Title: Virgin Birth: A genetic basis for facultative parthenogenesis

Authors: A. L. Braun[1*], D. K. Fabian[1], E. Garrison[2], and D. M. Glover[1,3*]

Affiliations:

[1]University of Cambridge; Department of Genetics, Cambridge, UK.

[2]University of Tennessee Health Science Center; Memphis, USA.

[3]California Institute of Technology; Pasadena, USA.

*Corresponding author. A. L. Braun (alb84@cam.ac.uk) D. M. Glover (dmglover@caltech.edu)

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,

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

Results

The parthenogenetic ability of *D. mercatorum*

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

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

The genome of *D. mercatorum*

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

We found 24.4% divergence between both parthenogenetically and sexually reproducing *D. meractorum* genome assemblies and the *D. melanogaster* reference genome (release 6).

There was clustering of each contig from both *D. meractorum* genomes to specific chromosome arms in *D. melanogaster* (Fig. S2A-B), indicative of the shuffling of genes, which largely remain on the same chromosome arms. We also confirmed chromosome arm contig matching by checking the DNA k-mers using Nucmer (Fig. S3A-B). This accords with long-held knowledge of how corresponding chromosome arms form a series of homologous genetic 'building blocks' in different *Drosophila* species within which synteny is lost [12, 13]. These chromosome arm 'building blocks' correspond to the six Muller elements (A-F) and are conserved across Diptera [14]. Together these analyses indicated that the chromosome-level genome assemblies for the sexually and parthenogenetically reproducing *D. mercatorum* strains were suited to detailed comparison between each other and with the *D. melanogaster* genome.

We next confirmed that the genome assemblies matched the karyotypes of the sexual and parthenogenetic of *D. mercatorum* strains by localising local sequence markers onto preparations of mitotic chromosomes from *D. mercatorum* third instar larval brains using a Hybridisation Chain reaction (HCR) fluorescence in situ hybridization (FISH) protocol that we developed for this purpose (see Supplementary text). We selected single genes within syntenic blocks that are conserved between *D. melanogaster* and *D. mercatorum* to serve as markers for each of the 6 chromosome arms of the mitotic karyotype (Fig. 2A). This allowed us to identify the Muller elements, A-F, for both sexual and parthenogenetic *D. mercatorum* strains (Fig. 2B-C). We found a fusion of the 2L/B and 3R/E (*D. melanogaster*/Muller) chromosome arms that was previously documented as unique to *D. mercatorum* within the *repleta* group [11] and the remaining chromosome arms were telocentric. We observed that the 4th chromosome of the parthenogenetic strain was substantially larger than the 4th chromosome in the sexual strain and we continue to investigate the underlying reason for this. We also used HCR FISH to physically position the 14 largest contigs from the

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

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

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

We then tested whether down-regulating the *D. melanogaster* homologs of genes showing reduced expression in parthenogenetic *D. mercatorum* strains would result in the production of offspring that died as embryos, larvae, pupae, or from old age as adult flies. To this end, we examined CRISPR knock-out alleles that we generated in candidate genes (Fig. S7, Table S6); publicly available mutants; or established lines in which candidate genes were down-regulated by RNAi. We also tested *D. melanogaster* constructs engineered to increase expression of genes whose homologues had elevated expression in the parthenogenetic *D. mercatorum* strains. In the case of variant alleles that were not homozygous viable, screening was carried out on heterozygotes. Together we screened a total of 44 genes (Fig. 3A-C, see Supplementary text) and identified 16 able to cause 0.1-0.4% parthenogenesis in *D. melanogaster* when their expression was either increased or decreased (Fig. S8A; Table S7 and see Supplementary text). The parthenogenesis observed resulted in the offspring developing to varying stages and dying as embryos or from old age as adult flies. The percentage given is relative to the number of adult flies screened. For this single mutant screen, we found largely only the generation of embryos that died before hatching.

The low level of parthenogenesis detected in this single mutant screen, where the expression of only a single gene was perturbed, is in line with earlier studies that had concluded that parthenogenesis was a polygenic trait [4]. This consideration led us to carry out a double mutant screen in which we combined pairs of variants in different genes into individual fly stocks that we then screened for parthenogenesis. This revealed several mutant combinations able to generate between 0.5-7% parthenogenetic offspring that died as embryos, larvae, pupae, or from old age as adult flies. From the more successful combinations, we found that one of the mutant genes either encoded a desaturase, *desat1* or *desat2*, or a protein predominantly involved in regulating cell division and proliferation, *Myc*, *slmb*, or *polo* (Fig. 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

Myc locus of the parthenogenetic strain showed many deletions and insertions leading to the changes in primary amino-acid sequence of the protein as indicated in Fig. S10A. None of these mutations affected either the basic Helix-Loop-Helix (bHLH) DNA-binding domain or the three Myc Box (MB) domains (1-3), or the three known phosphorylation sites of the Myc protein (Fig. S9E) [16-18]. There were also changes in genome organisation at the *Myc* locus of the *D. mercatorum* parthenogenetic genome, which has a 1.4kbp repetitive region between a *Drosophila* INterspersed Elements-1 (DINE-1/INE1) transposable element (TE) and the *Myc* coding region, which are 9.3kbp apart. A similar TE insertion in the classic mutant allele of *Myc* (*dm1*) causes the minute phenotype [16], therefore having a repressive effect on Myc expression. The 1.4kbp repetitive sequence present in the parthenogenetic genome between the gene and the TE could allow de-repression resulting in an increased expression of *Myc* relative to the sexually reproducing flies. Finally, we detected a 48bp deletion in the parthenogenetic genome 344bp up stream of the start site. The above mutations have the potential to affect the transcription, translations, or protein stability of Myc. Moreover, they might also perturb Myc's functions as a transcription factor to influence the expression of the other genes identified in our study [19]. Future studies will be required to distinguish these possibilities.

The development of parthenogenetic embryos

To understand how development might be initiated during parthenogenesis we first examined fertilised eggs from the sexually reproducing *D. mercatorum* strain that were initiating the mitotic nuclear division cycles (Fig 5C, Fig. S11A) to compare to the parthenogenetic eggs. The extent to which nuclear division cycles could take place in parthenogenetic *D. mercatorum* reflected the extent of parthenogenicity. We observed that 38% of unfertilised eggs from the parthenogenetic strain had one or more cell divisions (Fig 5A,C, Fig. S11E-G),

whereas 12% of unfertilised eggs from the partially parthenogenetic strain showed one or more cell divisions (Fig 5B,C, Fig. S11B-D). The sexually reproducing *D. mercatorum* embryos had timely cell divisions and no obvious nuclear defects. In contrast, the unfertilised parthenogenetic and partially parthenogenetic embryos developed with abnormal numbers of nuclei (Fig. S11D) or DNA abnormalities (Fig. S11G). All parthenogenetic *Drosophilids* appear to retain normal meiosis and rediploidise their genomes either by fusion of one or more of the four haploid nuclei arising from meiosis or by post-meiotic duplication of the haploid gamete [3, 5, 20-24]. All four meiotic products, three polar bodies and the female pronucleus, are present within the *Drosophilid* egg, and the three polar normally fuse and arrest in a mitotic-like state. We only observed the presence of polar bodies in 44% of parthenogenetic embryos that initiated the mitotic nuclear division cycles suggesting that the missing polar bodies may be participating in the mitotic nuclear division cycles in the developing embryos.

We then examined fertilised and unfertilised wildtype *D. melanogaster* eggs and compared their development to the parthenogenetic *D. melanogaster* eggs. All unfertilised wildtype *D. melanogaster* eggs completed meiosis and 70% appeared to have entered the first mitotic division and had condensed chromosomes (Fig 5N, Fig. S12A-B). In contrast, nearly all fertilised embryos had begun to undergo the mitotic nuclear division cycles (Fig 5N, Fig. S11C-D). Development of the induced parthenogenetic *D. melanogaster* embryos mirrored the findings from the parthenogenesis screens. We found nearly all unfertilised eggs laid by either *GFP-polo*$^{4+}$ or *desat2*$^{-/-}$ mothers were not able to undertake mitotic nuclear division cycles (Fig. 5D-E,G-J,O, Fig. S13A-D). By contrast, 6% of the unfertilised eggs laid by *GFP-polo*$^{4+}$; ; *desat2*$^{-/+}$ *D. melanogaster* mothers could undertake at least limited mitosis (Fig. 5F,K-M,O, Fig. S13E-G). Similar to the unfertilised parthenogenetic and partially parthenogenetic *D. mercatorum* embryos, the parthenogenetic *D. melanogaster* embryos had

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

Discussion

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

and this could explain the varying degree of parthenogenetic ability, not only within *D. mercatorum,* but also across the *Drosophila* genus. Given the polygenic nature of facultative parthenogenesis and the fact that there are multiple inputs into core cell cycle regulation, it may explain why no unifying signature of parthenogenesis has been found to date [31]. Thus, we anticipate that parthenogenesis might have different causal events in each species or even between individuals of the same species.

Some consider sporadic facultative parthenogenesis to be an unimportant accident. However, there could be a benefit of having sporadic facultative parthenogenesis inducing heterozygotic mutations floating around in the population, they may facilitate an 'extinction escape hatch', thus helping a lineage of the species stave off extinction in the face of isolation until an opportunity to mate arises again. Parthenogenesis is spread across the order Diptera and rare facultative parthenogenesis is prevalent in *Drosophila* [32] making it likely that the mechanism we propose is not restricted to *D. mercatorum*.

Acknowledgements: We would like to thank Jonathan Day (Frank Jiggens' Lab) and Bettina Fischer (Richard Durbin's Lab) for advice on library preparation, technical assistance, protocols, reagents, and endless patience. We would like to thank Harry Choi and Mike Liu, from Molecular Instruments for troubleshooting and for gifted reagents that were during the optimisation of the HCR FISH protocol for use on DNA. We would like to thank Paula Almeida-Coelho for technical advice on in situs and karyotyping. We would like to thank Richard Durbin for advice, discussions, and computation assistance. We would like to thank Frank Sprenger for flies, kindly brought to Cambridge. Finally, we would like to thank the Genetics Fly Facility for embryo injections, particularly Dr Alla Madich for multiple attempts at developing transgenics in *D. mercatorum*.

Funding: We gratefully acknowledge support from Leverhulme Trust Project Grant RPG-2018-229, (ALB, DKF and DMG). We gratefully acknowledge support from NIH/NIDA U01DA047638 (EG) and NIH/NIGMS R01GM123489 (EG)

Author contributions:

Conceptualization: ALB

Methodology: ALB, DKF, EG, DMG

Investigation: ALB, DKF, EG

Visualization: ALB, DKF, EG

Funding acquisition: ALB, DMG

Project administration: ALB, DMG

Supervision: DMG

Writing – original draft: ALB

Writing – review & editing: DKF, EG, DMG

Competing interests: Authors declare that they have no competing interests.

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.

References:

1. Nabours, R.K. (1919). Parthenogenesis and Crossing-Over in the Grouse Locust Apotettix. The American Naturalist *53*, 131-142.

2. Olsen, M.W., Wilson, S.P., and Marks, H.L. (1968). Genetic control of parthenogenesis in chickens. Journal of Heredity *59*, 41-42.

3. Stalker, H.D. (1954). Parthenogenesis in Drosophila. Genetics *39*, 4-34.

4. Carson, H.L. (1967). Selection for parthenogenesis in *Drosophila mercatorum*. Genetics *55*, 157-171.

5. Carson, H.L., Wei, I.Y., and Niederkorn Jr., J.A. (1969). Isogenicity in parthenogenetic strains of *Drosophila mercatorum*. Genetics *63*, 619-628.

6. Carson, H.L., Teramoto, L.T., and Templeton, A.R. (1977). Behavioral Differences Among Isogenic Strains of *Drosophila mercatorum*. Behavior Genetics *7*, 189-197.

7. Suvorov, A., Kim, B.Y., Wang, J., Armstrong, E.E., Peede, D., D'Agostino, E.R.R., Price, D.K., Waddell, P., Lang, M., Courtier-Orgogozo, V., et al. (2022). Widespread introgression across a phylogeny of 155 Drosophila genomes. Current biology : CB *32*, 111-123 e115.

8. Wasserman, M. (1960). Cytological and phylogenetic relationships in the *repleta* group of the genus *Drosophila*. Proceedings of the National Academy of Sciences of the United States of America *46*, 842-859.

9. Stouthamer, R., Breeuwert, J.A., Luck, R.F., and Werren, J.H. (1993). Molecular identification of microorganisms associated with parthenogenesis. Nature *361*, 66-68.

10. O'Neill, S.L., and Karr, T.L. (1990). Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. Nature *348*, 178-180.

11. Wasserman, M. (1963). Cytology and Phylogeny of *Drosophila*. The American Naturalist *97*, 333-352.

12. Sturtevant, A.H., and Novitski, E. (1941). The Homologies of the Chromosome Elements in the Genus *Drosophila*. Genetics *26*, 517-541.

13. Schaeffer, S.W., Bhutkar, A., McAllister, B.F., Matsuda, M., Matzkin, L.M., O'Grady, P.M., Rohde, C., Valente, V.L., Aguade, M., Anderson, W.W., et al. (2008). Polytene chromosomal maps of 11 Drosophila species: the order of genomic scaffolds inferred from genetic and physical maps. Genetics *179*, 1601-1655.

14. Muller, H.J. (1940). Bearings of the 'Drosophila' work on systematics. The New Systematics *edited by Huxley J. Clarendon Press, Oxford*, 185–268.

15. Templeton, A.R. (1979). The Parthenogenetic Capacities and Genetic Structures of Sympatric Populations of *Drosophila mercatorum* and *Drosophila hydei*. Genetics *92*, 1283-1293.

16. Gallant, P., Shiio, Y., Cheng, P.F., Parkhurst, S.M., and Eisenman, R.N. (1996). Myc and Max homologs in *Drosophila*. Science *274*, 1523-1527.

17. Galletti, M., Riccardo, S., Parisi, F., Lora, C., Saqcena, M.K., Rivas, L., Wong, B., Serra, A., Serras, F., Grifoni, D., et al. (2009). Identification of domains responsible for ubiquitin-dependent degradation of dMyc by glycogen synthase kinase 3beta and casein kinase 1 kinases. Mol Cell Biol *29*, 3424-3434.

18. Schwinkendorf, D., and Gallant, P. (2009). The conserved Myc box 2 and Myc box 3 regions are important, but not essential, for Myc function in vivo. Gene *436*, 90-100.

19. Grifoni, D., and Bellosta, P. (2015). Drosophila Myc: A master regulator of cellular performance. Biochim Biophys Acta *1849*, 570-581.

20. Carson, H.L. (1973). The Genetic System in Parthenogenetic Strains of *Drosophila mercatorum*. Proceedings of the National Academy of Sciences of the United States of America *70*, 1772-1774.

21. Eisman, R.C., and Kaufman, T.C. (2007). Cytological Investigation of the Mechanism of Parthenogenesis in *Drosophila mercatorum*. Fly *1*, 317-329.

22. Chang, C.C., Ting, C.T., Chang, C.H., Fang, S., and Chang, H.Y. (2014). The persistence of facultative parthenogenesis in Drosophila albomicans. PloS one *9*, e113275.

23. Murdy, W.H., and Carson, H.L. (1959). Parthenogenesis in D*rosophila mangabeirai Malog.* The American Naturalist *93*, 355-363.

24. Doane, W.W. (1960). Completion of meiosis in uninseminated eggs of *Drosophila melanogaster*. Science *132*, 677-678.

425 25. Marcillac, F., Bousquet, F., Alabouvette, J., Savarit, F., and Ferveur, J.F. (2005). A
426 mutation with major effects on Drosophila melanogaster sex pheromones. Genetics
427 *171*, 1617-1628.

428 26. Greenberg, A.J., Moran, J.R., Coyne, J.A., and Wu, C.-I. (2003). Ecological
429 Adaptation During Incipient Speciation Revealed by Precise Gene Replacement.
430 Science *302*, 1754-1757.

431 27. Kim, S.O., Herbst, A., Tworkowski, K.A., Salghetti, S.E., and Tansey, W.P. (2003).
432 Skp2 Regulates Myc Protein Stability and Activity. Molecular Cell *11*, 1177-1188.

433 28. de la Cova, C., Abril, M., Bellosta, P., Gallant, P., and Johnston, L.A. (2004).
434 *Drosophila* Myc Regulates Organ Size by Inducing Cell Competition. *117*, 107-116.

435 29. Templeton, A.R. (1979). The Unit of Selection in *Drosophila mercatorum*. II. Genetic
436 Revolution and the Origin of Coadapted Genomes in Parthenogenetic Strains.
437 Genetics *92*, 1265-1282.

438 30. Xiao, D., Yue, M., Su, H., Ren, P., Jiang, J., Li, F., Hu, Y., Du, H., Liu, H., and Qing,
439 G. (2016). Polo-like Kinase-1 Regulates Myc Stabilization and Activates a
440 Feedforward Circuit Promoting Tumor Cell Survival. Mol Cell *64*, 493-506.

441 31. Jaron, K.S., Bast, J., Nowell, R.W., Ranallo-Benavidez, T.R., Robinson-Rechavi, M.,
442 Schwander, T., and Orive, M. (2020). Genomic Features of Parthenogenetic Animals.
443 Journal of Heredity.

444 32. Braun, A.L., and Glover, D.M. (2022). Parthenogenesis in Diptera. In prep.

445

446

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

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

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

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

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

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

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

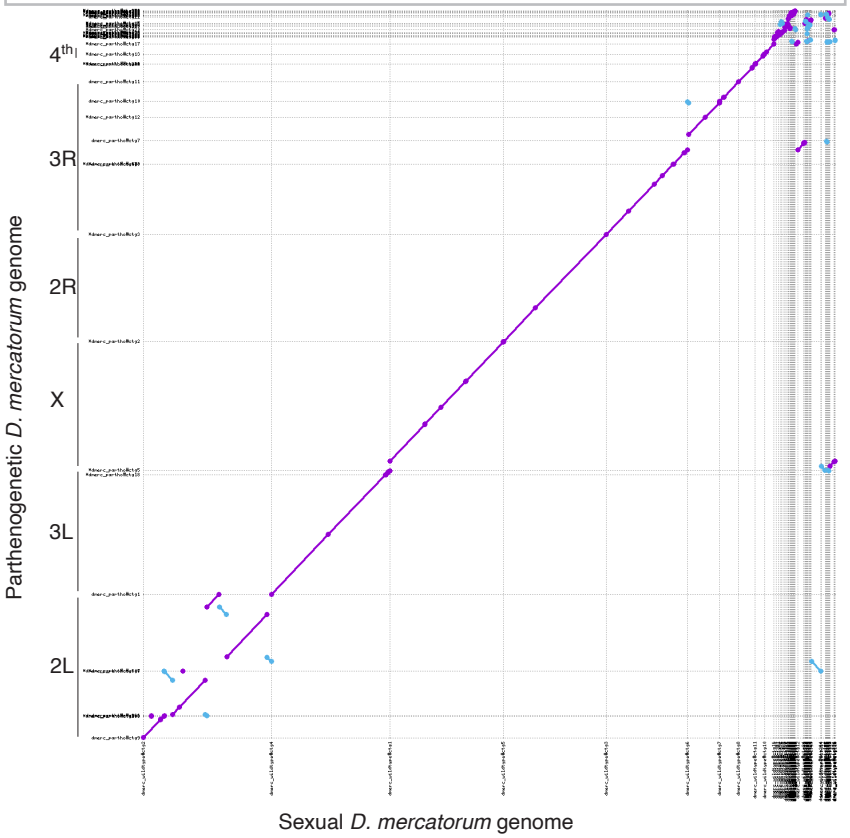


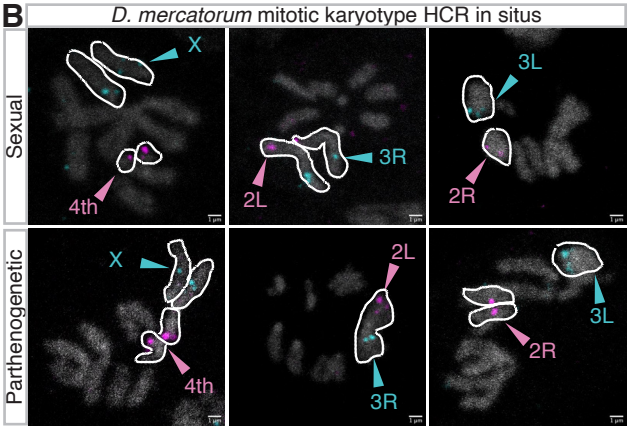
Figure 1

A

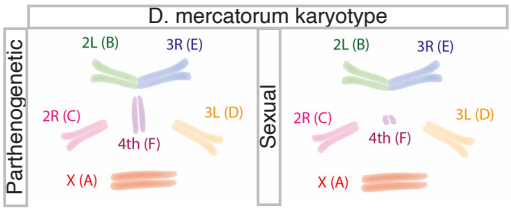
D. mercatorum mitotic karyotype HCR in situs

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



C

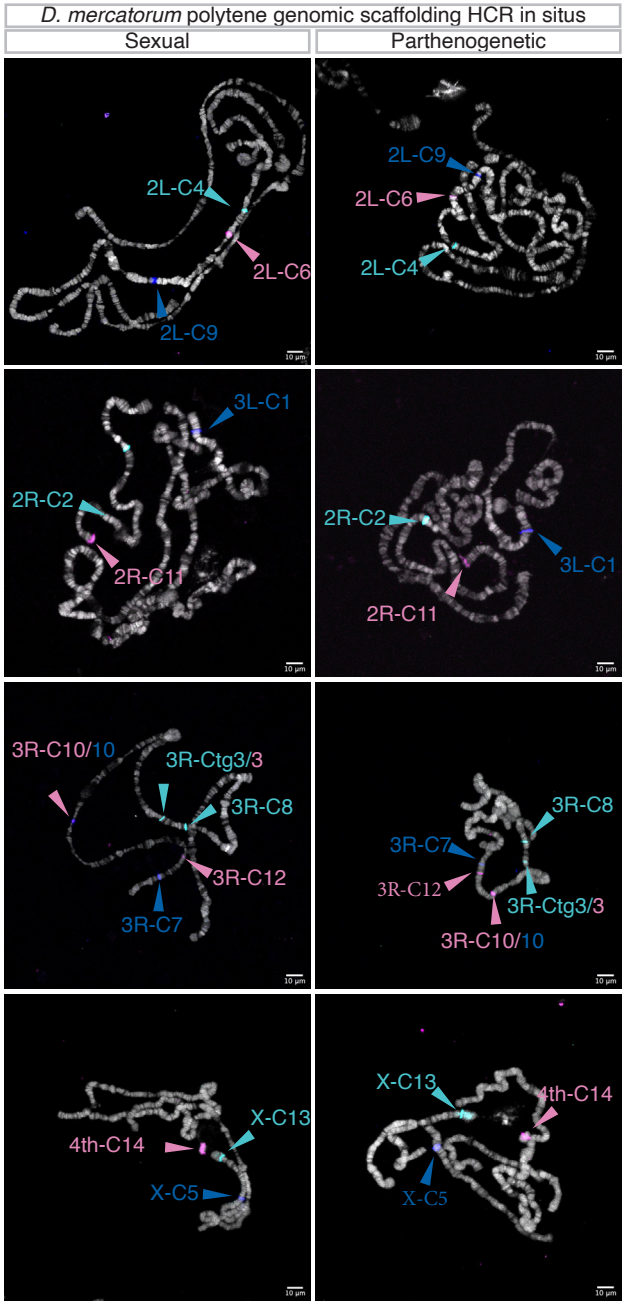


D

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



F

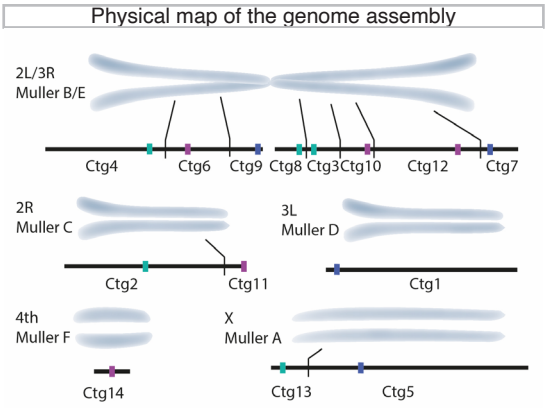


Figure 2

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*$^{i/-}$	0%	1
desat1$^{+/1}$ / *w-RNAi*$^{i/-}$	0%	1

Figure 4

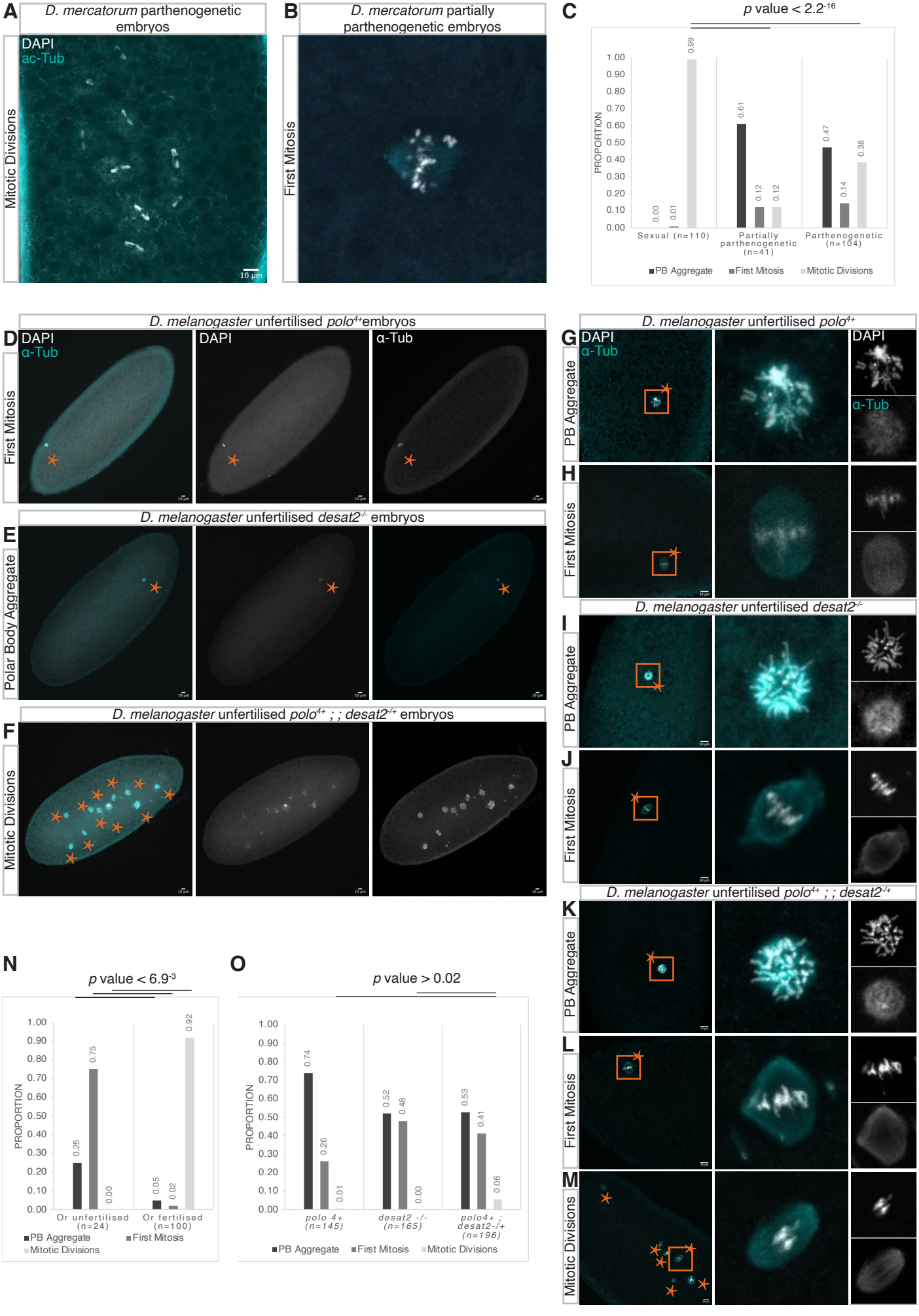


Figure 5

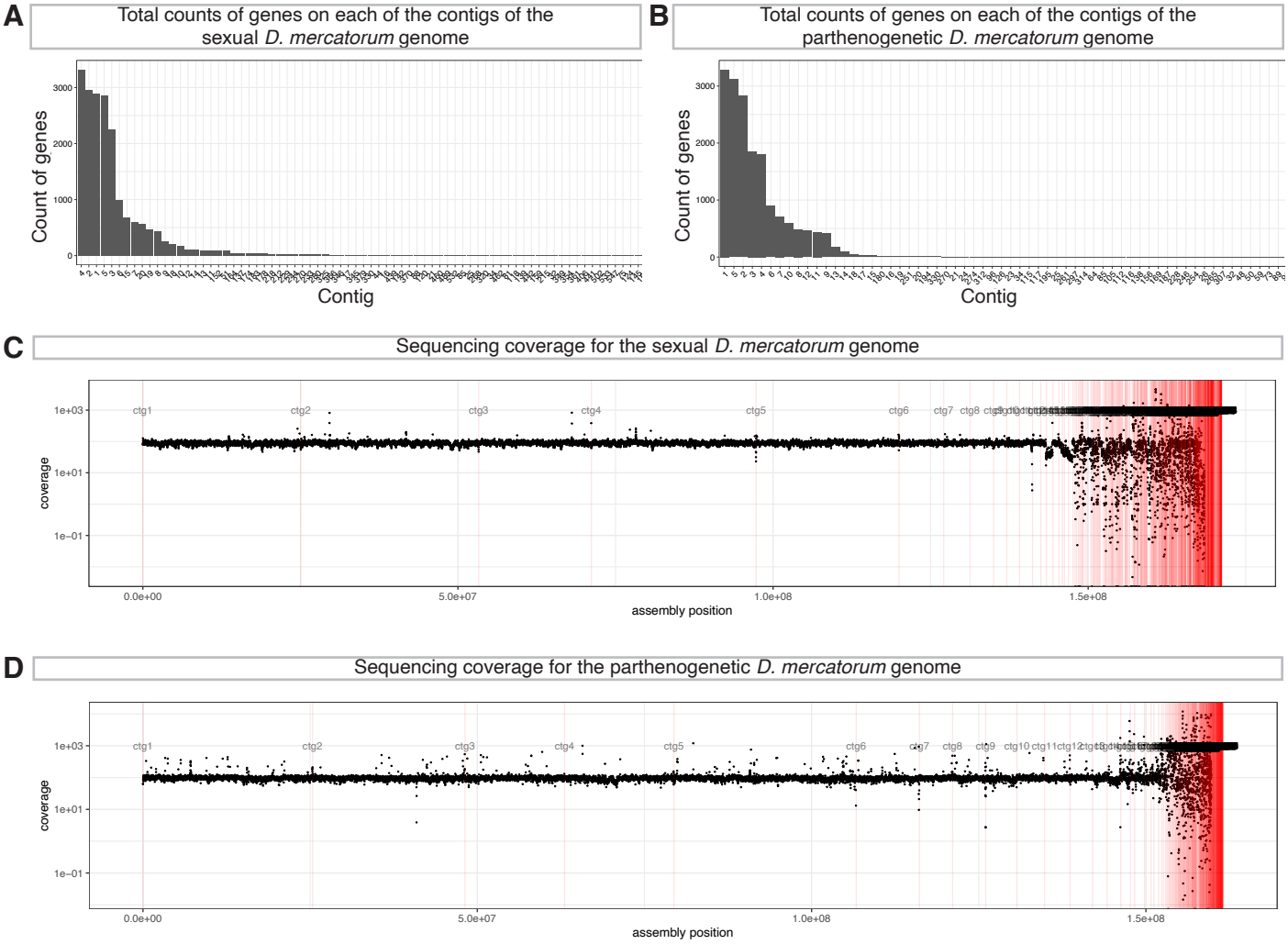


Figure S1:

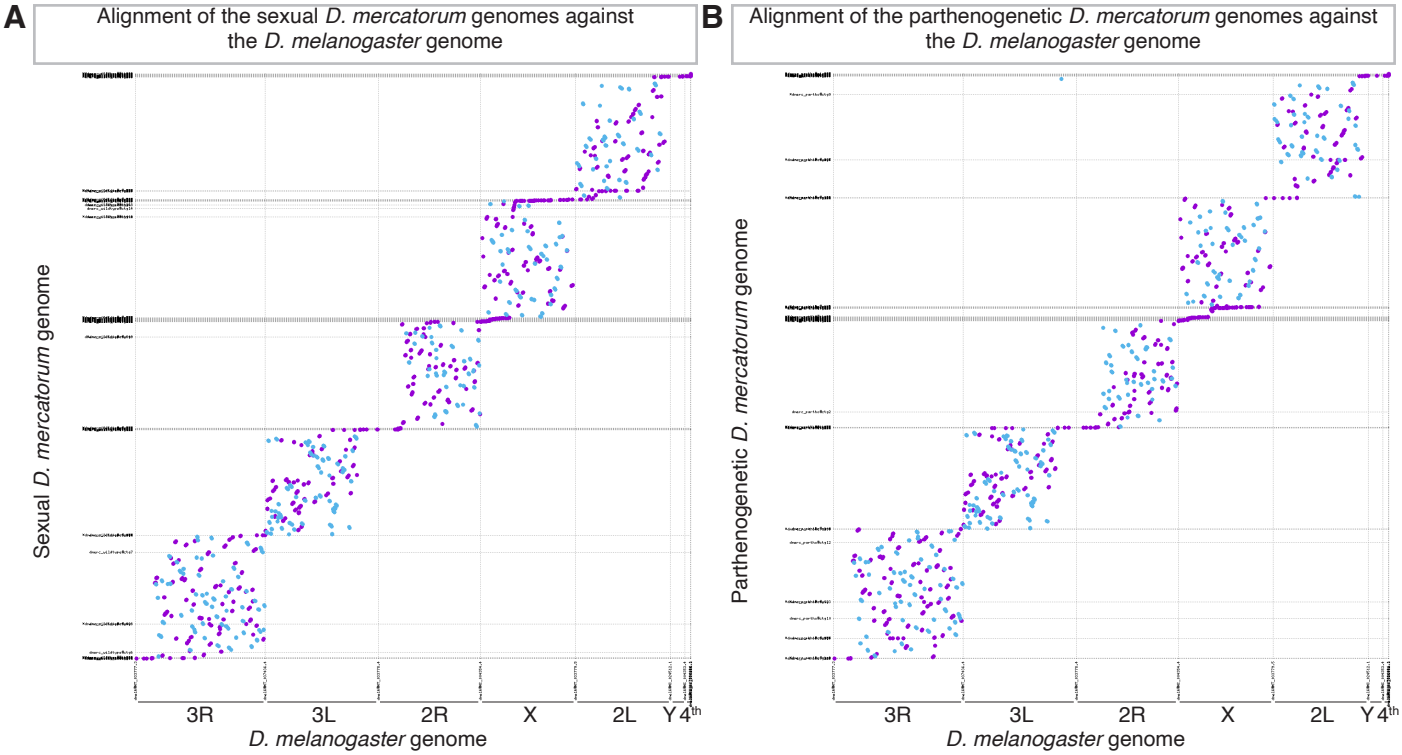
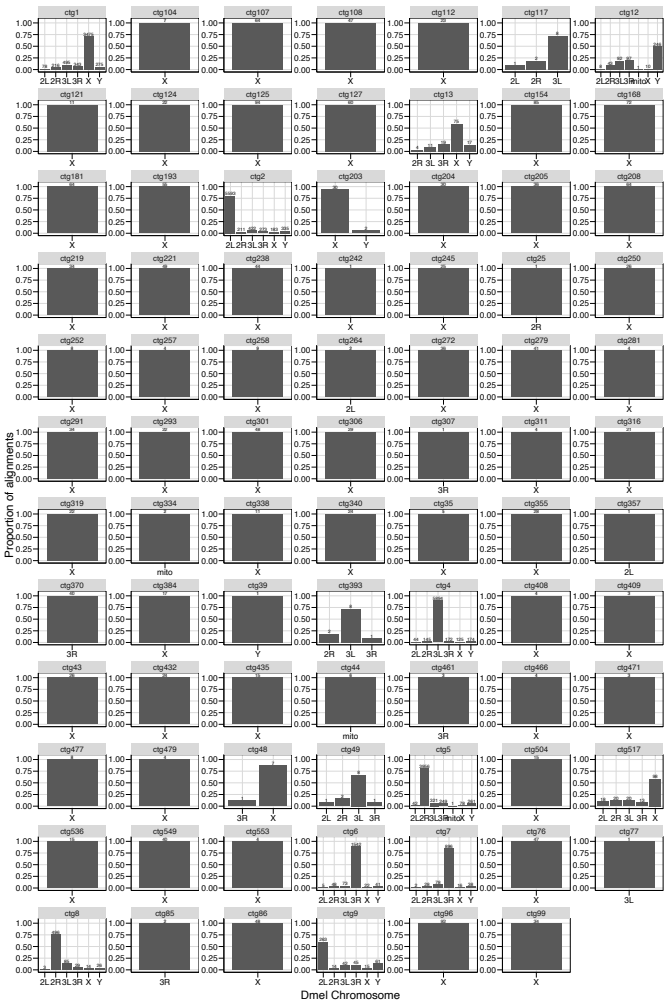


Figure S2:

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

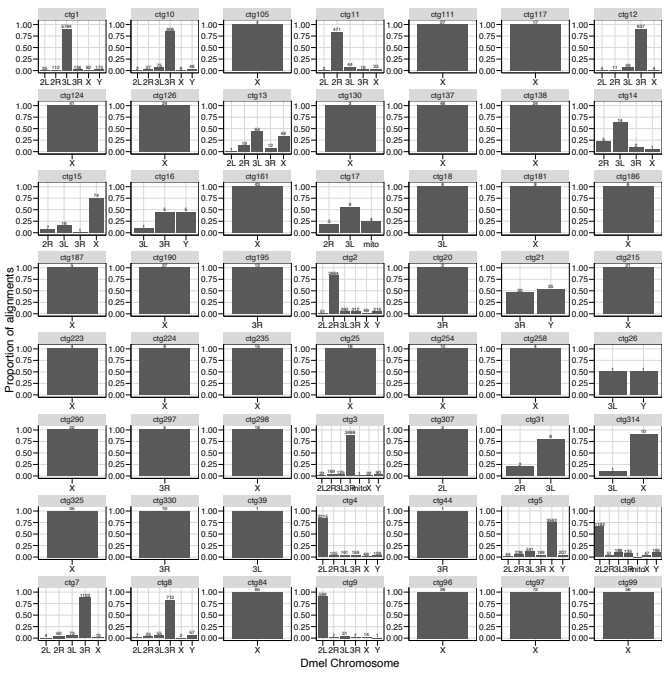
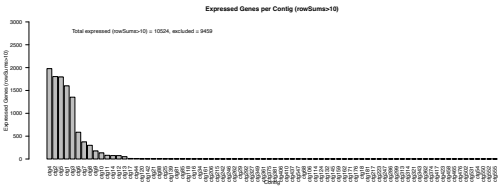
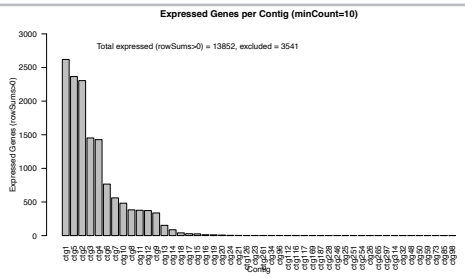


Figure S3:

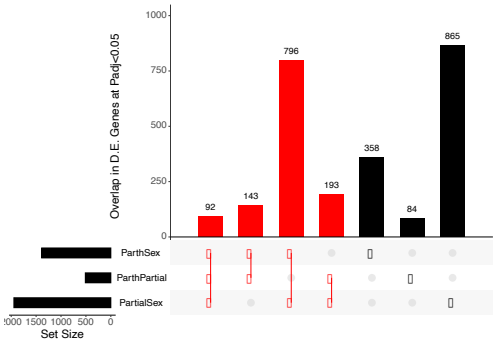
A Expressed genes per contig for the sexual *D. mercatorum* genome



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

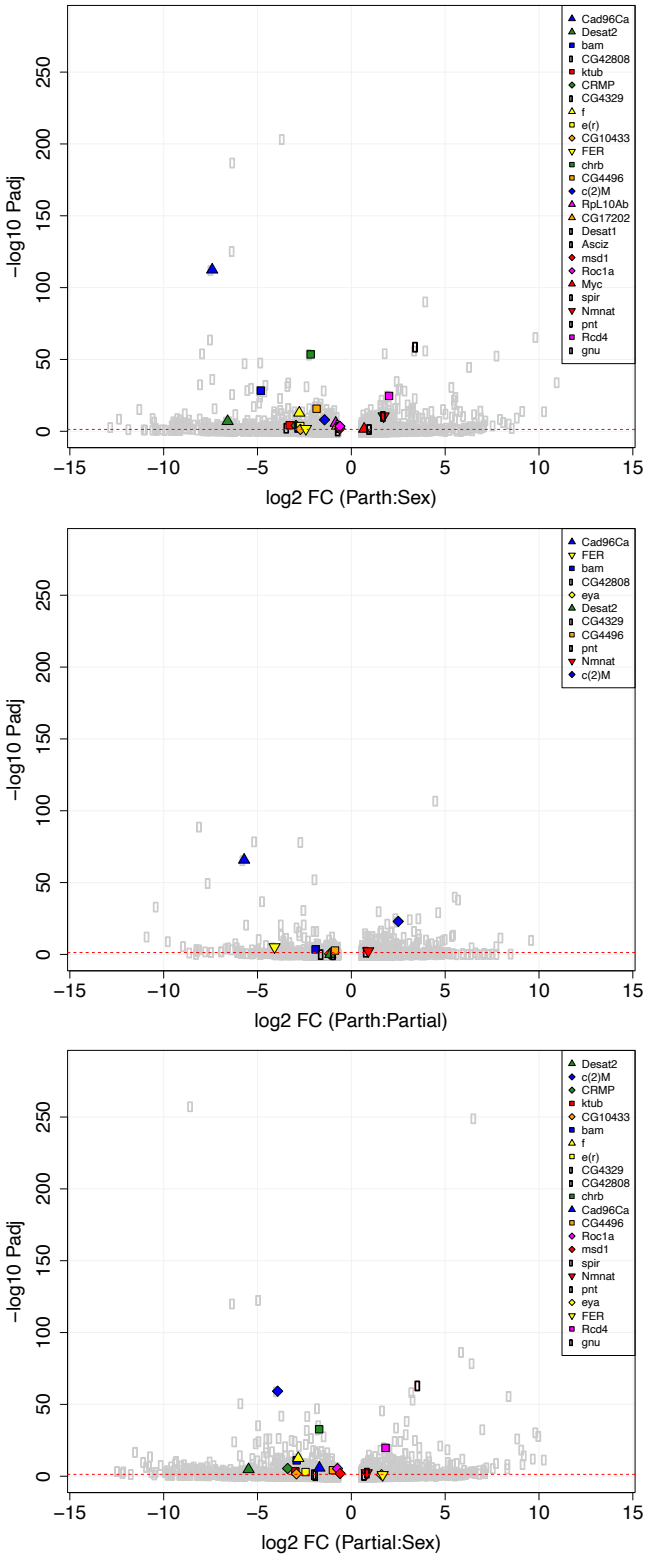


Figure S4:

A

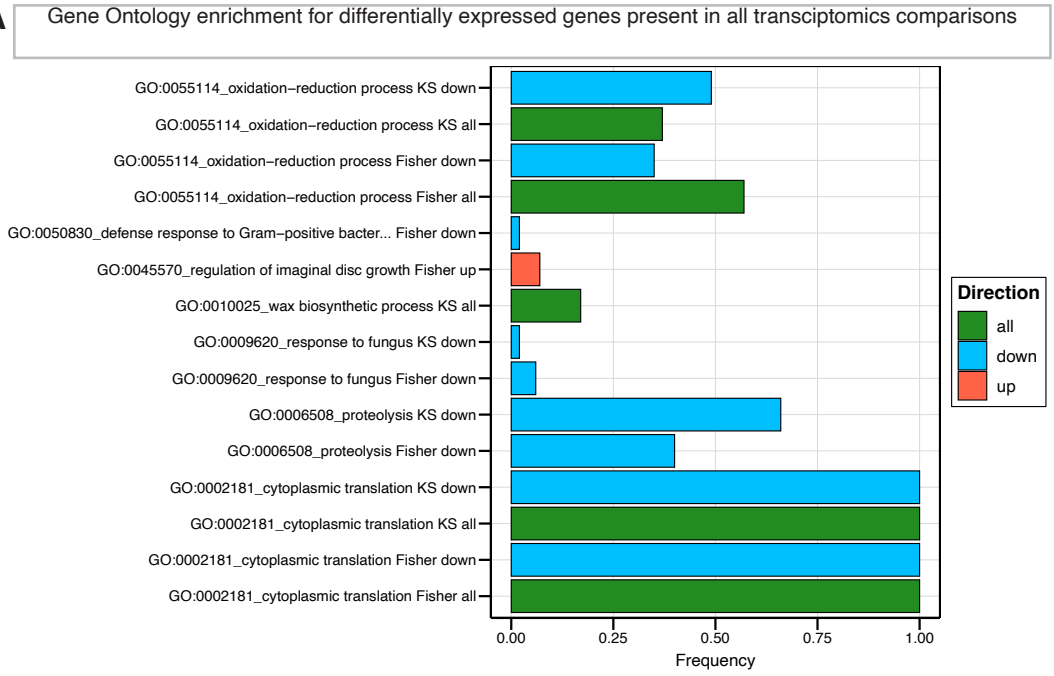
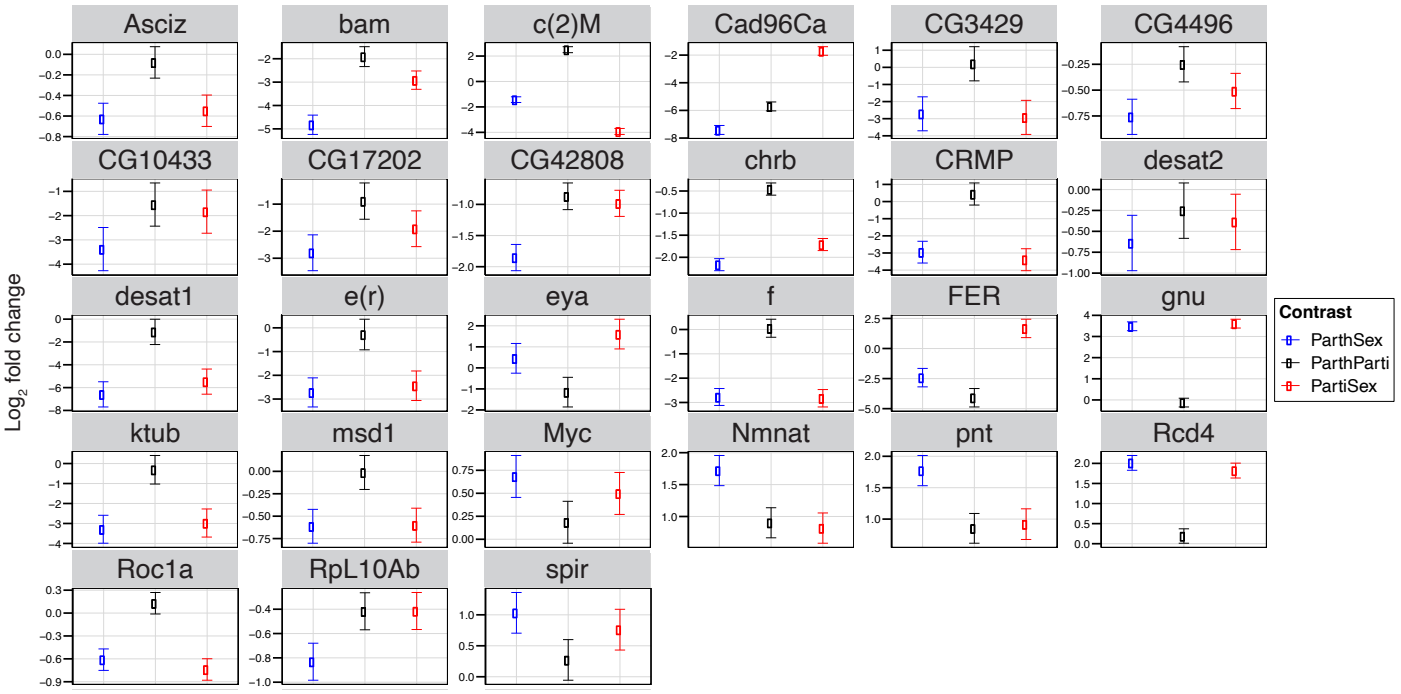


Figure S5:

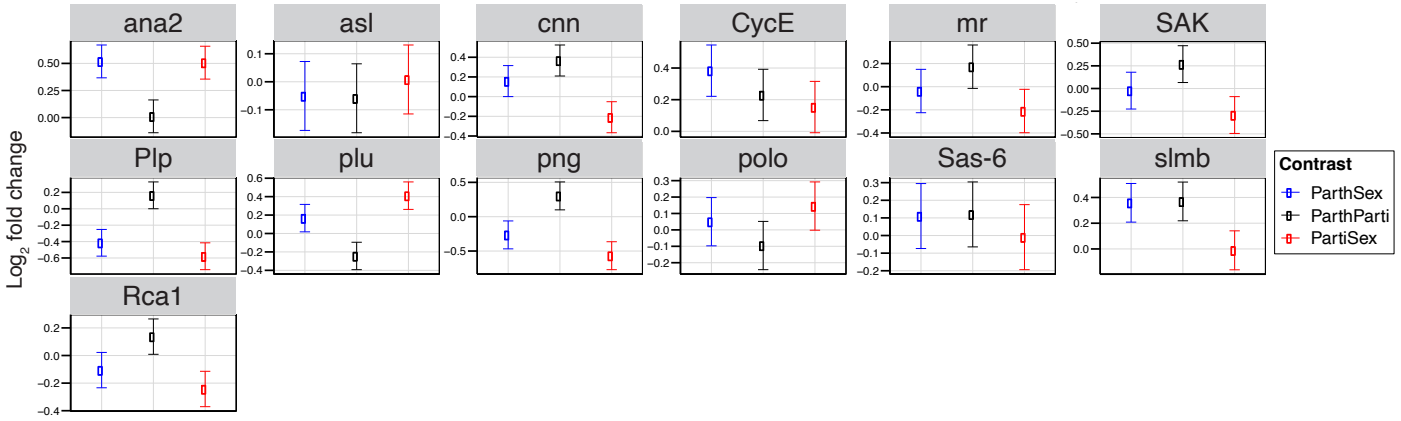
A

Candidates from the Transcriptomics Screen:
Differentially expressed genes between all three pairwise comparisons from the three different transcriptomes



B

Candidates from the biased screen:
Non-differentially expressed genes between all three pairwise comparisons from the three different transcriptomes



C

Controls:
Non-differentially expressed genes between all three pairwise comparisons from the three different transcriptomes that were used as controls

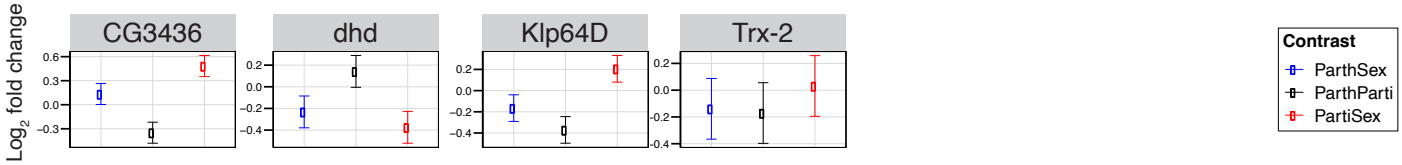
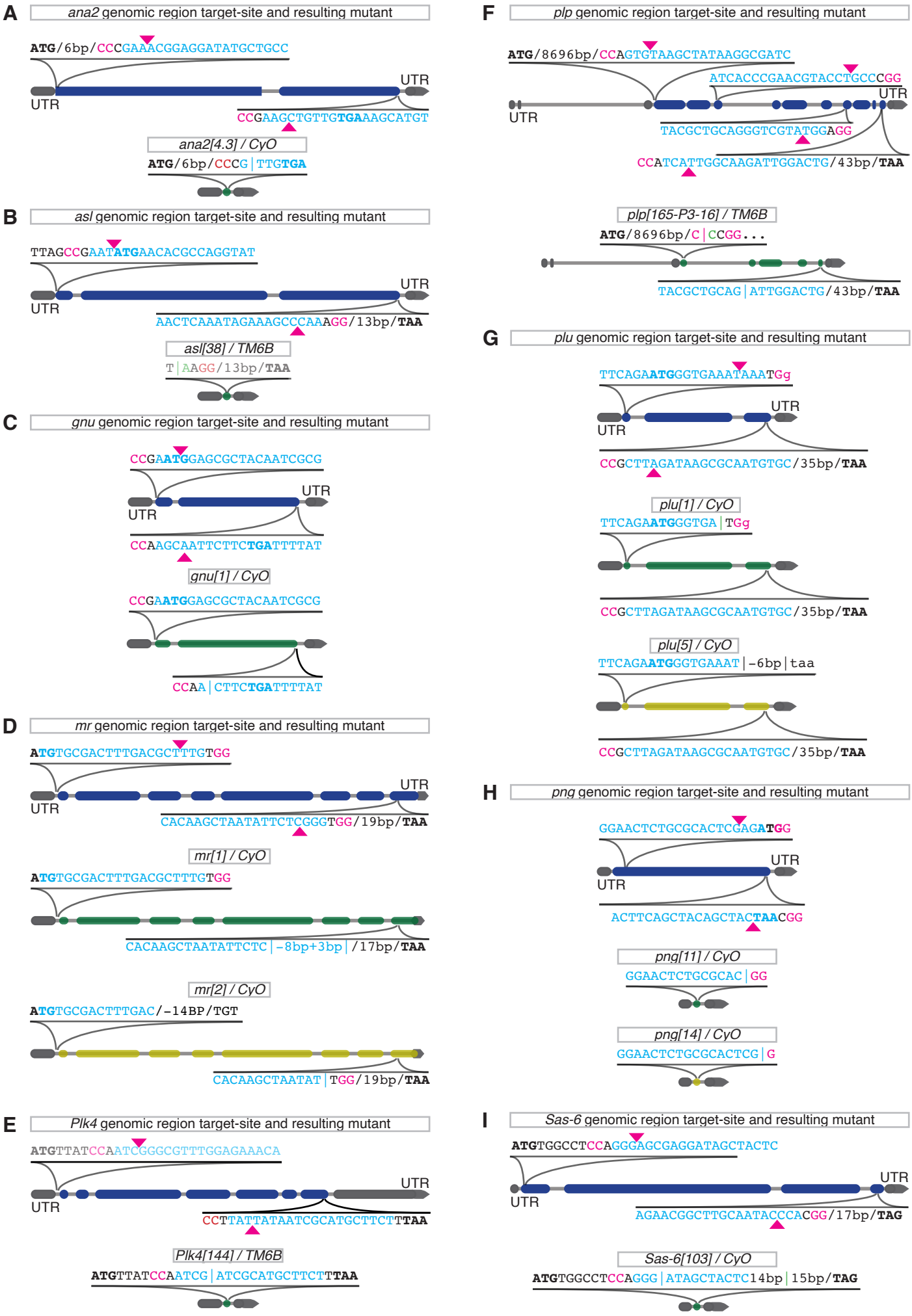


Figure S6:



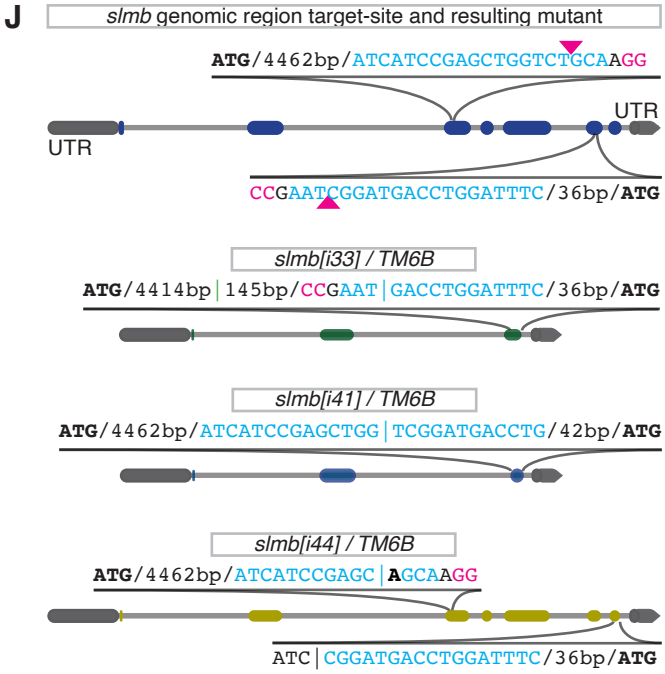


Figure S7:

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:

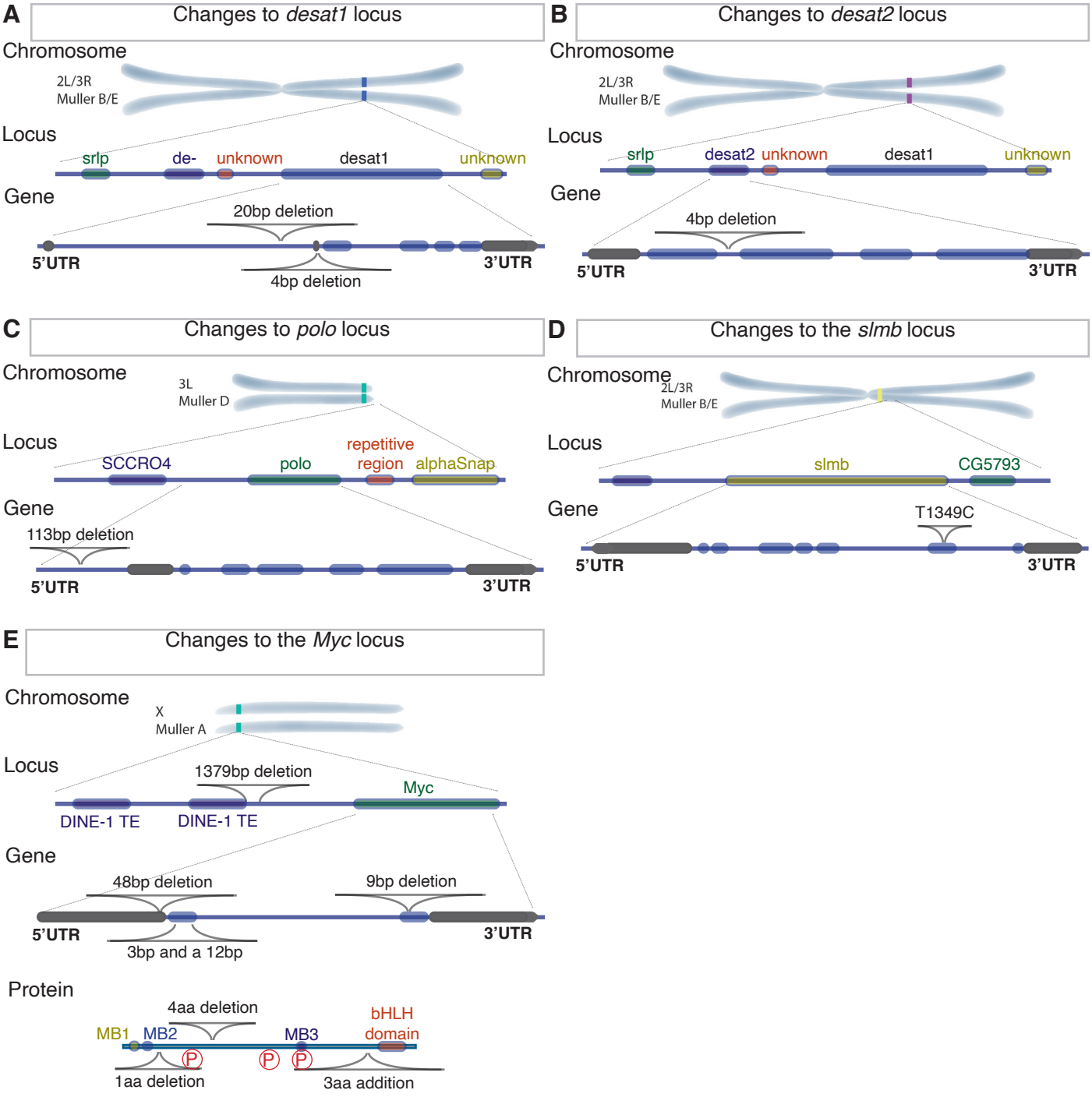


Figure S9:

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--SANGGVNGANNNNNNNSSNI  103
parthenogenetic_Dmercatorum          KIAAEHAHNMNSLALADDFDIKPEIRNGDCMWSAFGS--SANGGVNGANNNN-NNSSNI  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                   TQHKLQQQKLQQQQQQQQQRLTYLLSSNNYNNSNNNSNNNSYSMPEDEVLPVFRHNVDLRA  283
parthenogenetic_Dmercatorum          TQHKLQQQKLQQQQQQQQQRLTYLLSSNNYNNSNNNSNNNSYSMPEDEVLPVFRHNVDLRA  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
                                     *.:: :          ..:: :.  .*::  .*    ::   . .      :
```

Figure 10:

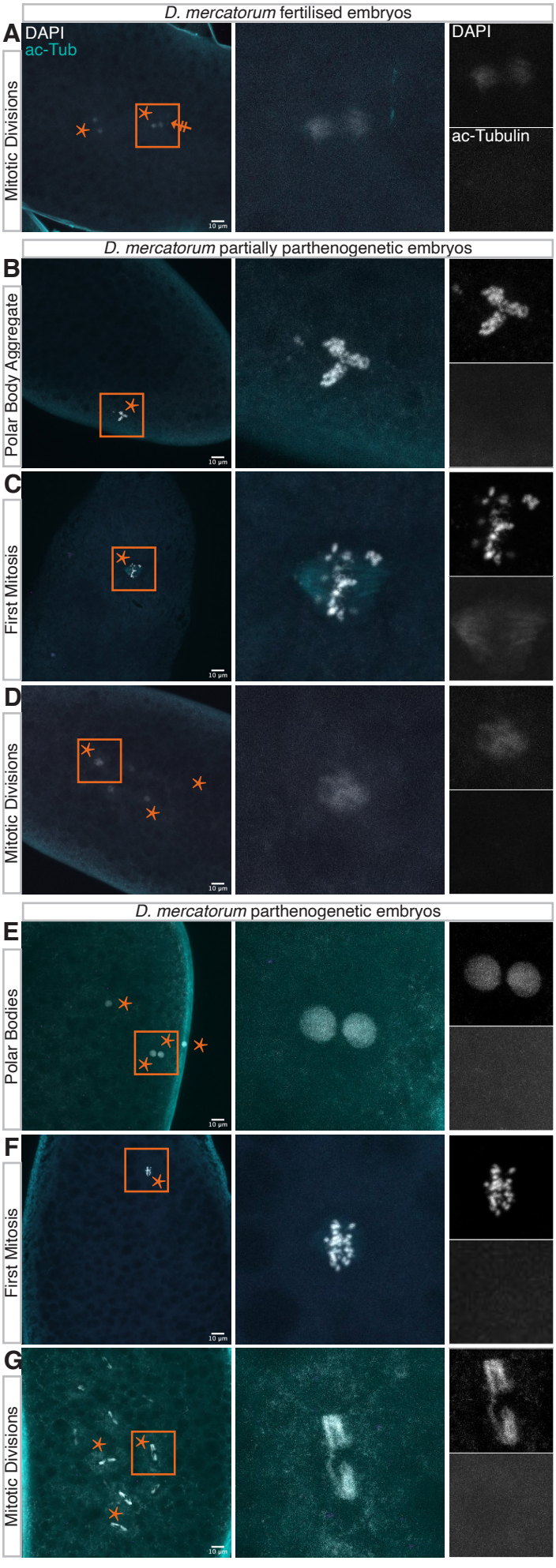


Figure S11:

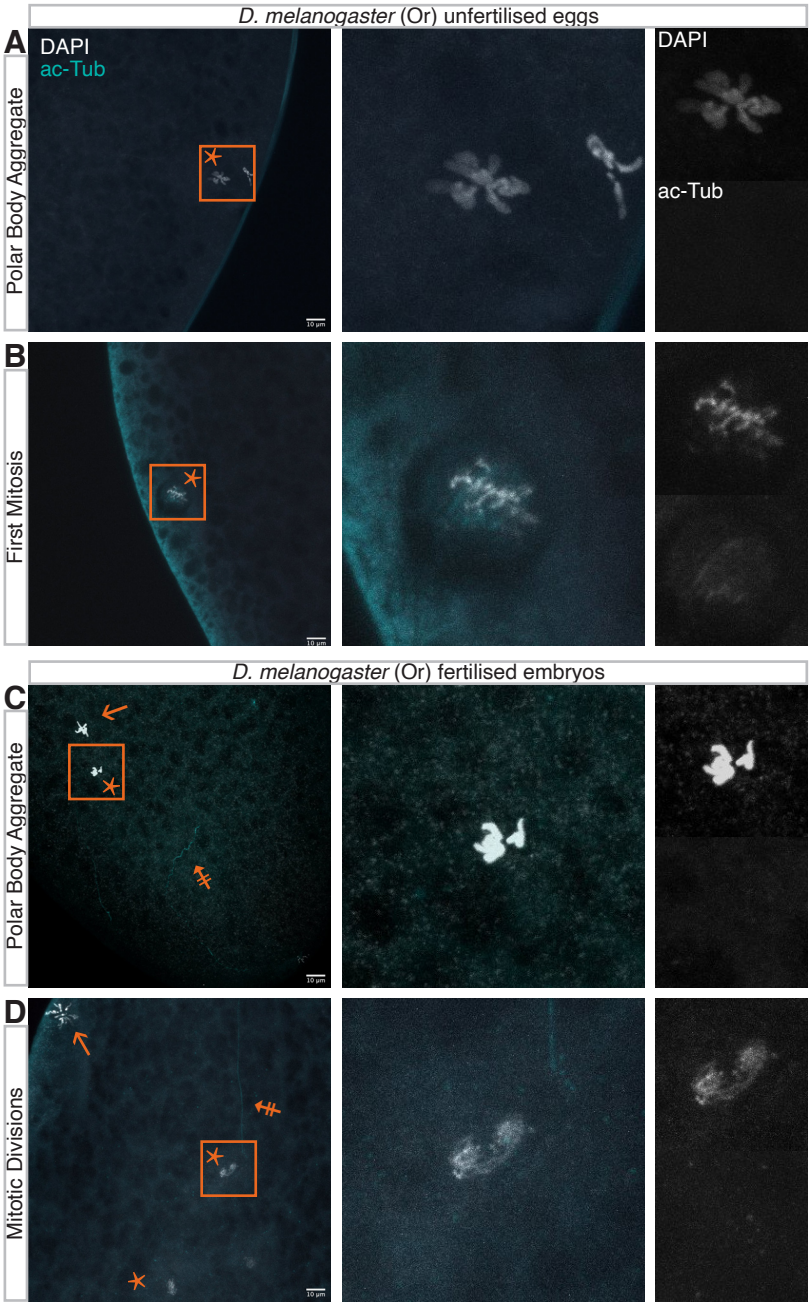


Figure S12:

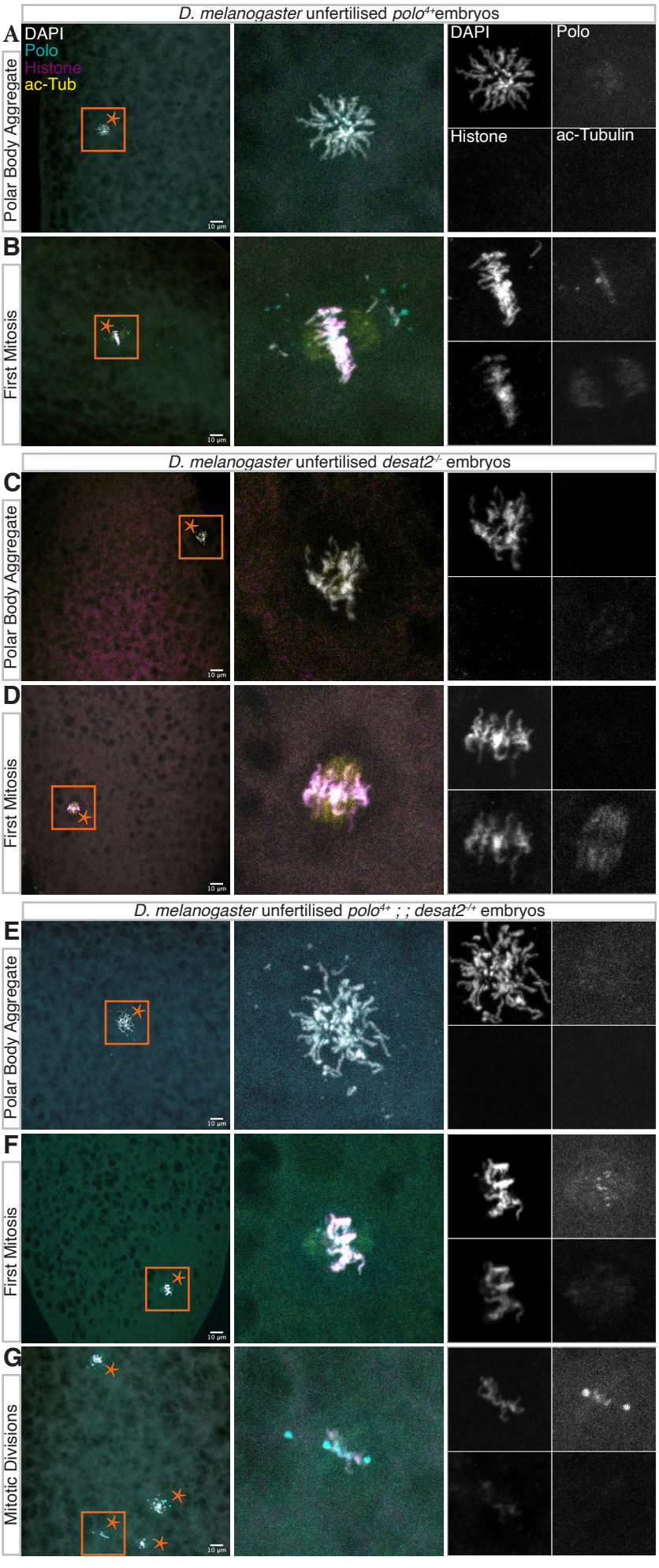


Figure S13