Genomic evidence of paternal genome elimination in globular springtails

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Abstract

Paternal genome elimination (PGE) - a type of reproduction in which males inherit but fail to pass on their father's genome - evolved independently in six to eight arthropod clades. Thousands of species, including several important for agriculture, reproduce via this mode of reproduction. While some of the clades are well established PGE systems, the evidence in globular springtails (Symphypleona) remains elusive, even though they represent the oldest and most species rich clade putatively reproducing via PGE. We sequenced genomic DNA from whole bodies of *Allacma fusca* males with sufficiently high fractions (31 - 38%) of sperm to conclusively confirm that all the sperm carry one parental haplotype only. Although it is suggestive that the single haplotype present in sperm is maternally inherited, definitive genetic proof of the parent of origin is still needed. The genomic approach we developed allows for detection of genotypic differences between germline and soma in all species with sufficiently high fraction of germline in their bodies. This opens new opportunities for scans for reproductive modes in small animals.

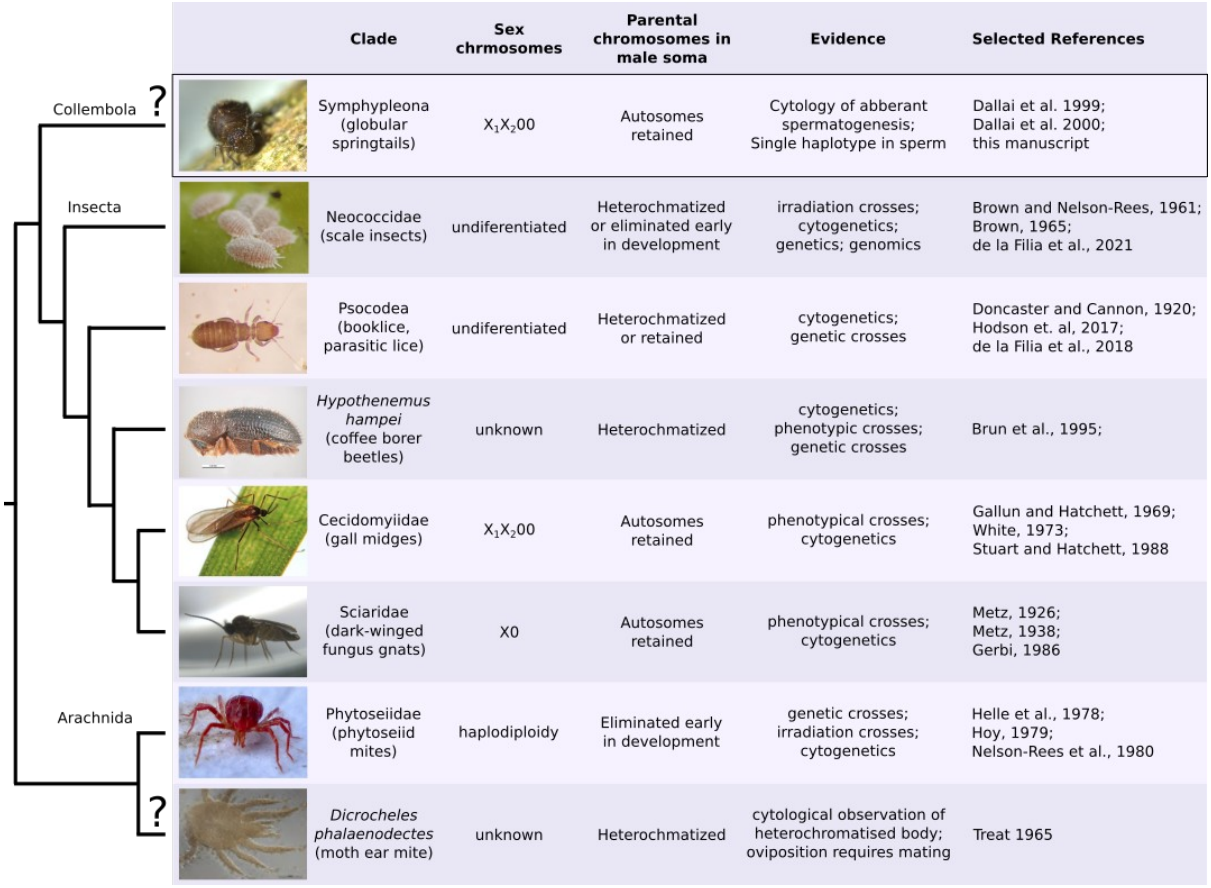
Introduction

The mechanism of reproduction varies considerably across the tree of life (Bachtrog et al., 2014; Normark, 2003). Historically, cytological comparisons of male and female karyotypes have been used to determine the mode of reproduction in a species. However, cytological studies are labour intensive and not all species have visible sex-specific karyotypes. As a consequence, many species still have undefined reproductive systems. On the other hand, genomic techniques have been successfully deployed to identify sex chromosomes in many taxa such as Diptera (Anderson et al., 2020; Vicoso & Bachtrog, 2015), and Lepidoptera (Fraïsse et al., 2017) and more recently to understand the exact form of parthenogenesis in species such as californian stick insects (Jaron et al., 2021), and bdelloid rotifers (Simion et al., 2021). Now, it is time to consider the ways we can use genomic techniques to study other modes of reproduction such as paternal genome elimination.

Paternal genome elimination is a reproduction system in which males develop from fertilised eggs, but pass to the next generation only the maternally inherited haplotype (see (Burt & Trivers, 2006) for a clear introduction to the topic). The inheritance pattern is exactly the same as in better known haplodiploidy (arrhenotoky), in which males develop from

unfertilized haploid eggs, but mechanistically they represent very different reproductive systems. Similar to haplodiploidy, there are only a few known transitions to PGE (six to eight), and PGE clades are frequently very old and diverse. Thousands of arthropod species reproduce via some form of paternal genome elimination including numerous agricultural pests (scale insects, Hessian fly, lucerne flea) and even pest control species (phytoseiid mites). However, the occurrence of PGE is likely significantly under-reported as it can be hard to confirm. It tends to occur in small arthropods that are poorly studied and hard to culture under laboratory conditions, making it challenging to study inheritance patterns. For example, PGE was only demonstrated in *Liposcelis* lice and human head and body lice (order Psocodea) very recently through genetic crosses tracking alleles over several generations, (de la Filia et al., 2018; Hodson et al., 2017) even though meiosis was known to be unusual in lice for decades prior to this (Cannon, 1922; Doncaster & Cannon, 1920). Because of the difficulty of inheritance studies, many of the reported cases are based on indirect evidence, usually cytogenetic observations of unusual chromosome behaviour (**Supplementary Table 1**).

Part of the reason PGE is difficult to identify, is that individual clades differ greatly in the mechanism of PGE, and hence require different types of evidence for confirmation (**Figure 1**). In all PGE species males develop from fertilized diploid eggs, and always exclusively transmit maternally inherited chromosomes to offspring. However, they differ in the processes leading to the elimination of paternal chromosomes. For example, in *Phytoseiidae* mites and some armored scale insects, the paternal genome is completely eliminated early in embryogenesis in a process called embryonic PGE (Brown, 1965; Nelson-Rees et al., 1980). The fact that males are completely haploid soon after fertilisation makes this type of PGE easy to detect in genetic and cytological studies, although it can be hard to distinguish from true haplodiploidy. The two can be distinguished, however, via carefully designed phenotypic or irradiation crosses (Helle et al., 1980; Hoy, 1979), by cytology of early embryogenesis (Nelson-Rees et al., 1980), or by observing whether unfertilised eggs develop into males (Häußermann et al., 2020).



Clade	Sex chrmosomes	Parental chromosomes in male soma	Evidence	Selected References
Symphypleona (globular springtails)	X_1X_200	Autosomes retained	Cytology of abberant spermatogenesis; Single haplotype in sperm	Dallai et al. 1999; Dallai et al. 2000; this manuscript
Neococcidae (scale insects)	undiferentiated	Heterochmatized or eliminated early in development	irradiation crosses; cytogenetics; genetics; genomics	Brown and Nelson-Rees, 1961; Brown, 1965; de la Filia et al., 2021
Psocodea (booklice, parasitic lice)	undiferentiated	Heterochmatized or retained	cytogenetics; genetic crosses	Doncaster and Cannon, 1920; Hodson et. al, 2017; de la Filia et al., 2018
Hypothenemus hampei (coffee borer beetles)	unknown	Heterochmatized	cytogenetics; phenotypic crosses; genetic crosses	Brun et al., 1995;
Cecidomyiidae (gall midges)	X_1X_200	Autosomes retained	phenotypical crosses; cytogenetics	Gallun and Hatchett, 1969; White, 1973; Stuart and Hatchett, 1988
Sciaridae (dark-winged fungus gnats)	X0	Autosomes retained	phenotypical crosses; cytogenetics	Metz, 1926; Metz, 1938; Gerbi, 1986
Phytoseiidae (phytoseiid mites)	haplodiploidy	Eliminated early in development	genetic crosses; irradiation crosses; cytogenetics	Helle et al., 1978; Hoy, 1979; Nelson-Rees et al., 1980
Dicrocheles phalaenodectes (moth ear mite)	unknown	Heterochmatized	cytological observation of heterochromatised body; oviposition requires mating	Treat 1965

Figure 1. Clades with suggested paternal genome elimination (PGE) and evidence supporting it. The cladogram shows the phylogenetic relationships between putative (marked by "?") and confirmed PGE clades. Although all PGE clades exhibit transmission dynamics where paternally inherited chromosomes are not transmitted to offspring through males, the sex chromosome system and the treatment/expression of paternally inherited chromosomes in male somatic cells can differ between and within clades. A more detailed list of relevant literature is in **SM Table 1.** Two taxa without definitive proofs of PGE are marked "?". Image credits: mealybugs (scale insects) by Andrew J. Mongue, coffee borer beetles by Walker, K., phytoseiid mite by Mick Talbot.

In other types of PGE, males fully or partially retain their paternal genome throughout development and paternal chromosomes are excluded during spermatogenesis only, hence these types are known as germline PGE. While paternal chromosomes are retained, they form a dense heterochromatic body at the periphery of the cell nuclei for most scale insects (Brown, 1965; Ross et al., 2012), the coffee borer beetle (Brun et al., 1995), booklice (Hodson et al., 2017), and potentially in some Leapideae mites (Treat, 1965). This distinctive feature is not a formal test of PGE, but allows potential PGE species to be easily detected using cytological observation. It also means that males in these clades are mostly haploid in terms of gene expression, despite their diploid karyotype (Brun et al., 1995; de la Filia et al., 2021). A combination of embryonic and spermatogenic elimination is found in two dipteran families: fungus gnats, and gall midges. Males of these clades exclude one or two paternal chromosomes in early embryogenesis (usually referred to as X chromosomes), while retaining all other chromosomes in their soma. The remaining paternal chromosomes are lost during aberrant spermatogenesis. In fungus gnats and gall midges it has been shown by

crosses that all the eliminated chromosomes in both embryogenesis and spermatogenesis are of paternal origin (Gallun & Hatchett, 1969; Metz, 1926, 1938; Stuart & Hatchett, 1988). Finally a similar type of PGE has been suggested to occur in globular springtails. However the evidence is solely based on unusual chromosome behaviour and no inheritance studies are available.

Globular springtails are a large and species-rich order with enormous importance for soil ecology (Hopkin, 1997). Their karyotype consists of four to five autosomes and two sex chromosomes reffered as X_1 and X_2 (Dallai et al., 2000, 2004). As in PGE flies, male globular springtail zygotes are initially fully diploid, but during very early embryogenesis males eliminate one copy of the X_1 and X_2 chromosomes (**Supplementary figure 1**) (Dallai et al., 2000). Furthermore, the aberrant spermatogenesis also shows a great similarity to the two dipteran families. During meiosis I of spermatogenesis the two X chromosomes cosegregate, hence half of the secondary spermatocytes carry all six chromosomes and the other half contain the four autosomes only (**Figure 2**). The X chromosome lacking spermatocytes immediately degenerate, and only the spermatocytes with the complete chromosome set undergo a second meiotic division to form two haploid spermatids (Dallai et al., 2000). In a series of papers, Dallai and colleagues described this type of aberrant spermatogenesis in five globular springtail families, namely Dicyrtomidae (Dallai et al., 1999), Sminthuridae (Dallai et al., 2000), Bourletiellidae (Dallai et al., 2001), Sminthurididae and Katiannidae (Dallai et al., 2004). This is likely the ancestral state of the Symphypleona order. Hence, it is clear that one full haploid set of chromosomes gets eliminated during development (X chromosomes) and spermatogenesis (autosomes) of males. However, it remains unclear whether the chromosome elimination is random during meiosis or systematically dependent on the parental origin (i.e. PGE).

There is no distinct name for the putative sex chromosome constelation in globular springtails. It is best described as PGE X0, although the absence of X chromosomes in males is not the primary sex determination. Other springtail orders in contrast have regular meiosis (Dallai et al., 1999) and X0 or XY sex determination (Hemmer, 1990; Núñez, 1962).

We investigated possible approaches to confirm PGE in globular springtails. First, we considered conducting genetic crosses of *Allacma fusca*, a relatively common and large globular springtail commonly found in woodland areas across Europe. However, wild-caught globular springtails are hard to maintain in lab conditions. Alternatively, genotyping a male and its sperm can at least inform us if all sperm contain a single haplotype only, presumably the maternal one. While investigating methods to efficiently sequence male sperm, we discovered male bodies contain a large fraction of sperm (up to 38% of cells) and sequencing whole bodies seems to be the most efficient way to sequence sperm, although it requires in-silico bioinformatics analysis to separate the effect of somatic and germline genomes in the sequencing library. With our innovative approach we demonstrated that the set of autosomes co-segregate with the X_1 and X_2 chromosomes, implying uniparental inheritance.

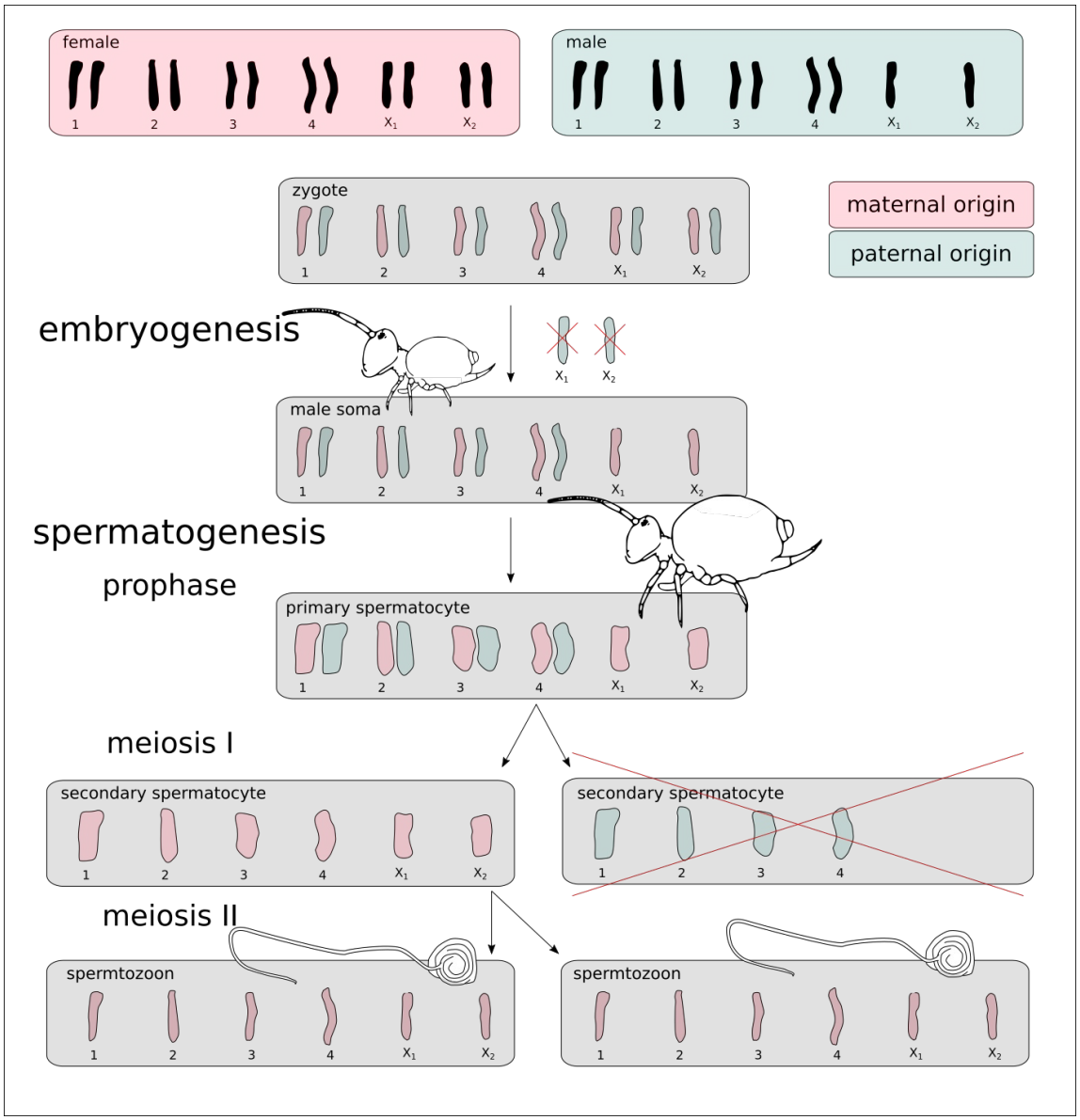


Figure 2: Scheme of PGE in globular springtails. Male springtail zygotes are initially diploid for all chromosomes. One copy of chromosomes X_1 and X_2 is excluded during early embryogenesis. Adult males then generate a half of their secondary spermatocytes with the remaining X chromosomes, and a half without X_1 and X_2 that degenerates immediately. The scheme and cartoonized shapes of chromosomes are based on (Dallai et al., 2000). Note the spermatozoon “tail” is not flagellum, as flagellum is densely coiled, see (Dallai et al., 2009) for details. The coloring is assuming reproduction via paternal genome elimination as tested in this study.

Materials and Methods

Springtails collected and sequenced

We used an assembly (GCA_910591605.1) and sequencing reads (sample accession ERS6488033) we previously generated for a male *Allacma fusca* individual (Anderson et al., 2020). We also collected 12 additional *A. fusca* samples for resequencing. The sex of individual samples was determined from the modality of sequencing coverage and revealed 11 of 12 resequenced samples were females (**SM Figure 1**). The resequenced male individual was sampled at Blackford Hill (sample id BH3-2, ERS6377982), Edinburgh, Scotland (55.924039, -3.196509). We isolated the DNA using Qiagen DNeasy Blood and tissue kit extraction protocol and sequenced using the Illumina HiSeq platform. The standard adapters and low quality bases were trimmed using skewer `v0.2.2` with options `-m pe -n -q 26 -l 21` (Jiang et al., 2014). We used both the male and all female libraries to identify X-linked scaffolds. Although the reference genome is fragmented, reliable chromosomal assignments are available for 170.6 Mbp, representing 40.1% of the assembly span. In total, 77.9 Mbp of scaffolds are X-linked, while 92.7 Mbp are autosomal (**SM Text 1**).

All analyses were also performed on the genome of an outgroup species *Orchesella cincta* (GCA_001718145.1, (Faddeeva-Vakhrusheva et al., 2016)). Both male *O. cinta* resequencing data (ERS7711323) and chromosomal assignments were taken from (Anderson et al., 2020). *Orchesella cincta* is a distantly related springtail with X0 sex determination (Hemmer, 1990) and therefore ideal as a negative control for this study.

Calculation of k-mer coverages

All male sequencing libraries were initially subjected to quality control using kmer spectra analysis. We calculated the k-mer coverage histogram with k = 21 using KMC3 (Kokot et al., 2017) and visualized it using GenomeScope 2.0 (Ranallo-Benavidez et al., 2020). GenomeScope fits a model estimating error rate, genome size and heterozygosity assuming all chromosomes are of the same ploidy. This assumption is clearly violated in male globular springtails, hence we disregarded the genome size and heterozygosity estimates for these samples and used the software only for characterising 1) the monoploid (1n) k-mer coverage representing the two X chromosomes and the autosomal heterozygous loci, 2) the diploid (2n) k-mer coverage representing homozygous autosomal regions and 3) the error peak indicating overall quality of the sequencing run.

In sequencing libraries of a tissue with AAX0 karyotype, the autosomes are expected to have exactly twice the coverage of X chromosomes (i.e. the library has evenly spaced peaks). However, k-mer coverages displayed unevenly spaced 1n and 2n peaks for the reference *A. fusca* male (**SM Figure 2A**), the BH3-2 *A. fusca* had insufficient coverage to confirm the pattern (**SM Figure 2C**), but the *O. cincta* male showed evenly spaced 1n and 2n peaks (**SM Figure 2E**). To clarify the signal and replicate the pattern we also estimated mapping coverage (see **Box 1** for the difference between the two).

Calculation of mapping coverages

We mapped trimmed sequencing libraries to the softmasked reference genomes of *Allacma fusca* (`GCA_910591605.1`) and *Orchesella cincta* respectively (`GCA_001718145.1`). The reads were mapped using bowtie2 with the parameter `--very-sensitive-local` (Langmead & Salzberg, 2012). We used samtools depth to extract per base coverage (Li et al., 2009), and calculated per scaffold mean coverage. Then we estimated 1n and 2n coverage peaks using kernel smoothing with kernel width chosen by Sheather and Jones method (`bw = "SJ"`) (Sheather & Jones, 1991) while weighted by scaffold length (`weights = scf_tab$len / sum(scf_tab$len)`).

This method resulted in both *A. fusca* males showing distinctively uneven peaks (**SM Figure 2B** and **D**), while the *O. cincta* male again showed the expected 1n and 2n coverage peaks. These findings prompted us to create a two tissue model that can explain the shift of relative positions of the two peaks.

Box 1: Different types of sequencing coverages used in this manuscript

Sequencing coverage is the mean number of times every position in a genome is represented in reads. Sequencing coverage is usually estimated by dividing the total sequencing yield by the haploid genome size. However, in many cases, for genome analyses of non-model organisms the level of contamination in sequencing libraries; sequencing errors; and genome size is unknown. This can make estimating sequencing coverage challenging. Hence there are multiple other ways to measure and estimate sequencing coverage. These different measures have different properties and are used for different purposes. **K-mer coverage** is the mean number of occurrences of each unique continuous genomic sequence of length k in reads. K-mer decomposition is independent of a reference genome and the coverage is estimated by fitting a model to k-mer coverage histogram (Ranallo-Benavidez et al., 2020). This reference-free technique is well suited to observing raw signals from data unbiased by complicated procedures such as genome assembly. **Mapping coverage** is the mean number of reads mapping to each position of a haploid reference genome. It is dependent on the quality of the reference and quality of mapping. The main advantage is that reads of heterozygous sites as well as infrequent sequencing errors still typically map to the same position on the reference. Hence, this coverage is suited the best to estimate ploidy of each reference genomic region. **Allelic coverage** is also derived from sequencing reads mapped to the genome. However, the mapped reads usually have PCR duplicates marked and are subsequently used for calling variants. The coverage is then the number of non-duplicated reads supporting individual alleles. Sequencing errors do not contribute to this coverage.

For the sake of completeness, in theory, k-mer coverage (C_k) is convertible to mapping coverage (C_m) by a simple approximation

$$C_k \approx C_m \frac{R-k+1}{R}$$

Where k is the length of k-mer, R is the length of reads. However, in practice there are usually too many issues - no haploid reference is perfect and the mapping process is also dependent on many assumptions. Hence in practice the two measures need to be calculated independently.

Two tissue model

The unevenly spaced mapping coverage (see **Box 1**) peaks of X chromosomes and autosomes implies the sequencing library contained tissues with various ploidies. A simple model that can explain the pattern is a two tissue mixture model - a mixture of a tissue with 1:2 X to autosome ratio (e.g. male soma) and a tissue with 1:1 X to autosome ratio (e.g. secondary spermatocytes or sperms). Using the X-chromosome and autosome mapping coverage peaks, we can estimate the relative contribution of the two tissue types to the sequencing library (**Supplementary figure 5A**) and the fraction of the two tissues. Assuming the 1:1 tissue is haploid, the relative fraction of that tissue (f_h) in the sequencing library is

$$f_h = 1 - \frac{(c_A - c_X)}{c_X},$$

where c_A is the mapping coverage of the autosomal peak, and c_X is the mapping coverage of the X chromosome peak.

The only described tissue with 1:1 X to autosome ratio in adult male globular springtails are primary and secondary spermatocytes, spermatids, and spermatozoon (Dallai et al., 2000). Hence, it is probably safe to assume this is the tissue that is causing the relative mapping coverage shift illustrated in **Supplementary figure 5B** (for alternative unsupported hypotheses tested to explain the 1n mapping coverage shift, see **SM Text 2**).

Testing PGE

Sequencing a mixture of sperm and soma provides us a chance to test previously suggested paternal genome elimination (PGE) in globular springtails. The PGE inheritance model (**Figure 2**) predicts that the sperm contain only the maternally inherited haploid set of chromosomes (A_mX_m). As all the autosomes present in the haploid sperm are of maternal origin, all the heterozygous autosomal loci should display a higher coverage support of maternal alleles compared to paternal. In the ideal case, we would like to compare coverage support of phased haplotypes, which is unfeasible with fragmented reference genomes and short read libraries. Instead, we separated the alleles of heterozygous autosomal variants to the “major” and “minor” alleles - representing the variants with higher and lower coverage support respectively. Under the PGE model the maternal and paternal alleles are expected to have vastly different coverage support, therefore the “major” alleles will be vastly of maternal origin, while the “minor” alleles will be vastly paternal. The fraction of possible misassigned variants was explored through modeling of sequencing coverages using negative binomial distributions with parameters estimated from expected sequencing coverages (**Figure 4**).

Furthermore, under the PGE model, the distribution of maternal allele coverage depths is expected to resemble the distribution of X-chromosome allele coverage depths. Due to a small fraction of misassigned alleles in males (as explained in the previous paragraph) the match is not expected to be exactly perfect. The expected levels of imperfect match were also estimated via the same set of simulated coverages.

We performed the same analysis on the genome of male *O. cincta* and two *A. fusca*

females. The two females only show the decomposition of autosomal heterozygous alleles in the case of frequent misassignment of maternal and paternal alleles (as they are generated from the same coverage distribution, **SM Figure 3**). The *O. cinca* male further allows the same comparison of decomposed allele coverages to the distribution of coverage of alleles found on the X chromosome.

Before calling variants we marked duplicates in the mapping files using picard MarkDuplicates (*Picard Toolkit*, 2019) and called variants using freebayes `v1.3.2-dirty` (Garrison & Marth, 2012) with stringent input base and mapping quality filters as well as required minimal allele coverage (`--standard-filters --min-coverage 5`), but we relaxed the priors of Hardy–Weinberg proportions are it might not be met in a PGE population (`--hwe-priors-off`), while assuming diploidy (`-p 2`). The raw variant calls were subsequently filtered for high quality variants (`-f "QUAL > 20"`) only using vcffilter from the vcflib library version `1.0.0_rc3` (Garrison et al., 2021) and sorted to autosomal and X-linked using a custom python script. The variants sorted to chromosomes were plotted using R scripts.

Independent estimate of the relative fraction of the haploid tissue

The described test for PGE requires moderate to high levels of heterozygosity to provide enough power to estimate the coverage distributions of the putatively maternal and paternal alleles. Low heterozygosity in combination with stringent parameters for SNP calling could cause low statistical power to test PGE via called variants.

To avoid the lack of power and any other potential biases introduced via SNP calling, we deployed an alternative approach as a supplementary analysis. For the two *Almaca* males we counted sequence states aligned under the `GCA_910591605.1` reference using samtools mpileup converted to matrix form with Popoolation2 mpileup2sync (Kofler et al., 2011). After filtering out scaffolds with evidence of copy-number variation between the males (**SM Figure 4**), we calculated minor frequencies p_p for all genomic positions with at least two states in the pileup. Then we examine the distribution of variant sites by minor allele frequency for both males. See **SM Text 4** for details.

As all the sperm is expected to contain only maternally inherited autosomes, the expected proportion $p_p$of paternal (green shaded in **Figure 2**) automosomes over all the body's cells is $\frac{1-f_h}{2-f_h}$. The expected allele coverage ratio (site frequency) of the paternal state is p_p, this is the minority state when $f_h > 0$, and

$$f_h = \frac{1-2\,p_p}{1-p_p}$$

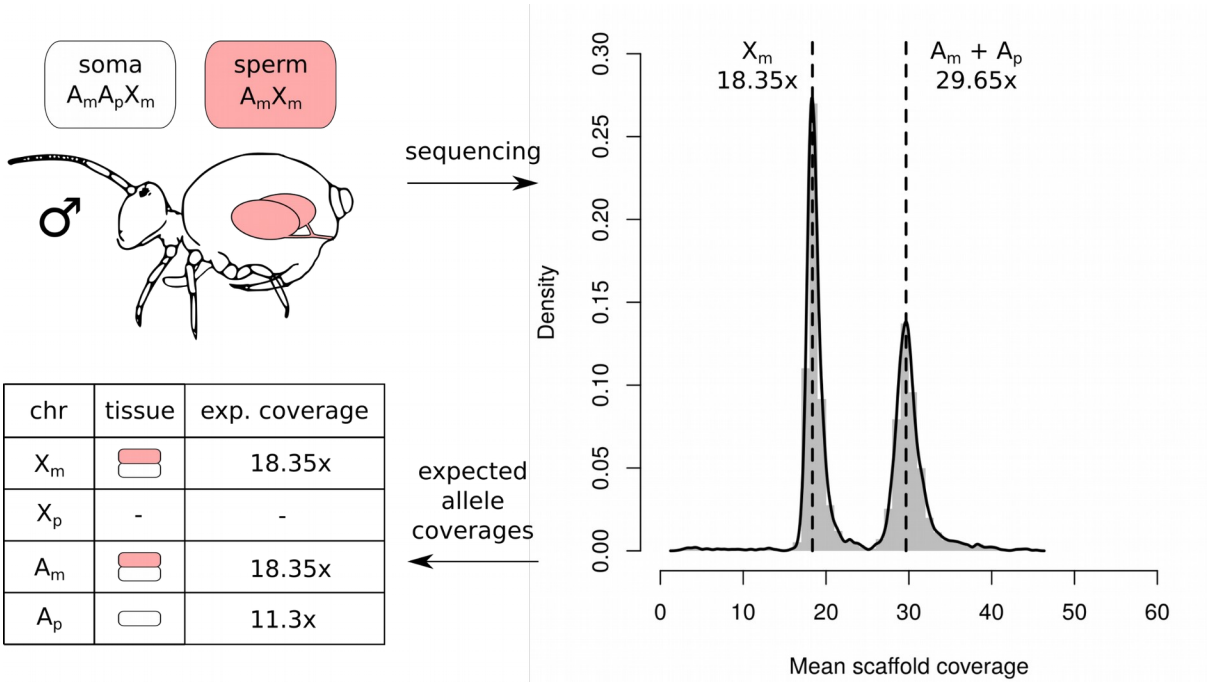
which allows us to estimate the relative fraction of haploid tissue from the estimated allele coverage ratios. We also present this analysis for heterozygous SNP calls, allowing comparison of the two methods.

All scripts and materials are available online at https://github.com/RossLab/genomic-evidence-of-PGE-in-globular-springtails.

Results

The analysis of trimmed sequencing libraries of the two *A. fusca* male individuals revealed that both have unexpected relative k-mer and mapping coverages of the X chromosomes compared to the autosomes (see **Box 1** for coverage definitions). In both cases the 1n coverage estimates were more than half of the diploid coverage estimate. We estimated the X-chromosome and autosomal mapping coverages in the BH3-2 male to be 18.35x and 29.65x respectively (**Figure 3**), deviating from the 1:2 ratio, under which we would expect the diploid coverage to be 36.7x (or haploid coverage of 14.8x). A remarkably similar uneven spacing of the X and autosomal peaks was observed in the reference *A. fusca* male for both k-mer and mapping coverages (57.7x and 95.2x), while in a strong contrast with a male sequencing library of a non-PGE species *O. cincta*, where the two coverage peaks were nearly perfectly spaced (**Supplementary figure 2**).

Using the coverage estimates and the two tissues mixture model (see **Methods** and **Supplementary figure 5**) we calculated the fraction of sperm cells in male *A. fusca* to be 35% (the reference male) and 38% (BH3-2). Furthermore, for the BH3-2 individual we estimated the expected allele coverages for paternal (11.3x) and maternal (18.35x) autosomes and X chromosomes (18.35x) (**Figure 3**).



chr	tissue	exp. coverage
X_m		18.35x
X_p	-	-
A_m		18.35x
A_p		11.3x

Figure 3: Overview of expected coverages potentially explaining the shift of the coverage peaks. PGE is expected to cause the shift of coverage peaks due to a significant proportion of sperm in the body, as indicated in cartoons (explained in greater detail in **Supplementary figure 5**). The table contains the expected paternal and maternal coverages of autosomes and X-chromosomes for the male resequencing individual BH3-2.

The expected coverages of maternal and paternal autosomes and X chromosomes were compared with the distribution of allelic coverages of variants on autosomes and X chromosomes. After quality filtering we identified 28,070 and 235,301 heterozygous variants anchored to chromosomes in the reference and BH3-2 individuals respectively (**SM Table**

2). The extremely low heterozygosity of the reference male reduces the power to use the sample for testing the PGE hypothesis and is discussed in **SM Text 3**. Of the BH3-2 anchored heterozygous variants 227,570 were located on autosomal scaffolds, while only 7,731 heterozygous variants on X-linked scaffolds, indicating low levels of false positives among variant calls (less than 100 false positives per 1Mbp). On the other hand, we identified 60,999 homozygous variant calls on the X linked scaffolds that were used for the comparison with allele coverages of the autosomal variants. The coverage supports of *A. fusca* male were contrasted to 1,959,258 heterozygous autosomal variants and 400,001 homozygous X-linked variants in the outgroup species *O. cincta* (non-PGE springtail).

We decomposed the male heterozygous autosomal variants in both samples to the “major” and “minor” alleles - representing the variants with higher and lower coverage support respectively. The mean coverage of maternal variants (18.35x) is expected to be higher compared to the coverage of paternal variants (11.3x), hence although it is possible some of the paternal variants will be by chance higher, this will affect only a very small fraction of the variants. To quantify the effect of misassigning variants by coverage, we simulated the coverage support of maternal and paternal alleles under the PGE model using the negative binomial distribution (**Figure 4A**). The simulation with 200000 variants showed that the probability of missassigment is 0.162 (Binom. CI: 0.1608748, 0.1641082). On the other hand, applying the same decomposition of heterozygous variants to “major” and “minor” in non-PGE species leads to ~0.5 of misassigned variants (by definition) (**Figure 4B**). In both cases the black distribution in the background represents the background distribution for the maternal variants. In the real data, we used the homozygous variants located on the X chromosomes to estimate the coverage distribution of monoploid maternal alleles. Under the PGE model, we expect it to roughly overlap with the “major” variant coverage peak (**Figure 4A**), contrasting to the non-PGE model where the expected distribution will be exactly in the middle of the “major” and “minor” coverage peaks (**Figure 4B**).

We confirm the coverage supports of “major” and “minor” autosomal variants in *A. fusca* male BH3-2 are close to the expected coverages (**Figure 4C**) generated from the two tissue mixture model (**Figure 3**). The fit is not perfect, probably due to problems with misassigned alleles. Furthermore, the distribution of “major” autosomal variants closely resembles the distribution of homozygous X-linked variants, with similar levels of disagreement compared to the simulated data (**Figure 4A**). Both comparisons together provide a strong support for the PGE model in *Allacma fusca*. The analysis of *A. fusca* shows a clearly different pattern to *O. cincta,* a springtail with standard spermatogenesis. The decomposed coverage supports display largely overlapping distributions and the coverage distribution of X-linked variants is nearly located intermediate between the peaks of “major” and “minor” allele coverages (**Figure 4D**), as predicted by the non-PGE model (**Figure 4B**). Note that the first coverage peak of X-linked variants displays spurious and unexpected coverages, which according to the genome profiling (**SM Figure 2E**) should be considered false positives.

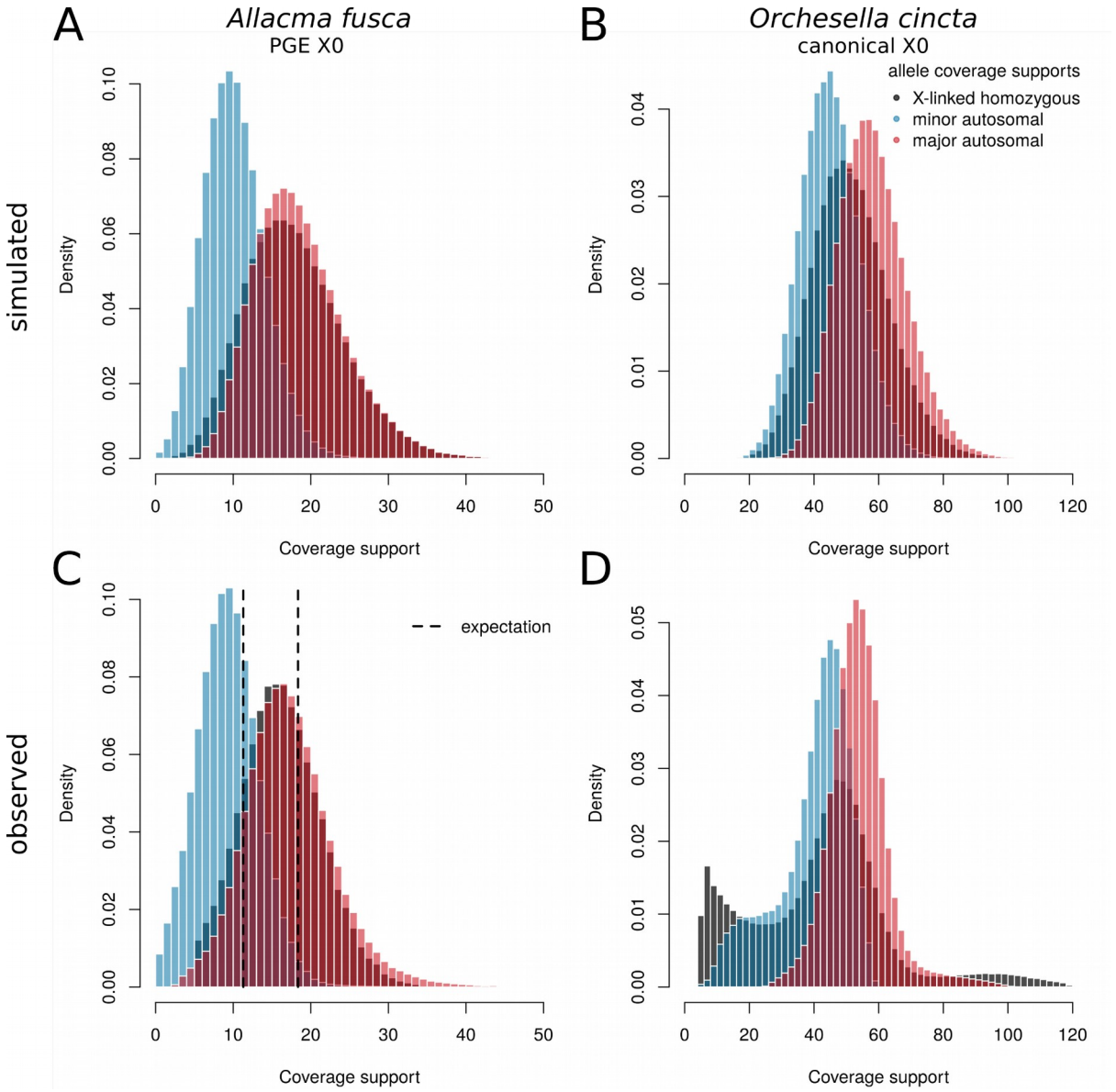


Figure 4: Decomposed heterozygous allele coverage supports. Coverage supports of the two alleles of heterozygous sites are decomposed to those with higher coverage ("major", in red) and lower coverage ("minor", in blue). These are compared to coverage supports of homozygous X-linked variants. Panels **A** and **B** are simulated allele coverages for a PGE X0 system and non-PGE X0 system. In PGE species (**A**), major alleles vastly represent maternal alleles and show similar coverage distributions to homozygous X-linked alleles (maternal haploid). In canonical X0 system (**B**) the decomposition also leads to bimodal distribution, however, the X-linked allele has an intermediate coverage peak in between of the two autosomal distributions. The observed coverage distributions in *Allacma fusca* (**C**) strongly support the PGE model. The major allele coverage support distribution closely reassembles the distribution of homozygous X-linked alleles as well as matching the expected coverage calculated from the 1n coverage shift (**Figure 3**). In contrast *Orchesella cincta* (**D**), a species with regular spermatogenesis and X0 sex determination, shows patterns consistent with the expected properties of canonical sexual X0 sex determination species with X-linked coverage support intermediate of the decomposed autosomal coverage supports.

We utilised an analysis of raw pileups to create an independent estimate of the fraction of the haploid tissue f_h from the estimated minor allele frequency of all genomic positions with two states located on scaffolds with no signs of copy number variation (See **SM Text 4** and **SM Figure 4**). This approach showed a higher abundance of bistates around coverage ratios 0.397 in Afus1 and 0.406 in BH3-2 (**SM Figure 5**), indicating that even the reference male shows some detectable heterozygous states, but with much noisier signal compared to BH3-2. The estimated fraction of sperm in the bodies from the paternal allele frequency p_p are 33.96% for Afus1 and 31.39% for BH3-2 individuals respectively.

Discussion

We estimated that a large proportion of a male adult *A. fusca* body (31 - 38%) consists of secondary spermatocytes, spermatids or mature sperm (from now on collectively referred to as sperm). Although the estimated fraction is relatively high, it is in agreement with high production of spermatophores by *Allacma fusca* (Dallai et al., 2009) and the estimate does not surpass that of other invertebrates. *Caenorhabditis elegans* can carry around 2000 germ cells, while their soma consist of precisely 959 cells (Sulston & Horvitz, 1977). Germ cells therefore represent ~67% of *C. elegans* cell count. A similar case is found among arthropods: Up to 75% of body cells in *Daphnia* males are sperm (Dufresne et al., 2019). It is important to note we specifically discuss the fraction of cells, not the biomass, as presumably, sperm are substantially smaller compared to other cell types in the body.

Taking advantage of the high sperm fraction, we demonstrated that all the sperm have exactly the same genotype which conclusively implies co-segregation of full chromosomal sets under the absence of recombination in this globular springtail. This conclusion was also supported by analyses of read pileups (**SM Text 4** and **SM Figure 5**). The analyses of pileups further revealed that the reference sample indeed also shows uneven coverage ratios of heterozygous alleles, although this signal was much weaker compared to the BH3-2 individual. We propose the reference individual could have reduced heterozygosity due to local inbreeding of the population that was sampled. Altogether, all results are in agreement with the PGE model (**Figure 2**) that has been previously proposed (Dallai et al., 2000).

We have shown a set of chromosomes is eliminated, but not whether the eliminated set is maternal or paternal. To provide definitive proof of PGE we would have to genotype both parents of a male as well as its sperm. Hypothetically, the eliminated chromosomes could be maternal. However, the elimination of maternal chromosomes during spermatogenesis has only even been observed in a rare form of androgenesis (Schwander & Oldroyd, 2016), a reproductive system in which males fertilize a female of a closely related sexual strain and cause elimination of the maternal genome as found in freshwater clam *Corbicula leana* (Komaru et al., 1998) or Australian carp gudgeons (Majtánová et al., 2021). However, this form of androgenesis requires a co-existence of lineages with canonical sexual reproduction with male androgenetic lineages, which is extremely unlikely in the case of globular springtails as the aberrant spermatogenesis seems to be present already in the common ancestor of globular springtails (Dallai et al., 1999, 2000, 2001, 2004). Paternal genome elimination on the other hand is a mode of reproduction that is conserved in at least six large

clades (**Figure 1**) and although with our data we also cannot completely exclude the possibility that the non-random chromosome elimination is associated with a different, as yet undescribed, evolutionary phenomena, paternal genome elimination is the only explanation compatible with known biology.

In particular, globular springtail reproduction most closely resembles the reproductive cycle of two dipteran families that also eliminate paternal chromosomes both in early spermatogenesis (what we call X chromosomes in these species) and during spermatogenesis (Gerbi, 1986; Metz, 1938). In both these two families females are frequently monogenic - each female produce broods of single sex only (Metz, 1931). So far this has not been tested in globular springtails, probably because they are both difficult to cultivate and show very little sexual dimorphism. Finally, the third genomic peculiarity found in both PGE fly families - they carry germ-line restricted chromosomes (Hodson et al., 2021; Metz, 1938), is a feature that is not shared in globular springtails as no differences between germ-line and soma karyotypes have been reported other than the aberrant spermatogenesis discussed in detail above.

Although we have tested this hypothesis in only a single globular springtail species *Allacma fusca*, the same type of aberrant spermatogenesis was demonstrated in seven species of five different families (**Supplementary table 1)** (Dallai et al., 1999, 2000, 2001, 2004). The most parsimonious explanation of the aberrant spermatogenesis in all the examined species is that PGE is the ancestral feature of globular springtails. Although we expect most of the globular springtails to retain this type of reproduction, there are multiple transitions to parthenogenetic reproduction (reviewed in (Chernova et al., 2010)). Other PGE clades usually show high conservation of this reproduction mode (Brown, 1965; Gerbi, 1986; Ross et al., 2012), the only known exception is found in louse. The human body louse seems to show a partial reversal to non-PGE sexual type of reproduction (de la Filia et al., 2018; McMeniman & Barker, 2005). Whether or not any globular springtail species have reverted to a more canonical type of reproduction is however an open question for further research.

Our study strongly suggests that globular springtails are the oldest and most species-rich clade reproducing via PGE. With 15,600 species estimated worldwide (Porco et al., 2014) globular springtails are a great clade to study the long term consequences of coping with PGE over hundreds of millions of years of evolution (Leo et al., 2019). This unusual mode of inheritance is likely to profoundly influence their evolutionary history. Recent theory suggests that haplodiploidy and PGE affect the evolution of reproductive isolation and increase diversification rates (Lohse & Ross, 2015; Patten et al., 2015). Springtails provide a great opportunity to test this theory as three of four springtail orders are species rich and allow us to estimate rates of diversification.

Paternal genome elimination also affects the dynamics of sexual conflict as shown in recently developed models (Hitchcock et al., 2021; Klein et al., 2021). Notably, it changes the relative role of X chromosomes and autosomes. Under PGE both X chromosomes and autosomes show bias in transmission between generations and sex alternation (see (Klein et al., 2021) for details), however, X chromosomes in globular springtails are also subjected to haploid selection in males. Unlike in species with normal diploid reproduction, the dominance of male beneficial alleles is the only factor that determines if they are more likely to get fixed on X chromosome (for recessive alleles) or anywhere in the genome (for dominant alleles)

(Klein et al., 2021). Therefore comparing the levels of sexual antagonism on X chromosomes and autosomes in globular springtails will allow the effect of dominance in sexual selection to be quantified, which has been a central question of sex chromosome evolution.

Besides rich biological interpretation, our study demonstrates the power of a careful bioinformatics analysis of whole genome sequencing data. The initial observation of suspiciously spaced coverage peaks was discovered during a routine quality control step using k-mer spectra analysis. Systematic scans for mixtures of two tissues in a single library could be more widely utilised to detect any peculiarities in reproduction that cause differences of karyotypes of male germline and soma such as the peculiar type of PGE in globular springtails.

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