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Paternal genome elimination in *Liposcelis* booklice (Insecta: Psocodea)

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ABSTRACT

How sex is determined in insects is diverse and dynamic, and includes male heterogamety, female heterogamety, and haplodiploidy. In many insect lineages, sex determination is either completely unknown or poorly studied. We studied sex determination in Psocodea, a species-rich order of insects that includes parasitic lice, barklice, and booklice. We focus on a recently discovered species of *Liposcelis* booklice (Troctomorpha: Psocodea), which are among the closest free-living relatives of parasitic lice. Using genetic, genomic, and immunohistochemical approaches, we show that this group exhibits paternal genome elimination (PGE), an unusual mode of sex determination that involves genomic imprinting. Controlled crosses, following a genetic marker over multiple generations, demonstrated that males only transmit genes they inherited from their mother to offspring. Immunofluorescence microscopy revealed densely packed chromocenters associated with H3K9me3, a conserved marker for heterochromatin, in males, but not in females, suggesting silencing of chromosomes in males. Genome assembly and comparison of read coverage in male and female libraries showed no evidence for differentiated sex chromosomes. We also found that females produce more sons early in life, consistent with facultative sex allocation. It is likely that PGE is widespread in Psocodea, including human lice. This order represents a promising model for studying this enigmatic mode of sex determination.

INTRODUCTION

Females and males are ubiquitous across the animal kingdom, yet how the sexes are determined is incredibly dynamic (Bachtrog *et al*. 2014; Beukeboom and Perrin 2014). Insects are an excellent demonstration of this diversity. For example, while the ancestral sex determination in insects is thought to be male heterogamety (i.e. XY or XO males), there have been several transitions to other modes, such as female heterogamety (i.e. ZW or ZO females, for example in butterflies and moths) and haplodiploidy (i.e. diploid females and haploid males, for example in wasps, bees, and thrips) (Blackmon *et al*. 2017). Even in lineages where the mode of sex determination is conserved, sex chromosomes and sex determining genes can change rapidly. For example, Vicoso and Bachtrog (2015) have recently found that although dipterans (i.e. flies) typically exhibit male heterogamety, there have been numerous gains and losses of sex chromosomes. Perhaps the most striking example of rapid evolution and diversity of sex determination systems in insects is that of the housefly, *Musca domestica,* which is polymorphic for male heterogamety, female heterogamety, and even temperature-dependent sex determination, driven largely by a highly mobile and variable master sex determining locus (Dübendorfer *et al*. 2002)

While there has been exciting progress on the genetics and evolution of sex determination in insects, there are enormous gaps in our knowledge. The factors that drive the rapid turnover of sex determination systems are not well

92 understood, although it is likely that conflicts over transmission and sexually
93 antagonistic genes both play important roles (Normark 2003; Kozielska *et al*.
94 2010; Bachtrog *et al*. 2014). The master sex determining gene has only been
95 identified in a handful of insects (Bell *et al*. 1988; Beye *et al*. 2003; Kiuchi *et*
96 *al*. 2014; Hall *et al*. 2015; Krzywinska *et al*. 2016). Furthermore, there remain
97 entire lineages of insects for which the mode of sex determination is not known
98 (Beukeboom and Perrin 2014).
99
100 In this paper, we fill this gap by studying sex determination in
101 Psocodea, a species-rich (~10,000 extant described species) order of insects that
102 includes parasitic lice, barklice, and booklice, and that is related to true bugs
103 and thrips (insects with incomplete metamorphosis and piercing, sucking
104 mouthparts) (Li *et al*. 2015). Until recently, Psocodea consisted of two separate
105 orders – Psocoptera (barklice and booklice) and Phthiraptera (parasitic lice).
106 However, molecular and morphological phylogenetic analyses clearly
107 demonstrate that Phthiraptera emerged from within Psocoptera (Yoshizawa and
108 Johnson 2003; Li *et al*. 2015), and are most closely related to Liposcelididae –
109 wingless, flattened booklice that include a number of cosmopolitan stored grain
110 pests.
111
112 Very little is known about sex determination in Psocodea. Cytological
113 studies concluded that male barklice have an XO (or rarely XY) karyotype
114 (Wong and Thornton 1966; Golub and Nokkala 2001, 2009). Nothing is known

about sex determination in booklice (Liposcelididae), and sex determination in parasitic lice is mysterious and as yet unresolved, but they do not appear to have heteromorphic sex chromosomes (Tombesi and Papeschi 1993; Golub and Nokkala 2004). Recently, the first genetic study of reproduction in parasitic lice (or in Psocodea for that matter) found a puzzling result. McMeniman and Barker (2006) followed the inheritance of microsatellite markers in human lice, *Pediculus humanus humanus*, and found that some heterozygous males transmit their genes in Mendelian fashion, while other males only transmit genes inherited from their mother.

We investigated the reproductive mode of a recently discovered species of *Liposcelis* (Liposcelididae), collected from the Chiricahua Mountains in Arizona (Perlman *et al*. 2015). *Liposcelis* occupies an interesting place in the psocodean evolutionary tree, as it is a member of the family that is the closest free-living relative (and sister group) of parasitic lice (Yoshizawa and Johnson 2003; Li *et al*. 2015). We used controlled crosses, immunohistochemistry, and genomic analysis to demonstrate that this lineage exhibits paternal genome elimination (PGE), an unusual mode of reproduction that has evolved independently in at least six clades of arthropods, including scale insects, phytoseiid mites, and fungus gnats and their relatives (Blackmon *et al.* 2017). We also show that females produce more sons early in life, consistent with the facultative sex allocation found in other species that exhibit paternal genome elimination. In organisms with paternal genome elimination, males arise from

fertilized eggs (in contrast to arrhenotokous haplodiploidy) but only transmit the genes they inherited from their mother. Much is still unknown about the mechanism of paternal genome elimination; however, genomic imprinting seems to be at the heart of this unusual form of reproduction (Herrick and Seger 1999). Altogether, this study fills a large gap in the insect tree of life in terms of how sex is determined, and documents a new case of paternal genome elimination, an interesting and unusual mode of sex determination.

MATERIALS AND METHODS

Culture information

Individuals of *Liposcelis* sp. were initially collected from the Chiricahua Mountains, Arizona, in 2010 (Perlman *et al*. 2015), and lab cultures were established. Individuals from our lab culture have been deposited in the insect collection at the Royal British Columbia Museum, Victoria, BC, while this species awaits formal description. (A maternally transmitted sex ratio distortion was previously reported in this species [Perlman *et al*. 2015], but note that this polymorphism is not present in the cultures used in this study.)

Colonies are maintained at approximately 27° and 75% relative humidity. We keep *Liposcelis* sp. in small glass canning jars (125ml) with the lid replaced with 70mm Whatman filter paper (Sigma-Aldrich). We rear them on a diet of 1:10 (weight: weight) mixture of Rice Krispies (Kellogg's) to cracked red wheat (Planet Organic). We check the colonies every second week

and replace food with new food as needed to avoid crowding in the colonies. It takes approximately 40 days for individuals to be reproductively mature. To obtain virgin females, we isolate them at their final nymphal stage (they are larger and have a rounder abdomen than males at this point). Males develop faster than females so we collect virgin males by isolating them before females of the same age develop into adults.

Inheritance experiment

We used controlled crosses over two successive generations to test for paternal genome elimination and departures from Mendelian inheritance. Our crossing scheme took advantage of a two allele polymorphism in the cAMP-specific IBMX-insensitive 3',5'-cyclic phosphodiesterase gene (Phos1 for short) in our lab culture of *Liposcelis* sp. (Perlman *et al*. 2015). By following the inheritance of Phos1 alleles, we were able to test two specific predictions: a) heterozygous females will transmit both alleles, and b) heterozygous males will only transmit the allele they inherited from their mother. We extracted DNA from single booklice using 30µl Prepman Ultra (ThermoFisher Scientific) according to manufacturer instructions (to yield 15µl of product). Individuals were genotyped after PCR amplification with the primers Phos1F (TCCCTTCCGTCAATAAATGC) and Phos1R (AATGTTCGAAATGCCGAGTC) using the following thermocycling conditions: 95°C×3min, (94°C×1min, 56°C×1min, 72°C×2min)×35, 72°C×10min. Sequencing was performed by Sequetech (California, USA). We

184 scored individuals as either homozygous or heterozygous by examining
185 chromatograms for double peaks, using Geneious 6.1.8. See Figure S1 and
186 Figure S2 for visualization of Phos1 alleles and an example of our crossing
187 setup.
188
189 For the first generation of the experiment, we set up 15 small petri
190 dishes, each containing one virgin male and three virgin females, along with
191 0.5g of food. Females were left with the male for two weeks, after which the
192 male was removed and his DNA extracted. We transferred the females into
193 individual dishes with the same amount of food and left them for two weeks to
194 lay eggs when we transferred them into new dishes and left them for another
195 two weeks before extracting their DNA. We sequenced the Phos1 region of
196 each male and female and noted the possible offspring genotypes each cross
197 could produce.
198
199 We sequenced the F1 offspring from several types of parental crosses to
200 determine whether all expected offspring genotypes were present in the F1
201 generation. The three types were: 1) heterozygous male mated to homozygous
202 female, 2) homozygous male mated to heterozygous female, and 3)
203 heterozygous male mated to heterozygous female. Offspring from pairings in
204 which the male parent was heterozygous (type 1 and 3) should be missing an
205 expected genotype if the male is only transmitting one allele, as expected if
206 PGE is present in the system. Pairings in which the male parent was

homozygous but the female parent was heterozygous (type 2) were screened to assess whether the female is transmitting both alleles.

Finally, we set up crosses between F1 individuals, ensuring that they were isolated before they mated. Here, we only used males that were potentially informative, i.e. we did not use males whose parents had the same homozygous genotype. We checked dishes weekly, removing the F1 father once F2 nymphs were observed, and preserving him in 95% EtOH. We left the F1 female in the dish for another two weeks, then removed and preserved her in 95% EtOH. We allowed the offspring to develop for another 2 weeks. We then sequenced the Phos1 region of all of the F2 offspring whose F1 fathers were heterozygous. We only sequenced individuals from crosses that had produced more than 8 offspring. We analyzed whether the F1 males transmitted both the alleles they inherited to F2 offspring. To do this, we determined which allele each F1 male inherited from his mother or father, and used Fisher's exact tests to determine whether F2 offspring exhibited deviations in the expected allele frequencies inherited from their paternal grandmother and grandfather. Crosses were pooled based on the expected genotypes in the F2 offspring (i.e. whether three or two genotypes were possible in the F2 generation).

Screening for differentiated sex chromosomes in *Liposcelis* sp.

We compared read coverage from high-throughput sequencing of males and females to test for the presence of differentiated sex chromosomes in

Liposcelis sp., and to ensure the Phos1 marker used for the inheritance study is not associated with a sex chromosome. To do this, we assembled a draft genome of *Liposcelis* sp., and mapped reads to the assembled contig set (GenBank accession: BioProject ID PRJNA355858). Briefly, DNA was extracted from separate pools of male and female *Liposcelis* sp. (~80 individuals; Qiagen DNEasy kit) and sequenced using 100 bp PE Illumina HiSeq following library construction at Genome Quebec; these reads were combined with previously generated sequence (Perlman *et al*. 2015) for assembly. Assembly was done using Ray v 2.2.0 (k = 31; Boisvert *et al*. 2012), with ~123 M 100 bp PE reads to generate an assembly of ~264 Mb and a contig N_{50} of 4,617 bp. Raw reads from female-specific (~44 M) and male-specific (~53 M) libraries were mapped to the assembly using bwa mem (Li 2013) and high quality read mappings (mapq > 10) retained and quantified using samtools (Li *et al*. 2009). Raw read mappings were normalized as counts per million mapped reads (CPM), with contigs > 1000 bp retained in the analysis (Vicoso and Bachtrog 2015).

Immunofluorescence microscopy

Paternal genome elimination often results in condensation of paternal chromosomes in male somatic and/or germ tissue (Brun *et al*. 1995; Bongiorni *et al*. 2004, 2007). To test for the presence of condensed chromosomes in male booklice, we conducted immunofluorescence microscopy with an antibody for H3K9me3, a conserved marker for heterochromatin (Cowell *et al*. 2002). We

253 conducted immunofluorescence staining on female and male *Liposcelis*
254 abdominal tissue. The abdomen was used for staining since we wanted to
255 include both reproductive tissue and somatic tissue in the preparations to
256 explore the specificity of heterochromatinization.
257
258 The immunofluorescence protocol we used was adapted from Bongiorni
259 *et al.* (2007), who previously used this approach to study paternal genome
260 elimination in the mealybug *Planococcus citri*. Briefly, virgin female and male
261 *Liposcelis* were collected in Bradley-Carnoy fixative (4:3:1 chloroform:
262 ethanol: acetic acid), followed by fixation and dissection in a drop of 45%
263 glacial acetic acid on siliconized coverslips. After dissection to isolate
264 abdominal tissues, siliconized coverslips were squashed on poly-L-lysine
265 (Sigma, P8920) coated microscope slides (which transferred the tissue to the
266 slide) followed by freezing in liquid nitrogen. Coverslips were removed with a
267 razor blade and tissues permeabilized by incubating the slide in 1xPBS
268 containing 1% Triton X-100 and 0.5% acetic acid. Slides were washed three
269 times in 1xPBS for 5 minutes and blocked in 1% BSA in PBST (1xPBS +0.1%
270 Tween 20) for 30 minutes at room temperature, followed by incubation with a
271 rabbit primary antibody targeting H3K9me3 (Cell Signaling Technology
272 9754S- 1:200) in 1% BSA in PBST for 1 hour in a humid chamber. Slides were
273 then washed 3 times in 1xPBS followed by incubation with anti-rabbit Alexa
274 Fluor 488 secondary antibody (Invitrogen A-11008- 1:500) for 1 hour in a
275 humid chamber and 3 washes in 1xPBS as above. DAPI containing mounting

media (Sigma F6057) was used to counterstain for DNA and slides were sealed with nail polish. Slides were imaged on a Leica DM IRE2 inverted fluorescent microscope.

Sex allocation in *Liposcelis* sp. females

A major prediction of systems with paternal genome elimination is maternal control over offspring sex ratio (Haig 1993; Varndell and Godfray 1996; Nagelkerke and Sabelis 1998; Sanchez 2010). We set up an experiment to test whether females exhibit facultative sex allocation by examining whether female age and rearing condition affect sex ratio. We placed approximately 200 late instar female nymphs and 200 males into jars (125ml, 70 mm diameter) containing a small amount of food. We left females for 7 days so they had an opportunity to mature and mate before transferring them into petri dishes (35mm in diameter) containing 1.7g of food. The experiment consisted of three treatments: a low, medium, and high-density treatment with 2, 10, or 20 females in each dish, and 5 replicate dishes for each treatment. We also kept 3 males in each dish to ensure females were not sperm limited, replacing males when necessary. Adults were transferred into new dishes weekly for 4 weeks, upon which the experiment was terminated.

We measured the sex ratio (measured as the number of offspring of each sex reaching adulthood) produced by females in each replicate each week, which allowed us to measure both the total sex ratio for each treatment and also

how sex ratio changed over time. If more than 20% of the females in a replicate died we stopped recording data from that replicate. This occurred for one replicate in the low-density treatment in week three and one replicate in the medium-density treatment in week four. We analyzed data in RStudio v3.1.0 (R Core Team 2014) using a generalized linear mixed model with a binomial error distribution and logit link. We used a model selection process, choosing the model that minimized the AIC and including female density and the week the data was collected as explanatory variables, and replicate as a random variable.

Data availability

File S1 contains supplementary information including information on genotypes in the inheritance study and additional results from the immunohistochemistry staining. Illumina sequence data is deposited in GenBank (NCBI) under BioProject ID PRJNA355858 and allele inheritance study sequence data under accessions KY454577 and KY454578.

RESULTS

Transmission distortion of Phos1 allele in males

We sequenced 155 F1 offspring from 14 crosses with 10 males mated to up to three different females (Table 1). We found that heterozygous females mated with homozygous males (i.e. type 2 crosses 11-1, 9-1, and 6-3) produced offspring with both of the expected genotypes, indicating that females transmit both of their alleles. On the other hand, crosses involving heterozygous males

(type 1 and 3) did not produce genotypes that would be expected under standard diploid Mendelian inheritance. These crosses were always missing one of the expected offspring genotypes. Heterozygous males mated to more than one female (for example in crosses 12-1 and 12-2) always transmitted the same allele to offspring.

Table 1. F1 offspring genotypes. Only crosses that produced more than six offspring were included in the table. Cross type indicates whether only the male (type 1), the female (type 2), or both parents (type 3) were heterozygous. Dashes indicate genotypes that are not expected to be present in the offspring. Every cross in which the male parent is heterozygous is missing an expected offspring genotype.

Parents					**F1 Offspring**			
Male	Male Genotype	Female	Female Genotype	Cross Type	AA	Aa	Aa	Total
1	Aa	1-2	Aa	3	0	3	6	9
4	Aa	4-1	AA	1	0	6	−	6
		4-2	Aa	3	0	4	4	8
		4-3	Aa	3	0	6	9	15
5	Aa	5-2	AA	1	0	6	−	6
6	AA	6-3	Aa	2	6	2	−	8
8	Aa	8-1	Aa	3	0	5	5	10
9	aa	9-1	Aa	2	−	2	7	9
10	Aa	10-3	Aa	3	0	8	6	14
11	AA	11-1	Aa	2	7	9	−	16
12	Aa	12-1	AA	1	10	0	−	10
		12-2	aa	1	−	15	0	15
14	Aa	14-1	AA	1	14	0	−	14
		14-3	Aa	3	6	9	0	15

Our F2 crosses, using heterozygous F1 males, confirmed that males only transmit one allele to offspring, and allowed us to determine that allele's parent-of-origin. We sequenced 115 F2 offspring from 11 crosses and found

that in all cases males transmitted exclusively the allele that they inherited from their mother to offspring (Table 2; Figure 1) ($p<0.0001$ for all comparisons).

Table 2. F2 offspring genotypes produced by heterozygous F1 males mated to F1 females. Each male parent received the allele in red from his mother and the one in blue from his father. Dashes indicate offspring genotypes not expected to be present under standard diploid Mendelian inheritance. In every case, the male only transmitted the allele he inherited from his mother to offspring. (Note that cross 6-2 is not included in Table 1 as all offspring from this cross were expected to be heterozygous – parents were AA*aa).

F1 Parents			**F2 Offspring**			
Male	Male Genotype	Female Genotype	AA	Aa	aa	Total
4-1M2	Aa	aa	–	9	0	9
4-1M4	Aa	aa	–	13	0	13
4-3M5	Aa	AA	10	0	–	10
5-2M1	Aa	AA	6	0	–	6
5-2M4	Aa	Aa	5	6	0	11
6-2M1	Aa	aa	–	0	10	10
8-1M2	Aa	AA	14	0	–	14
9-1M1	Aa	AA	10	0	–	10
12-2M5	Aa	Aa	0	3	7	10
12-2M9	Aa	Aa	0	7	8	15
14-3M6	Aa	Aa	0	1	6	7

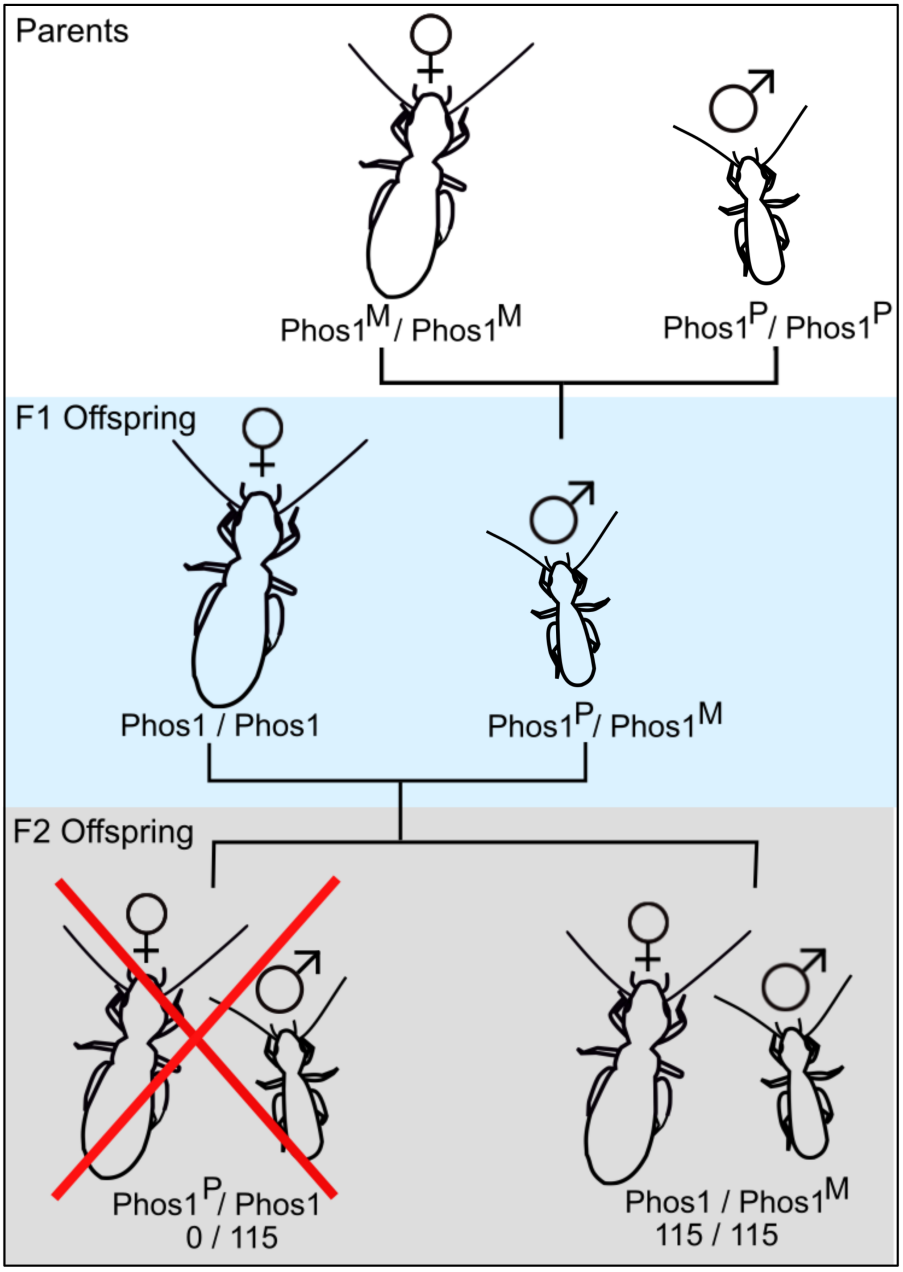

Figure 1. Schematic of cross experiment design, as well as the results from the F2 generation. Phos1 indicates the cAMP-specific IBMX-insensitive 3',5'-cyclic phosphodiesterase gene region used for sequencing and the superscripts M and P indicate that the allele is maternal or paternal in the parental generation, respectively. All offspring in the F2 generation carry the allele transmitted to them from their paternal grandmother.

No evidence for a differentiated sex chromosome in *Liposcelis* sp.

Following the logic of recent studies using next-generation sequencing approaches to characterize sex-determination systems (Vicoso and Bachtrog 2015), we assembled a genome combining female and male derived reads. We mapped raw reads to this assembly to identify contigs at ½ the coverage in males relative to females (and vice versa) that may represent portions of sex chromosomes. A histogram of the log2 male/female read coverage for contigs in this assembly (as read counts per million reads mapped) had a single discernible peak with a median near 0 (Figure 2; median = -0.03), representing equal read coverage in male and female libraries, lending little support to the existence of a differentiated sex chromosome. Importantly, our Phos1 marker does not show differential read coverage between males and females, suggesting that it does not lie in an atypical (or sex-linked) part of the genome.

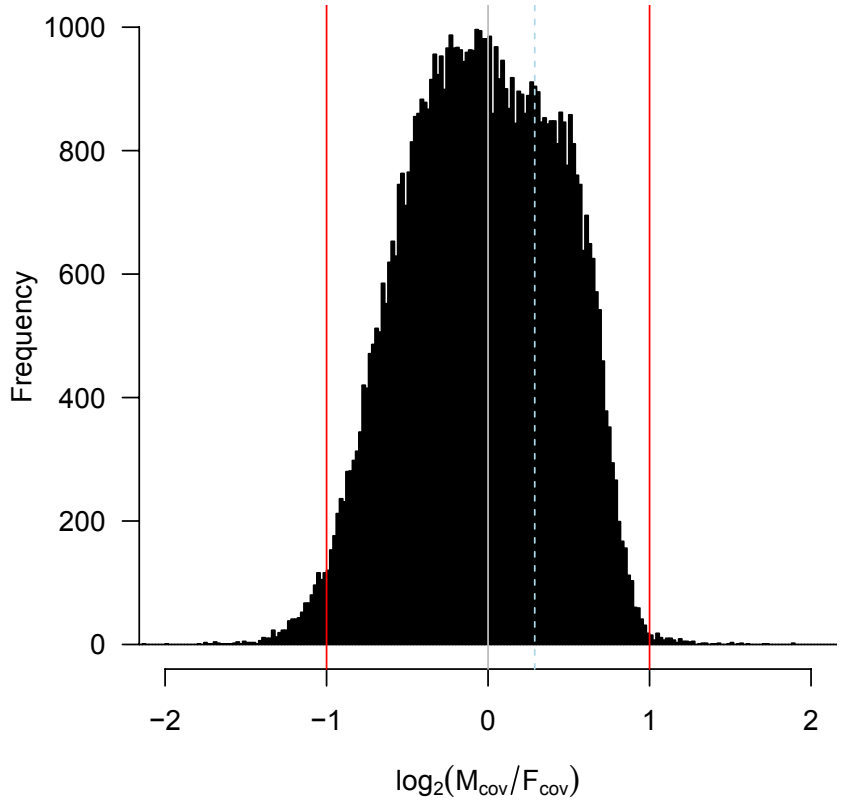

Figure 2. Histogram comparing the coverage of male to female reads mapping back to the *Liposcelis* sp. genome contigs. Reads at zero have the same coverage in males and females. Reads mapping to -1 are found at double the frequency in females than males (as would be expected for sex-restricted contigs under male heterogamety). The dashed line represents the position of the Phos1 marker used in inheritance experiments.

Heterochromatic chromocenters are present in males

DAPI staining revealed condensed regions of intense fluorescence (i.e. chromocenters) present throughout male abdominal tissue but not female abdominal tissue (Figure 3; Figure S3). Additionally, H3K9me3 fluorescence that colocalized with DAPI staining was present in male but not female cells (Figure 3). This indicates that these regions are likely heterochromatinized in

males. Condensed heterochromatic regions were also present in head and thoracic tissue in *Liposcelis* sp. (Figure S4).

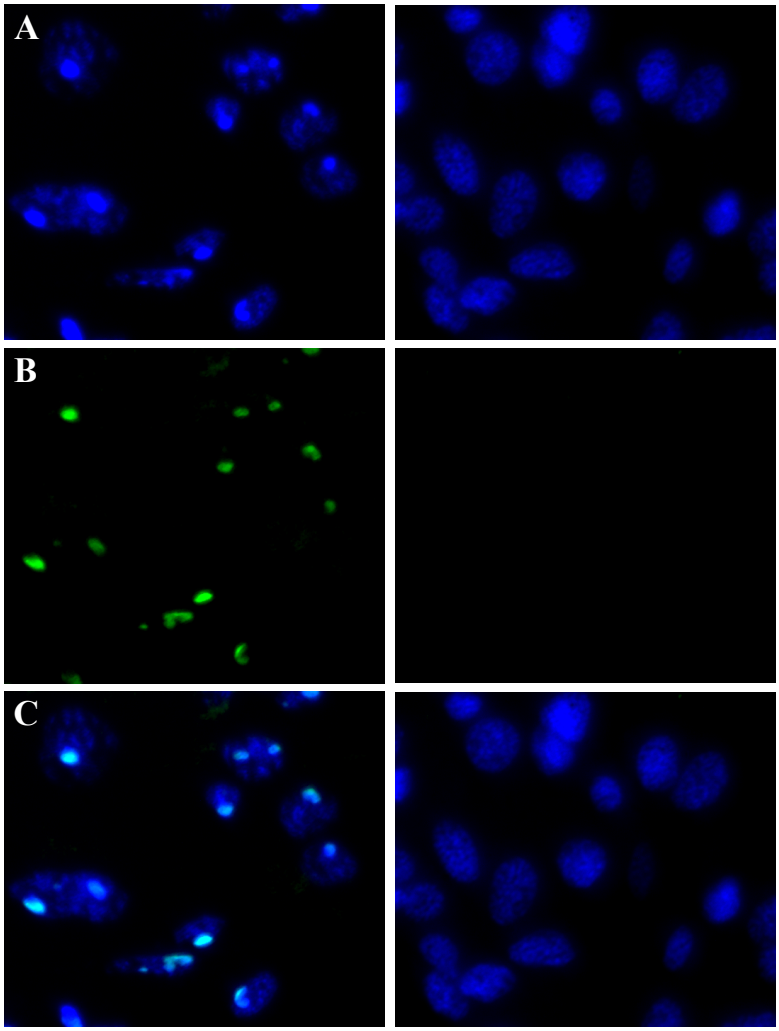

Figure 3. DAPI (A), H3K9me3 (B), and merged (C) images of male (left panels) and female (right panels) *Liposcelis* sp. abdominal tissue. Condensed regions of DAPI staining that colocalize with H3K9me3 staining are present in male tissue but absent from female tissue indicating chromocenters are present in male cells. The scale bars represent 5um.

Sex ratio varies with female age

Females in all treatments produced offspring with a female biased sex ratio (Figure 4) (Sex ratio (# males/ total offspring)= 0.40±0.12, 0.32±0.08, 0.30±0.08 for low, medium and high density treatments respectively). However, offspring sex ratio varied with maternal age. In all treatments, when females were young they produced more sons compared to when they aged (generalized linear model: $p<0.001$). For example, in the first week of the experiment, when females had just become adults, the offspring sex ratio was 0.59 for all treatments, as opposed to the last week of the experiment when it averaged 0.13. These differences were unlikely to be due to differential offspring mortality, as females produced comparable numbers of offspring across treatments (mean offspring produced per female per week: 4.9, 5.4, 7.1 for high, medium, and low density treatments respectively) and over time (mean offspring produced per female per week: 4.5, 4.2, 5.4, 5.9 for weeks 1-4 respectively). Finally, density had a small but significant effect on sex ratio with females in the low density treatment producing a slightly more male biased sex ratio than females in the other density treatments (generalized linear model: $p=0.015$).

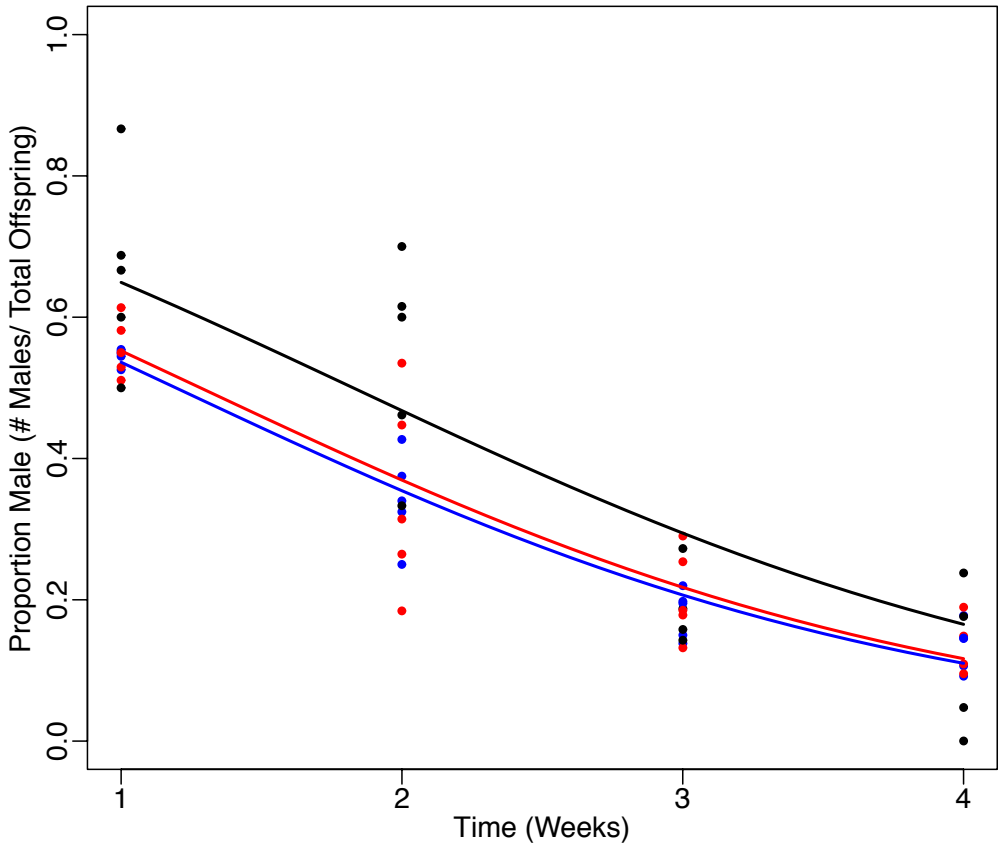

Figure 4 Sex ratio (# males/ total offspring) produced by *Liposcelis* sp. females as they aged. Females produce a female biased sex ratio overall, which varied as females aged, with a more male biased sex ratio when females were young compared to when they were older. Black, red, and blue data points indicate the low, medium, and high density treatments respectively.

DISCUSSION

We explored the mode of reproduction and sex determination in *Liposcelis* sp., and found that this species exhibits paternal genome elimination. Within males, paternally-inherited chromosomes were never transmitted to offspring. Also, immunofluorescence microscopy revealed the presence in males, but not in females, of densely packed chromocenters associated with

H3K9me3 (an epigenetic mark associated with heterochromatinization), suggesting that paternal chromosomes are silenced in males. This is an exciting finding as this is the first species in the order Psocodea in which paternal genome elimination has been conclusively demonstrated. An earlier study found that some, but not all, male human body lice, *Pediculus humanus*, transmit only the genes that they inherit from their mother (McMeniman and Barker 2006), suggesting that paternal genome elimination may be widespread in this order, although in *P. humanus* it is not clear why all males did not exhibit this chromosome inheritance pattern.

PGE has been documented in five other arthropod orders: mites (Phytoseiidae, Otopheidomenidae, and Ascoidea), flies (it has evolved twice, in Sciaridae - fungus gnats and Cecidomyiidae - gall midges), springtails (Symphypleona), beetles (Cryphalini - bark beetles), and scale insects (Neococcoidea) (Metz 1938; Helle *et al*. 1978; Nur 1980; Stuart and Hatchett 1988; Brun *et al*. 1995; Dallai *et al*. 2000). In all of these lineages, males develop from fertilized eggs but fail to transmit chromosomes they inherited from their fathers. However, how paternal genome elimination occurs in these lineages is quite different. In sciarid and cecidomyiid flies, and in symphyleonan springtails, paternally-inherited sex chromosomes (but not autosomes) are ejected during male development, often in complex combinations (Metz 1938; Stuart and Hatchett 1988; Dallai *et al*. 2000). On the other hand, mites, bark beetles and scale insects do not have sex chromosomes

463 at all. Instead, the entire paternal chromosome complement is eliminated or
464 inactivated in males (Nur 1980; Nelson-Rees *et al*. 1980; Brun *et al*. 1995).
465 Paternal chromosomes can be heterochromatinized early in development and
466 excluded from viable sperm during spermatogenesis (e.g. Lecanoid and
467 Comstockiella scale insects), or they can be lost entirely in early development
468 in males (Diaspidid scale insects) (Ross *et al*. 2010a). The lack of consistent
469 molecular features makes it difficult to diagnose paternal genome elimination in
470 species without extensive investigation into male meiosis or crossing
471 experiments that follow alleles in males over several generations. Because of
472 this, it is likely that PGE is present in more species than it has been identified in
473 to date.
474
475 Our finding of heterochromatinization occurring throughout male but
476 not female abdominal tissue, and the lack of an obvious sex chromosome in our
477 genomic analysis, suggests that paternal genome elimination in *Liposcelis*
478 booklice is likely similar to the Lecanoid/Comstockiella systems in scale
479 insects, with paternal chromosomes being heterochromatinized in male body
480 tissues as well as the germline, rather than being eliminated in somatic tissue, or
481 present but not heterochromatinized (Ross *et al*. 2010a). In many species that
482 exhibit PGE, paternal chromosomes are epigenetically silenced in males
483 through heterochromatinization and form a large chromocenter; this has been
484 best studied in scale insects, particularly the citrus mealybug *P. citri* (Bongiorni
485 *et al*. 2004, 2007). Heterochromatinization is thought to occur through

imprinting, as paternal chromosome heterochromatinization occurs soon after fertilization, before embryonic genes are highly expressed (Sabour 1972). Paternal genome heterochromatinization in males involves many of the same components that are involved in facultative heterochromatinization in other animals. For instance, H3K9me3 is involved in paternal chromosome heterochromatinization in *P. citri,* and *Liposcelis*, and also in X-chromosome inactivation in mammals (Cowell *et al*. 2002).

Although the lineages in which PGE occurs are taxonomically widespread, they share some striking similarities in their ecology. Species that exhibit PGE are typically small and have limited dispersal throughout their life, resulting in a high degree of mating between close relatives. The reason for the association between PGE and inbreeding remains unclear. Several theoretical studies have proposed that inbreeding promotes the evolution of PGE and other asymmetric genetic systems (Hamilton 1967; Haig 1993; Gardner and Ross 2014; alternatively see Bull 1979); however, there has been little empirical work quantifying the level of inbreeding in PGE species and related taxa. *Liposcelis* exhibit many of the ecological factors that are associated with PGE, being small, wingless, and with limited dispersal, which may result in a high degree of inbreeding. Obtaining estimates of sex ratio and inbreeding in wild *Liposcelis* may help elucidate why there is an association between inbreeding and PGE.

Additionally, species with PGE often have female biased sex ratios with maternal control over the offspring sex ratio. This has been studied best in mites (Helle *et al*. 1978, Nagelkerke and Sabelis 1998) and scale insects (Varndell and Godfray 1996; Ross *et al*. 2010b, 2012). The results from our controlled lab experiments point towards maternal control of sex ratio in *Liposcelis* sp. We found highly female-biased sex ratios in *Liposcelis* sp., which altered as a female aged, with a more male biased sex ratio produced when females were young. The finding that females produce more males early in reproduction is intriguing, as something similar was found in *P. citri* (Ross *et al*. 2012); we speculate that this might be driven by the need to ensure mating in groups with little dispersal. It is unlikely that the sex ratio differences we observed were due to differential mortality, as females produced approximately the same amount of offspring each week in the experiment. To confirm that females are able to control offspring sex ratio, it would be interesting to conduct similar experiments in more natural settings, and alter other ecological factors such as relatedness of individuals and resource availability.

It is likely that paternal genome elimination is widespread in Psocodea, and is perhaps the mode of sex determination for the entire lineage that includes Liposcelididae and Phthiraptera (Yoshizawa and Johnson 2010). A number of features strongly suggest that human parasitic lice, *P. humanus* (and probably other parasitic lice) exhibit PGE. First, as mentioned earlier, a previous study found that some, but not all, male *P. humanus* only transmitted their maternal

532 copy of microsatellite markers (McMeniman and Barker 2006). Additionally,
533 human lice do not have sex chromosomes (Tombesi and Papeschi 1993; Golub
534 and Nokkala 2004; Bressa *et al*. 2015) and exhibit highly female-biased sex
535 ratios (Buxton 1941).
536
537 Parasitic lice also have unusual spermatogenesis and sperm morphology
538 that have been suggested to be linked to PGE (Ross and Normark 2015;
539 Blackmon *et al*. 2017). Spermatogenesis is highly distinctive in parasitic lice,
540 consisting of several mitotic divisions at the end of spermatogenesis, the last
541 one being unequal and resulting in half the products of the mitotic division
542 forming functional sperm and the other half forming non-functional pycnotic
543 nuclei (Hindle and Pontecorvo 1942; Tombesi and Papeschi 1993; Golub and
544 Nokkala 2004). Although little is currently known about spermatogenesis in
545 Liposcelididae, this group is known to have an unusual sperm morphology
546 which is also present in *P. humanus* and other lice species (Dallai and Afzelius
547 1991; Ross and Normark 2015) where sperm contain two axonemes rather than
548 the usual single one (King and Ahmed 1989).
549
550 Even if PGE is widespread in Psocodea, it is likely to be quite different
551 between parasitic lice and booklice. Cytogenetic studies of parasitic lice (Golub
552 and Nokkala 2004; Bressa *et al*. 2015) report males and females having the
553 same number of chromosomes, and do not mention any differences in the
554 appearance of chromosomes in males and females, suggesting that male

555 chromosomes may not be heterochromatinized. Thus Psocodea represents an
556 exciting new model for studying the evolution, ecology, and genetics of
557 paternal genome elimination, an enigmatic and interesting mode of sex
558 determination. The ease with which booklice can be maintained in the
559 laboratory compared to other arthropods with paternal genome elimination
560 makes them especially promising for study.
561
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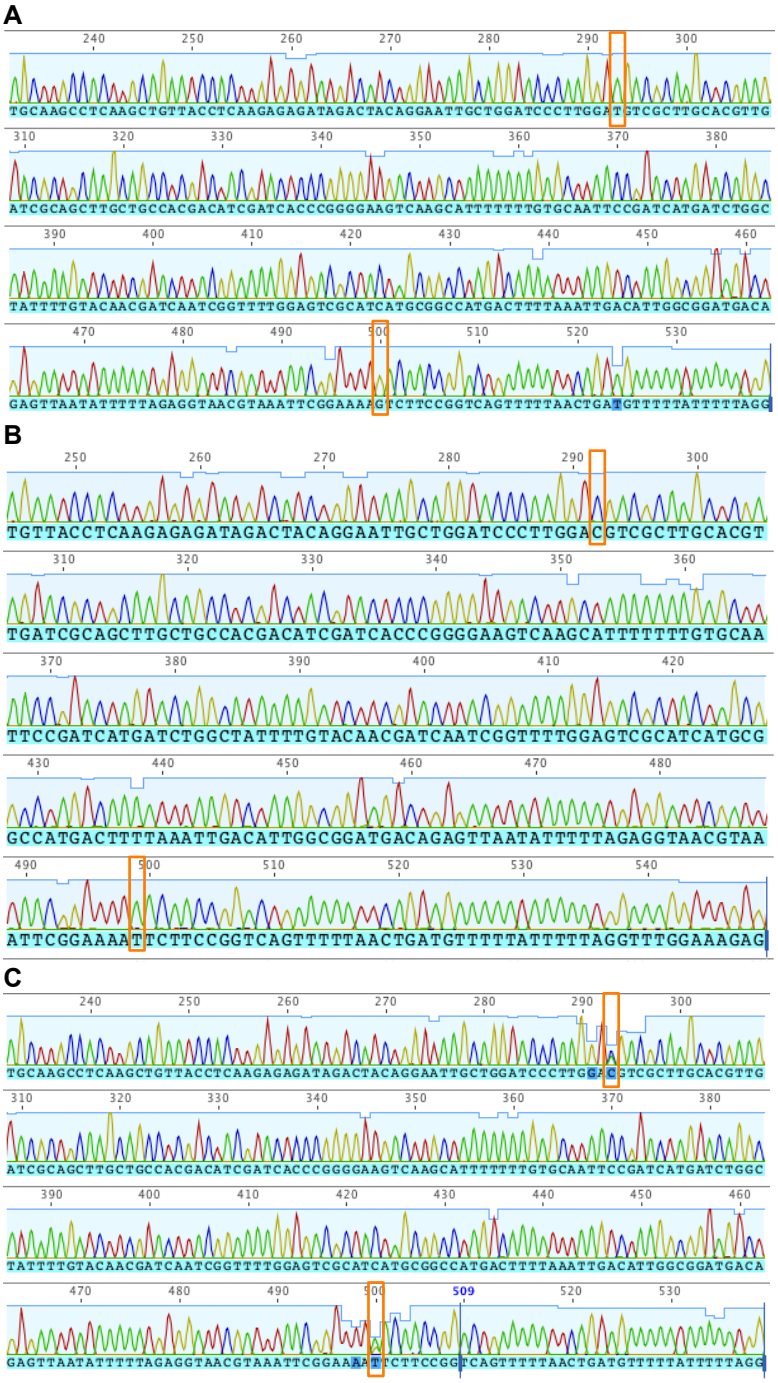
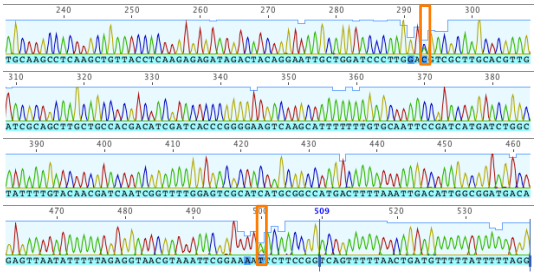


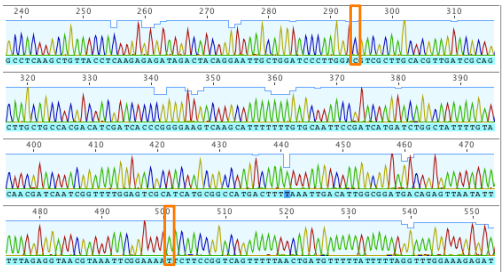
Figure S1. Chromatograms of genotypes ‘aa’ (A), ‘AA’ (B), and ‘Aa’ (C) from the allele inheritance experiment. Orange boxes indicate regions that differ between the genotypes.

A

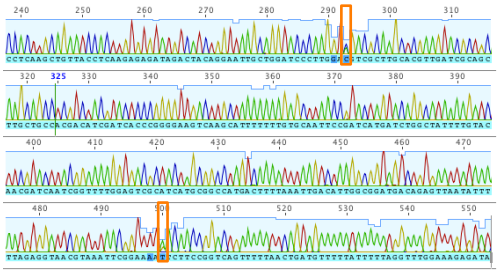
Father 4M: Aa



Mother 4-1: AA

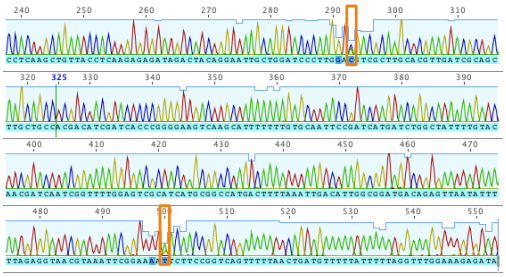


4-1 F1 Offspring: Aa N=6

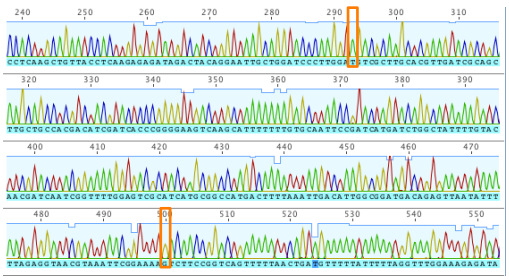


B

F1 Father 4-1M2: Aa (A from mother, a from father)



F1 Mother 4-3F5: aa



F2 Offspring Aa N=9

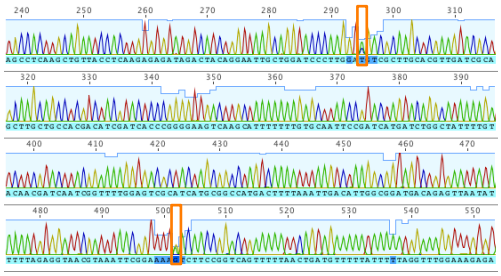


Figure S2. Schematic of the results of the allele inheritance experiment for family 4-1. The first generation cross (A) generated heterozygous F1 offspring. F1 males from this family (e.g. 4-1M2) were crossed to F1 females to generate F2 offspring (B). The F1 male 4-1M2 transmitted the allele he inherited from his mother to all F2 offspring.

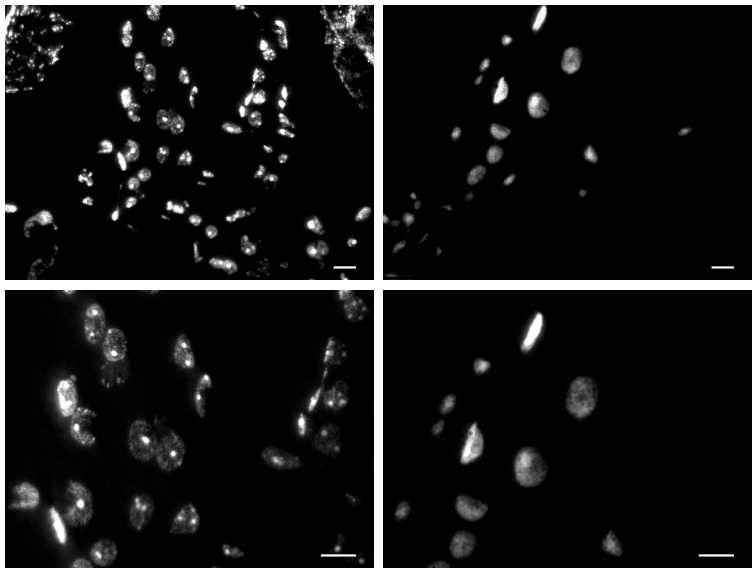


Figure S3. DAPI stained male (left panels) and female (right panels) abdominal tissue in *Liposcelis* sp. Male tissue contains dense regions of staining within cells indicating the presence of condensed chromosomes in these cells. These condensed regions are absent in female cells. The scale bars represent 10μm.

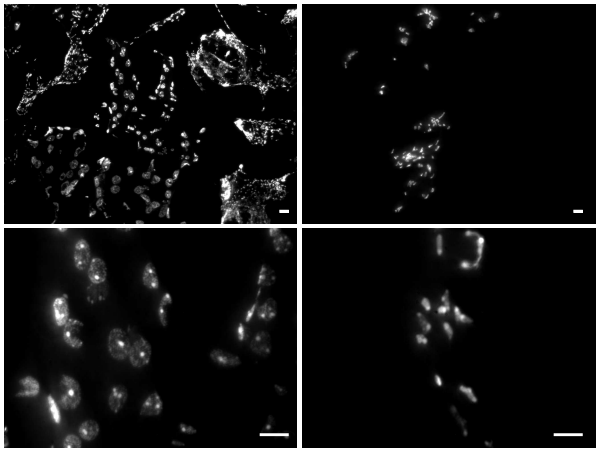


Figure S4. Male *Liposcelis* sp. abdominal (left panels) and head/thorax (right panels) tissue stained with DAPI. Both abdominal and head/thorax tissues contain regions of condensed staining in cells (chromocenters). Scale bars represent 5μM.