Research article

Disentangling the causes for faster-X evolution in aphids

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Running title: X chromosome evolution in aphids

Abstract

Faster evolution of X chromosomes has been documented in several species and results from the increased efficiency of selection on recessive alleles in hemizygous males and/or from increased drift due to the smaller effective population size of X chromosomes. Aphids are excellent models for evaluating the importance of selection in faster-X evolution, because their peculiar life-cycle and unusual inheritance of sex-chromosomes lead to equal effective population sizes for X and autosomes. Because we lack a high-density genetic map for the pea aphid whose complete genome has been sequenced, we assigned its entire genome to the X and autosomes based on ratios of sequencing depth in males and females. Unexpectedly, we found frequent scaffold misassembly, but we could unambiguously locate 13,726 genes on the X and 19,263 on autosomes. We found higher non-synonymous to synonymous substitutions ratios *(dN/dS*) for X-linked than for autosomal genes. Our analyses of substitution rates together with polymorphism and expression data showed that relaxed selection is likely to contribute predominantly to faster-X as a large fraction of X-linked genes are expressed at low rates and thus escape selection. Yet, a minor role for positive selection is also suggested by the difference between substitution rates for X and autosomes for male-biased genes (but not for asexual female-biased genes) and by lower Tajima's D for X-linked than for autosomal genes with highly male-biased expression patterns. This study highlights the relevance of organisms displaying alternative inheritance of chromosomes to the understanding of forces shaping genome evolution.

Keywords: Sex chromosome, sex-biased expression, evolutionary rates, hemizygosity, selection, drift.

Introduction

Sex chromosomes are major players in evolution. Besides their role in sex determination, sex chromosomes contribute to genomic conflicts (Rice 1984; Meiklejohn and Tao 2010; Soh et al. 2014), genetic incompatibilities and reproductive isolation (Coyne and Orr 2004; Saether et al. 2007; Kitano et al. 2009; Johnson and Lachance 2012). A pair of sex-determining chromosomes typically evolves toward reduced recombination rates (crossing overs), which eventually causes one of the sex chromosomes to gradually lose most of the chromosomal regions (loci) present in the alternate one (Charlesworth et al. 2005). These loci will thus be found in single copy in the sex that carries the degenerate, smaller sex chromosome. When the heterogametic sex is the male, sex chromosomes are denoted X and Y (e.g., in mammals), whereas when it is the female, sex chromosomes are noted W and Z (e.g., in birds). Alleles of loci present only on the X (W) are more exposed to selection in individuals of the heterogametic sex, facilitating the fixation of beneficial mutations and the purging of deleterious ones (Charlesworth et al. 1987). On the other hand, because males (XY) bear and transmit a single X chromosome, effective population size is smaller for the X compared to autosomes (Wright 1931; Caballero 1994; Caballero 1995). This increases the rate of fixation of slightly deleterious mutations on the X by genetic drift (Kimura 1983) (the same principles apply to ZW systems, so we ignore these in the following). Consequently, X-linked genes may evolve faster than autosomes ("faster-X" evolution) due to higher positive selection (rate of fixation of beneficial mutations) and/or higher genetic drift (rate of fixation of slightly deleterious mutations) (Vicoso et al. 2009, Mank et al. 2010). Faster evolution of X-linked proteins is supported by observations in a large panel of species (e.g., *Drosophila*, nematodes, mammals, birds, see Meisel and Connallon 2013 for a review). In some, a preponderant effect

of drift was demonstrated (Mank et al. 2010; Avila et al. 2014), while positive selection would play a predominant role in other species (Baines et al. 2008; Hvilsom et al. 2012; Langley et al. 2012; Mackay et al. 2012; Kousathanas et al. 2014; Sackton et al. 2014; Avila et al. 2015).

In this context, organisms with atypical inheritance of sex chromosome can greatly facilitate inferences about the processes contributing to the evolution of sex chromosomes (Bachtrog et al. 2011). Among those, aphids, which present X0 males and XX females, have equal effective population sizes for X chromosomes and autosomes (Jaquiéry et al. 2012a). This eliminates one possible confounding factor of faster-X evolution, the smaller effective population size of the X, helping to properly test its causes. Aphids reproduce by cyclical parthenogenesis, such that males and sexual females constitute only a short part of their life-cycle, which is dominated by apomictic parthenogenetic (clonal) XX females (figure 1). Males are produced asexually via the elimination of one X from the germ line (Wilson et al. 1997; Caillaud et al. 2002). As a result, X-linked recessive alleles are exposed to selection in male aphids, just like in other X0 or XY males. However, because all sexually-produced aphid eggs are XX females, all the progeny inherit their X from males and sexual females in equal proportions. This difference from other heterogametic systems, where progeny present even sex ratios, has deep consequences for the evolutionary trajectory of the aphid X chromosome (Jaquiéry et al. 2012a). Furthermore, in contrast to standard systems, variance in reproductive success between sexes, population expansion, bottlenecks and sex-biased dispersal should not differentially affect sex chromosomes and autosomes in aphids (Jaquiéry et al. 2012a). Mutation and recombination rates are also expected to be equal across chromosomes because of their similar mode of inheritance and the complete absence of crossing overs in males (Jaquiéry et al. 2012a). These similarities between X chromosomes

and autosomes make aphids exceptionally useful to pinpoint the causes of faster-X evolution, since the factors mentioned above need not be accounted for. Still, a notable difference between X and autosome in aphids is the theoretical propensity of the X to accumulate sexually antagonistic mutations beneficial for males and detrimental to asexual females, which also is the consequence of cyclical parthenogenesis combined with the inheritance of the X (Jaquiéry et al. 2013).

Empirical analyses on a small subset of genes of the pea aphid (*Acyrthophon pisum*) showed that X-linked genes evolve faster than autosomal genes (Jaquiéry et al. 2012a) and that genes expressed predominantly in males (hereafter "male-biased" genes) predominantly locate on the X (Jaquiéry et al. 2013). Subsequent genome-wide analyses did not however support faster-X evolution (Purandare et al. 2014), and mitigated the degree of enrichment of male-biased genes on the X (Purandare et al. 2014; Pal and Vicoso 2015). These discrepancies however likely stem from the fact that these two studies did not assign individual genes to chromosome types, but entire scaffolds, which present assembly errors (as shown by Bickel et al. 2013 and suggested by Jaquiéry et al. 2013). Misassignment of genes to chromosomes would indeed artificially decrease the contrast between X-linked and autosomal genes.

Here, we aimed to go past these shortcomings in order to fully disentangle the causes for faster-X evolution in aphids. For this, we first attributed genes to the X or to autosomes at the scale of the entire genome in the pea aphid. On a large set of genes, we combined estimates of substitution rates at the interspecific level with polymorphism data in pea aphid populations and gene expression levels in the various genders and morphs. This allowed assessing how relaxed selection (genetic drift) and adaptation contribute to the faster evolution of the X chromosome in this system.

Material and Methods

Assignment of scaffold regions to the X and autosomes

Full-genome sequencing of females and males

An asexual aphid mother has the same diploid autosomal genome as her sons, but has two X chromosomes instead of just one (figure 1). We took advantage of this XX/X0 system to assign the pea aphid genome sequence (Acyr 2.0, Genbank accession GCA_000142985.2, IAGC 2010) to the X or to the autosomes by comparing sequencing depth along assembled scaffolds between mapped reads from females and from males of the same parthenogenetic lineage (clone). DNA from five asexual females and five winged and five wingless males of clone P123 (Simon et al. 2011) was extracted with the Qiagen DNeasy Blood and Tissue kit, following the manufacturer's protocol. Wing male polymorphism in this clone was used to determine the X copy that each male carried, based on the knowledge that the locus that controls this trait is X-linked (Caillaud et al. 2002) and is heterozygous in clone P123 (Frantz et al. 2010). Each individual was genotyped at seven polymorphic microsatellite markers (Peccoud et al. 2008) to confirm its identity. One of those markers, which is known to be X-linked (Caillaud et al. 2002), allowed us to confirm the nature of the X copy inherited by each male. Three DNA extracts were obtained: one from five females and one per genotype of males, using five individuals in each. These samples were sequenced on the Illumina Hiseq 2000 platform yielding 100 bp pair-end reads at ~43X coverage for the females sample and 25 to 30X coverage for each male type. Reads from each sample were mapped on scaffolds of the pea aphid genome assembly (Acyr 2.0) and on genome sequences of the bacterial symbionts of this pea aphid clone using the method described in Gouin et al. (2015) using Bowtie 2 (Langmead and Salzberg 2012) with proper insert sizes and parameters set as

default. Depth of coverage at each nucleotide position of the reference genome was recorded and single nucleotide polymorphisms (SNPs) were identified using GATK's Haplotype Caller (McKenna et al. 2010; DePristo et al. 2011). The raw sequence data has been deposited in the SRA division of Genbank (project accession: ERP022905 and PRJNA385573).

Comparison of sequencing depth between males and females

The following analysis was performed in R (R Development Core Team 2015). We analyzed genome positions covered by 20 to 70 reads in the asexual female sample, a range chosen to eliminate regions with low-coverage and regions with suspiciously high coverage (potentially duplicated or repeat-rich regions). Since overall coverage was slightly higher for one of the male types (~30X) than for the other (~25X), we normalized the depth of coverage data of the second male type (multiplying coverage estimates by a 30/25 ratio). We then averaged depth of coverage at each base position over male types. The ratio of median coverage depth of males to median coverage depth of the female sample was calculated on 10-kilobases (kb) scaffold windows sliding by 2-kb steps. A single window was used for scaffolds shorter than 10 kb. We expect the ratio of median coverage depth to be twice larger for autosomal regions than for the X chromosome. Accordingly, this ratio had a clearly bimodal distribution (supplementary figure S1), with modes at 0.34 and 0.66. We assigned a 10-kb window to the X if its ratio ranged between 0.2 and 0.445, to autosomes if it ranged between 0.53 and 1, whereas the region was tagged as "ambiguous" if it ranged between 0.445 and 0.53. Windows assigned to the same chromosome type and which were separated by less than four consecutive "ambiguous" windows were aggregated into a scaffold region we call a "block". A whole block, including its "ambiguous" windows, was assigned to the corresponding chromosome type.

Comparison of male and female genotypes

Inheritance of single nucleotide polymorphisms (SNPs) also informs on the type of chromosome carrying a scaffold block. In fact, SNPs that are heterozygous in females but are also heterozygous in males are necessarily located on autosomes. Conversely, SNPs which are heterozygous in females but homozygous in males must be on the X. This SNP-based approach is however expected to be less powerful than the depth of coverage-based method for genomic regions with low heterozygosity. Thus, we only used SNP data to validate X/A assignments based on depth of coverage ratio (supplementary figure S2). A position was determined as heterozygous if the rarest allele was represented in at least 25% of reads, otherwise it was considered as homozygous. Assignment of SNPs to chromosome types was performed according to the genotypes of males, as described above. SNPs showing inconsistent genotypes (e.g., females and males of one type are both heterozygous while males of the other type are homozygous) were not assigned. SNP-based assignments were then visually compared to assignments based on depth of coverage (supplementary figure S2).

Assignment of predicted genes to the X and autosomes

We used the 36,990 genes that constitute the gene prediction v2.1 for the Acyr 2.0 genome assembly available at http://bipaa.genouest.org/is/aphidbase/. Each of these genes was determined as X-linked or autosomal if the full length of its coding sequence (CDS) was comprised in a single scaffold block or was spread over several scaffold blocks assigned to the same type (either X, or A). Genes that could not be unambiguously assigned (mainly

because they located on "ambiguous" blocks) were removed from further analyses. We also excluded 589 predicted genes that corresponded to rRNA (non-coding DNA).

Sex-biased gene expression

We used the eight RNAseq libraries from Jaquiéry et al. (2013) to characterize gene expression patterns between morphs. Briefly, these eight libraries correspond to whole insects, with three male libraries, three parthenogenetic female libraries and two sexual female libraries – different libraries in each morph representing biological replicates – using adults of a single clone of *A. pisum* (clone LSR1). Details regarding aphid rearing, library preparation and sequencing are provided in Jaquiéry et al. (2013). Libraries were mapped on Acyr 2.0 as described previously. The number of reads covering each CDS was then counted. Read counts were normalized with the R package DESeq with default parameters (Anders and Huber 2010). For each gene, the effect of the morph (a three-level factor comprising male, sexual female and asexual female) on expression was tested with a GLM (R package MASS, Venables et al. 2002) with a quasi-poisson distribution of residuals, considering the different libraries for each morph as replicates. *P*-values were corrected for multiple testing using the Benjamini-Hochberg method implemented in R. Genes differentially expressed between morphs ($p<0.05$ after adjusting for multiple testing) were then categorized according to their pattern of expression in the different morphs as described in table 1.

Evolutionary rates

To assess substitution rates in X-linked and autosomal genes, sequences from another aphid species were necessary. *Acyrthosiphon svalbardicum* was chosen, ensuring sufficient genetic divergence, and reducing the risk of mutational saturation and of chromosomal

218 rearrangements between the two species. Asexual females of *A. svalbardicum* were collected
219 in Svalbard in 2009, and were then reared in the lab under 10:14 light:dark and 15°C on
220 *Dryas octopetala*. Ten females were then frozen into liquid nitrogen and kept for subsequent
221 RNA extraction performed using the RNeasy plant mini kit (Qiagen) according to
222 manufacturer's instructions. Two separate RNA extractions of 5 adults were performed. RNA
223 quality was checked on Bioanalyzer (Agilent) and quantified on Nanodrop (Thermo
224 Scientific). One sample made of a pool of 2 µg of the two independent RNA extractions was
225 subsequently sent to GATC Company for RNA paired-end sequencing. The raw sequence
226 data has been deposited in the SRA division of Genbank (project accession: PRJNA385897).
227 A *de novo* transcriptome assembly for *A. svalbardicum* was obtained following the
228 methods of Rispe et al. (2016). Low quality parts of the reads were trimmed from the right
229 ends with prinseq-lite (http://prinseq.sourceforge.net/) when the mean of phred score in a 20-
230 bp window was below 20. Reads longer than 20 bp after trimming were re-organized by pairs
231 (orphans were suppressed) and assembled with Trinity (Grabherr et al. 2011) using default
232 parameters. Coding regions were predicted using FrameDP (Gouzy et al. 2009). Reciprocal
233 BLASTN (Altschul et al. 1990) searches between CDSs of *A. svalbardicum* and of *A. pisum*
234 were carried out with an e-value threshold of 10^{-8}. The following steps were performed with
235 an R script. Reciprocal best hit criterion was used to identify putative orthologous genes
236 between the two species. These were aligned by the pairWiseAlignment function of the
237 Biostrings package (Pages et al. 2016). Indels were inspected to flag CDS regions where the
238 two species did not present the same reading frame. Bases in these regions were replaced with
239 Ns, and were therefore trimmed by the Gblocks program (Castresana 2000; Talavera and
240 Castresana 2007), alongside regions of unreliable alignment. We then estimated pairwise
241 synonymous (*dS*) and nonsynonymous (*dN*) substitution rates for each gene, using the codon-

242 based method of Li (1993), as implemented in the R package seqinR (Charif and Lobry
243 2007). Only the 9,696 genes (out of 9,924) with an alignment length of >90 nucleotides, *dN*
244 <0.3 and *dS* <2 were kept. We also truncated *dN/dS* ratios to a maximal value of 2.5.
245

246 **Estimates of selection intensity based on intraspecific polymorphism**

247 Polymorphism data for *A. pisum* was obtained from 60 genotypes originating from
248 three *Medicago sativa* fields located in France and Switzerland (Jaquiéry et al. 2012b). These
249 fields can be considered to harbour a single large population of pea aphids (Jaquiéry et al.
250 2012b, Peccoud et al. 2009a). DNA was individually extracted from four asexual females of
251 each clone using the method described above. Because the approach described below does not
252 require reconstructing allele sequences or individual genotypes, sequencing the pooled
253 individuals (Gautier et al. 2013) was used to save costs. After RNAse treatment on each
254 sample and DNA dosage with Pherastar, DNA samples were pooled to attain equimolar
255 proportions. Paired-end libraries were then sequenced on two lanes of Illumina HiSeq 2000
256 using the Illumina Sequencing Kit v3 (producing 100-bp reads) by Beckman Coulter
257 Genomics (Danvers MA, USA). This yielded ~85X of sequencing coverage, hence an
258 expectation of 0.71X per individual chromosome. Reads were mapped on Acyr 2.0 and
259 symbiont genome sequences as described previously. The two alignment (BAM) files (one
260 per sequencing lane) were filtered from PCR duplicates using SAMtools rmdup (Li et al.
261 2009) and reads realigned near indels using the Genome Analysis Toolkit (McKenna et al.
262 2010). The raw sequence data have been deposited in the SRA division of Genbank (project
263 accession: PRJNA385905).

264 The two BAM files were merged and converted as pileup format using SAMtools
265 (options -B -Q 0 –R) (Li et al. 2009). A modified estimator of Tajima's D (Tajima
266 1989) which takes into account sequencing errors (Achaz 2008) was then calculated from
267 this mpileup with Popoolation 1.2.2 (Kofler et al. 2011), after subsampling at a uniform
268 coverage (subsample-pileup.pl, options : --target-coverage 30 --max-coverage 120 --method
269 withoutreplace). Computations were performed for each gene including introns (Variance-at-
270 position.pl --pool-size 120). Tajima's D allows evaluating the type of selection at work, since
271 selective sweeps and/or purifying selection tend to decrease it, and balancing selection tends
272 to increase it.
273 The McDonald and Kreitman (1991) approach, which compares fixed mutations to
274 polymorphic mutations in CDS, was adopted to further evaluate selection pressures on these
275 different categories of genes, using the DoS estimate (Direction of Selection, Stoletzki and
276 Eyre-Walker 2011). Positive, null and negative values of DoS respectively suggest adaptive
277 evolution, neutral evolution, and purifying selection. Fixed mutations between species were
278 counted from alignments we previously generated for *A. svalbardicum* and *A. pisum* CDSs.
279 We restricted the analysis to regions of reliable alignments, as given by the Gblocks txts
280 outputs. In these regions, we called SNPs on the BAM files with LoFreq (Wilm et al. 2012),
281 which offers a good compromise between speed, sensitivity and accuracy in pools of multiple
282 individuals (Huang et al. 2015). We used SAMtools mpileup (Li et al. 2009) to assess depth
283 of coverage at all positions in these regions, polymorphic or not. We instructed mpileup to
284 discard reads with mapping quality 0. The following was done in R. We discarded all
285 positions covered by less than three reads (both BAM files combined). At each SNP, the
286 number of polymorphic mutations was the number of different bases (alleles) found in the pea
287 aphid population minus one. A fixed difference was counted if no base was shared at a

position between the pea aphid population and *A. svalbardicum*. The number of polymorphic non-synonymous mutations per codon was taken as the number of amino acids found in the pea aphid populations for that codon minus one. To count the number of fixed non-synonymous differences per codon, we considered that a codon might differ between the two species by up to three mutations. Any of these may involve a change in protein sequence that we cannot ascertain without knowledge on the order of appearance of the mutations. We adopted parsimony and considered the minimum number of mutations required between the two codons. If several codons were present in the pea aphid population (due to a SNP), we considered the minimum number of coding changes that any pair of codons between the pea aphid and *A. svalbardicum* involves. For all these counts, we discarded rare codons showing more than one SNP, because the actual codons (and amino acids) present in the pea aphid population cannot be determined without phasing. We counted the following for each gene: the number of polymorphic non-synonymous changes (*Pn*), the number of all polymorphic changes minus *Pn* (which is the number of polymorphic synonymous mutations, noted *Ps*), the number of fixed non-synonymous differences (*Dn*), the number of all fixed differences minus *Dn* (which is the number of fixed synonymous changes, noted *Ds*). DoS was then calculated as $Pn/(Pn+Ps) - Dn(Dn+Ds)$ by concatenating all genes from a given class of expression to avoid divisions by zero, due to genes without polymorphism or divergence, as done in Burgarella et al. (2015). We considered only genes whose average depth of coverage, as given by mpileup, was between 20 and 150 (expected coverage was ~85) to avoid including genes presenting multiple collapsed copies that could artificially inflate polymorphism.

Statistical analyses

Differences in expression levels between X-linked and autosomal genes in the different morphs, as well as differences in *dN/dS*, *dN* and *dS* between X-linked and autosomal genes were tested with Mann-Whitney U tests. The latter analysis was done on all genes, and on genes grouped based on average expression over the three morphs. To evaluate evolutionary forces responsible for faster-X evolution, we then compared *dN/dS*, Tajima's D and DoS between X-linked and autosomal genes for classes of genes with different expression patterns (unbiased, male-biased, sexual female-biased and asexual female-biased genes). For biased genes, we considered different fold changes in expression (2- to 5-fold, and > 5-fold). Statistical significance was evaluated with Mann-Whitney U tests.

Results

Gene assignment to the X and autosomes

Based on the ratio of depth of coverage by reads from males over reads from females, 64% of the nucleotides assembled in the pea aphid reference genome (Acyr 2.0) were assigned to autosomes, 31% to the X chromosome while only 5% could not be assigned (supplementary figure S1). Genotypes of males at SNPs that were heterozygous in the female generally confirmed the assignment from coverage depth data (supplementary figure S2), though confirmation was not possible in regions lacking such SNPs. These estimates roughly correspond to the expected size of the X chromosome in the pea aphid, which represents ~30% of the chromosome content based on karyotypes (Mandrioli and Borsatti 2007). This assignment revealed a very high rate of misassembly in Acyr 2.0: 56% of scaffolds of size ≥150 kb (which represent 80% of the assembly length) comprised blocks assigned to different

334 chromosome types (supplementary figure S3 and table S1). Based on assigned scaffold
335 blocks, 19,263 predicted genes were located on autosomes, 13,726 on the X chromosome
336 while 4,001 genes could not be unambiguously assigned. The X chromosome contains a
337 higher fraction of predicted genes than expected from its relative size (42%, test of
338 proportion, $p<10^{-15}$).
339

340 **Gene evolutionary rates**

341 By comparing pea aphid gene sequences to transcripts sequenced from a related species (*A.*
342 *svalbardicum*), we assessed the rates of substitution. Results confirm faster-X evolution, as X-
343 linked genes present almost a twice higher non-synonymous substitution rate, *dN* (mean dN_X=
344 0.034; dN_A= 0.019, Mann-Whitney U = 4839860, $p <10^{-15}$, n = 9096) and only slightly higher
345 synonymous substitution rate, *dS* (mean dS_X=0.101 ; dS_A= 0.085, Mann-Whitney U =
346 6190137, $p <10^{-6}$). As a result, the evolution of X-linked genes involves more protein-
347 sequence changes (in proportion) than the evolution of autosomal genes (mean dN_X/dS_X =
348 0.390; dN_A/dS_A = 0.237; Mann-Whitney U = 5026170, $p <10^{-15}$, figure 2A and
349 supplementary table S2).
350

351 **Causes of Faster-X evolution**

352 High ratios of non-synonymous to synonymous rates, *dN/dS*, can result from a decreased
353 influence of selection - and thus an increased influence of drift - on amino acid substitutions
354 (i.e., relaxed negative selection) and/or from more efficient selection of adaptive changes in
355 the protein sequence (i.e., increased positive selection). To balance these two hypotheses, we
356 used the expression levels of genes as proxies for their possible impact on fitness. Expression

357 levels of X-linked genes are lower than those of autosomal genes in all three main aphid
358 morphs: males, sexual females and parthenogenetic females (figure 2B). Expression level
359 averaged over the 13,726 X-linked genes ranges from ~52 reads/kb in sexual and asexual
360 females to 155 reads/kb in males, while expression level averaged over the 19,263 autosomal
361 genes varies from 575 (in asexual females) to 648 reads/kb in males. Genes that are not
362 expressed or expressed at a low level may have a reduced effect on the phenotype and may
363 therefore accumulate non-synonymous substitutions faster (reduced purifying selection). We
364 indeed observe that, for both X and autosomes, *dN/dS* ratios decrease with increasing
365 expression levels (averaged over the three morphs), and that the contrast between X and
366 autosomes tends to decline for highly expressed genes (figure 2A, supplementary table S2).
367 Slight contrast in *dN* ratios is still maintained for highly expressed genes, though
368 (supplementary table S2). Therefore, low expression levels of X-linked genes may not
369 entirely account for faster X-evolution in aphids.
370 Selection may also be relaxed in genes that are predominantly expressed in rare
371 morphs (males and sexual females), which constitute a minor fraction of the annual life cycle
372 of aphids dominated by asexual females. Relaxed selection on mutations affecting male-
373 biased genes (Purandare et al. 2014, Brisson and Nuzhdin 2008), combined with the tendency
374 of such genes to locate on the X (Jaquiéry et al. 2013; Pal and Vicoso 2015) could contribute
375 to faster-X evolution. However, the influence of X-linkage could not properly be evaluated in
376 Purandare et al. (2014) because misassembled scaffolds, rather than individual genes, were
377 assigned to chromosomes. Our new dataset of X-linked and autosomal genes unambiguously
378 confirms that the X is largely enriched in genes over-expressed in males, and to a smaller
379 extent in those over-expressed in sexual females (table 1). Like Purandare et al. (2014), we
380 observe higher *dN/dS* ratios in genes over-expressed in the rarer morphs (i.e. males and sexual

381 females, figure 3B-C), but not in genes over-expressed in the common morph
382 (parthenogenetic females, figure 3D) when considering X-linked and autosomal genes
383 together. Tajima's D also tends to increase in genes over-expressed in the sexual morphs
384 compared to unbiased genes (significantly so for all male-biased and for 2- to 5-fold female-
385 biased genes, figure 4B), but not in genes over-expressed in the common morph (where D is
386 significantly lower compared to unbiased genes, figure 4D), a pattern compatible with relaxed
387 selection on genes expressed mainly in the rare morphs. However, the DoS did not differ
388 significantly between these categories of genes, except for strongly female-biased genes
389 (supplementary figure S4).

390 When analyses are done by chromosome type, *dN*/*dS* ratios of X-linked genes are
391 significantly higher than those of autosomal genes for both sexual female and male-biased
392 genes (figure 3E-3H), but not for asexual females. Contrastingly, Tajima's D differs between
393 chromosome types only for strongly male-biased genes (being lower for X-linked genes,
394 suggesting more positive selection, figure 4F) and for unbiased genes (being larger for X-
395 linked genes, possibly revealing more balanced selection, figure 4E). As a result, the
396 enrichment of the X with genes expressed in the rare male morph (which have been
397 hypothesized to evolve under relaxed selection) may not strongly contribute to faster-X
398 evolution. No signal was detected between chromosome types based on the DoS index
399 (supplementary figure S4).

400 Alternatively, the faster evolution of X-linked male-biased genes compared to
401 autosomal male-biased genes could result from the fact that the former are present in
402 hemizygous state in males. Non-synonymous mutations on the X are thus more exposed to
403 selection in males, since they are not masked by other potentially dominant alleles, such that
404 adaptive mutations on the X should more rapidly and more likely reach fixation than adaptive

405 mutations on autosomes. This hypothesis predicts that the contrast between substitution rates
406 of X-linked genes and autosomal genes will be highest for male-biased genes, and lowest for
407 sexual- and asexual female-biased genes, because in these morphs, the X is always diploid
408 and adaptive mutations can be recessive. We indeed observe these patterns (figure 3F), and
409 the significantly lower Tajima's D for X-linked male-biased genes compared to autosomal
410 genes provides some support to this hypothesis (figure 4F).
411

412 # Discussion

413 Here we performed a genome-wide identification of X-linked genes enabling to locate a large
414 number (13,726) and proportion (42%) of predicted genes on the X chromosome. We
415 demonstrated that these genes tend to evolve faster than autosomal genes on average,
416 confirming earlier results based on a much smaller set of coding genes (Jaquiéry et al. 2012a).
417 Faster-X evolution mainly results from low expression of a large fraction of X-linked genes,
418 which would less affect the phenotype and may accumulate non-synonymous mutations. Our
419 analyses also suggest that higher exposure of recessive X-linked alleles to selection in
420 hemizygous males might also partially contribute to faster-X evolution via more efficient
421 positive selection.

422 We demonstrate clear faster-X evolution in the pea aphid based on a large set of X-
423 linked and autosomal genes. The non-synonymous to synonymous substitution ratio (*dN/dS*)
424 for X-linked genes is 1.69 times greater than for autosomal genes. This clearly places aphids
425 among species showing strong contrast between evolution of X-linked and autosomal genes.
426 Excluding sex-specific genes (e.g. Torgerson 2003; Kousathanas et al. 2014) and genes that
427 escape post-meiotic silencing (Sin et al. 2012) for which X-linked sequences evolve much
428 faster than autosomal ones, the *dN/dS* for X-linked genes is between ~0.9 and ~1.8 times that

429 of autosomes in most species studied (i.e., *Drosophila*, mammals, birds and moths, review in
430 Meisel and Connallon 2013; see also Sackton et al. 2014).

431 Remarkably, the pea aphid presents the same effective population size for the X and
432 autosomes (Jaquiéry et al. 2012a), such that hemizygosity in males should be the only
433 differentiating factor affecting the evolution of genes located on different chromosome types.
434 However, our analyses revealed another key difference between X-linked and autosomal
435 genes, in that the former are on average 4 to 10 times less expressed than the latter (figure
436 2B), and expectedly show higher rates of substitution. Such negative correlations between
437 substitution rates and expression level have already been observed in several species (e.g.,
438 Drummond et al. 2005, Nguyen et al. 2015, Zhang and Yang 2015). Therefore, enrichment of
439 the X with lowly expressed genes explains faster-X evolution in aphids to a large extent.

440 Gene expression differs between chromosome types in another dimension, as the X is
441 enriched in genes that are mostly expressed in the rare morphs (i.e., males and sexual females)
442 (Jaquiéry et al. 2013, Pal and Vicoso 2015). Such genes should also evolve under more
443 relaxed constraints as they are exposed to the selective environment only during a short period
444 of the aphid life cycle (Brisson and Nuzhdin 2008, Purandare et al. 2014). However, our
445 analyses revealed that X-linked male-biased genes show lower Tajima's D than autosomal
446 male-biased genes (figure 4F-4G). As a result, enrichment of the X chromosome with genes
447 expressed in the rare male morph is unlikely to be an important contributor to faster-X
448 evolution in the pea aphid.

449 This leaves hemizygosity of the X chromosome in males, which exposes all X-linked
450 alleles expressed in males to the selective environment, as another contributing cause. This
451 hypothesis is supported by the contrast in *dN/dS* between X-linked genes and autosomal
452 genes, which is larger for male-biased genes than for sexual and asexual females-biased genes

453 (figure 3F-3H), and by the lower Tajima's D on male-biased X-linked genes than for those on
454 autosomes (figure 4F-4G), but not by the DoS, which shows no sign of selection.
455 Mean expression levels of X-linked genes measured in the whole body are strikingly
456 lower than those of autosomal genes, in all morphs studied. The difference we found is more
457 pronounced than in previous observations (Jaquiéry et al. 2013, Pal and Vicoso 2015)
458 probably due to more reliable gene assignments to chromosomes (we found that 10% of the
459 3,712 genes used in Jaquiéry et al. 2013 had been misassigned because of scaffold
460 misassembly). Lower expression of X-linked genes compared to autosomal genes is observed
461 in mammals (Nguyen et al. 2015), but not in *Drosophila* (Zhang and Presgraves 2016). To our
462 knowledge, no other taxon displays such a strong contrast between the X and autosomes gene
463 expression levels as does the pea aphid. This raises the question as to why genes on the X are
464 so little expressed in this species. A theoretical model (Jaquiéry et al. 2013) predicts that the
465 X chromosome is more easily invaded by sexually antagonistic alleles beneficial to males and
466 deleterious to females than autosomes. This may have favored a global decrease in gene
467 expression of this chromosome in the common morph (the asexual females) for which it could
468 be harmful. Pseudogenisation on the X chromosome would have ensued if genetic variation in
469 lowly expressed genes has little effect on fitness. Yet, this chromosome carries one third of
470 the genome and contains a higher fraction of genes than predicted by its relative size. Insights
471 into the role of sexual antagonism on the peculiar expression patterns observed here could be
472 gained by studying gene expression of this chromosome in different male and female tissues.
473 Particularly, transcriptomes of tissues subject to contrasted sex-specific selection pressures
474 (e.g. Parisi 2003; Khil et al. 2004; Yang et al. 2006; Huylmans and Parsch 2015) could help
475 examine this hypothesis.

476 Assignments of scaffold blocks to chromosomes revealed widespread errors in the pea
477 aphid genome assembly (Acyr 2.0). More than half of scaffolds larger than 150kb are clear
478 chimeras of X and autosomes. This is a minimal estimate for the rate of misassembly, since
479 our method only detects breakpoints between X and autosomes. Given that the X represents
480 30% of the pea aphid genome, assembly errors involving two X-linked genomic regions, and
481 those involving two autosomal regions should respectively represent 0.3^2 and 0.7^2 (58% in
482 total) of all assembly errors, a significant number of events that we could not detect.
483 Moreover, assembly errors involving fragments of less than 10 kb could not be detected given
484 the resolution of our analyses (see methods). Other lines of evidence argue for frequent errors
485 in the current pea aphid genome assembly. First, a genetic map of 305 microsatellite markers
486 (Jaquiéry et al. 2014) validates our assignment to chromosomes, and also demonstrates high
487 rates of scaffold misassembly (see supplementary file S1). Second, X-A breakpoints co-
488 localize with sharp variations in genetic differentiation (F_{ST}) between pea aphid populations
489 from different host plants, as determined by high-density genome scans (Nouhaud et al.
490 submitted). Third, synteny between Acyr 2.0 scaffolds and scaffolds from two *Myzus persicae*
491 genomes (Mathers et al. 2017, available at www.aphidbase.com) stops at X-A breakpoints
492 (data not shown). Fourth, entire scaffolds assigned by Bickel et al. (2013) to the X
493 chromosome account for 11% of Acyr 2.0, much lower than the ~30% of the genome that this
494 chromosome represents, based on karyotypes (Mandrioli and Borsatti 2007). Bickel et al.
495 (2013) explained this discrepancy by assembly errors combined with methodological bias
496 towards assigning misassembled scaffolds to autosomes (a hypothesis we confirm by the
497 comparison of scaffold assignments to chromosomes by Bickel et al. 2013 and the present
498 study, see supplementary figure S5). By contrast, our new assignments to chromosomes
499 provide estimates corresponding to the expected relative size of the X. Consequently, we

confidently conclude that the genome of the pea aphid presents considerable assembly problems, to a degree that goes far beyond what current assembly pipelines typically yield (e.g. Salzberg et al. 2004; Muggli et al. 2015). While the cause of these errors remains undetermined, they have important drawbacks for genomic studies on a species that is currently considered the model aphid, in particular those relying on the physical organization of the genome, ranging from high-resolution genome scans to studies of chromatin conformation, genomic rearrangements, etc. We therefore urge for a reassembly of the pea aphid genome based on additional data. The results presented here should however not be affected by misassembly as we were able to assign unambiguously almost 90% of the 36,990 predicted gene of the pea aphid. This means that at fine-grain level, that of a few kilobases to few tens of kilobases (the range within which most genes are contained), the chimerism problem is not a major issue.

In conclusion, we have shown here that faster-X evolution of proteins in the pea aphid is principally explained by relaxed selection on lowly expressed genes, a class of genes that is more frequent on the X chromosome than on autosomes. Exposure of X-linked recessive alleles to selection in hemizygous males could play an additional, yet marginal, role in faster-X evolution. Importantly, the pea aphid complex, which includes races and cryptic species at different stages of divergence (Peccoud et al. 2009a, 2009b, 2014), offers excellent opportunities to investigate mechanisms driving genome evolution through comparative genomics. In particular, characterizing gene expression in morphs of closely and distantly related species could help disentangling the role of drift and selection in the low expression of the X and its masculinization.

References

Achaz G. 2008. Testing for neutrality in samples with sequencing errors. Genetics, 179:1409-1424.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.

Anders S, Huber W. 2010. Differential expression analysis for sequence count data. Genome Biol. 11:R106.

Avila V, Campos JL, Charlesworth B. 2015. The effects of sex-biased gene expression and X-linkage on rates of adaptive protein sequence evolution in *Drosophila*. Biol. Lett. 11:20150117.

Avila V et al. 2014. Faster-X effects in two *Drosophila* lineages. Genome Biol. Evol. 6:2968–2982.

Bachtrog D et al. 2011. Are all sex chromosomes created equal? Trends Genet. 27:350–357.

Baines JF, Sawyer SA, Hartl DL, Parsch J. 2008. Effects of X-linkage and sex-biased gene expression on the rate of adaptive protein evolution in *Drosophila*. Mol. Biol. Evol. 25:1639–1650.

Bickel RD, Dunham JP, Brisson JA. 2013. Widespread selection across coding and noncoding DNA in the pea aphid genome. G3 3:993–1001.

Brisson JA, Nuzhdin SV. 2008. Rarity of males in pea aphids results in mutational decay. Science 319:58–58.

Burgarella C et al. 2015. Molecular evolution of freshwater snails with contrasting mating systems. Mol. Biol. Evol. 32:2403–2416.

Caballero A. 1994. Developments in the prediction of effective population size. Heredity 73:657–679.

Caballero A. 1995. On the effective size of populations with separate sexes, with particular reference to sex-linked genes. Genetics 139:1007–1011.

Caillaud MC, Boutin M, Braendle C, Simon J-C. 2002. A sex-linked locus controls wing polymorphism in males of the pea aphid, *Acyrthosiphon pisum* (Harris). Heredity 89:346–352.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17:540–552.

Charif D, Lobry JR. 2007. SeqinR 1.0-2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis. In: Bastolla U, Porto M, Roman HE, Vendruscolo M, editors. Structural approaches to sequence evolution: Molecules, networks, populations. Biological and Medical Physics, Biomedical Engineering. New York: Springer Verlag. p. 207–232.

Charlesworth B, Coyne JA, Barton NH. 1987. The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130:113–146.

Charlesworth D, Charlesworth B, Marais G. 2005. Steps in the evolution of heteromorphic sex chromosomes. Heredity 95:118–128.

Coyne JA, Orr, H. A. 2004. Speciation. Sinauer Associates. Sunderland, MA

Drummond DA, Bloom JD, Adami C, Wilke CO, Arnold FH. 2005. Why highly expressed proteins evolve slowly. Proc. Natl. Acad. Sci. 102:14338–14343.

DePristo MA et al. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43:491–498.

Frantz A, Plantegenest M. & Simon JC. 2010. Host races of the pea aphid *Acyrthosiphon pisum* differ in male wing phenotypes. Bull. Entomol. Res. 100:59-66.

Gautier M et al. 2013. Estimation of population allele frequencies from next-generation sequencing data: pool-versus individual-based genotyping. Mol. Ecol. 22:3766–3779.

565 Gouin A et al. 2015. Whole-genome re-sequencing of non-model organisms: lessons from unmapped
566 reads. Heredity 114:494–501.

567 Gouzy J, Carrere S, Schiex T. 2009. FrameDP: sensitive peptide detection on noisy matured
568 sequences. Bioinformatics 25:670–671.

569 Grabherr MG et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference
570 genome. Nat. Biotechnol. 29:644–652.

571 Huang HW, Mullikin JC, Hansen NF. 2015. Evaluation of variant detection software for pooled next-
572 generation sequence data. BMC Bioinformatics 16:1–9.

573 Huylmans AK, Parsch J. 2015. Variation in the X:Autosome distribution of male-biased genes among
574 *Drosophila melanogaster* tissues and its relationship with dosage compensation. Genome
575 Biol. Evol. 7:1960–1971.

576 Hvilsom C et al. 2012. Extensive X-linked adaptive evolution in central chimpanzees. Proc. Natl.
577 Acad. Sci. 109:2054–2059.

578 International Aphid Genome Consortium. 2010. Genome sequence of the pea aphid *Acyrthosiphon*
579 *pisum*. Plos Biol. 8:e1000313.

580 Jaquiéry J et al. 2012a. Accelerated evolution of sex chromosomes in aphids, an X0 system. Mol. Biol.
581 Evol. 29:837–847.

582 Jaquiéry J et al. 2012b. Genome scans reveal candidate regions involved in the adaptation to host plant
583 in the pea aphid complex. Mol. Ecol. 21:5251–5264.

584 Jaquiéry J et al. 2013. Masculinization of the X chromosome in the pea aphid. Plos Genet.
585 9:e1003690.

586 Jaquiéry J et al. 2014. Genetic control of contagious asexuality in the pea aphid. PLoS Genet.
587 10:e1004838.

588 Johnson NA, Lachance J. 2012. The genetics of sex chromosomes: evolution and implications for
589 hybrid incompatibility. Ann. N. Y. Acad. Sci. 1256:E1–E22.

590 Khil PP, Smirnova NA, Romanienko PJ, Camerini-Otero RD. 2004. The mouse X chromosome is
591 enriched for sex-biased genes not subject to selection by meiotic sex chromosome
592 inactivation. Nat. Genet. 36:642–646.

593 Kimura M. 1983. The neutral theory of molecular evolution. Univ. press. Cambridge

594 Kitano J et al. 2009. A role for a neo-sex chromosome in stickleback speciation. Nature 461:1079–
595 1083.

596 Kofler R et al. 2011. PoPoolation: a toolbox for population genetic analysis of next generation
597 sequencing data from pooled individuals. *PloS one*, *6:*e15925.

598 Kousathanas A, Halligan DL, Keightley PD. 2014. Faster-X adaptive protein evolution in house mice.
599 Genetics 196:1131–1143.

600 Langley CH et al. 2012. Genomic variation in natural populations of *Drosophila melanogaster*.
601 Genetics 192:533–598.

602 Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9:357–
603 359.

604 Li H et al. 2009. The sequence alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

605 Li WH. 1993. Unbiased estimation of the rates of synonymous and nonsynonymous substitution. J.
606 Mol. Evol. 36:96–99.

607 Mackay TFC et al. 2012. The *Drosophila melanogaster* genetic reference panel. Nature 482:173–178.

608 Mandrioli M, Borsatti F. 2007. Analysis of heterochromatic epigenetic markers in the holocentric
609 chromosomes of the aphid *Acyrthosiphon pisum*. Chromosome Res. 15:1015–1022.

610 Mank JE, Nam K, Ellegren H. 2010. Faster-Z evolution is predominantly due to genetic drift. Mol.
611 Biol. Evol. 27:661–670.

612 Mank JE, Vicoso B, Berlin S, Charlesworth B. 2010. Effective population size and the faster-X effet:
613 empirical results and their interpretation. Evolution 64:663–674.

614 Mathers TC et al. 2017 Rapid transcriptional plasticity of duplicated gene clusters enables a clonally
615 reproducing aphid to colonise diverse plant species. Genome Biology, 18:27.

616 McDonald JH, Kreitman M. 1991. Adaptive protein evolution at the Adh locus in *Drosophila*. Nature
617 351:652–654.

618 McKenna A et al. 2010. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-
619 generation DNA sequencing data. Genome Res. 20:1297–1303.

620 Meiklejohn CD, Tao Y. 2010. Genetic conflict and sex chromosome evolution. Trends Ecol. Evol.
621 25:215–223.

622 Meisel RP, Connallon T. 2013. The faster-X effect: integrating theory and data. Trends Genet.
623 29:537–544.

624 Muggli MD, Puglisi SJ, Ronen R, Boucher C. 2015. Misassembly detection using paired-end sequence
625 reads and optical mapping data. Bioinformatics 31:i80–i88.

626 Nguyen L-P, Galtier N, Nabholz B. 2015. Gene expression, chromosome heterogeneity and the fast-X
627 effect in mammals. Biol. Lett. 11:20150010.

628 Pages H, Aboyoun P, Gentleman R, DebRoy S. 2016. Biostrings: String objects representing
629 biological sequences, and matching algorithms. R package version 2.40.2.

630 Pal A, Vicoso B. 2015. The X chromosome of hemipteran insects: conservation, dosage compensation
631 and sex-biased expression. Genome Biol. Evol. 7:3259–3268.

632 Parisi M. 2003. Paucity of genes on the *Drosophila* X chromosome showing male-biased expression.
633 Science 299:697–700.

634 Peccoud J et al. 2008. Host range expansion of an introduced insect pest through multiple
635 colonizations of specialized clones. Mol. Ecol. 17:4608–4618.

636 Peccoud J, Ollivier A, Plantegenest M, Simon J-C. 2009a. A continuum of genetic divergence from
637 sympatric host races to species in the pea aphid complex. Proc. Natl. Acad. Sci. 106:7495–
638 7500.

639 Peccoud J, Simon J-C, McLaughlin HJ, Moran NA. 2009b. Post-Pleistocene radiation of the pea aphid
640 complex revealed by rapidly evolving endosymbionts. Proc. Natl. Acad. Sci. U. S. A.
641 106:16315–16320.

642 Peccoud J et al. 2014. Widespread host-dependant hybrid unfitness in the pea aphid species complex:
643 postzygotic isolation in pea aphids. Evolution 68:2983–2995.

644 Purandare SR, Bickel RD, Jaquiéry J, Rispe C, Brisson JA. 2014. Accelerated evolution of morph-
645 biased genes in pea aphids. Mol. Biol. Evol. 31:2073–2083.

646 R Development Core Team 2015: A language and environment for statistical computing. Vienna,
647 Austria Available from: URL http://www.R-project.org.

648 Rice WR. 1984. Sex Chromosomes and the Evolution of Sexual Dimorphism. Evolution 38:735–742.

649 Rispe C et al. 2016. De novo transcriptome assembly of the grapevine phylloxera allows identification
650 of genes differentially expressed between leaf- and root-feeding forms. BMC Genomics 17:1–
651 15.

652 Sackton TB et al. 2014. Positive selection drives faster-Z evolution in silkmoths. Evolution 68:2331–
653 2342.

Saether SA et al. 2007. Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers. Science 318:95–97.

Salzberg SL, Church D, DiCuccio M, Yaschenko E, Ostell J. 2004. The genome assembly archive: a new public resource. PLoS Biol 2:e285.

Simon J-C et al. 2011. Facultative symbiont infections affect aphid reproduction. PLoS ONE 6:e21831.

Sin H-S, Ichijima Y, Koh E, Namiki M, Namekawa SH. 2012. Human postmeiotic sex chromatin and its impact on sex chromosome evolution. Genome Res. 22:827–836.

Soh YQS et al. 2014. Sequencing the mouse Y chromosome reveals convergent gene acquisition and amplification on both sex chromosomes. Cell 159:800–813.

Stoletzki N, Eyre-Walker A. 2011. Estimation of the neutrality index. Mol. Biol. Evol. 28:63–70.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:485–595.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56:564–577.

Torgerson DG. 2003. Sex-linked mammalian sperm proteins evolve faster than autosomal ones. Mol. Biol. Evol. 20:1705–1709.

Venables WN, Ripley BD. 2002. Modern applied statistics with S. 4th ed. New York: Springer

Vicoso B, Charlesworth B. 2009. Effective population size and the faster-X effect: an extended model. Evolution 63:2413–2426.

Wilm A et al. 2012. LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res. 40:11189-11201.

Wilson ACC, Sunnucks P, Hales DF. 1997. Random loss of X chromosome at male determination in an aphid, *Sitobion* near *fragariae*, detected using an X-linked polymorphic microsatellite marker. Genet. Res. 69:233–236.

Wright S. 1931. Evolution in mendelian populations. Genetics 16:97–159.

Yang X et al. 2006. Tissue-specific expression and regulation of sexually dimorphic genes in mice. Genome Res. 16:995–1004.

Zhang Z, Presgraves DC. 2016. *Drosophila* X-linked genes have lower translation rates than autosomal genes. Mol. Biol. Evol. 33:413–428

Zhang J, Yang J-R. 2015. Determinants of the rate of protein sequence evolution. Nat. Rev. Genet. 16:409–420.

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Figure legends

Fig. 1. Life-cycle of the pea aphid and ploidy levels for autosomes (A) and the sex-chromosome (X) (adapted from Jaquiéry et al. 2013).

Fig. 2. Evolutionary rates for autosomal and X-linked genes and gene expression in males, sexual and asexual females. A) Evolutionary rates (*dN/dS*) are shown for all genes (expressed or not in *A. pisum*) and for genes expressed at different levels (when averaged over male, sexual and asexual females): lowly expressed genes (i.e. covered by < 100 reads/kb of exon model); moderately expressed (from 100 to 1000 reads/kb), highly expressed genes (>1000 reads/kb). The number of genes per category is shown above each boxplot. B) Expression level for X-linked (n = 13613) and autosomal genes (n = 18812) in males, sexual females and asexual females. It should be noted that males carry only one X chromosome per cell and females carry two. Significance was tested with Mann-Whitney U tests.

Fig. 3. Substitution rates of genes (*dN/dS*, measured between *A.pisum* and *A. svalbardicum*) according to the ratios of expression levels between morphs. Panels A-D consider all genes together (X-linked and autosomal), and panels E-H consider X-linked (dark grey) and autosomal (light grey) genes separately. The number of genes in each class is shown above each boxplot. Only genes supported by at least 100 reads/kb of exon model were retained. ub: unbiased genes (padj > 0.1 for morph effect on expression), 2-5: levels of gene expression are two to five times higher in the specified morph (padj <0.05 for morph effect), >5: levels of gene expression are at least five times higher in the specified morph (padj <0.05). Significance of differences: ns: $p > 0.05$; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Mann-Whitney U tests). For panels B-D, differences correspond to comparisons with genes of the "unbiased" category, while X and autosomes were compared in panels F-H.

723 **Fig. 4.** Tajima's D according to the ratios of gene expression levels between morphs. Panels
724 A-D consider all genes together (X-linked and autosomal) and panels E-H consider X-linked
725 (dark grey) and autosomal (light grey) genes separately. Terms are defined as in Figure 3.
726 Dashed lines show median values for unbiased genes. Significance of differences was tested
727 with Mann-Whitney U tests.

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731 # Tables

732 **Table 1.** Number of X-linked and autosomal genes and frequency of X-linkage for classes of
733 genes with contrasted patterns of expression between morphs.

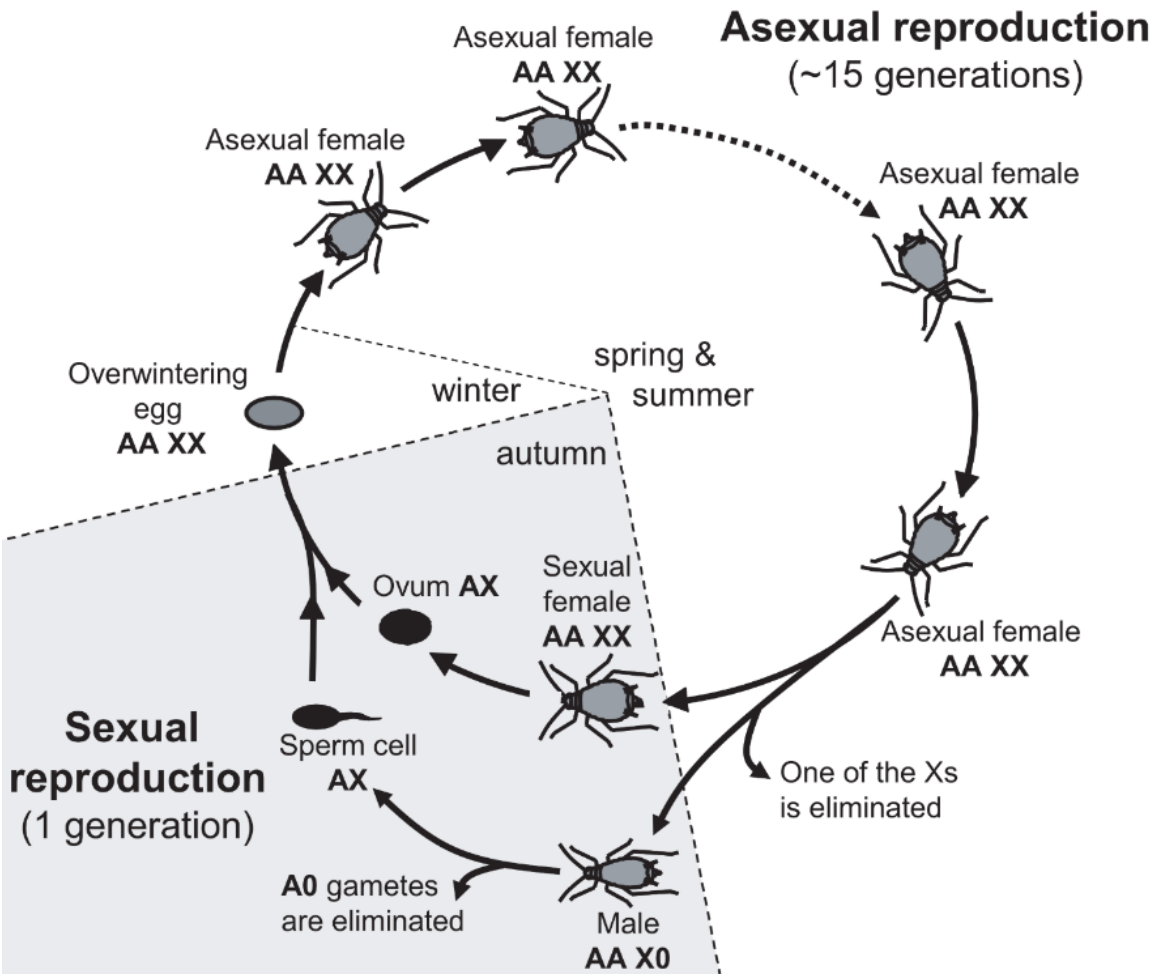
734

Category of genes	number of X-linked genes	number of autosomal genes	Frequency of X-linkage	*p* value [6]
All[1]	13726	19263	0.42	10^{-16}
Low expression[2] (less than 10 reads/kb)	10995	8136	0.57	10^{-16}
Expressed[3] (at least 10 reads/kb)	2771	11127	0.20	0.0001
Unbiased[4] (>10 reads/kb & padj>0.1)	697	3355	0.17	na
2-fold male-biased[5]	1546	2245	0.41	10^{-16}
5-fold male-biased[5]	962	948	0.50	10^{-16}
2-fold sexual female-biased[5]	448	1369	0.25	10^{-10}
5-fold sexual female-biased[5]	148	407	0.27	10^{-7}
2-fold asexual female-biased[5]	244	1023	0.19	0.10
2-fold asexual female-biased[5]	93	423	0.18	0.68

[1]all predicted genes that were assigned to the X or autosomes are included
[2]genes with on average <10 reads/kb of exon (average over the 3 morphs)
[3]genes with on average ≥10 reads/kb of exon (average over the 3 morphs)
[4]genes with on average ≥10 reads/kb of exon (average over the 3 morphs) and with an adjusted p-value ≥ 0.1 when tested for morph-biased expression.
[5]A gene was included in the morph-biased category (either male-, female- or asexual-biased) if the adjusted p-value for a morph effect was <0.05 and if it was at least x-fold (2 or 5) more expressed in one of the morph compared to the two other morphs.
[6]Deviation from expectation (given by the "unbiased" category) was evaluated with a test of proportion.

742 # Figures

743 ## Figure 1



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747 **Figure 2**

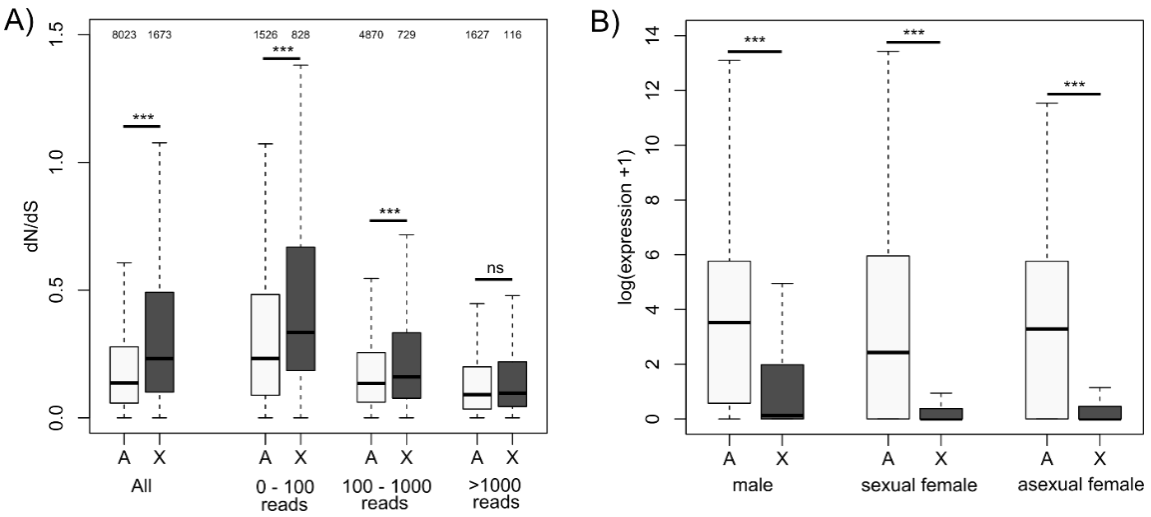

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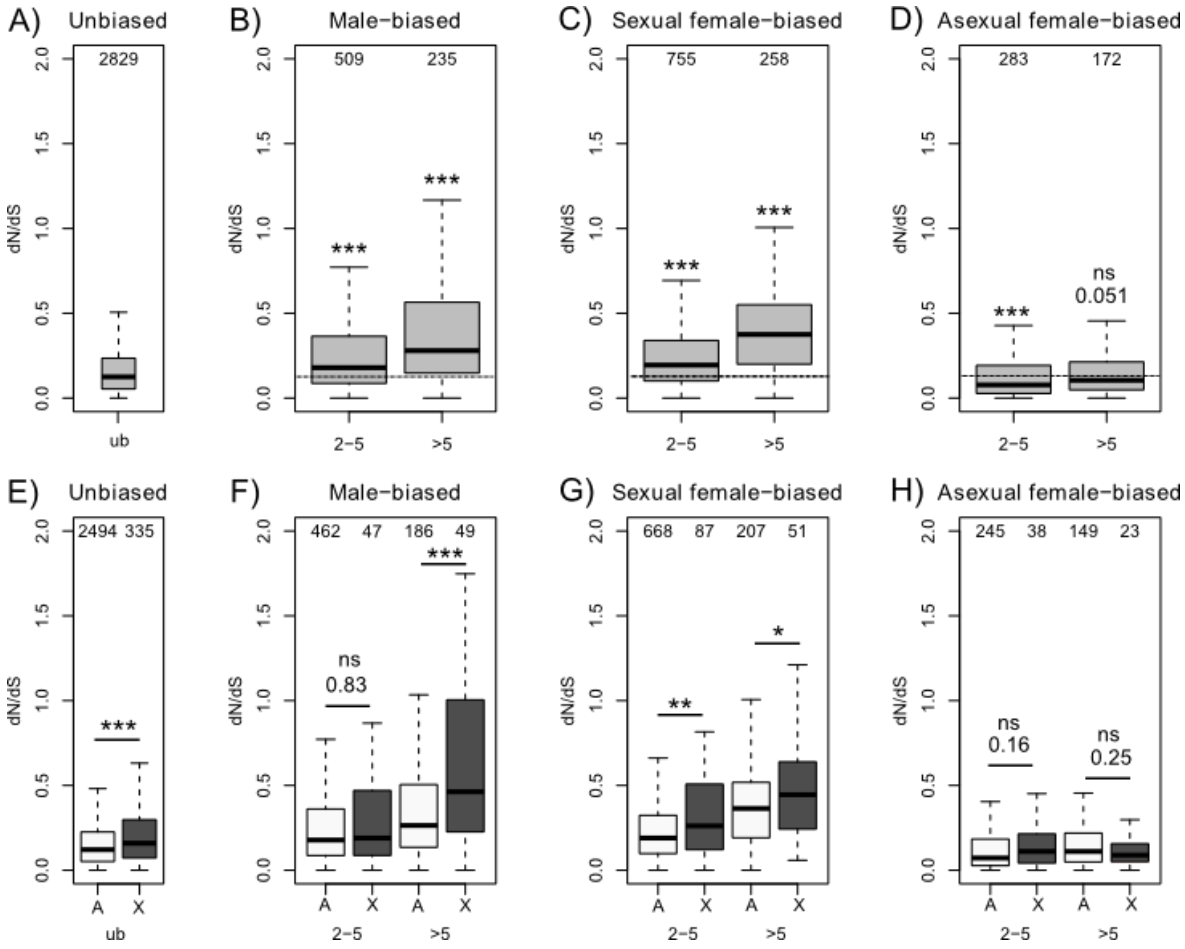
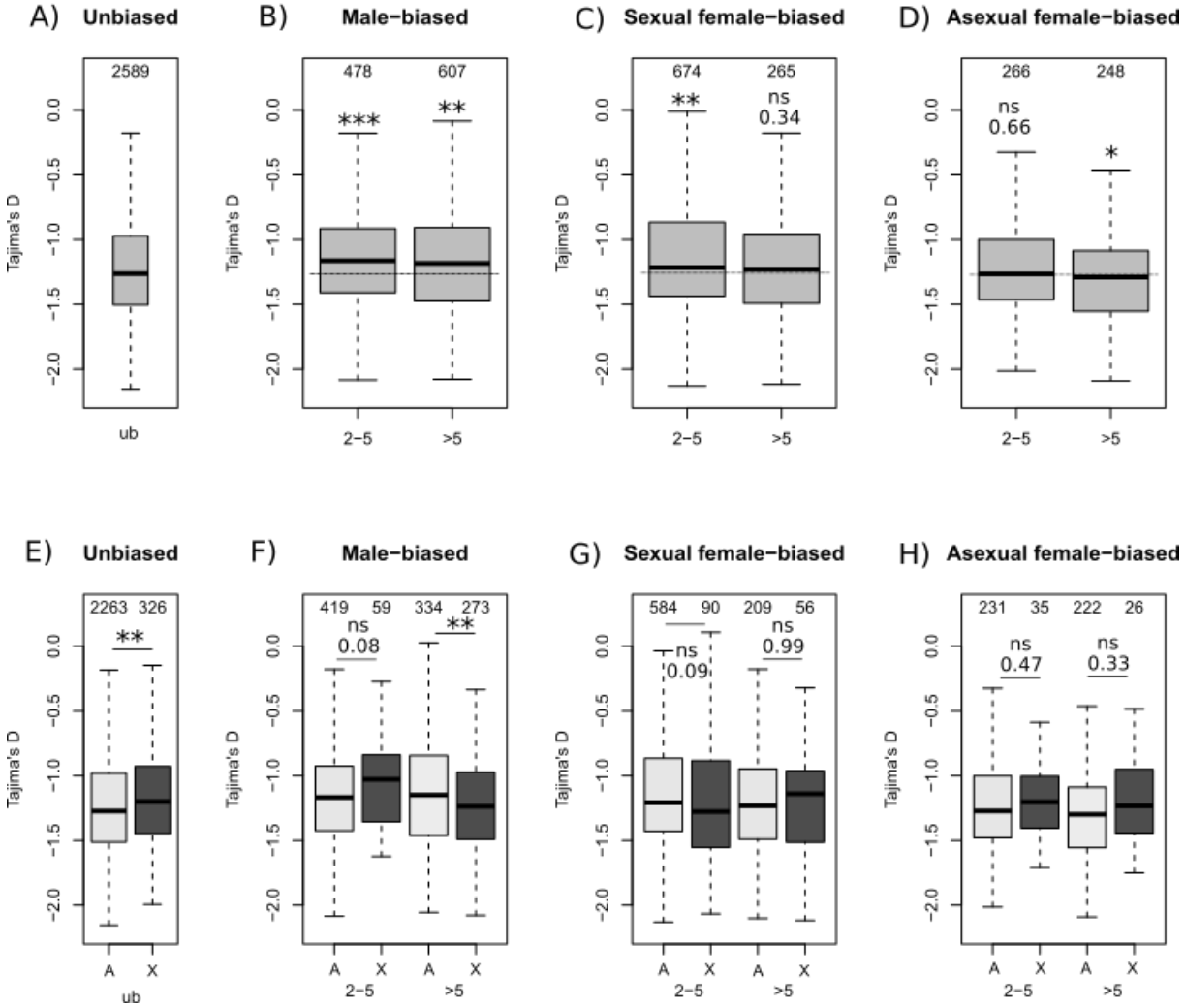
Figure 3



753 **Figure 4**

754



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