genes,
99 which largely remain on the same chromosome arms. We also confirmed chromosome arm
100 contig matching by checking the DNA k-mers using Nucmer (Fig. S3A-B). This accords with
101 long-held knowledge of how corresponding chromosome arms form a series of homologous
102 genetic 'building blocks' in different *Drosophila* species within which synteny is lost [12,
103 13]. These chromosome arm 'building blocks' correspond to the six Muller elements (A-F)
104 and are conserved across Diptera [14]. Together these analyses indicated that the
105 chromosome-level genome assemblies for the sexually and parthenogenetically reproducing
106 *D. mercatorum* strains were suited to detailed comparison between each other and with the *D.*
107 *melanogaster* genome.
108 We next confirmed that the genome assemblies matched the karyotypes of the sexual and
109 parthenogenetic of *D. mercatorum* strains by localising local sequence markers onto
110 preparations of mitotic chromosomes from *D. mercatorum* third instar larval brains using a
111 Hybridisation Chain reaction (HCR) fluorescence in situ hybridization (FISH) protocol that
112 we developed for this purpose (see Supplementary text). We selected single genes within
113 syntenic blocks that are conserved between *D. melanogaster* and *D. mercatorum* to serve as
114 markers for each of the 6 chromosome arms of the mitotic karyotype (Fig. 2A). This allowed
115 us to identify the Muller elements, A-F, for both sexual and parthenogenetic *D. mercatorum*
116 strains (Fig. 2B-C). We found a fusion of the 2L/B and 3R/E (*D. melanogaster*/Muller)
117 chromosome arms that was previously documented as unique to *D. mercatorum* within the
118 *repleta* group [11] and the remaining chromosome arms were telocentric. We observed that
119 the 4th chromosome of the parthenogenetic strain was substantially larger than the 4th
120 chromosome in the sexual strain and we continue to investigate the underlying reason for
121 this. We also used HCR FISH to physically position the 14 largest contigs from the

122 parthenogenetic genome onto the 3rd instar larval salivary gland polytene chromosomes of
123 both the sexual and parthenogenetic strains of *D. mercatorum*. We found that each contig
124 mapped to the chromosome arm as predicted by the annotation and nucleotide sequence (Fig.
125 2D-F). Notably the polytene 4th (F) chromosome of sexually and parthenogenetically
126 reproducing strains of *D. mercatorum* appeared of similar size suggesting that the increased
127 size of their corresponding diploid chromosomes is due to acquisition of satellite,
128 heterochromatic sequences that do not undergo endoreduplication in the generation of
129 polytene chromosomes. We conclude that the two chromosome-level genome assemblies of
130 the sexually and parthenogenetically reproducing *D. mercatorum* represent the protein coding
131 part of these genomes and accurately reflects chromosome organisation in this species.
132

133 **Gene expression differences between sexual and parthenogenetic *D. mercatorum***

134 We argued that genomic changes with potential to lead to changes in reproductive ability
135 should reveal themselves as gene expression changes late in female germline development.
136 We therefore used RNA sequencing to characterise the transcriptomes of mature eggs (Stage
137 14 egg chambers) isolated from the sexual, parthenogenetic, and a 'partially parthenogenetic'
138 strain of *D. mercatorum*. The partially parthenogenetic strain reproduces sexually but has an
139 enhanced ability to switch to parthenogenetic reproduction. From the three transcription
140 profiles we identified 7656 genes that were expressed in mature eggs with the same
141 distribution as the annotated genes (Fig. S4A-B, Fig. S1A-B). There were 92 genes
142 differentially expressed in all three pairwise transcriptome comparisons (Fig. S4C, Tables
143 S3). However, there were few strongly and significantly differentially expressed genes and
144 after manual curation a subset were selected for further study, highlighted in the volcano
145 plots (Fig. S4D). We analysed the significantly differentially expressed genes from all
146 pairwise comparisons and found gene ontology (GO) enrichment of genes involved in redox,

147 immune function, wing disc growth, biosynthesis, proteolysis, and translation (Fig. S5A).
148 Following consideration of the GO analysis and the pairwise comparisons of expression, we
149 selected a further set of genes whose mis-regulation could potentially result in
150 parthenogenesis (Fig. 3A). Genes were selected that exhibited significant differential
151 expression ($p$adj<0.05) at a level equivalent to heterozygosis ($\log_2$ fold change ± 0.6) or
152 greater and were involved in common cellular processes, based upon the gene ontology
153 analysis.
154

155 **Functional screens for parthenogenesis**

156 We decided to take a two-pronged approach in an effort to identify genes that could lead to
157 parthenogenesis in a sexually reproducing fly: an unbiased screen of candidate genes from
158 the transcriptomics (Fig. 3A), a biased screen of candidate genes based on their cell
159 cycle/centriole functions (Fig. 3B), and a series of controls (Fig. 3C). For the first group, our
160 objective was to replicate, as far as possible, the degree of differential expression seen
161 between *D. meractorum* strains (Fig. S6). Since all strains of *D. mercatorum* we screened
162 were already parthenogenetic to some degree, we carried out this screen in the non-
163 parthenogenetic species *D. melanogaster*. Using 13 different *Drosophila* species, we first
164 determined that a baseline indicator of parthenogenesis could be given by testing the ability
165 of approximately 500 virgin female flies to generate progeny (Table S4). Strong levels of
166 parthenogenesis could be detected with as little as 30 flies. Using these criteria, we found that
167 two typical laboratory strains of *D. melanogaster* (w^- and *Oregon-R*) showed no
168 parthenogenesis whatsoever, whereas a strain caught in the wild (*CB1*) produced a small
169 number of embryos that showed restricted development before dying (Table S5). This
170 accords with previous findings that *D. melanogaster* strains caught in the wild have slight
171 parthenogenetic ability [3].

172 We then tested whether down-regulating the *D. melanogaster* homologs of genes showing
173 reduced expression in parthenogenetic *D. mercatorum* strains would result in the production
174 of offspring that died as embryos, larvae, pupae, or from old age as adult flies. To this end,
175 we examined CRISPR knock-out alleles that we generated in candidate genes (Fig. S7, Table
176 S6); publicly available mutants; or established lines in which candidate genes were down-
177 regulated by RNAi. We also tested *D. melanogaster* constructs engineered to increase
178 expression of genes whose homologues had elevated expression in the parthenogenetic *D.*
179 *mercatorum* strains. In the case of variant alleles that were not homozygous viable, screening
180 was carried out on heterozygotes. Together we screened a total of 44 genes (Fig. 3A-C, see
181 Supplementary text) and identified 16 able to cause 0.1-0.4% parthenogenesis in *D.*
182 *melanogaster* when their expression was either increased or decreased (Fig. S8A; Table S7
183 and see Supplementary text). The parthenogenesis observed resulted in the offspring
184 developing to varying stages and dying as embryos or from old age as adult flies. The
185 percentage given is relative to the number of adult flies screened. For this single mutant
186 screen, we found largely only the generation of embryos that died before hatching.
187 The low level of parthenogenesis detected in this single mutant screen, where the expression
188 of only a single gene was perturbed, is in line with earlier studies that had concluded that
189 parthenogenesis was a polygenic trait [4]. This consideration led us to carry out a double
190 mutant screen in which we combined pairs of variants in different genes into individual fly
191 stocks that we then screened for parthenogenesis. This revealed several mutant combinations
192 able to generate between 0.5-7% parthenogenetic offspring that died as embryos, larvae,
193 pupae, or from old age as adult flies. From the more successful combinations, we found that
194 one of the mutant genes either encoded a desaturase, *desat1* or *desat2*, or a protein
195 predominantly involved in regulating cell division and proliferation, *Myc*, *slmb*, or *polo* (Fig.
196 4A, and Table S8). Notably, 0.8% of the offspring derived from females heterozygous for a

197 mutation in *desat2* and carrying two extra copies of a *polo* transgene expressed from its
198 endogenous promotor (*GFP-polo*$^{4+}$;; *desat1*$^{-/+}$) developed to adulthood (Table S8). This level
199 of *D. melanogaster* parthenogenesis is a comparable the 'partially parthenogenetic' strain of
200 *D. mercatorum* used in generating the transcriptomics data. Parthenogenesis results from
201 decreased expression of either *desat1* or *desat2*. Since the *desat2* allele is known to be a
202 natural variant present in most populations, we determined whether our *desat1* stock carries
203 the *desat2* allele and indeed it does. Therefore, the *desat1* stock is a double mutant for *desat1*
204 and *desat2*, accounting for its stronger phenotype.
205 We then asked whether the parthenogenetic offspring obtained from these screens for
206 parthenogenesis in *D. melanogaster* were themselves able to carry out parthenogenetic
207 reproduction and found that none of them could (Tables S7-8). We did, however, find that the
208 parthenogenetic *D. melanogaster* were still able to mate with males and produce fertile
209 offspring (Fig. S8B), similar to previous findings [4]. The parthenogenetic *D. mercatorum*
210 offspring from the sexually reproducing stocks could not be established as a lab stock and did
211 not survive beyond the 7th generation of parthenogenesis as also found previously [15]. Even
212 our long-held stocks of fully parthenogenetic females were able to mate with males (Table
213 S2). Therefore, we have not found a genetic combination that leads to obligate-like
214 parthenogenesis, but we have identified key genes for facultative parthenogenesis.
215 Having identified *D. mercatorum* genes whose homologues led to a degree of
216 parthenogenetic development when mis-expressed in *D. melanogaster,* we looked for
217 genomic differences in these genes in sexual and parthenogenetic strains of *D. mercatorum*.
218 We found no substantial changes in gene organisation of *desat1/2*, *polo*, or *slmb,* although we
219 cannot exclude the possibility of changes in distal enhancer elements that have not been
220 mapped (Fig. S9A-D, Supplementary text). There were several changes to the *Myc* locus that
221 could affect the expression of the protein and change its downstream function (Fig. S9E). The

222 Myc locus of the parthenogenetic strain showed many deletions and insertions leading to the
223 changes in primary amino-acid sequence of the protein as indicated in Fig. S10A. None of
224 these mutations affected either the basic Helix-Loop-Helix (bHLH) DNA-binding domain or
225 the three Myc Box (MB) domains (1-3), or the three known phosphorylation sites of the Myc
226 protein (Fig. S9E) [16-18]. There were also changes in genome organisation at the *Myc* locus
227 of the *D. mercatorum* parthenogenetic genome, which has a 1.4kbp repetitive region between
228 a *Drosophila* INterspersed Elements-1 (DINE-1/INE1) transposable element (TE) and the
229 *Myc* coding region, which are 9.3kbp apart. A similar TE insertion in the classic mutant allele
230 of *Myc* (*dm1*) causes the minute phenotype [16], therefore having a repressive effect on Myc
231 expression. The 1.4kbp repetitive sequence present in the parthenogenetic genome between
232 the gene and the TE could allow de-repression resulting in an increased expression of *Myc*
233 relative to the sexually reproducing flies. Finally, we detected a 48bp deletion in the
234 parthenogenetic genome 344bp up stream of the start site. The above mutations have the
235 potential to affect the transcription, translations, or protein stability of Myc. Moreover, they
236 might also perturb Myc's functions as a transcription factor to influence the expression of the
237 other genes identified in our study [19]. Future studies will be required to distinguish these
238 possibilities.
239
240 **The development of parthenogenetic embryos**
241 To understand how development might be initiated during parthenogenesis we first examined
242 fertilised eggs from the sexually reproducing *D. mercatorum* strain that were initiating the
243 mitotic nuclear division cycles (Fig 5C, Fig. S11A) to compare to the parthenogenetic eggs.
244 The extent to which nuclear division cycles could take place in parthenogenetic *D.*
245 *mercatorum* reflected the extent of parthenogenicity. We observed that 38% of unfertilised
246 eggs from the parthenogenetic strain had one or more cell divisions (Fig 5A,C, Fig. S11E-G),

247 whereas 12% of unfertilised eggs from the partially parthenogenetic strain showed one or
248 more cell divisions (Fig 5B,C, Fig. S11B-D). The sexually reproducing *D. mercatorum*
249 embryos had timely cell divisions and no obvious nuclear defects. In contrast, the unfertilised
250 parthenogenetic and partially parthenogenetic embryos developed with abnormal numbers of
251 nuclei (Fig. S11D) or DNA abnormalities (Fig. S11G). All parthenogenetic *Drosophilids*
252 appear to retain normal meiosis and rediploidise their genomes either by fusion of one or
253 more of the four haploid nuclei arising from meiosis or by post-meiotic duplication of the
254 haploid gamete [3, 5, 20-24]. All four meiotic products, three polar bodies and the female
255 pronucleus, are present within the *Drosophilid* egg, and the three polar normally fuse and
256 arrest in a mitotic-like state. We only observed the presence of polar bodies in 44% of
257 parthenogenetic embryos that initiated the mitotic nuclear division cycles suggesting that the
258 missing polar bodies may be participating in the mitotic nuclear division cycles in the
259 developing embryos.
260 We then examined fertilised and unfertilised wildtype *D. melanogaster* eggs and compared
261 their development to the parthenogenetic *D. melanogaster* eggs. All unfertilised wildtype *D.*
262 *melanogaster* eggs completed meiosis and 70% appeared to have entered the first mitotic
263 division and had condensed chromosomes (Fig 5N, Fig. S12A-B). In contrast, nearly all
264 fertilised embryos had begun to undergo the mitotic nuclear division cycles (Fig 5N, Fig.
265 S11C-D). Development of the induced parthenogenetic *D. melanogaster* embryos mirrored
266 the findings from the parthenogenesis screens. We found nearly all unfertilised eggs laid by
267 either *GFP-polo*$^{4+}$ or *desat2*$^{-/-}$ mothers were not able to undertake mitotic nuclear division
268 cycles (Fig. 5D-E,G-J,O, Fig. S13A-D). By contrast, 6% of the unfertilised eggs laid by
269 *GFP-polo*$^{4+}$; ; *desat2*$^{-/+}$ *D. melanogaster* mothers could undertake at least limited mitosis
270 (Fig. 5F,K-M,O, Fig. S13E-G). Similar to the unfertilised parthenogenetic and partially
271 parthenogenetic *D. mercatorum* embryos, the parthenogenetic *D. melanogaster* embryos had

272 abnormal numbers of nuclei and/or DNA abnormalities. Although there are abnormalities in
273 these parthenogenetic embryos, during early embryogenesis there only need be one normal
274 nucleus dividing to produce an animal. We were unable to observe polar bodies in the
275 unfertilised *GFP-polo*$^{4+}$; ; *desat2*$^{-/+}$ - derived eggs that had initiated the mitotic cell divisions
276 and found that nuclei were not present in the expected ratio, suggesting that the polar bodies
277 participate in the nuclear division cycles. This leads us to propose that the recapture of polar
278 bodies contributes to rediploidisation in these induced parthenogenetic *D. melanogaster*
279 embryos.
280

281 **Discussion**

282 Our study offers the first account of a molecular basis underlying the evolution of any type of
283 parthenogenesis in any animal. Our findings relate specifically to the Dipteran *D.*
284 *mercatorum* and suggest a route through which parthenogenesis could arise in this species.
285 Key to this is the differential expression of *desat* and cell cycle genes between
286 parthenogenetically and sexually reproducing strains. The ability of *desat1* mutants to
287 enhance the phenotype of *desat2* mutants in driving parthenogenesis when heterozygous in
288 *D. melanogaster* is likely a consequence of overlapping function between their encoded
289 proteins, which show 85% identity in amino-acid sequence. Both *desat1* and *desat2* encode
290 desaturases that generate double bonds in hydrocarbons and have roles in lipid metabolism.
291 Desat1 also generates double bonds during sex pheromone biogenesis [25], and *desat1*
292 mutations can result in female resistance to mating. Desat2 desaturates cuticular
293 hydrocarbons; it has been associated with increased cold tolerance [26] and is credited with
294 imparting the ability *of D. melanogaster* to become invasive and colonise cosmopolitan
295 habitats. The highly pleiotropic nature of *desat1* and *desat2* makes it difficult to determine
296 how their down-regulation relates to the increased incidence of parthenogenesis. As their

297 mutation leads to a higher fluidity of membranes, it is possible that this could influence a
298 wide range of membrane associated trafficking events associated with the completion of
299 female meiosis, behaviour of the polar bodies, and the onset of zygotic mitoses. The potential
300 effects of these mutations upon such events will require detailed future studies.
301 The other group of genes involved in enabling parthenogenesis regulate some aspect of cell
302 cycle progression. Slmb is a subunit of the SCF, the Skp, Cullin, F-box containing ubiquitin
303 ligase complex that regulates S phase entry by targeting multiple substrates. One of the
304 multiple targets of the SCF is Myc [27]. There was only a modest increase in Myc transcripts
305 (0.6 Log_2 fold change) in the parthenogenetic strain, however, this expression level change
306 was highly significant (padj<0.001) and is equivalent to having one extra copy of the gene.
307 The Myc bHLH transcription factor has been shown to give *D. melanogaster* cells a
308 competitive growth advantage [28] and could account for the finding that parthenogenetic
309 offspring are physically larger than the sexually reproducing animals [29]. Myc is known to
310 promote Polo-like-kinase1 expression in mammalian cells that in turn destabilises SCF [30].
311 If a similar mechanism were to act in *Drosophila*, this could account for the relationships we
312 observe here to promote parthenogenesis. Our findings suggest that an effective step towards
313 establishing parthenogenesis is the heterozygosity of *desat1* or *desat2* coupled to *Myc* over-
314 expression or heterozygosity of the SCF (slmb).
315 Although we have shown that we can induce parthenogenesis in a sexually reproducing line
316 of *D. melanogaster* to a similar degree as a partially parthenogenetic strain of *D. mercatorum*,
317 we are not able to maintain these animals as a parthenogenetic stock. Therefore, although we
318 identify a significant step towards heritable parthenogenesis, this is not the end of the story
319 and additional changes would be required for parthenogenesis to become fixed in a
320 population and transit to more obligate-like parthenogenetic reproduction. Moreover, there
321 are likely to be many alternative paths to the devolution of sexual reproduction in animals

322 and this could explain the varying degree of parthenogenetic ability, not only within *D.*
323 *mercatorum,* but also across the *Drosophila* genus. Given the polygenic nature of facultative
324 parthenogenesis and the fact that there are multiple inputs into core cell cycle regulation, it
325 may explain why no unifying signature of parthenogenesis has been found to date [31]. Thus,
326 we anticipate that parthenogenesis might have different causal events in each species or even
327 between individuals of the same species.
328 Some consider sporadic facultative parthenogenesis to be an unimportant accident. However,
329 there could be a benefit of having sporadic facultative parthenogenesis inducing
330 heterozygotic mutations floating around in the population, they may facilitate an 'extinction
331 escape hatch', thus helping a lineage of the species stave off extinction in the face of isolation
332 until an opportunity to mate arises again. Parthenogenesis is spread across the order Diptera
333 and rare facultative parthenogenesis is prevalent in *Drosophila* [32] making it likely that the
334 mechanism we propose is not restricted to *D. mercatorum*.
335
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Data and materials availability: All raw and analyzed *D. mercatorum* genomic and transcriptomic data generated by this study will be provided on public databases, all code used for analysis in on GitHub via FabianDK and ekg, all *Drosophila strains/stocks* will be offered to Bloomington Stock Centre or made available upon request.

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445

446

447 **Figure 1**: **Sexual and Parthenogenetic *D. mercatorum* genome analysis.** A) Genome
448 assembly data metrics, quality control, and annotation metrics B) Alignment of the
449 parthenogenetic *D. mercatorum* genome against the sexual genome.

450

451 **Figure 2: *D mercatorum* karyotyping and genome assembly physical mapping.** A) Select
452 genes used for probes to identify the chromosome arms. B) Mitotic chromosomes with the
453 chromosome arm indicated for the parthenogenetic and sexually reproducing *D. mercatorum*
454 3rd instar neuroblasts stained with DAPI and the probes indicated in 'A', chromosomes are
455 outlined with a white dashed line. The indicated chromosome was marked in 100% of typical
456 karyotypes analysed (n≥42, N≥3). The scale is 1µm. C) Cartoon of the parthenogenetic and
457 sexual *D. mercatorum* karyotype with the analogous *D. melanogaster* chromosomes
458 indicated along with the Muller Element letter in brackets. D) Select genes that have
459 chromosome level synteny across the genus *Drosophila* that were used to make probes for
460 mapping contigs to the polytene chromosomes. E) Images of polytene chromosomes used for
461 mapping of the largest 14 contigs of the sexual and parthenogenetic *D. mercatorum* genomes,
462 chromosomes were stained with DAPI and HCR in situ DNA probes matching the genes
463 listed in 'B'. The scale is 10µm. F) Schematic of the mapping of the first 14 Contigs.

464

465 **Figure 3: Unbiased and biased candidate genes that were screened for parthenogenesis.**
466 A) Differentially expressed genes between the parthenogenetic, partially parthenogenetic, and
467 sexual transcriptomes, the function was assigned from flybase.org, the $\log_2$ fold change and
468 the *p*adj are given for only the parthenogenetic vs sexual comparison except those with a (*)
469 are from the partial parthenogenetic vs sexual comparison, the screening was performed with
470 the listed genetic tools. B) Biased screen of cell cycle and centrosome genes that were not

471 differentially expresses, the function was attributed from flybase.org and the screening was
472 performed with the listed genetic tools. D) Negative controls that were not differentially
473 expressed, the screening was performed with the listed genetic tools.

474

475 **Figure 4: Double mutant screen results.** A) Positive results from the functional screens of
476 candidate genes that may cause parthenogenesis and two controls, the *p*-value was calculated
477 using the Fisher's exact test the control used for these calculations was the combined results
478 from the primary screen for the two genes used.

479

480 **Figure 5: Parthenogenetic *D. mercatorum* and *D. melanogaster* embryos**.
481 A-B) Unfertilised parthenogenetic and partially parthenogenetic *D. mercatorum* embryos.
482 The parthenogenetic embryo has entered the mitotic divisions. The partially parthenogenetic
483 embryo has initiated the first mitosis. Refer to Fig. S11. C) Histogram of the proportion of
484 sexually and parthenogenetically reproducing *D mercatorum* eggs/embryos that have
485 aggregated polar bodies, initiated the first mitosis, or have entered the mitotic cell divisions.
486 E-D) *GFP-polo*$^{4+}$, *desat2*$^{-/-}$, and *GFP-polo*$^{4+}$*; desat2*$^{-/+}$ *D. melanogaster* embryos that have
487 initiated the first mitosis, aggregated polar bodies, and have entered the mitotic cell divisions,
488 respectively. G-M) *GFP-polo*$^{4+}$, *desat2*$^{-/-}$, and *GFP-polo*$^{4+}$*; desat2*$^{-/+}$ *D. melanogaster*
489 embryos that have initiated the first mitosis, aggregated polar bodies, and have entered the
490 mitotic cell divisions. N) Histogram of the proportion of wildtype unfertilised and fertilised
491 *D. melanogaster* eggs/embryos that have aggregated polar bodies, initiated the first mitosis,
492 or have entered the mitotic cell divisions, refer to Fig. S12 for examples. O) Histogram of the
493 proportion of unfertilised *GFP-polo*$^{4+}$, *desat2*$^{-/-}$, and *GFP-polo*$^{4+}$*; desat2*$^{-/+}$ *D. melanogaster*
494 embryos that have aggregated polar bodies, initiated the first mitosis, or have entered the

495 mitotic cell divisions. The Fisher's exact test was used to calculate all *p*-values. The nuclei

496 are marked with asterisks. The scale is 10μm.

A Assembly metrics and annotation of the *D. mercatorum* genomes

Metric		Sexual	Parthenogenetic
Genome size		171,182,504bp	161,570,079bp
Contig number		556	330
ContinuityContig	Mean contig Length	307,882bp	489,606bp
	Scaffolds NG50	22,671,956bp	16,356,382bp
Repeat Content	Bases in repetitive regions	34,751,631bp	29,808,408bp
	Percent of genome repetitive	20.30%	18.45%
	GC Content	40.65%	40.33%
Functional completeness	Genes	19,983	17,611
	Uniquely mapped reads (STAR)	80-91%	74-89%

B Alignment of the sexual and parthenogenetic *D. mercatorum* genomes against each other

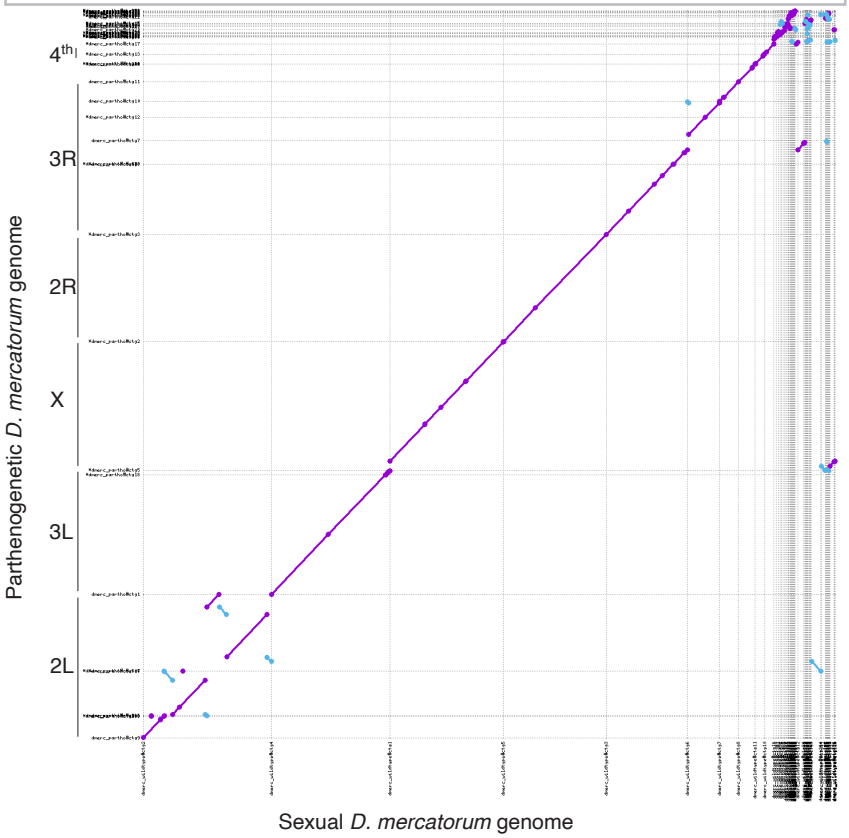
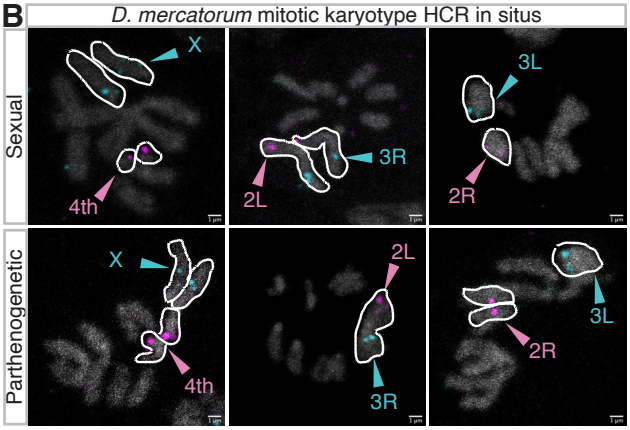


Figure 1

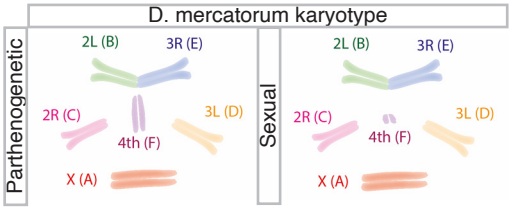
A *D. mercatorum* mitotic karyotype HCR in situs

D. mel	Contig (C)	Gene	Wavelength
X	5/13	*f/sdt*	488
2L	6	*fic*	594
2R	2/11	*Fili/Dgkε*	594
3L	1	*wit*	488
3R	12	*Rpn2*	488
4th	14	*apolpp*	594

B *D. mercatorum* mitotic karyotype HCR in situs



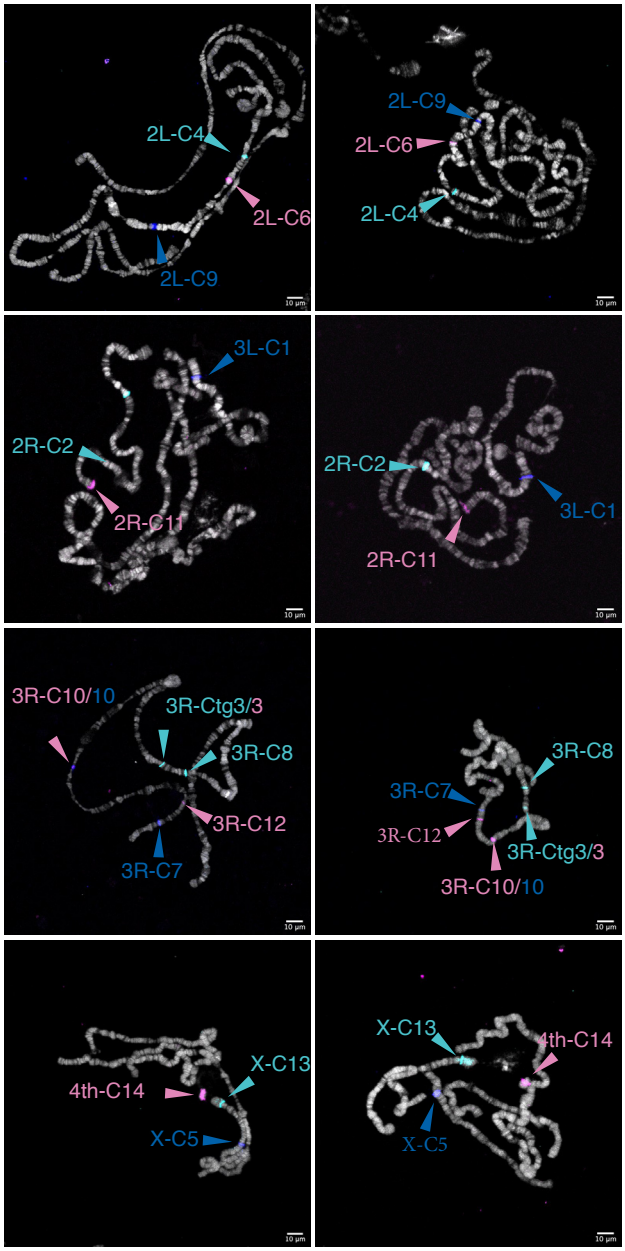
C D. mercatorum karyotype



D Karyotyping and mapping genes

D. mel	Contig (C)	Gene	Wavelength
X	5	*f*	647
	13	*sdt*	488
2L	4	*cort*	488
	6	*fic*	546
	9	*nesd*	647
2R	2	*Fili*	488
	11	*Dgkε*	546
3L	1	*wit*	647
3R	3	*SIDL*	488/546
	7	*pic*	647
	8	*asp*	488
	10	*desat2*	546/647
	12	*Rpn2*	546
4th	14	*apolpp*	546

E *D. mercatorum* polytene genomic scaffolding HCR in situs



F Physical map of the genome assembly

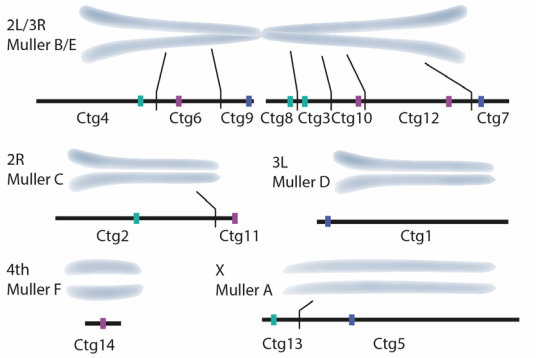


Figure 2

A Candidate genes from the transcriptomics screens

Gene	Function	Log_2 Fold Change	p_{adj}	Screened with
Asciz	Transcription	-0.6	1.3^{-4}	RNAi
bam	Cell fate	-4.8	8.4^{-25}	RNAi/mutant
c(2)M	Female meiosis	-1.4	1.7^{-8}	RNAi
Cad96Ca	Receptor tyrosine kinase	-7.4	2.5^{-126}	RNAi
CG4329	Cilia/flagella	-2.8	2.6^{-3}	RNAi
CG4496	Transcription	-1.8	3.2^{-15}	RNAi
CG10433	Female receptivity	-2.7	7.0^{-2}	RNAi
CG17202	Myc-binding	-0.8	3.5^{-5}	mutant
CG42808	Unknown	-3.4	2.5^{-3}	RNAi
chrb	Cell growth inhibition	-2.2	2.9^{-71}	RNAi
CRMP	Pyrimidine catabolism	-3.0	5.2^{-4}	RNAi/mutant
desat1	Fatty acid desaturase	-0.6	1.7	RNAi/mutant
desat2	Fatty acid desaturase	-6.6	1.3^{-7}	RNAi/mutant
e(r)	Pyrimidine biosynthesis	-2.7	1.5^{-3}	mutant
*eya**	Transcription	1.6	0.12	UAS/Gal4 overexpression
f	Actin filament	-2.8	3.7^{-11}	RNAi/mutant
FER	Tyrosine kinase	-2.4	1.5^{-3}	RNAi/mutant
gnu	Translation	3.5	1.7^{-73}	mutant/UAS/Gal4 overexpression
ktub	Endocytosis	-3.3	5.7^{-4}	RNAi/mutant
msd1	Mitotic spindle	-0.6	6.2^{-3}	mutant
Myc	Transcription	0.7	9.2^{-3}	UAS/Gal4 overexpression
Nmnat	Adenylyltransferase	1.7	1.9^{-10}	UAS/Gal4 overexpression
pnt	Transcription	1.8	1.7^{-9}	UAS/Gal4 overexpression
Rcd4	Centrosome	2.0	2.3^{-27}	UAS/Gal4 and ubiquitous expression
Roc1a	SCF complex/ Cell cycle	-0.6	2.3^{-6}	mutant UAS/Gal4 overexpression
RpL10Ab	Ribosome	-0.8	1.6^{-8}	mutant
spir	Actin nucleation	1.0	2.0^{-2}	UAS/Gal4 overexpression

B Candidate Cell cycle and centrosome: centriole/ pericentriolar material (PCM) genes

Gene	Function	Screened with
ana2	Centriole	CRISPR
asl	Centriole/PCM	CRISPR/ubiquitous expression
cnn	PCM	mutant
cyclinE	Cell cycle	UAS/Gal4 overexpression
morula	Cell cycle	CRISPR
Plk4	Centriole/ Centrosome	CRISPR/UAS/Gal4 overexpression
Plp	PCM	CRISPR
plu	Translation	CRISPR
png	Translation	CRISPR
polo	Cell cycle	mutants/endogenous promotor overexpression
Sas-6	PCM	CRISPR/ubiquitous expression
slmb	SCF complex/ Cell cycle	CRISPR/mutant
Rca1	Cell cycle	UAS/Gal4 overexpression
tefu/atm	Serine/threonine kinase	RNAi/mutant

C Negative controls

Gene	Function	Screened with
CG3436	Cell cycle	mutant
dhd	embryonic development	mutant
Klp64D	Motor protein	RNAi/mutant
Trx-2	Redox	RNAi/mutant
w	Eye pigment transporter	RNAi/mutant

Figure 3

A **Summary of the double mutant screen:** Genes that cause parthenogenesis when combined and select controls

Genotype	Percent parthenogenetic offspring	*p* value
desat1$^{+/-}$ / *desat2*$^{+/-}$	0.4%	1
desat2$^{+/-}$ / *slmb*$^{+/-}$	1.2%	0.13
Myc$^{+/+}$; *desat1*$^{+/-}$	0%	1
Myc$^{+/+}$; *desat2*$^{+/-}$	0%	1
Plk4$^{+/-}$ / *slmb*$^{+/-}$	0.9%	0.33
polo^{4+} ; ; *desat1*$^{+/-}$	1.7-5.2%	<0.003
polo^{4+} ; ; *desat2*$^{+/-}$	1.6-7.4%	<0.003
polo^{4+} ; *Myc*$^{+/+}$	0.6%	0.1217
polo^{4+} ; ; *slmb*$^{+/-}$	3.1%	3.2^{-08}
polo^{4+} ; ; *w-RNAi*$^{/-}$	0%	1
desat1$^{+/1}$ / *w-RNAi*$^{/-}$	0%	1

Figure 4

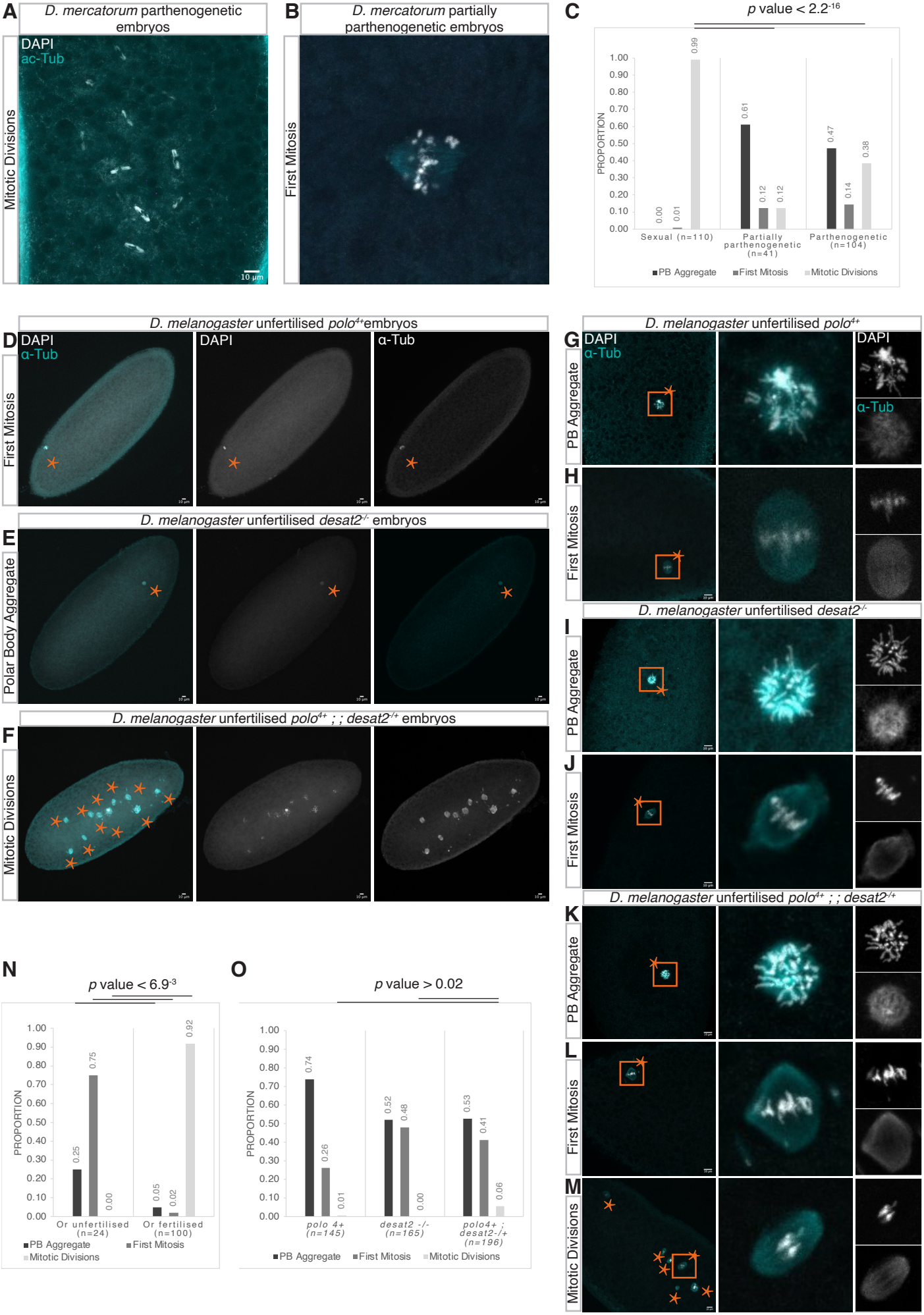

A
D. mercatorum parthenogenetic embryos
DAPI
ac-Tub
Mitotic Divisions
10 µm
B
D. mercatorum partially parthenogenetic embryos
First Mitosis
C
p value < 2.2-16
PROPORTION
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.01
0.99
0.61
0.12
0.12
0.47
0.14
0.38
Sexual (n=110)
Partially parthenogenetic (n=41)
Parthenogenetic (n=104)
PB Aggregate
First Mitosis
Mitotic Divisions
D
D. melanogaster unfertilised polo4+ embryos
DAPI
α-Tub
First Mitosis
E
D. melanogaster unfertilised desat2-/- embryos
Polar Body Aggregate
F
D. melanogaster unfertilised polo4+ ; ; desat2-/+ embryos
Mitotic Divisions
G
D. melanogaster unfertilised polo4+
PB Aggregate
H
First Mitosis
I
D. melanogaster unfertilised desat2-/-
PB Aggregate
J
First Mitosis
K
D. melanogaster unfertilised polo4+ ; ; desat2-/+
PB Aggregate
L
First Mitosis
M
Mitotic Divisions
N
p value < 6.9-3
PROPORTION
0.25
0.75
0.00
0.05
0.02
0.92
Or unfertilised (n=24)
Or fertilised (n=100)
PB Aggregate
First Mitosis
Mitotic Divisions
O
p value > 0.02
PROPORTION
0.74
0.26
0.01
0.52
0.48
0.00
0.53
0.41
0.06
polo 4+ (n=145)
desat2 -/- (n=165)
polo4+ ; desat2-/+ (n=196)
PB Aggregate
First Mitosis
Mitotic Divisions

Figure 5

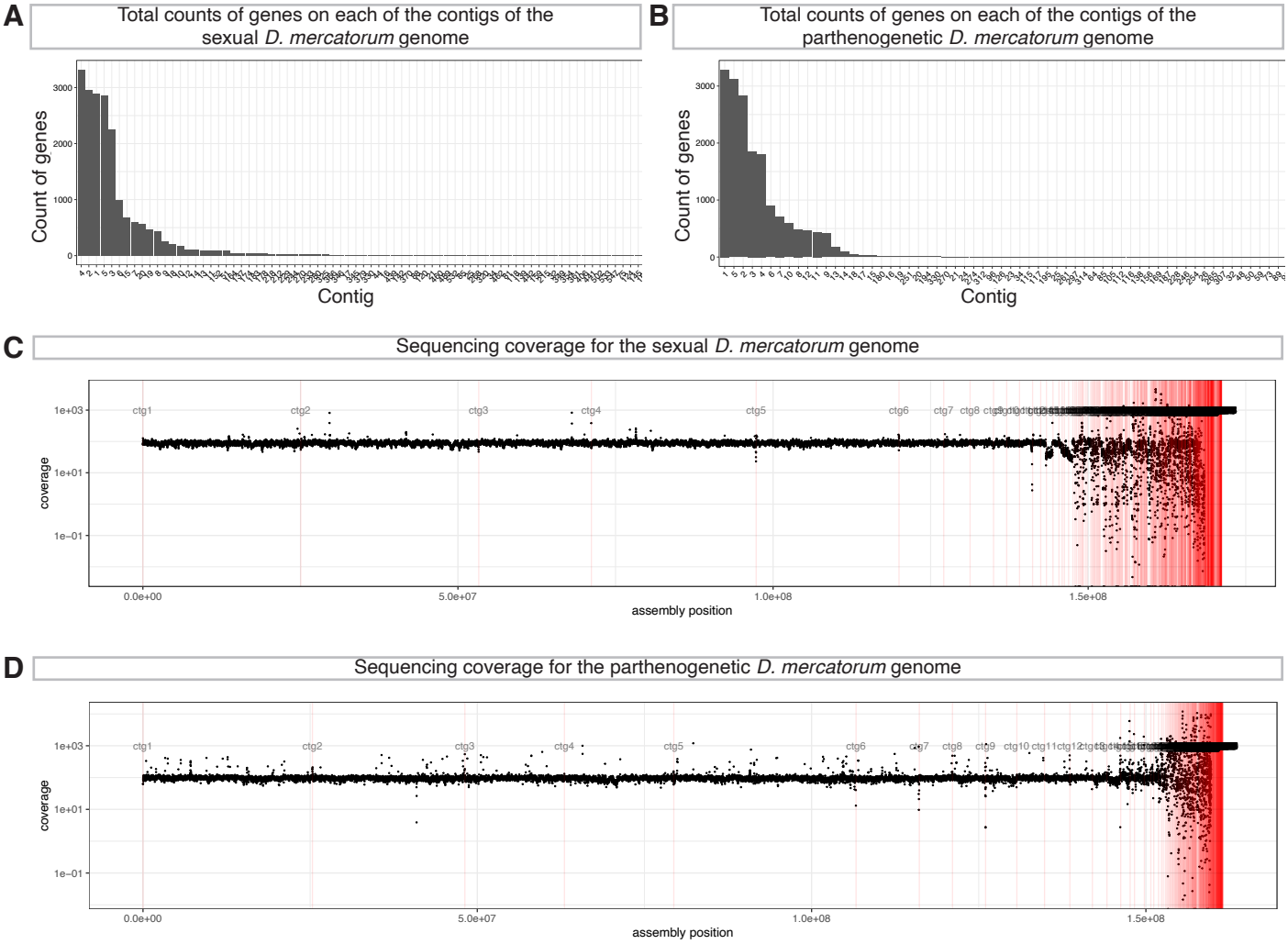

A
Total counts of genes on each of the contigs of the sexual D. mercatorum genome
Count of genes
Contig
B
Total counts of genes on each of the contigs of the parthenogenetic D. mercatorum genome
Count of genes
Contig
C
Sequencing coverage for the sexual D. mercatorum genome
coverage
assembly position
D
Sequencing coverage for the parthenogenetic D. mercatorum genome
coverage
assembly position

Figure S1:

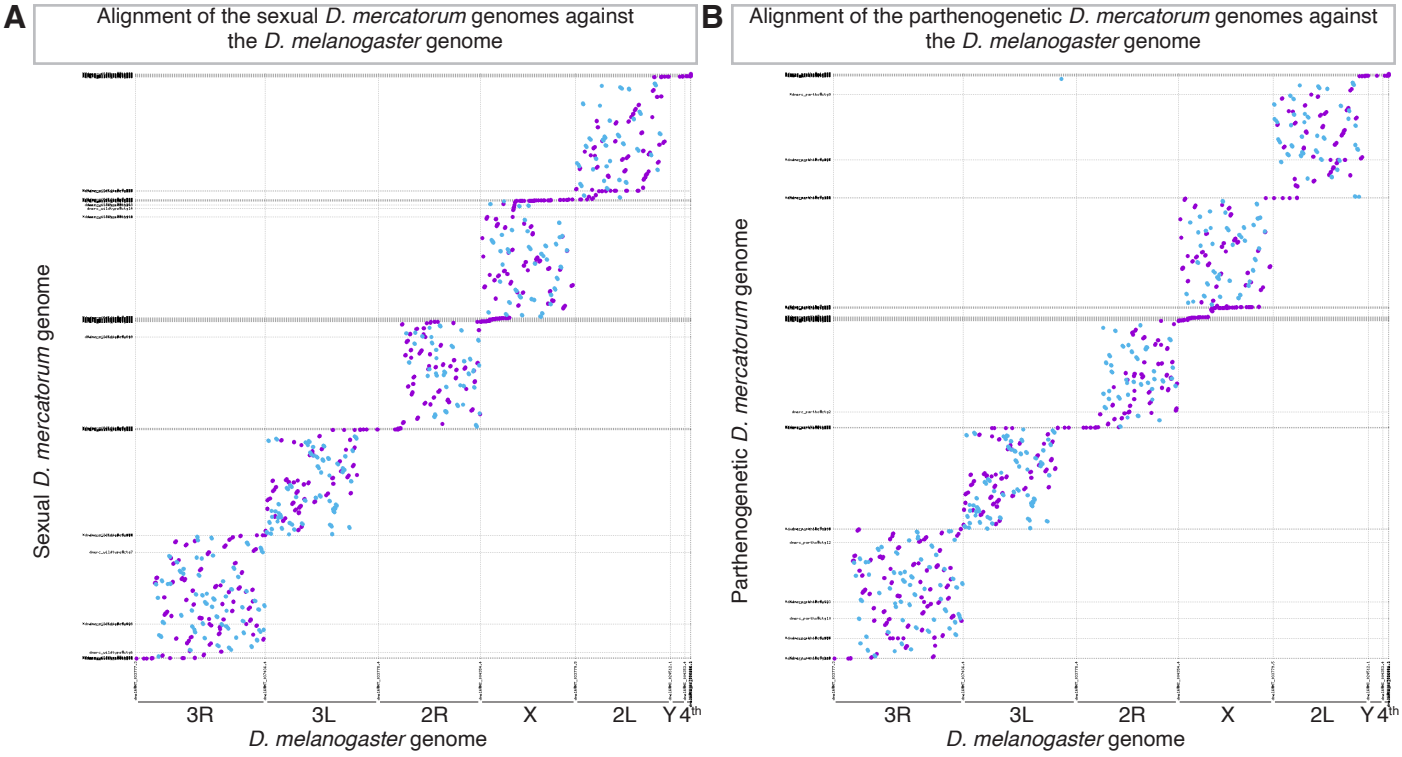

A
Alignment of the sexual D. mercatorum genomes against the D. melanogaster genome
Sexual D. mercatorum genome
3R
3L
2R
X
2L
Y
4th
D. melanogaster genome
B
Alignment of the parthenogenetic D. mercatorum genomes against the D. melanogaster genome
Parthenogenetic D. mercatorum genome
3R
3L
2R
X
2L
Y
4th
D. melanogaster genome

Figure S2:

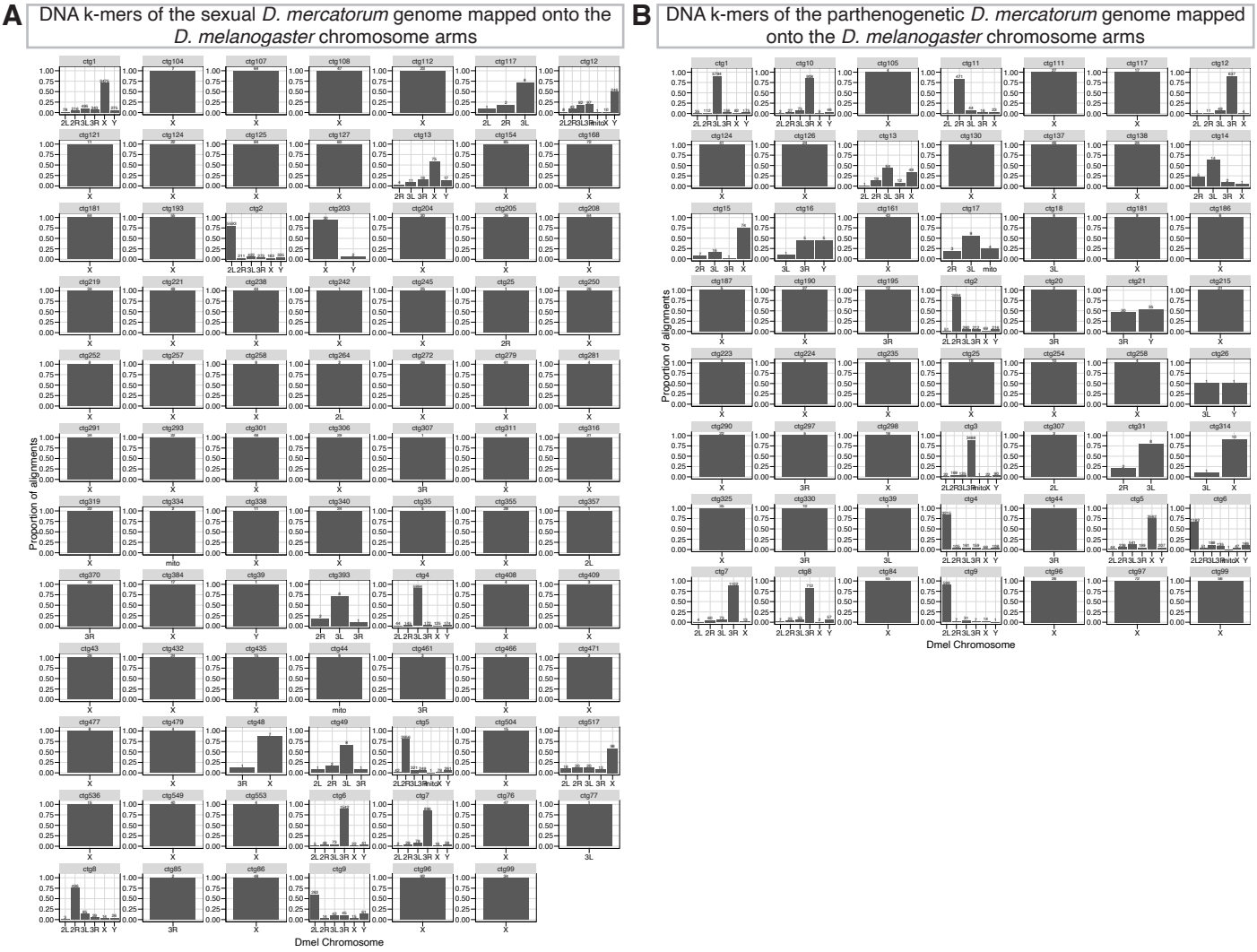
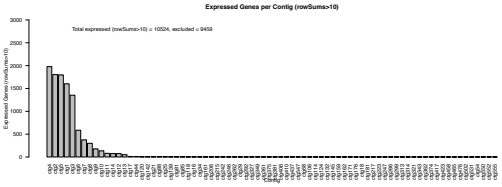
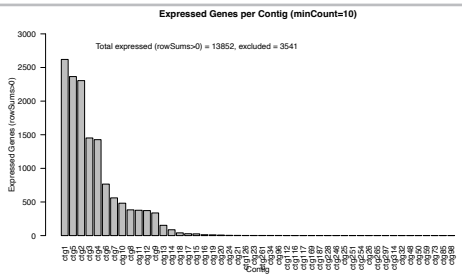

A
DNA k-mers of the sexual *D. mercatorum* genome mapped onto the *D. melanogaster* chromosome arms
B
DNA k-mers of the parthenogenetic *D. mercatorum* genome mapped onto the *D. melanogaster* chromosome arms
Proportion of alignments
Dmel Chromosome

Figure S3:

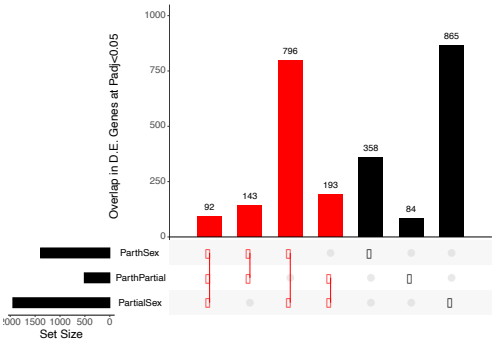
A Expressed genes per contig for the sexual *D. mercatorum* genome



B Expressed genes per contig for the Parthenogenetic *D. mercatorum* genome



C Upset plot of the overlapping differentially expressed genes between pairwise comparisons \ of *D. mercatorum* transcriptomes



Candidate genes at Padj<0.05
Red bars = significant according to Super Exact Test

D Volcano plots: three pairwise comparisons of *D. mercatorum* transcriptomes

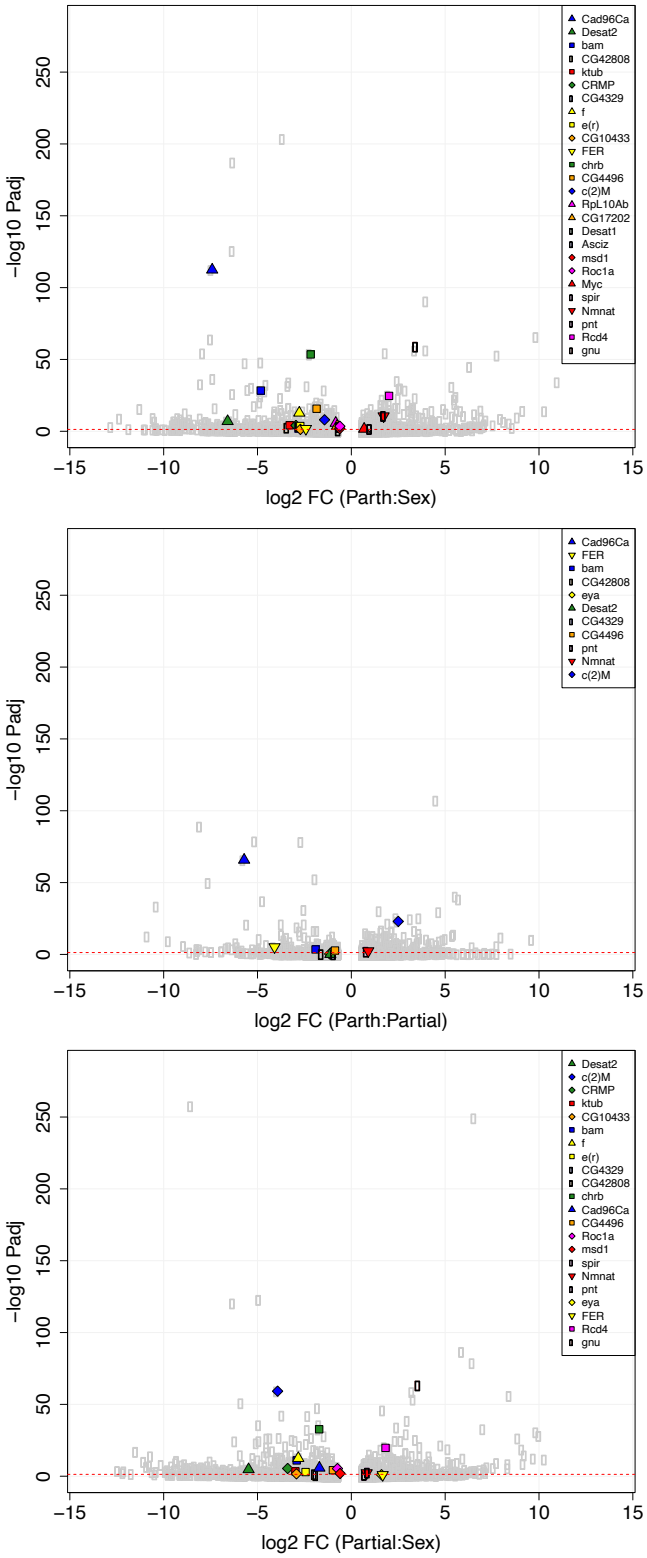


Figure S4:

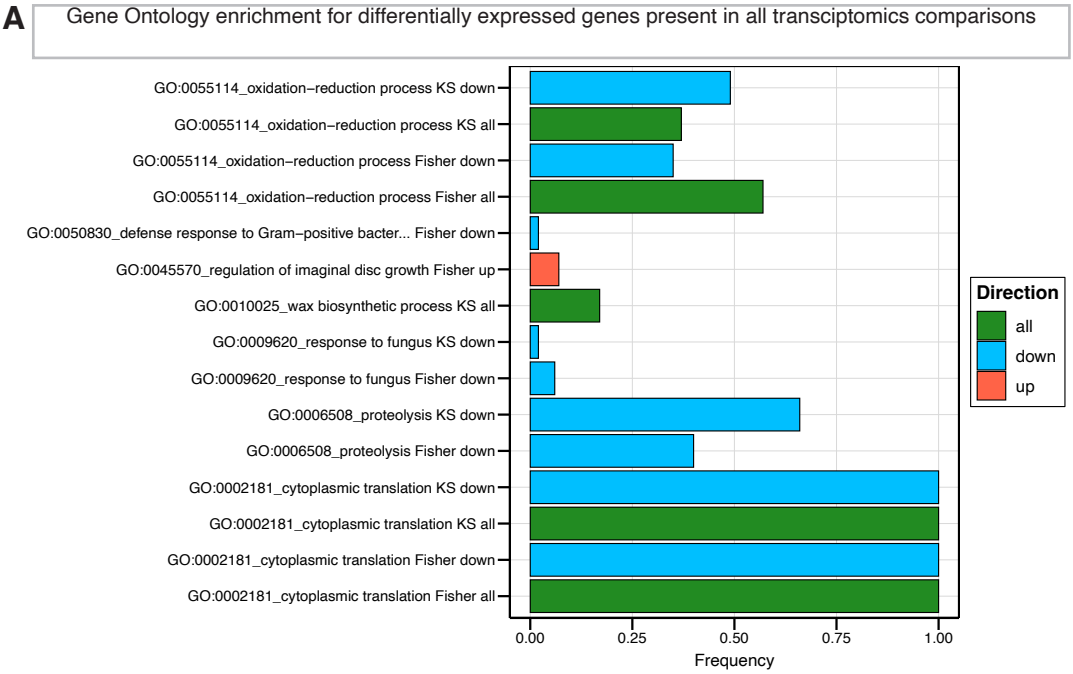
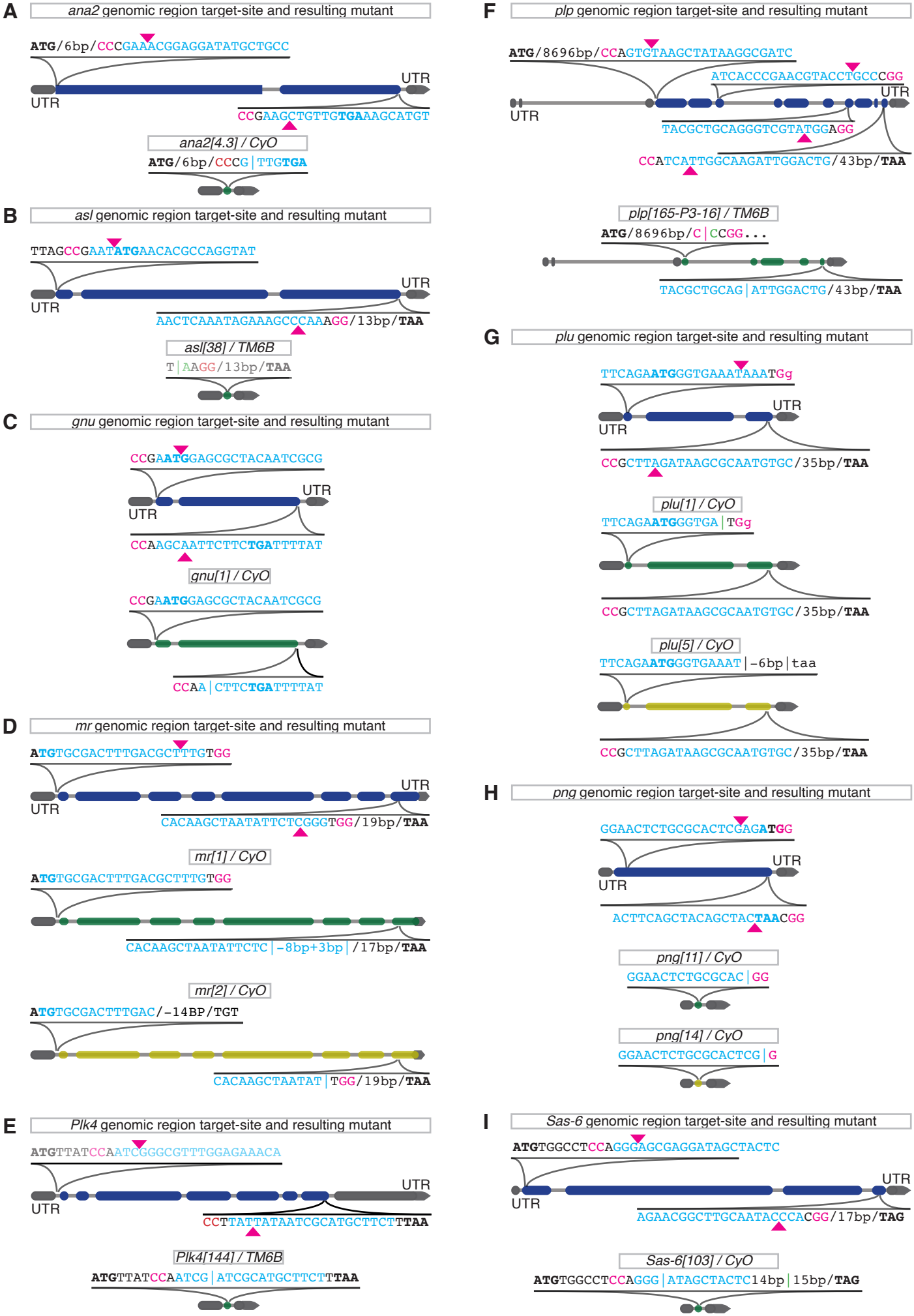

A
Gene Ontology enrichment for differentially expressed genes present in all transciptomics comparisons
GO:0055114_oxidation−reduction process KS down
GO:0055114_oxidation−reduction process KS all
GO:0055114_oxidation−reduction process Fisher down
GO:0055114_oxidation−reduction process Fisher all
GO:0050830_defense response to Gram−positive bacter... Fisher down
GO:0045570_regulation of imaginal disc growth Fisher up
GO:0010025_wax biosynthetic process KS all
GO:0009620_response to fungus KS down
GO:0009620_response to fungus Fisher down
GO:0006508_proteolysis KS down
GO:0006508_proteolysis Fisher down
GO:0002181_cytoplasmic translation KS down
GO:0002181_cytoplasmic translation KS all
GO:0002181_cytoplasmic translation Fisher down
GO:0002181_cytoplasmic translation Fisher all
0.00
0.25
0.50
0.75
1.00
Frequency
Direction
all
down
up

Figure S5:

A
Candidates from the Transcriptomics Screen:
Differentially expressed genes between all three pairwise comparisons from the three different transcriptomes
Asciz
bam
c(2)M
Cad96Ca
CG3429
CG4496
CG10433
CG17202
CG42808
chrb
CRMP
desat2
desat1
e(r)
eya
f
FER
gnu
ktub
msd1
Myc
Nmnat
pnt
Rcd4
Roc1a
RpL10Ab
spir
Log2 fold change
Contrast
ParthSex
ParthParti
PartiSex
B
Candidates from the biased screen:
Non-differentially expressed genes between all three pairwise comparisons from the three different transcriptomes
ana2
asl
cnn
CycE
mr
SAK
Plp
plu
png
polo
Sas-6
slmb
Rca1
Log2 fold change
Contrast
ParthSex
ParthParti
PartiSex
C
Controls:
Non-differentially expressed genes between all three pairwise comparisons from the three different transcriptomes that were used as controls
CG3436
dhd
Klp64D
Trx-2
Log2 fold change
Contrast
ParthSex
ParthParti
PartiSex

Figure S6:


A
ana2 genomic region target-site and resulting mutant
ATG/6bp/CCCGAAACGGAGGATATGCTGCC
UTR
UTR
CCGAAGCTGTTGTGAAAGCATGT
ana2[4.3] / CyO
ATG/6bp/CCCG|TTGTGA
B
asl genomic region target-site and resulting mutant
TTAGCCGAATATGAACACGCCAGGTAT
UTR
UTR
AACTCAAATAGAAAGCCCAAAGG/13bp/TAA
asl[38] / TM6B
T|AAGG/13bp/TAA
C
gnu genomic region target-site and resulting mutant
CCGAATGGAGCGCTACAATCGCG
UTR
UTR
CCAAGCAATTCTTCTGATTTTAT
gnu[1] / CyO
CCGAATGGAGCGCTACAATCGCG
CCAA|CTTCTGATTTTAT
D
mr genomic region target-site and resulting mutant
ATGTGCGACTTTGACGCTTTGTGG
UTR
UTR
CACAAGCTAATATTCTCGGGTGG/19bp/TAA
mr[1] / CyO
ATGTGCGACTTTGACGCTTTGTGG
CACAAGCTAATATTCTC|-8bp+3bp|/17bp/TAA
mr[2] / CyO
ATGTGCGACTTTGAC/-14BP/TGT
CACAAGCTAATAT|TGG/19bp/TAA
E
Plk4 genomic region target-site and resulting mutant
ATGTTATCCAATCGGGCGTTTGGAGAAACA
UTR
UTR
CCTTATTATAATCGCATGCTTCTTTAA
Plk4[144] / TM6B
ATGTTATCCAATCG|ATCGCATGCTTCTTTAA
F
plp genomic region target-site and resulting mutant
ATG/8696bp/CCAGTGTAAGCTATAAGGCGATC
ATCACCCGAACGTACCTGCCCGG
UTR
UTR
TACGCTGCAGGGTCGTATGGAGG
CCATCATTGGCAAGATTGGACTG/43bp/TAA
plp[165-P3-16] / TM6B
ATG/8696bp/C|CCGG...
TACGCTGCAG|ATTGGACTG/43bp/TAA
G
plu genomic region target-site and resulting mutant
TTCAGAATGGGTGAAATAAATGg
UTR
UTR
CCGCTTAGATAAGCGCAATGTGC/35bp/TAA
plu[1] / CyO
TTCAGAATGGGTGA|TGg
CCGCTTAGATAAGCGCAATGTGC/35bp/TAA
plu[5] / CyO
TTCAGAATGGGTGAAAT|-6bp|taa
CCGCTTAGATAAGCGCAATGTGC/35bp/TAA
H
png genomic region target-site and resulting mutant
GGAACTCTGCGCACTCGAGATGG
UTR
UTR
ACTTCAGCTACAGCTACTAACGG
png[11] / CyO
GGAACTCTGCGCAC|GG
png[14] / CyO
GGAACTCTGCGCACTCG|G
I
Sas-6 genomic region target-site and resulting mutant
ATGTGGCCTCCAGGGAGCGAGGATAGCTACTC
UTR
UTR
AGAACGGCTTGCAATACCCACGG/17bp/TAG
Sas-6[103] / CyO
ATGTGGCCTCCAGGG|ATAGCTACTC14bp|15bp/TAG

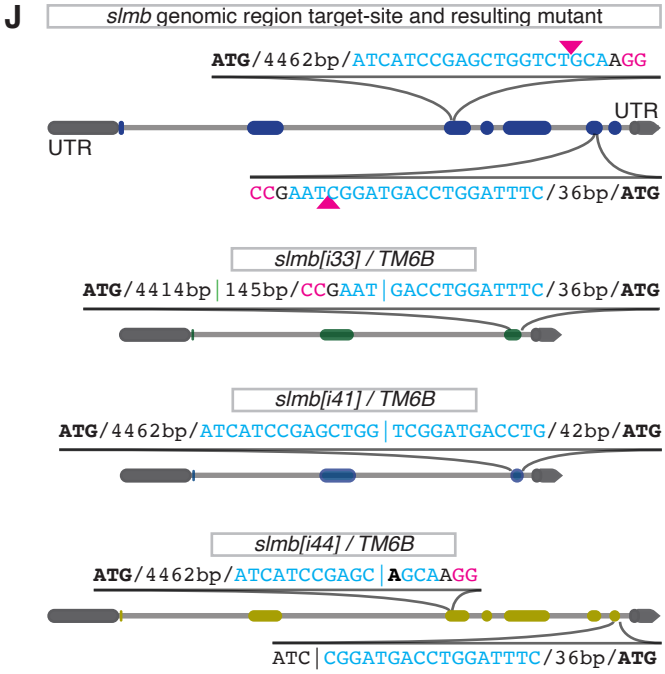

J
slmb genomic region target-site and resulting mutant
ATG/4462bp/ATCATCCGAGCTGGTCTGCAAGG
UTR
UTR
CCGAATCGGATGACCTGGATTTC/36bp/ATG
slmb[i33] / TM6B
ATG/4414bp|145bp/CCGAAT|GACCTGGATTTC/36bp/ATG
slmb[i41] / TM6B
ATG/4462bp/ATCATCCGAGCTGG|TCGGATGACCTG/42bp/ATG
slmb[i44] / TM6B
ATG/4462bp/ATCATCCGAGC|AGCAAGG
ATC|CGGATGACCTGGATTTC/36bp/ATG

Figure S7:

A

Single Mutant Screen:
Genes that cause a small percentage of parthenogenesis

Genotype	Screened with	Percent parthenogenetic offspring	*p* value
asl$^{-/+}$	CRISPR	0.1%	0.50
cnn$^{-/+}$	Mutant	0.1%	0.38
CRMP$^{-/+}$	Mutant	0.2%	0.50
desat1$^{-/-}$	RNAi/Mutant	0.2-0.4%	>0.035
desat2$^{-/-}$	Mutant	0.3%	0.40
f$^{-/-}$	RNAi/Mutant	0.1%	0.50
gnu$^{-/+}$	CRISPR	0.2%	0.50
ktub$^{-/+}$	RNAi/Mutant	0.1-0.4%	0.20
mr$^{-/+}$	CRISPR	0.1%	0.40
Myc$^{+/+}$	Over-expression	0.3%	0.23
Plp$^{-/+}$	CRISPR	0.1%	0.24
plu$^{-/+}$	CRISPR	0.1%	0.50
polo^{4+}	Over-expression	0.1%	0.49
Sas-6$^{-/+}$	Over-expression	0.1%	0.40
slmb$^{-/+}$	CRISPR	0.3%	0.17
Trx-2$^{-/-}$	RNAi	0.4%	0.11

B

Parthenogenetic Female Mating

***Drosophila* species/genotype**	**Successful F2**	**No F2**
melanogaster		
polo^{4+} ; *desat1*$^{-/+}$	1	0
polo^{4+} ; *desat2*$^{-/+}$	2	0
mercatorum		
partially parthenogenetic	9	5

Figure S8:

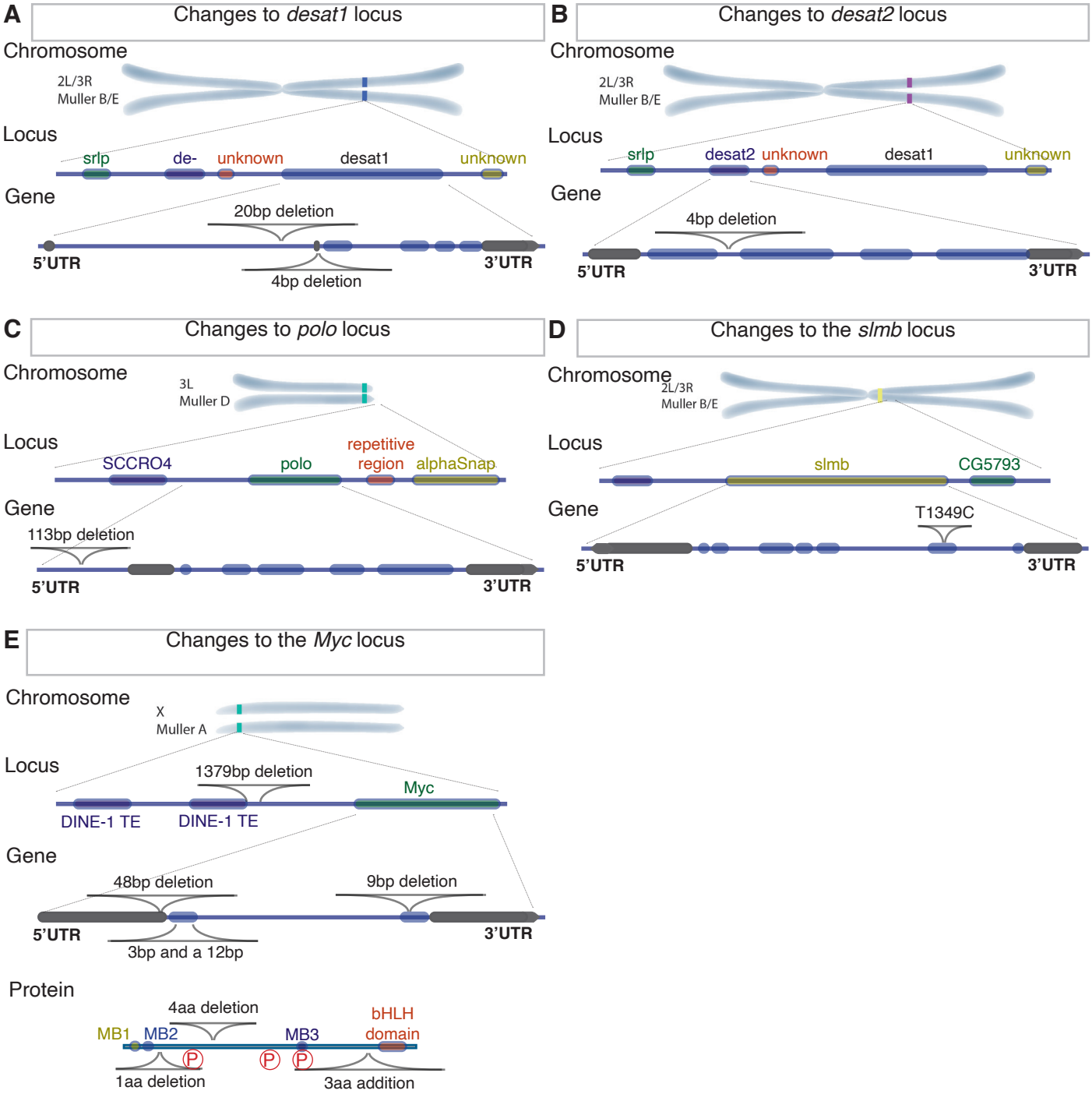

A
Changes to desat1 locus
Chromosome
2L/3R
Muller B/E
Locus
srlp
de-
unknown
desat1
unknown
Gene
20bp deletion
5'UTR
3'UTR
4bp deletion
B
Changes to desat2 locus
Chromosome
2L/3R
Muller B/E
Locus
srlp
desat2
unknown
desat1
unknown
Gene
4bp deletion
5'UTR
3'UTR
C
Changes to polo locus
Chromosome
3L
Muller D
Locus
SCCRO4
polo
repetitive
region
alphaSnap
Gene
113bp deletion
5'UTR
3'UTR
D
Changes to the slmb locus
Chromosome
2L/3R
Muller B/E
Locus
slmb
CG5793
Gene
T1349C
5'UTR
3'UTR
E
Changes to the Myc locus
Chromosome
X
Muller A
Locus
1379bp deletion
Myc
DINE-1 TE
DINE-1 TE
Gene
48bp deletion
9bp deletion
5'UTR
3'UTR
3bp and a 12bp
Protein
4aa deletion
bHLH
domain
MB1
MB2
MB3
P
P
P
1aa deletion
3aa addition

Figure S9:

A Protein sequence comparison between mouse, human, mosquito, *D. melanogaster*, sexual *D. mercatorum*, and parthenogenetic *D. mercatorum*

```
mouse                          ------------------------------------------------------------   0
human                          ------------------------------------------------------------   0
mosquito                       MVSIKQEPSCWDDIKTISIKQELSNWDDSH--NMDIDWEQDIGIQFMDLPTSEFLTSAVE   58
Dmelanogaster                  -----------MAL---YRSDPYSIMDDQLFSNISIFDMDNDLYDMDKLLSSSTIQSDLE   46
Sexual_Dmercatorum             -----MTTACSSGI---CISGEFDLMDEMGFDLLE-FNVQDIGY------RLPSIQNDLE   45
parthenogenetic_Dmercatorum    -----MTTACSSGI---CISGEFDLMDEMGFDLLE-FNVQDIGY------RLPSIQNDLE   45

                                       Myc box 1                Myc box 2
mouse                          ------------------------------------------------------------   0
human                          ------------------------------------------------------------   0
mosquito                       LEQTYGSATCPANGWEQPASSKTQIRNHDCMWSGTCFDQSHPGKMGCGTNHGPANTTQDQ   118
Dmelanogaster                  KIEDMESV-F--QDYDLEEDMKPEIRNIDCMWPAMSS--CLTSGNGNGIE----------   91
Sexual_Dmercatorum             KIAAEHAHNMNSLALADDFDIKPEIRNGDCMWSAFGS--SANGGVNGANNNNNNNNSSNI   103
parthenogenetic_Dmercatorum    KIAAEHAHNMNSLALADDFDIKPEIRNGDCMWSAFGS--SANGGVNGANNNN-NNNSSNI   102
                                                                                 1 aa deletion

mouse                          ------------------------------------------------------------   0
human                          ------------------------------------------------------------   0
mosquito                       S--EVSNKFSTVTAVAAASLNNNVV----------------VS---------QKPILT--   149
Dmelanogaster                  SGNSAASSYSETGAVSLAMVSGSTNLYSAYQRSQT-TDNTQSNQQHVVNSAENMPVIIKK   150
Sexual_Dmercatorum             NSLSAASSYSESSAVPPAFVSGSTLHIKRELEDEVQLEEVHDQDQDNDSNSENCPVNSNS   163
parthenogenetic_Dmercatorum    NSLSAASSYSESSAVPPAFVSGSTLHIKRELEDEVQLEEVHDQDQDNDSNSENCPVNSN-   161

mouse                          ------------------------------------------------------------   0
human                          ------------------------------------------------------------   0
mosquito                       -------------------------PANTSAMNINNNLTAKMATVKQQIPAGRSLLISSR   184
Dmelanogaster                  ELADLDYTVCQKRLRLSGGDKKSQ-----------------IQDEVHLIPPGGSLLRKRN   193
Sexual_Dmercatorum             GSNSSGIRKRTNSCRSTGGSSKVAAATATPTISIPSRMIHRDPVIEPYIPPGGSLLRKSN   223
parthenogenetic_Dmercatorum    ---SSGIRKRTNSCRSTGGSSKVAAATATPTISIPSRMIHRDPVIEPYIPPGGSLLRKSN   218
                               4 aa deletion

mouse                          -------------------MDFLWALET--------PQTATTM-----------------   16
human                          -------------------MDFFRVVEN--------QQPPATM-----------------   16
mosquito                       IKQQMNRIPTVST---S---DFLR--ERETAVPLHRPDTPLSL--DEDPPEFKHNIDLAT   234
Dmelanogaster                  NQDIIRKSGELSG---SDSIKY------------QRPDTPHSLTDEVAASEFRHNVDLRA   238
Sexual_Dmercatorum             TQHKLQQQKLQQQQQQQQRLTYLLSSNNYNNSNNNSNNNSYSMPEDEVLPVFRHNVDLRA   283
parthenogenetic_Dmercatorum    TQHKLQQQKLQQQQQQQQRLTYLLSSNNYNNSNNNSNNNSYSMPEDEVLPVFRHNVDLRA   278
                                                     :               :   ::

mouse                          ----PLNVNFTNRNYDLDYDS------------------------VQPY------------   37
human                          ----PLNVSFTNRNYDLDYDS------------------------VQPY------------   37
mosquito                       CTIGSNRLSLTGHSRHYKNHQSHHDDPSSHRIINMLKEHLEDNESSSFRTCMASSTGEVG   294
Dmelanogaster                  CVMGSNNISLTGNDSDVNY-------------IKQISRELQNTGKDPLPVR-YIP-----   279
Sexual_Dmercatorum             CVMGSNNISLTN-SSDANI-------------IDLLSRELQNTSKERIDLP-YRIPGDPP   328
parthenogenetic_Dmercatorum    CVMGSNNISLTN-SSDANI-------------IDLLSRELQNTSKERIDLP-YRIPGDPP   323
                                     .:.:*. . . .                          .

mouse                          FICDEEENFYHQQQQSE---------LQPPAPSEDIWKKFELLPTPPLSPSRRSGLCSPS   88
human                          FYCDEEENFYQQQQQSE---------LQPPAPSEDIWKKFELLPTPPLSPSRRSGLCSPS   88
mosquito                       SLTDLLNDLEEMEEM--------------------------------ES-R----DGDD   316
Dmelanogaster                  PINDVLDVLNQHSNSTGGQQQLNQQQLDEQQQAIDIATGRNTVDSPPTTG-S----DSDS   334
Sexual_Dmercatorum             IITDVLEVLNQQAQQSASSAA----------AAAAAAAAAATLSPPATTA-T----SSDS   373
parthenogenetic_Dmercatorum    IITDVLEVLNQQAQQSASSAA----------AAAAAAAAAATLSPPATTA-T----SSDS   368
                                  *  : : .  :                                          . .

mouse                          YVAVATSFSPRE--------DDDGGGG--NFSTADQLEMM--TELLGGDMVNQSFICDPD   136
human                          YVAV-TPFSLRG--------DNDGGGG--SFSTADQLEMV--TELLGGDMVNQSFICDPD   135
mosquito                       SHGEELDSDTDSNADSSSRSSSKGGGISGGYTHAHNQEMSPSSSSSSSSSSYEYQGTHVG   376
Dmelanogaster                  DDGEPLNFDLRHH------RTSKSGSNASITTNNNNSN--NKNNKLKNNSNGMLHMMHIT   386
Sexual_Dmercatorum             D-----------------------------------SD--YGDCSMGESSCSASIMRHIS   396
parthenogenetic_Dmercatorum    D-----------------------------------SD--YGDCSMGESSCSASIMRHIS   391
                                                                     :          .        .
                                                      Myc box 3
mouse                          DETFIKNIIIQDCMWSGFSAAAKLVSEKL--ASYQAARKDSTSLSPARGHSVCST-----   189
human                          DETFIKNIIIQDCMWSGFSAAAKLVSEKL--ASYQAARKDSGSPNPARGHSVCST-----   188
mosquito                       DHSYTRPKARYNLAELGVQTPSDSEDEEIDVVSIGE-KNLPTNPTPRDKRHVESRVALKI   435
Dmelanogaster                  DHSYTRCNDMVD-DGPNLETPSD-SDEEIDVVSYTD-KKLPTNPSCHLMGALQFQMAHKI   443
Sexual_Dmercatorum             DHSYTRCNEVE----ANLDTPSD-SDEEIDVVSLND-KKLPTNPSDRDRRVLQTKVANKI   450
parthenogenetic_Dmercatorum    DHSYTRCNEVE----ANLDTPSD-SDEEIDVVSLND-KKLPTNPSDRDRRVLQTKVANKI   445
                               *.:: :          ...: :.  .*::  .*    ::   . .      :
```

```
mouse                          -----------SSLYLQDLTAAASECIDP--SVVFPYPLNDSSSPKSCTSSDSTAFSPSS     236
human                          -----------SSLYLQDLSAAASECIDP--SVVFPYPLNDSSSPKSCASQDSSAFSPSS     235
mosquito                       RKHPQGNPSHHH--------------RRRHSGEDYPSHHGMSSSSSQHSPSKSYGYSPNY     481
Dmelanogaster                  SIDHMK-QKPRYNNFNLPYTPASSSPVKSVANSRYPSPSST---PYQNCSSASPSYSPLS     499
Sexual_Dmercatorum             SSDNRIVAHRSSRRYELPYTPASSSPVKSVANSRYPSPSST---PYQGAATGPATYSPES     507
parthenogenetic_Dmercatorum     SSDNRIVAHRSSRRYELPYTPASSSPVKSVANSRYPSPSST---PYQGAATGPATYSPES     502
                                                                .  :*   .      .        :**

mouse                          DSLLSS-ESS----------------------------------------------PRAS     249
human                          DSLLSSTESS----------------------------------------------PQGS     249
mosquito                       LTPASSTSIS----------------G---------------SNTPLPPNSSSISNPR--     508
Dmelanogaster                  VDSSNVSSSSSSSSSQSSFTTSSSNKGRKRSSLKDPGLLISSSSVYLPGVNNKVTH----     555
Sexual_Dmercatorum             SSSSSDCTTP-------SIALGVGAGGKK-----------NRKPFYMPDCNDDLLTAKRQ     549
parthenogenetic_Dmercatorum    SSSSSDCTTP-------SIALGVGAAGKK-----------NRKPFYMPDCNDDLLTAKRQ     544
                                   .                  G526A

mouse                          PEPLVLHEETPPTTSSDSEEEQEDEEEIDVVSVEKRQTPAKRSESGSSPS--RGHSKPPH     307
human                          PEPLVLHEETPPTTSSDSEEEQEDEEEIDVVSVEKRQAPGKRSESGSPSA--GGHSKPPH     307
mosquito                       --------KR-----------------------------------------------PSK     513
Dmelanogaster                  ------------------------------SSMMSKKSRGK-KVVGTSSGNTSPIS-SGQ     583
Sexual_Dmercatorum             PRGYLLSKKRPLKRTHYSSYGF-DAKE--VRSVLSHASNSV-STIGSSSS---NSSKSGH     602
parthenogenetic_Dmercatorum    PRGYLLSKKRPLKRTHYSSYGF-DAKE--VRSVLSHASNSV-STIGSSSSNSSNSSKSGH     600
                                                                                3 aa addition   :

mouse                          SPLVLKRCHVSTHQHNYAAPPSTRKDYPAAKRAKLDSGRVLKQISNNR----KCSSPRSS     363
human                          SPLVLKRCHVSTHQHNYAAPPSTRKDYPAAKRVKLDSVRVLRQISNNR----KCTSPRSS     363
mosquito                       DDRSKNRH----HQH------------------RNKKQRIPG-------KTIARSPESSE     544
Dmelanogaster                  DVDAMDRN----WQR------------------R--SGGIATSTSSNSSVHRKDFVLGFD     619
Sexual_Dmercatorum             SNG----S----HSS------------------N--SGHSNGSISNGSGINSLKRHLSID     634
parthenogenetic_Dmercatorum    SNG----S----HSS------------------N--SGHSNGSISNGSGINSLKRHLSID     632
                               .           .                   .  .                      .
                                                Helix-loop-helix DNA-binding domain
mouse                          DTEENDKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSIQA     423
human                          DTEENVKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQA     423
mosquito                       EQETLEKRNLHNDMERQRRIGLKNLFEELKRQIPNLRDKERAPKVNILREAAVLCTRLNR     604
Dmelanogaster                  EADTIEKRNQHNDMERQRRIGLKNLFEALKKQIPTIRDKERAPKVNILREAAKLCIQLTQ     679
Sexual_Dmercatorum             EADTIEKRNLHNDMERQRRIGLKNLFEALKTQIPNIRDKERAPKVNILREAARLCEQLTS     694
parthenogenetic_Dmercatorum    EADTIEKRNLHNDMERQRRIGLKNLFEALKTQIPNIRDKERAPKVNILREAARLCEQLTS     692
                               : :   **. ** :*****  **. *  *: *** :.::*:**** **::*:     :

mouse                          DEHKLTSEKDLLRKRREQLKHKLEQLRNSGA--------------     454
human                          EEQKLISEEDLLRKRREQLKHKLEQLRNSCA--------------     454
mosquito                       EQEQLNALRKQ----QQRLYARVRQLRTSLHTQ-------RRVMD     638
Dmelanogaster                  EEKELSMQRQL-------LSLQLKQRQDTLASYQMELNESRSVSG     717
Sexual_Dmercatorum             EERDLNVKRQL-------LKAKLKQQQEQLARMRLNLSKNE----     728
parthenogenetic_Dmercatorum    EERDLNVKRQL-------LKAKLKQQQEQLARMRLNLSKNE----     726
                               ::..*   ..        *  ::.* :
```

Figure 10:

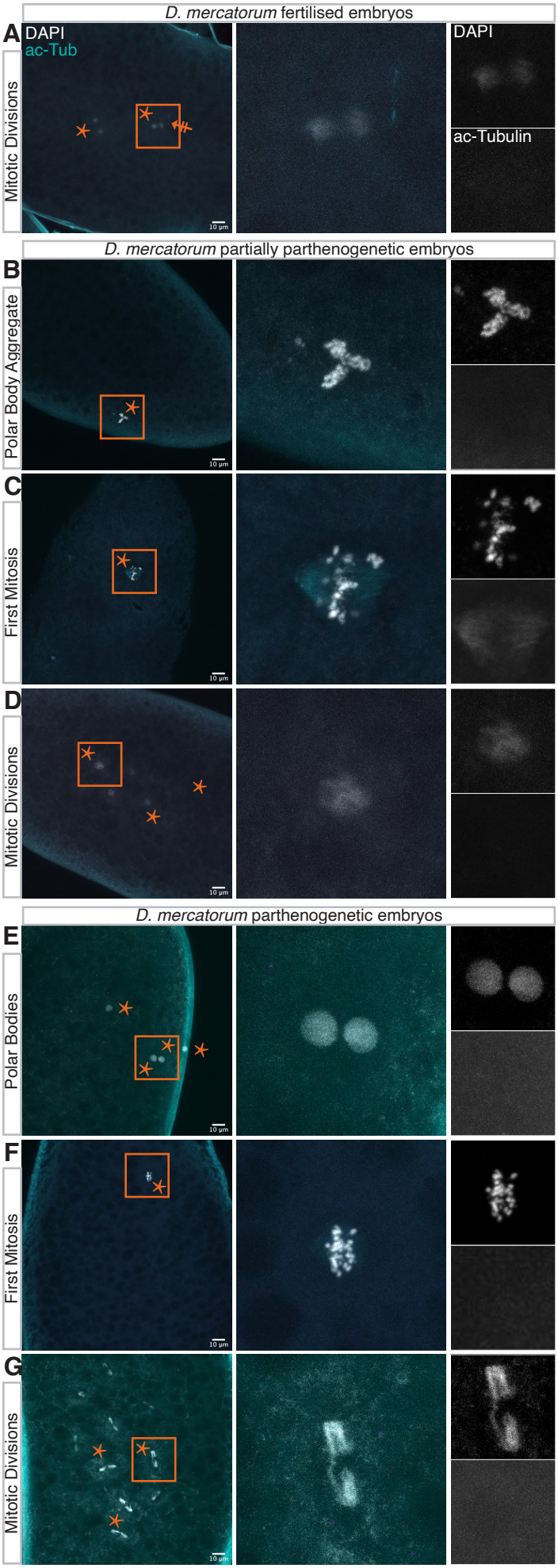

D. mercatorum fertilised embryos
A
DAPI
ac-Tub
DAPI
ac-Tubulin
Mitotic Divisions
D. mercatorum partially parthenogenetic embryos
B
Polar Body Aggregate
C
First Mitosis
D
Mitotic Divisions
D. mercatorum parthenogenetic embryos
E
Polar Bodies
F
First Mitosis
G
Mitotic Divisions

Figure S11:

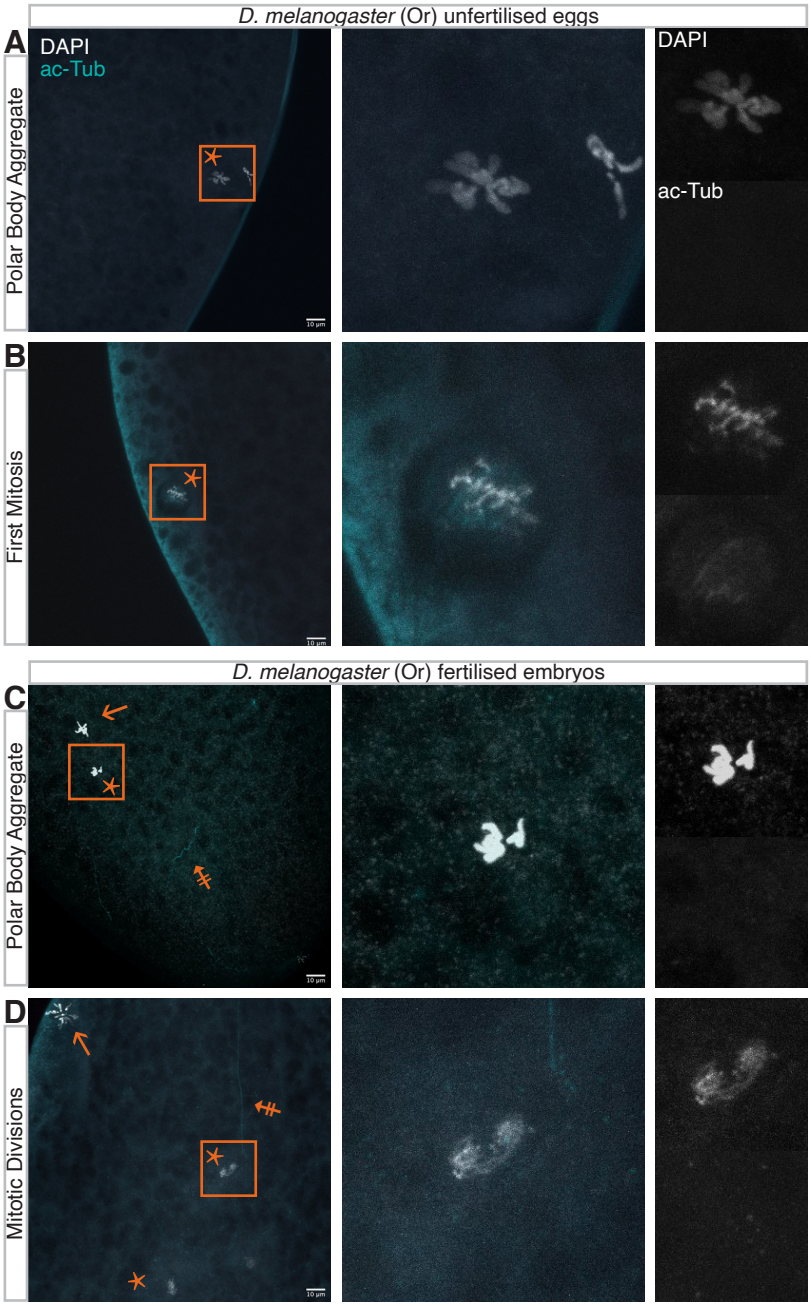

D. melanogaster (Or) unfertilised eggs
A
Polar Body Aggregate
DAPI
ac-Tub
DAPI
ac-Tub
B
First Mitosis
D. melanogaster (Or) fertilised embryos
C
Polar Body Aggregate
D
Mitotic Divisions

Figure S12:

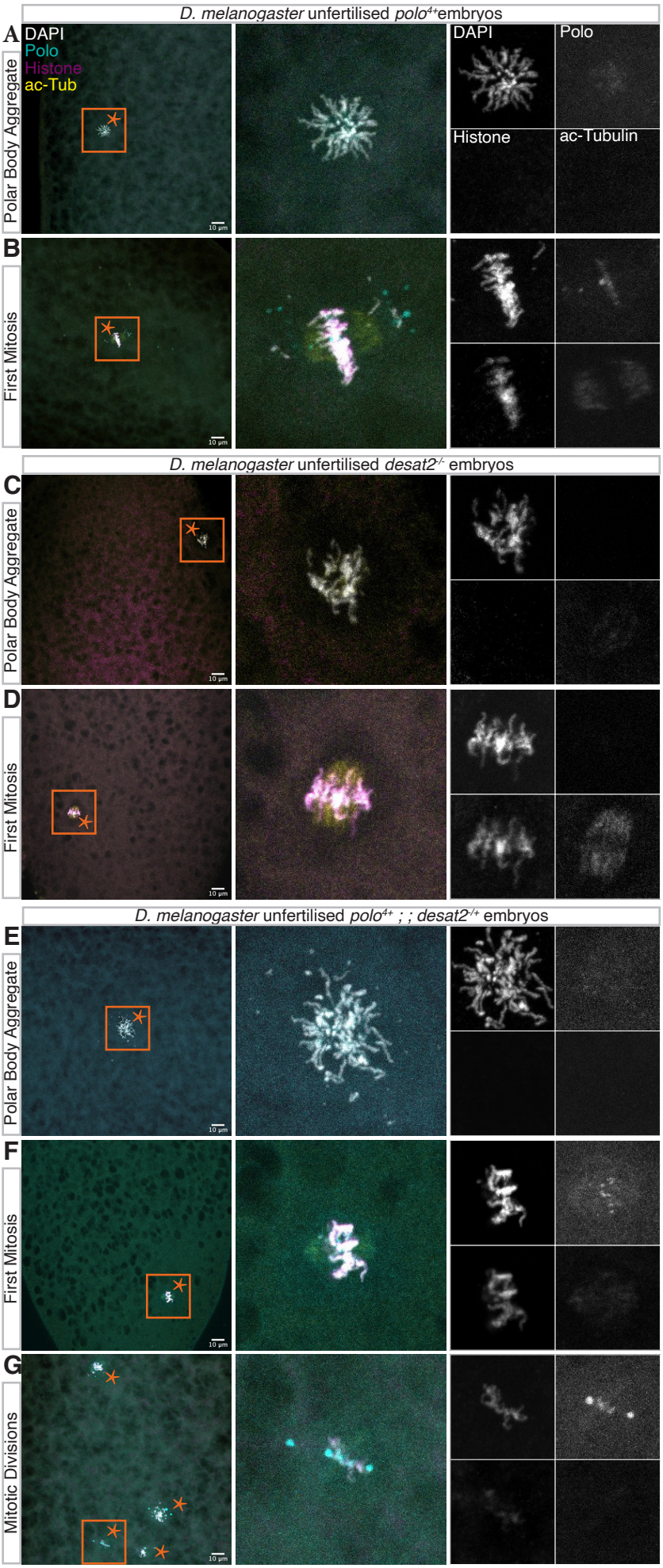

D. melanogaster unfertilised polo4+ embryos
A
DAPI
Polo
Histone
ac-Tub
Polar Body Aggregate
DAPI
Polo
Histone
ac-Tubulin
B
First Mitosis
D. melanogaster unfertilised desat2-/- embryos
C
Polar Body Aggregate
D
First Mitosis
D. melanogaster unfertilised polo4+ ; ; desat2-/+ embryos
E
Polar Body Aggregate
F
First Mitosis
G
Mitotic Divisions

Figure S13