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1 Characterization of a sex-determining region and its genomic context via
2 statistical estimates of haplotype frequencies in daughters and sons
3 sequenced in pools

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14 Sequences data are available from Genbank with accession numbers SRR8238987 and
15 SRR8238986 [Other data are being submitted].

16

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18

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20

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26

27 Abstract

28 Sex chromosomes are generally derived from a pair of autosomes that have acquired a locus
29 controlling sex. Sex chromosomes usually evolve reduced recombination around this locus and
30 undergo a long process of molecular divergence. Although sex chromosomes have been intensively
31 studied in several model taxa, the actual loci controlling sex are difficult to identify in highly
32 diverged sex chromosomes, hence they are known in relatively few species. Taxa with
33 evolutionarily young sex chromosomes can help fill this gap in knowledge. Here we aimed at
34 pinpointing the sex-determining region (SDR) of *Armadillidium vulgare*, a terrestrial isopod with
35 female heterogamety (ZW females and ZZ males) and which presumably presents evolutionarily
36 young sex chromosomes. To locate the SDR, we assessed SNP allele frequencies in F1 daughters
37 and sons sequenced in pools (pool-seq) in several families. We developed a Bayesian method that
38 uses the SNP genotypes of individually sequenced parents and poolseq data from F1 siblings to
39 estimate the genetic distance between a given genomic region (contig) and the SDR. This allowed
40 us to assign more than 43 Megabases of contigs to sex chromosomes. By taking advantage of the
41 several F1 families, we delineated a very short genomic region (~65 kilobases) that did not show
42 evidence for recombination with the SDR. In this region, the comparison of sequencing depths
43 between sexes outlined female-specific genes that may be involved in sex determination. Overall,
44 our results provide strong evidence for an extremely low divergence of sex chromosomes in *A.*
45 *vulgare*.

46 Introduction

47 The existence of males and females (gonochorism) constitutes a phenotypic variation found in
48 many taxa and which has a profound impact on their evolution. Despite gonochorism being
49 common and ancient, the mechanisms initiating the developmental cascade toward the male or
50 female phenotype appear to be highly variable (BACHTROG *et al.* 2014; BEUKEBOOM AND PERRIN
51 2014). In some species, sex is solely or partially determined by environmental factors such as
52 temperature (MERCHANT-LARIOS AND DIAZ-HERNANDEZ 2013) and social interactions (BRANTE *et*
53 *al.* 2016) while in many others, sex is entirely determined by genotype (review in BACHTROG *et al.*
54 (2014); BEUKEBOOM AND PERRIN (2014)).

55 When the sex of individuals is genetically determined, it is generally under the control of sex
56 chromosomes. In the strict sense, sex chromosomes are chromosomes that carry a gene (in the
57 mendelian sense) whose genotype determines the sex of its carrier. Sex chromosomes are not
58 required to be a pair of chromosomes that look different from each other under the microscope, a
59 property that is referred to as heteromorphy. In fact, sex chromosomes should initially be

60 homomorphic as they are usually derived from a pair of autosomes that have acquired a sex
61 determining locus (WRIGHT *et al.* 2016; FURMAN *et al.* 2020). As opposed to homologous
62 autosomes however, sex chromosomes generally diverge under balancing selection and the
63 influence of alleles with sex-antagonistic effects (BACHTROG *et al.* 2014; WRIGHT *et al.* 2016). This
64 evolution generally leads to reduced crossing over rates around the master sex-determining gene
65 (BERGERO AND CHARLESWORTH 2009). In effect, the non-recombining chromosomal region that
66 associates with the sex phenotype (hereafter referred to as the sex-determining region or SDR),
67 tends to increase in size. The reduction of recombination leads to the divergence of the two
68 chromosomes, due to inability to efficiently purge deleterious mutations (BERGERO AND
69 CHARLESWORTH 2009; BACHTROG 2013). Through this incessant divergence process, sex
70 chromosomes may become visually recognizable in a karyotype, which enabled the identification of
71 different genetic sex-determining systems (reviewed in BACHTROG *et al.* (2014)). The most
72 notorious ones are the XY system where males are heterogametic (i.e., they are heterozygous at the
73 sex-determining gene) (as in therian mammals and *Drosophila*), and the ZW system where females
74 are heterogametic (as in birds and lepidopterans). The ease to identify a pair of heteromorphic sex
75 chromosomes is however balanced by the difficulty to locate the master sex-determining gene, as
76 this gene is only part of a large non-recombining SDR that may occupy most of the chromosome
77 length. This difficulty may explain why the identified sex-determining genes are still few in
78 comparison to the diversity of taxa with chromosomal sex determination, as most model organisms
79 (mammals, birds, fruit flies, etc.) harbor highly heteromorphic sex chromosomes.

80 In taxa where sex chromosomes appear to undergo rapid turnover, such as teleost fishes (MANK
81 AND AVISE 2009), sex chromosomes are evolutionarily young, hence SDRs likely to be short. Short
82 SDRs have helped to pinpoint several sex-determining genes (e.g. KAMIYA *et al.* (2012); AKAGI *et al.*
83 *et al.* (2014)), some of which vary among related species within the same genus (MATSUDA *et al.*
84 2002; NANDA *et al.* 2002). These taxa therefore emerge as useful models to study the appearance
85 and early evolution of sex chromosomes (CHARLESWORTH *et al.* 2005) and to better characterize the
86 nature and diversity of sex-determining genes, hence the reaction cascades leading to the
87 development of sex phenotypes.

88 Terrestrial isopods, also known as woodlice or pillbugs, are good biological models for this
89 endeavor. These crustaceans indeed appear to undergo a dynamic evolution in their sex
90 chromosomes (JUCHAULT AND RIGAUD 1995), as evidenced by multiple transitions between XY
91 systems and ZW systems (BECKING *et al.* 2017). These recurrent transitions imply that sex
92 chromosomes of several isopod species may be evolutionarily young. Among these species, the
93 common pillbug *Armadillidium vulgare* has been the most studied with respect to sex determination

94 (CORDAUX *et al.* 2011). Although sex chromosomes are visually undistinguishable among the 27
95 chromosome pairs composing the *A. vulgare* genome (ARTAULT 1977), crossing experiments using
96 genetic females masculinized via hormones (JUCHAULT AND LEGRAND 1972) have demonstrated
97 that this species presents ZW sex chromosomes. Homomorphy of the *A. vulgare* sex chromosomes
98 is also consistent with the reported viability and fertility of WW individuals generated through these
99 crossing experiments. The molecular similarity between the Z and W chromosomes of *A. vulgare*
100 has more precisely been evaluated through the assembly and analysis of its ~1.72 Gbp genome
101 (CHEBBI *et al.* 2019). More specifically, comparison of sequenced depths from data obtained from
102 males and females allowed locating W-specific sequences (those with no similarity found in ZZ
103 males) in 27 contigs representing less than 1 Mbp (CHEBBI *et al.* 2019). However, the *A. vulgare*
104 SDR might not correspond to a region of absence of similarity between Z and W chromosomes. In
105 some species, sex has been shown to be controlled by a single nucleotide polymorphism (SNP)
106 (KAMIYA *et al.* 2012), a hypothesis that cannot be excluded in *A. vulgare*. In effect, very little is
107 known about the *A. vulgare* sex chromosomes beyond the 27 contigs containing W-specific
108 sequences.

109 In this situation, methods based on SNPs can be useful as they can locate SDRs, and genetically
110 linked loci, in sex chromosomes presenting very low molecular divergence. Researchers would
111 locate loci for which individuals of a given sex all present expected genotypes (e.g., loci presenting
112 SNPs that are heterozygous in all ZW daughters and homozygous in all ZZ sons). However, when
113 no information on the location of the SDR is available (e.g., in the absence of genetic map) and if
114 no candidate genes for sex determination are suspected, the whole genome must be analyzed, hence
115 re-sequenced or scanned. Such endeavor can be very costly if whole-genome sequencing is
116 undertaken on many individuals, especially considering the sample size required for reliable
117 statistical inference. These considerations motivated the use of techniques permitting partial
118 genome sequencing (PALMER *et al.* 2019), such as Restriction-site Associated DNA markers (RAD
119 sequencing) (BAIRD *et al.* 2008), which reduce sequencing costs at the expense of an increase in
120 DNA library preparation costs. An alternative strategy consists of pooling the DNA from multiple
121 individuals prior to sequencing (FUTSCHIK AND SCHLÖTTERER 2010), a method referred to as "pool-
122 seq". Pool-seq drastically cut DNA library preparation costs, and generally sequencing costs as well
123 given that the sequencing effort per individual is generally lower than in the case of individual
124 whole genome sequencing. Pool-seq substitutes the obtention of individual genotypes with
125 estimates of allele frequencies within pools. The main drawback is that allele frequencies among the
126 pooled individuals are inferred from allele frequencies among sequenced fragments ("reads")
127 covering a SNP, adding some degree of uncertainty that diminishes with increased sequencing

128 effort (GAUTIER *et al.* 2013). As each SNP is analyzed separately, somewhat arbitrary thresholds on
129 estimated frequencies among reads and on sequencing depths are used to determine whether
130 individual SNPs are associated to the sex phenotype (e.g., PAN *et al.* (2019)). Also, the absence of
131 individual genotypes prevents the construction of a genetic map, limiting knowledge about the
132 genomic context of loci of interest.

133 Here, we developed a statistical approach that tackles these limitations, based on the individual
134 genotyping of parents and pool-seq of progeny from several crosses. This method allowed us to
135 identify a short genomic region that likely contains the SDR of *A. vulgare*. Beyond the SDR, our
136 approach allowed us to assign more than 43 Mb of contigs to sex chromosomes, even though these
137 chromosomes appeared no different from autosomes with respect to molecular divergence and
138 showed uniform recombination rates.

139 Materials and methods

140 General approach

141 We developed a pool-seq based approach to locate a ZW-type SDR by considering that a W-linked
142 allele (where “linked” here denotes the absence of recombination with the SDR) should have
143 frequencies of 0.5 in females and 0 in males. This W-linked allele has a Z-linked counterpart with
144 frequencies of 0.5 in females and 1 in males. We estimate allele frequencies in daughters and sons
145 from the F1 progeny of two parents through whole-genome sequencing of pools of individuals of
146 the same sex, followed by read mapping on a genome assembly (Figure 1). Our analysis relies on
147 biallelic SNPs for which the mother is heterozygous and the father homozygous, hereafter called
148 “informative SNPs”. Allele frequencies at SNPs that are heterozygous in the father depend on
149 random sampling of paternal gametes, which do not determine the sex of the progeny.

150 Rather than estimating allele frequencies in F1s for each individual SNP, we estimate the frequency
151 of haplotypes combining the alleles of all informative SNPs of a given locus (genomic contig). This
152 allows much greater precision in our estimates since SNP alleles of the same haplotype have the
153 same frequency in a pool, assuming the absence of crossing over within the contig. We aim at
154 identifying the haplotypes carried by maternal chromosomes and whose frequencies in an F1 pool
155 depend on the pool’s sex.

156 Maternal haplotypes are defined as follows. We first consider that the allele that is specific to the
157 mother at an informative SNP, hereafter called “maternal allele”, can be carried either by the W or
158 Z chromosome (Figure 1). We define as “type-1” a SNP whose maternal allele is carried by the
159 maternal W chromosome, and as “type-2” a SNP whose maternal allele is carried by the maternal Z.
160 Hence, the W-linked haplotype carries maternal alleles of type-1 SNPs and paternal alleles of type-

161 2 SNPs. To reconstruct this haplotype, we use the fact that W-linked maternal alleles are
162 predominantly inherited by daughters, and that Z-linked maternal alleles are predominantly
163 inherited by sons (Figure 1). Hence, we classify a SNP as type 1 whenever the maternal allele is
164 more frequent in daughters than in sons, and as type 2 in the opposite situation. If the maternal
165 allele is equiproportional in both sexes, we attribute types at random.
166 To enable the same mathematical development for both sexes, we introduce the notion of SNP
167 "group". SNP group is the same as SNP type in daughters, while we assign every type-1 SNP to
168 group 2 and every type-2 SNP to group 1 in sons. Each SNP is thus assigned to different groups in
169 different sexes of the progeny. This allows us to define the "focal haplotype" as the one that
170 combines the maternal alleles of group-1 SNPs and the paternal alleles of group-2 SNPs, regardless
171 of the sex. The focal haplotype is thus the W-linked haplotype in daughters and the Z-linked
172 maternal haplotype in sons. In the absence of recombination with the SDR, the focal haplotype must
173 be inherited by all the descendants of a given sex, hence its frequency must be 0.5 in a pool (the
174 remainder represents paternal DNA).

175 Statistical estimation of haplotype frequencies

176 In a given pool i of n_i individuals born from n_i oocytes, we denote as f the unknown frequency of
177 the focal haplotype at a locus, with potential values in the set $F_i = \{0 \dots n_i / (2n_i)\}$. To put f in
178 perspective, the proportion of oocytes that underwent recombination between the contig and the
179 SDR, which is the distance to the SDR in Morgans, is $1 - 2f$. We then let c_{1i} denote the number of
180 sequenced DNA fragments (e.g., read pairs) from pool i carrying the maternal alleles of all group-1
181 SNPs of the locus. We let r_{1i} represent the number of fragments from pool i covering group-1 SNPs
182 (both alleles combined). We finally let c_{2i} and r_{2i} denote equivalent variables for group-2 SNPs. To
183 shorten the notation, we refer to the set of variables $\{c_{1i}, r_{1i}, c_{2i}, r_{2i}\}$ as \mathbf{cr}_i .
184 These specifications allow expressing the posterior probability of the focal haplotype frequency
185 given the observed data, via Bayes' theorem:

186

$$187 \quad P(f \mid \mathbf{cr}_i) = \frac{P(f) \cdot P(c_{1i}, c_{2i} \mid f, r_{1i}, r_{2i})}{P(c_{1i}, c_{2i} \mid r_{1i}, r_{2i})} \quad (1)$$

188

189 We use a uniform prior for f , hence $P(f) = 1/(n_i + 1) \forall f \in F_i$. Although a prior based on
190 $\text{Binomial}(n_i, 0.5)$ would more accurately represent the inheritance of maternal haplotypes for most
191 contigs, such prior would not be suited for contigs at less than 50 centiMorgans (cM) to the SDR,
192 the frequency of which is unknown. In particular, a binomial prior assumes that f is quite unlikely to
193 equal 0.5 (with a probability of 0.5^{n_i}), increasing the risk of false negatives when it comes to the

194 selection of contigs linked to the SDR. We would rather include false positives in our selection of
 195 candidates, as this selection is only a first step in the search for the sex-determining gene(s).
 196 To specify $P(c_{1i}, c_{2i} | f, r_{1i}, r_{2i})$, we assume the sequencing of the different SNP groups of a contig to
 197 be independent. Although this is not true if SNPs of different groups are covered by the same read
 198 pairs, we consider our approximation to be acceptable and stress the difficulty of taking this
 199 dependence into account. Hence,

$$201 \quad P(c_{1i}, c_{2i} | f, r_{1i}, r_{2i}) \approx P(c_{1i} | f, r_{1i})P(c_{2i} | f, r_{2i}) \quad (2)$$

202
 203 We then consider c_{1i} as the realization of $Binomial(r_{1i}, f)$, i.e., that the c_{1i} read pairs carrying the
 204 maternal allele of group-1 SNPs result from r_{1i} independent draws among DNA molecules
 205 containing the focal haplotype, which has frequency f in the pool. c_{2i} is the realization of
 206 $Binomial(r_{2i}, 0.5 - f)$ as the maternal alleles group-2 SNPs are carried by the non-focal maternal
 207 haplotype, which constitutes the rest of the maternally inherited chromosomes, themselves
 208 constituting half of the chromosomes in the F1. For a contig linked to the SDR, c_{2i} should be zero
 209 (Figure 1). However, a nucleotide indicating the maternal allele in a read may result from an
 210 “error”. Errors combine mutations between parents and offspring, *in vitro* mutations and sequencing
 211 or mapping errors. An error causing c_{2i} to be positive would nullify the posterior probability that $f =$
 212 0.5 , hence that no crossing over occurred between the locus and the SDR. To avoid this, we
 213 introduce a constant ε to represent the rate of error leading to a maternal allele appearing in a read.
 214 The probability that a read carries the maternal allele of a group-2 SNP becomes $(1 - \varepsilon)(1/2 - f) + \varepsilon$
 215 $= (1 + \varepsilon)/2 + \varepsilon f - f$.

216 We do not apply this correction to group-1 SNPs. A group-1 SNP of a contig linked to the SDR
 217 should present both alleles at the same frequency, such that errors leading to the detection of
 218 maternal alleles are compensated by error leading to the detection of non-maternal alleles.

219 From the above considerations,

$$220 \quad P(c_{1i} | f, r_{1i}) = \binom{r_{1i}}{c_{1i}} f^{c_{1i}} (1 - f)^{r_{1i} - c_{1i}}$$

221 and

$$222 \quad P(c_{2i} | f, r_{2i}) = \binom{r_{2i}}{c_{2i}} \left(\frac{1 + \varepsilon}{2} + \varepsilon f - f \right)^{c_{2i}} \left(\frac{1 - \varepsilon}{2} - \varepsilon f + f \right)^{r_{2i} - c_{2i}}$$

223
 224 The marginal probability of the maternal allele read counts, $P(c_{1i}, c_{2i} | r_{1i}, r_{2i})$, integrates over all the
 225 values that f can take, hence

226

$$227 \quad P(c_{1i}, c_{2i} | r_{1i}, r_{2i}) = \sum_{f \in F_i} P(f) \cdot P(c_{1i}, c_{2i} | f, r_{1i}, r_{2i}) \approx \sum_{f \in F_i} P(f) \cdot P(c_{1i} | f, r_{1i}) P(c_{2i} | f, r_{2i})$$

228

229 Replacing terms in equation 1 yields the following, after cancellation of terms present in the
230 numerator and denominator:

$$231 \quad P(f | \mathbf{c}r_i) \approx \frac{f^{c_{1i}}(1-f)^{r_{1i}-c_{1i}} \left(\frac{1+\varepsilon}{2} + \varepsilon f - f\right)^{c_{2i}} \left(\frac{1-\varepsilon}{2} - \varepsilon f + f\right)^{r_{2i}-c_{2i}}}{\sum_{\varphi \in F_i} \varphi^{c_{1i}}(1-\varphi)^{r_{1i}-c_{1i}} \left(\frac{1+\varepsilon}{2} + \varepsilon \varphi - \varphi\right)^{c_{2i}} \left(\frac{1-\varepsilon}{2} - \varepsilon \varphi + \varphi\right)^{r_{2i}-c_{2i}}}$$

232

233 These posterior probabilities were used to estimate the expected number of recombination events,
234 denoted here as n_{rec} , that occurred between a given contig and the SDR in the oocytes of several
235 pools. This was done by calculating the sum of the expected number of recombination events over
236 pools as follows:

237

$$238 \quad n_{\text{rec}} = \sum_{i=1}^p \sum_{f \in F_i} n_i (1-2f) P(f | \mathbf{c}r_i), \quad (3)$$

239

240 where p is the number of pools. This formula accounts for variable levels of uncertainty in the true
241 value of f among pools. Note that while the true number of recombination events is a discrete
242 random variable, its expected value n_{rec} is a weighted average and is therefore a continuous
243 variable.

244 Importantly, the accuracy of n_{rec} depends on the ability to reconstruct the maternal haplotypes, via
245 inference of SNP types (see previous section). This ability decreases with the genetic distance to the
246 SDR, which leads to underestimating of the number of recombination events (Supplementary text,
247 Figure S1). In our experiment, this bias becomes noticeable at about 8 recombination events,
248 corresponding to ~ 20 cM to the SDR, but it is minimal on contigs locating the closest to the SDR
249 (Figure S1).

250 [Crosses, sequencing and mapping](#)

251 We applied our approach to three *A. vulgare* lines: WXa, ZM and BF (Table 1), which have been
252 shown to have homologous sex chromosomes (CHEBBI *et al.* 2019). For each line, a single virgin
253 female was crossed with a single male until it showed evidence for gravidity and then isolated to lay
254 progeny. DNA was extracted from gonads, heads and legs of $n = 10$ descendants of each sex with
255 the Qiagen blood and tissue kit, according to the protocol for animal tissues (3h of incubation in
256 proteinase K at 56°C and 30 min of RNase treatment at 37°C). Absence of *Wolbachia*

257 endosymbionts and the *f* element in all samples was confirmed by PCR, as described previously
258 (LECLERCQ *et al.* 2016). DNA concentration was estimated for each sample by Qubit fluorometric
259 quantification, to enable pooling DNA samples in equimolar proportions. DNA samples from 10
260 individuals per sex constituted a pool containing 7 µg of DNA. Each pool was sequenced on an
261 Illumina HiSeq2500 platform (125-bp paired ends) by Beckman Coulter Genomics. We aimed at a
262 sequencing depth of 30X per pool to ensure that most parts of the 20 chromosome doses (i.e. from
263 10 diploid individuals) in the pools were sequenced. The DNA of individual parents from lines
264 WXA and ZM was extracted as described above and sequenced on an Illumina HiSeqX platform
265 (150-bp paired ends) by Génome Québec. To enable reliable genotyping, we targeted an average
266 sequencing depth of 30X per parent. However, technical reasons unrelated to our approach
267 prevented the sequencing of parents from line BF.

268 Sequencing reads were trimmed from low-quality parts using trimmomatic version 0.33 (BOLGER *et*
269 *al.* 2014). For each F1 pool and parent, trimmed reads were aligned on the female reference *A.*
270 *vulgare* genome (CHEBBI *et al.* 2019) using bwa_mem (LI 2013) with default settings. In the
271 resulting alignment (bam) file, reads sequenced from the same original DNA fragments (PCR or
272 optical duplicates) were flagged by picardtools MarkDuplicates version 2.12.0
273 (<http://broadinstitute.github.io/picard/>, last accessed June 9 2020). Reads containing indels were
274 realigned on the reference genome using GATK's IndelRealigner.

275 To establish the whole-genome genotype of each parent, we followed the GATK best practices
276 (VAN DER AUWERA *et al.* 2013) as described in CHEBBI *et al.* (2019). This involved recalibrating
277 base quality scores of mapped reads to reduce the risk of considering sequencing errors as variants,
278 followed by SNP genotype calling with HaplotypeCaller. Genotyping was performed independently
279 on each parent, recording all positions in a gvcf file. The four gvcf files were merged using
280 GenotypeGvcf in a single vcf file, discarding putative SNPs not passing quality check. We used this
281 file to select informative SNPs, excluding positions with indels, lack of sequence data in any
282 individual or with more than two alleles.

283 Estimation of haplotype frequencies within F1 pools

284 We used a custom R script (R DEVELOPMENT CORE TEAM 2020) that scans each F1 bam file via
285 samtools version 1.10 (LI *et al.* 2009) to retrieve the base carried by each read at informative SNPs,
286 associated with a unique read-pair identifier. Read pairs marked as duplicates were ignored as well
287 as secondary alignments and those with mapping quality score <20. For each pool and SNP, we
288 counted reads carrying the maternal and paternal alleles. Reads carrying other alleles were ignored.
289 At each informative SNP, the frequency of the maternal allele in the pool was estimated by the

290 proportion of reads carrying this allele. By comparing this frequency between sexes of the same
291 family, we inferred SNP type and group (1 or 2) using the principles described earlier.
292 For each contig in each pool i , we established the read count data (variables of the cr_i set) by
293 counting read pairs according to the definition for these variables. We then computed $P(f | cr_i)$ for
294 every possible value of f . We set the error rate ε at 0.01, which is higher than the typical
295 sequencing error rate of the Illumina technology. Using results from the four pools of the WXA and
296 ZM lines (for which we sequenced the parents), we estimated the number of recombination events
297 between the contig and the SDR, n_{rec} . For these computations, we excluded any SNP that failed to
298 pass the following criteria, which we applied independently for both families. Genotyping quality in
299 the mother (determined by GATK's haplotype caller) had to be higher than 10 and that of the father
300 higher than 40 (the presence of the rarer maternal allele in the F1 allowed us to be less restrictive on
301 the mother's genotype quality, while we wanted to ensure that the father was not homozygous). In
302 addition, at least one read had to carry the maternal or paternal allele in each F1 pool, the total
303 number of reads carrying either allele in both F1 pools combined had to not exceed its 95% quantile
304 (excessive sequencing depth may reflect the alignment of reads from several loci on the same
305 genomic region, due to paralog collapsing during genome assembly). Finally, the maternal allele
306 had to be present in at least one F1 read and both parental alleles had to be present in at least 75%
307 of the F1 reads covering the SNP (both pools combined).
308 Finally, preliminary results revealed a problem in which some contigs showed very low probability
309 of absence of recombination with the SDR in a given pool i , $P(f = 0.5 | cr_i)$, due to rare group-2
310 SNPs leading to aberrant c_{2i}/r_{2i} ratios. Accurate estimate of $P(f = 0.5 | cr_i)$ is critical as we use it to
311 select contigs that may contain the SDR (see section "Localization of genomic regions that may
312 contain the SDR"). We attribute the negative influence of "suspicious" SNPs on $P(f = 0.5 | cr_i)$ to
313 mapping or assembly errors (c_{2i} being much too high to result from sequencing errors). These errors
314 would lead to reads from different loci aligning on the same genomic region. Hence, the apparent
315 variation between reads would not represent allelic variation (SNPs), but another type of variation.
316 To locate these suspicious SNPs, we computed $P(f = 0.5 | cr_i)$ on each individual SNP as if it
317 constituted its own haplotype, and ignored SNPs yielding much lower posterior probability than the
318 rest of the SNPs of each contig (see Supplementary text, Figure S2).

319 [Contig assignment to sex chromosomes and analysis of recombination](#)

320 We used our estimate of the number of oocytes that underwent recombination between a target
321 contig and the SDR during both crosses (n_{rec} , equation 3) to isolate contigs that are significantly
322 closer to the SDR than expected assuming an autosomal location. To account for the uncertainty in
323 n_{rec} and approximations of our method (namely in equation 2), we contrasted the observed values of

324 n_{rec} to those obtained by simulating sequencing data in the F1 pools. These simulations used the
325 actual genomic positions of the retained informative SNPs and the identifiers of reads covering
326 these SNPs, only changing the bases that reads carried at SNPs to reflect a given genetic distance to
327 the SDR.

328 The simulations consisted in the following procedure, which we applied to every contig. First, we
329 assigned the contig a genetic distance (in Morgans) to the SDR, which we call d . We then applied
330 the following in each family independently. We designated the two homologous maternal
331 haplotypes at the contig with letters Z and W, denoting the sex chromosome hosting these
332 haplotypes in the mother. For each SNP that was informative in the family, the maternal allele was
333 randomly attributed to haplotype Z or W (i.e., the SNP is attributed to type 2 or 1) with the same
334 probability. We then applied the following to each of pool i of the family. We defined as n_{Zi} the
335 number of chromosomes carrying haplotype Z in the pool of $2n_i$ chromosomes. To simulate linkage
336 to the SDR, n_{Zi} was sampled from $\text{Binomial}(n_i, d)$ if the pool contained daughters or from $n_i -$
337 $\text{Binomial}(n_i, d)$ if the pool contained sons. To simulate the sequencing of the maternal haplotypes,
338 each read of maternal origin was randomly attributed to haplotype Z or W with probabilities
339 $n_{Zi}/(2n_i)$ and $0.5 - n_{Zi}/(2n_i)$, respectively. At each SNP, each read was set to carry the maternal allele
340 if the read and the maternal allele of this SNP were both attributed to the same haplotype (Z or W).
341 Otherwise, the read was set to carry the paternal allele. Based on these artificial reads, we inferred
342 SNP type and computed $P(f | cr_i)$ as for the real data. We repeated the procedure 1000 times for
343 every contig, with d set to 0.5 to simulate autosomal contigs. If, for any contig, the value of n_{rec}
344 obtained from the real data was lower than the 1/1000th quantile of values obtained from
345 simulations, we considered the contig as located on the sex chromosomes with a 1/1000 risk of false
346 positive.

347 We also performed simulations to investigate a potential reduction of recombination rate near the
348 SDR, which may evolve during sex chromosome divergence. To this end, we assessed how the
349 cumulated length of contigs (a proxy for physical distance on chromosomes) varies according to the
350 inferred distance to the SDR (n_{rec}). We did so for observed data and for simulated data assuming
351 uniform recombination rates along sex chromosomes. Uniformity in recombination rates was
352 ensured by attributing every contig a value of d sampled from $\text{Uniform}(0, 0.5)$. We assigned these
353 "simulated contigs" to sex chromosomes as we did for observed data. After discarding contigs not
354 assigned to sex chromosomes, we randomly sampled a number of simulated contigs, ensuring that
355 their total length was as close as possible to the total length of observed contigs assigned to sex
356 chromosomes. We repeated this sampling 1000 times to build confidence intervals of the cumulated
357 length of contigs as a function of the inferred genetic distance to the SDR.

358 Investigation of heterozygosity

359 The SDR and its genomic context are predicted to show increased allelic divergence compared to
360 autosomal regions due to balancing selection combined with potentially reduced recombination. We
361 investigated this hypothesis by measuring female heterozygosity in the two individually genotyped
362 mothers. For each mother, we ignored SNPs of genotype quality <40, those of sequencing depth <5
363 or higher than the 95% quantile for the genotyped individual (only considering positions reported in
364 the vcf file). For each contig, we counted the number of unique heterozygous positions passing
365 these filters when considering both mothers combined. To estimate SNP density given the
366 sequencing effort, we recorded the number of unique contig positions belonging to the
367 aforementioned range of sequencing depths for each contig, again combining both mothers.
368 Sequencing depth was computed with samtools, excluding duplicate reads, secondary alignments,
369 alignments with mapping quality zero and bases with PHRED score <10, to mimic the parameters
370 used by the SNP caller as much as possible.

371 We then analyzed how female heterozygosity varied according to the inferred genetic distance to
372 the SDR (n_{rec}). For these analyses, we opted to ignore contigs for which n_{rec} could not be inferred
373 with sufficient certainty. We did so by discarding contigs for which the highest posterior probability
374 of f , $\max(P(f | \mathbf{cr}_i))$, was lower than 0.5 in any pool i . Doing so considers that contigs with fewer
375 informative SNPs, hence with lower female heterozygosity on average, were less likely to be
376 assigned to sex chromosomes due to reduced statistical power. This bias might lead to a spurious
377 correlation between female heterozygosity and assignment to sex chromosomes. Selecting contigs
378 for which that data allowed estimating n_{rec} with a certain level of confidence should mitigate this
379 bias.

380 Localization of genomic regions that may contain the SDR

381 Our crossing scheme enabled a two-level selection of genomic regions that may contain the SDR.
382 The first level consisted in selecting contigs that showed little evidence for recombination with the
383 SDR during our crosses. We based our selection on the posterior probability of absence of
384 recombination, which we multiplied across pools for each contig as follows

$$385 \quad \prod p = \prod_{i=1}^p P(f = 0.5 | \mathbf{cr}_i). \quad (4)$$

386 We selected contigs for which $\prod p$ exceeded 0.5. We also considered selecting contigs that were
387 more likely to have undergone 0 recombination with the SDR, i.e. contigs for which n_{rec} was closer
388 to zero than to one ($n_{\text{rec}} < 0.5$). This criterion was fulfilled by all the contigs retained by the former
389 except three (109 vs. 112) and did not retain any additional contig. We opted for the more inclusive
390 criterion.

391 We then used our ability to reconstruct the parental haplotypes of the selected contigs for the WXa
392 and ZM lines to assess whether a target genomic region recombined with the SDR during the
393 divergence of the two lines (Figure 2). This task relied on SNPs whose alleles can be assigned to
394 parental W or Z chromosomes. These were the informative SNPs that we retained, whose alleles
395 were assigned to maternal chromosomes via inference of SNP type, or SNPs that were homozygous
396 in mothers (Figure 2). For each SNP that was not informative in at least one family, we imposed a
397 minimal genotype quality of 40 and a maximum sequencing depth equaling the 95% quantile of this
398 variable, for each parent of the family (or families). We then discarded SNPs for which the rarer
399 allele was carried by only one parental chromosome among the four parents, as such SNPs cannot
400 inform on recombination. We refer to the set of retained SNPs as "selected SNPs". Recombination
401 between a selected SNP and the SDR was inferred if these two loci constituted four different
402 haplotypes in the parents, considering the SDR as a biallelic locus with Z and W alleles. We refer to
403 selected SNPs that recombined with the SDR as "recombinant SNPs" (Figure 2).

404 Among selected SNPs, we looked for non-recombinant SNPs that were informative in both families
405 (e.g., SNPs #1 and #4 on Figure 2). We reasoned that close linkage to the SDR should maintain
406 female heterozygosity by balancing selection, hence favor this category of SNPs. Within this
407 category, we more specifically looked for SNPs that may directly contribute to sex determination,
408 i.e., SNPs that could compose the sex-controlling locus. Such a SNP must be heterozygous in all
409 females and homozygous in all males, for the same bases and in all families, considering both
410 parents and F1s. These SNPs were easily identified as being of type 1 and presenting the same
411 maternal and paternal alleles across families (e.g., SNP #1 on Figure 2). Hereafter, we call them
412 "causal SNPs".

413 To more reliably determine that a given SNP is causal, we estimated the probability that this SNP
414 has not recombined with the SDR in our third family (BF). Because parental genotypes were
415 missing, we assumed that the candidate SNP was informative in this family, was of type 1, and
416 presented the same maternal and paternal alleles as the WXa and ZM families. We computed $P(f=$
417 $0.5 | cr_i)$ in each BF pool i for each selected SNP as if it constituted its own haplotype. Not fulfilling
418 the assumptions or having recombined with the SDR would result in a low posterior probability.

419 We then multiplied the probabilities obtained from the two BF pools and discarded the SNP as
420 causal if this product was <1%. This threshold was chosen after considering that 98.9% of the
421 informative SNPs carried by the studied contigs in the WXa and ZM families had a value exceeding
422 1% at this variable. We therefore considered the 1% threshold as rather permissive in the selection
423 of causal SNPs.

424 Beyond SNPs, we aimed at defining larger regions that may or may not have recombined with the
425 SDR. To do so, we delineated contig regions (hereafter called "blocks") within which no SNP
426 showed evidence for recombination with any other, by applying the aforementioned four-haplotype
427 criterion on every possible pair of selected SNPs (see Supplementary text for details). Note that a
428 causal SNP and a recombinant SNP cannot be in the same block as the former behaves exactly as
429 the ZW SDR.

430 We ignored every block containing a single selected SNP, unless this SNP was causal, as we
431 considered such a short block as possibly resulting from a genotyping error rather than from
432 recombination. As blocks were initially delineated by SNP coordinates, we extended block
433 boundaries up to contig edges, or up to midpoints between consecutive blocks, as appropriate. We
434 then considered any block harboring at least two recombinant SNPs or at least 50% of recombinants
435 among selected SNPs as having recombined with the SDR.

436 [Search of W-specific sequences](#)

437 Z-linked and W-linked alleles may not show detectable homology due to excessive molecular
438 divergence. This possibility implies that the most divergent parts of the W and Z chromosomes,
439 potentially including the SDR, constitute different contigs in the reference genome assembly, with
440 some contig(s) containing W-specific regions and other contigs (or another contig) containing Z-
441 specific regions. Such regions would not present informative SNPs since W-derived and Z-derived
442 reads would not map on the same locations. However, the sequencing depth of a W-specific region
443 should be close to zero for male-derived sequencing reads and it should be higher for female-
444 derived reads. We used this criterion to locate such regions.

445 Sequencing depth of all six F1 pools was measured with samtools, using the same mapping quality
446 threshold as that used for the SNP analysis. For each pool, sequencing depth was averaged over 2-
447 kb sliding windows that moved by 500 bp, leading to a 1500-bp overlap between successive
448 windows. To standardize results given differences in sequencing effort, we multiplied depths by the
449 highest mean sequencing depth over the six pools (averaged over all windows) and divided them by
450 the mean sequencing depth of the pool under consideration. We then computed the "Chromosome
451 Quotient" (CQ) (HALL *et al.* 2013) by dividing the sequencing depth obtained from sons by that
452 obtained from daughters, for each window in each family.

453 [Results](#)

454 [Sex chromosomes constitute at least 43 Mbp of the *A. vulgare* genome](#)

455 For the WXa and ZM families combined, more than 5.1 million individual positions of the genome
456 were homozygous in fathers and heterozygous in mothers, constituting potentially informative

457 SNPs. The ~3.7 million SNPs that passed our filters were carried by 40640 contigs constituting
458 ~96.7% of the genome assembly length (1.72 Gbp). Among these, 30875 contigs (~82.2% of the
459 genome assembly length) carried informative SNPs in both families.
460 Sequence data from the F1 pools at retained SNPs were used to compute n_{rec} (equation 3), the
461 estimated number of oocytes that have recombined with the SDR in our crosses. Detailed results for
462 each contig are provided in File S1. Figure 3 shows the distribution of the percentage of
463 recombinant oocytes ($n_{rec}/40 \times 100$), which is the inferred distance to the SDR in cM. The
464 distribution obtained from real data is similar to that obtained from simulations assuming autosomal
465 contigs but is slightly shifted to the right, possibly due to errors that we did not simulate
466 (Supplementary text). Despite this slight right shift, a tail is visible to the left, denoting contigs
467 located closer to the SDR than expected for autosomes. In particular, 1004 contigs, representing the
468 pale blue distribution in Figure 3 and totaling ~43.6 Mbp, present significantly lower distances to
469 the SDR than expected from autosomal contigs and were assigned to sex chromosomes.
470 Considering that these results implicate ~82.2% of the genome assembly (contigs showing
471 informative SNPs in both families), we extrapolate that ~53 Mbp of contigs ($43.6/0.822$) locate on
472 sex chromosomes. These contigs should include about one thousandth of the autosomal contigs
473 (false positives) at our significance level. However, false negatives are likely to be more frequent
474 than 1/1000 according to additional simulations we performed to evaluate our method
475 (Supplementary text, Figure S3). Therefore, the estimated length of *A. vulgare* sex chromosomes
476 can be considered as conservative.

477 The sex determining region of *A. vulgare* is located within less than 1 Mb

478 The sex chromosomes of *A. vulgare* appear to show relatively uniform crossing over rates. The
479 cumulated length of contigs assigned to sex chromosomes indeed increases regularly with the
480 inferred genetic distance to the SDR and remains within the 99% confidence intervals obtained
481 from simulations assuming uniform crossing over rates (Figure 4). There is consequently no
482 evidence for a reduction in crossing over rates near the SDR, in which case the cumulated length of
483 contigs would have been higher than expected at short genetic distances.

484 Among the 1004 contigs assigned to sex chromosomes, 112 collectively accounting for ~5.1 Mbp
485 (Figure 5) present little evidence of recombination with the SDR during our crosses ($\chi^2 p > 0.5$,
486 equation 4). However, our SNP-based analysis leveraging the use of the two *A. vulgare* lines (WXa
487 and ZM) indicated that most of these contigs have recombined with the SDR at some point after the
488 divergence of the WXa and ZM chromosomes. Indeed, the 112 selected contigs were largely
489 composed of genomic blocks harboring recombinant SNPs. Overall, few genomic regions (~895
490 kbp total) did not show evidence of recombination with the SDR. We detected only ten SNPs that

491 could directly contribute to sex determination (i.e. potential causal SNPs for which all males appear
492 to be homozygous and females heterozygous for the same bases) in all three families (120 SNPs if
493 we ignore the BF family). The 10 SNPs correspond to five genomic blocks located on as many
494 different contigs, and total ~64 kbp. None of these SNPs locates in an exon.

495 The SDR had a modest impact on the molecular divergence of the Z and W chromosomes. Indeed,
496 female heterozygosity did not significantly decrease with the inferred genetic distance to the SDR
497 (one-sided Spearman's rank sum test $S = 74540329$, $p = 0.3416$) (Figure 6). Yet, female
498 heterozygosity increased at the closest distance to the SDR. Its median was indeed significantly
499 higher for the 112 contigs that showed little evidence of recombination with the SDR than the other
500 contigs assigned to the sex chromosomes (~10.32 SNPs/kbp vs ~9.01 SNPs/kbp, one sided Mann-
501 Whitney's $U = 41839$, $p \approx 0.005$). Despite this difference, the median female heterozygosity of the
502 contigs assigned to sex chromosomes did not differ from that of the other contigs (two-sided $U =$
503 5598974 , $p = 0.3253$). It should be kept in mind that these comparisons do not include the whole
504 genome as we discarded contigs for which f could not be inferred with a posterior probability of at
505 least 0.5 (see section "Investigation of heterozygosity").

506 [W-specific genomic regions are rare](#)

507 The ratios of sequencing depth obtained from sons to that obtained from daughters (CQ scores)
508 presented distributions that are typical of autosomes for all three families (Figure S4), showing little
509 evidence for sex chromosomes heteromorphy. Only seven contigs contained W-specific sequences,
510 as defined by genomic windows with CQ <0.3 and female sequencing depth >5 in all three families.
511 These windows added up to ~92 kbp. Informative SNPs present in these seven contigs indicated
512 that six have recombined with the SDR during the crosses, with a minimum n_{rec} of ~3.5. The one
513 exception was contig 20397 (Figure 7). Remarkably, it is also the contig that possesses the most
514 potentially causal SNPs ($n = 4$) in a single block spanning the whole contig (Figure 5).

515 Information about the two genes in contig 20397 (Figure 7) is provided in Table 2. A DNA
516 sequence similarity search using blastn (CAMACHO *et al.* 2009) with default settings showed that the
517 exons of these genes were similar to exons of other annotated genes of the *A. vulgare* genome.
518 These genes therefore present paralogs. The sequence identity of the most similar copy of each gene
519 (Table 2) was higher than the identity measured with the most similar annotated gene of *A. nasatum*
520 (BECKING *et al.* 2019), which was inferred to have diverged from the *A. vulgare* lineage ~25 million
521 years ago (BECKING *et al.* 2017). This result suggests relatively recent duplications of the two
522 genes.

523 Discussion

524 Benefits of our method

525 Segregation analysis methods aimed at locating sex-associated regions generally rely on individual
526 genotyping by partial genome sequencing, using RAD tags or similar techniques as cost-saving
527 measures (PALMER *et al.* 2019). Pool-seq appears to be less utilized for this task, especially
528 compared to its popularity in population genomics analyses using field samples (KOFLENER *et al.*
529 2011). However, when obtaining a genetic map is not essential, whole-genome pool-seq can be
530 advantageous, not only because of its ease of use and applicability to any species, but mostly
531 because of the millions of SNPs this approach can yield. In comparison, partial genome sequencing
532 using RAD tags yields tens of thousands of reliable SNPs at most. If this number does not largely
533 exceed the number of contigs in the genome assembly, as might be the case for a non-model
534 organism, a high risk of false negative may affect the selection of contigs that can contain the SDR.
535 RNA sequencing (as used in (MUYLE *et al.* 2016)) would also suffer this problem if exon density is
536 low (~1.4% of the genome assembly in the case of *A. vulgare*), added to the fact that only a subset
537 of genes is expressed during an experiment. The millions of SNPs yielded by whole-genome
538 sequencing is leveraged by our approach through the combination of all the informative SNPs of a
539 contig into haplotypes, whose frequencies are more precisely inferred than allele frequencies based
540 on single SNPs. A high SNP density is also particularly useful to pinpoint the SDR within contigs
541 that did not recombine with this locus during the crosses, thanks to a multi-family setup (Figure 2,
542 Figure 5). Here, the resolution corresponds to the typical distance between SNPs that inform on
543 recombination with the SDR and would be quite limited if partial genome sequencing were
544 employed. In fact, our approach can be used to locate a genomic region controlling any qualitative
545 trait that depends on a single locus. Doing so would simply require treating the phenotype that
546 associates with the heterozygous genotype as the ZW females of our study.

547 We emphasize that the number of read pairs covering all the informative SNPs of a contig
548 (variables r_{1i} and r_{2i}), not sequencing depth per se, determines the certainty of the estimated allele
549 frequencies. Contigs with more informative SNPs, hence longer contigs for a given SNP density,
550 thus require lower average sequencing depth for a similar degree of certainty in estimated haplotype
551 frequencies. While larger F1 pools should yield improved imprecision, the size of a pool, n , must
552 ensure that $1/n$ is at least twice as high as the sequencing error rate, to clearly differentiate rare
553 alleles from errors. Cost considerations aside, a larger number of families (hence of pools) can
554 circumvent this limitation and also increase the ability to detect past recombination with the SDR
555 during the divergence of studied lineages, hence to exclude genomic regions as containing the SDR.

556 The precision yielded by whole-genome pool-seq extends to the location of regions harboring
557 sequences that are specific to the rarer sex chromosomes (here, the W) via the CQ analysis. The use
558 of sliding windows, which would not be permitted under partial genome sequencing, allows low-
559 CQ regions to be shorter than contigs. In this case, the statistical analysis of nearby SNPs provides a
560 useful complement to the CQ scores. Indeed, the absence of reads having a particular DNA
561 sequence from one sex (CQ=0) may not imply the complete absence of this sequence from the
562 genome analyzed individual(s), due to the odds of DNA sequencing. Also, it is unclear whether a
563 low CQ value indicates low genetic similarity between sex chromosomes at the focal genomic
564 window (allowing a small portion of reads from a chromosome to map on the sequence of the other)
565 or the rare occurrence of an allele in the sex where this allele is supposed to be absent. The analysis
566 of nearby SNPs reduces these uncertainties by providing a probabilistic assessment of the
567 association between genetic variants and sexes, which may extend to low-CQ regions of the same
568 contig. This combination of approaches allowed us to outline contig 20397 among the seven contigs
569 showing low-CQ windows in *A. vulgare*.

570 *Extremely low divergence of A. vulgare sex chromosomes*

571 Seven contigs of the *A. vulgare* genome assembly contain ~92 kbp of potential W-specific
572 sequences, and only contig 20397 was inferred to not have recombined with the SDR during our
573 crosses. Our previous study (CHEBBI *et al.* 2019) outlined 27 contigs containing W-specific
574 sequences, only two of which (including contig 20397) presented low-CQ windows in the present
575 study. This difference can be explained by the fact that CHEBBI *et al.* (2019) investigated a single
576 family, reducing the probability of recombination with the SDR, and computed CQ at the scale of
577 whole contigs rather than genomic windows. Indeed, 14 of the 27 contigs outlined by CHEBBI *et al.*
578 (2019) are among those we assigned to sex chromosomes (considering that four of the 27 contigs
579 did not have informative SNPs and could not be assigned). Due to partial linkage to the SDR, these
580 14 contigs would have harbored genetic differences that associated with the sex of individuals
581 studied in CHEBBI *et al.* (2019), but this association did not hold in the three lines we analyzed,
582 except for contig 20397. The low CQ scores reported by CHEBBI *et al.* (2019) at these contigs
583 therefore do not amount to sex chromosome divergence, but to polymorphism in these
584 chromosomes. The other nine contigs that we did not assign to sex chromosomes may be more
585 distant to the SDR. Their median inferred genetic distance to the SDR of ~31.5 cM is still
586 significantly lower than the median of other contigs not assigned to sex chromosomes (39.3 cM)
587 (two-sided Mann & Whitney's $U = 64752$, $p \approx 8.5 \times 10^{-4}$). Thus, some of the nine contigs may
588 belong to sex chromosomes despite not having passed the assignment test.

589 The scarcity of W-specific sequences in the *A. vulgare* genome extends to Z-specific sequences.
590 Indeed, the CQ distributions obtained from the three studied lines (Figure S4) show no particular
591 bump near 2, which is the expected CQ value for Z-specific regions. We did not specifically study
592 regions with high CQ, because elevated CQ on short genomic windows is subject to high sampling
593 variance, hence to false positives/negatives, as opposed to low CQ which involves low sequencing
594 depth, hence low variance. At any rate, these results demonstrate the very low divergence of the *A.*
595 *vulgare* sex chromosomes. In our previous study (CHEBBI *et al.* 2019), the inference of low
596 divergence was not definitive as the size of sex chromosomes was undetermined. Here we show
597 that the sex chromosomes have a minimal size of 53 Mbp, that is, 83% the average size of *A.*
598 *vulgare* chromosomes (~64 Mbp) based on genome size and number of chromosomes. Even though
599 our estimate of sex chromosome length is conservative, it is orders of magnitude that of W-specific
600 sequences.

601 The reduced divergence of *A. vulgare* sex chromosomes is consistent with their recombination
602 rates. Only ~5.1 Mbp of contigs would not have recombined with the SDR during our crosses, and
603 most have recombined with the SDR since the divergence of the ZM and WXa lines (Figure 5). The
604 fact that sex chromosomes did not show evidence for uneven crossover rates (Figure 4) and are not
605 distinguishable from autosomes in terms of female heterozygosity (Figure 6) suggests comparable
606 levels of recombination between chromosome types. The higher density of heterozygous SNPs in
607 contigs locating the closest to the SDR (Figure 6) could just be the byproduct of balancing
608 selection, increasing coalescent times of SDR-linked alleles over a relatively short genomic region.
609 Hence, we do not consider this observation as conclusive evidence for a reduction of crossing over
610 rate around the SDR.

611 The apparent absence of recombination reduction in *A. vulgare* sex chromosomes could reflect their
612 recent origin, considering the apparent rapid turnover of sex chromosomes in terrestrial isopods
613 (BECKING *et al.* 2017). An evolutionary scenario for this renewal involves feminizing bacterial
614 endosymbionts of the genus *Wolbachia* (RIGAUD *et al.* 1997; CORDAUX *et al.* 2011). *Wolbachia*
615 endosymbionts that infect terrestrial isopods can improve their maternal transmission by feminizing
616 their carriers, as commonly observed in *A. vulgare* populations (JUCHAULT *et al.* 1993; VERNE *et al.*
617 2012; VALETTE *et al.* 2013). Theoretical models and field surveys on *A. vulgare* indicate that
618 invasion of a ZW population by feminizing *Wolbachia* leads to the loss of W chromosomes, as
619 feminized ZZ individuals take the role of mothers (reviewed in CORDAUX AND GILBERT (2017)).
620 Sex then becomes entirely determined by the presence of *Wolbachia* and is generally biased
621 towards females, reflecting the prevalence of the feminizing bacteria. New sex chromosomes may
622 emerge via the selection of masculinizing nuclear genes that may reestablish an even sex ratio

623 (CAUBET *et al.* 2000; BECKING *et al.* 2019) or through horizontal gene transfer of feminizing
624 *Wolbachia* genes into host genomes, producing new W-type chromosomes (LECLERCQ *et al.* 2016;
625 CORDAUX AND GILBERT 2017).

626 With these considerations in mind, we cannot strictly exclude that the ZW chromosomes of *A.*
627 *vulgare* are old, as female heterogamety prevails among Armadillidiidae and related families
628 (BECKING *et al.* 2017). The *A. vulgare* sex chromosomes may have been maintained as
629 homomorphic for a long period through sustained recombination, similarly to what is observed in
630 palaognaths (XU *et al.* 2019), European tree frogs (STÖCK *et al.* 2011) or guppies (DAROLTI *et al.*
631 2020). Characterizing the SDRs of ZW species that are closely related to *A. vulgare* should allow
632 evaluating the homology, hence the age, of sex chromosomes among these lineages.

633 The sex determining region of *A. vulgare*

634 Barring false negatives, the sex-controlling locus of *A. vulgare* should lie within the ~0.9 Mbp of
635 genomic blocks that did not show evidence for recombination with the SDR (Figure 5). As these
636 blocks span several contigs that also harbor recombinant SNPs, the total length of the SDR, as
637 defined by the non-recombining chromosomal region surrounding the master sex-determining
638 gene(s), is likely to be much shorter than 0.9 Mbp. Where the SDR locates in these 0.9 Mbp cannot
639 yet be determined, but we expect this locus to harbor Z-specific and W-specific alleles that have
640 been maintained for a long time by balancing selection. We therefore do not favor the 489 kpb of
641 genomic regions harboring no SNP whose alleles could be associated to the W and Z chromosomes
642 in both *A. vulgare* lines. On the opposite, the 64 kbp of genomic regions containing causal SNPs are
643 of greater interest as they contain sex-associated variation in all three investigated families.

644 Among the candidate genomic regions, contig 20397 clearly emerges. This contig harbors the
645 region with the highest number of causal SNPs, no SNP indicating past recombination with the
646 SDR and a low-CQ region encompassing two annotated genes. The low CQ on these genes
647 indicates the presence of large indels and/or the accumulation of smaller mutations that make male
648 reads unable to align on the reference sequence (i.e., the allele on the W chromosome). As the two
649 annotated genes of contig 20397 appear to be female-specific, their expression may be required for
650 development into a female. Interestingly, these genes present highly similar paralogs. Evolution of
651 master sex-determination genes by duplication of existing genes has been reported in diverse taxa,
652 such as medaka fish (MATSUDA *et al.* 2002; NANDA *et al.* 2002) and clawed frog (YOSHIMOTO *et al.*
653 2008). Unfortunately, the available functional annotation for the two genes on contig 20397 (Table
654 2) precludes any speculation about possible mechanisms of action. Moreover, the coding sequences
655 of the two female-specific genes in *A. vulgare* present at most six substitutions with their closest
656 paralog, preventing any reliable inference about natural selection acting on their evolutionary

657 branch. We therefore cannot exclude that these genes are redundant copies that happen to be linked
658 to a nearby feminizing allele, and whose absence in males has no consequence. We also keep in
659 mind that a ZW sex determining system may not necessarily involve feminizing gene transcripts or
660 proteins, and that sex determination in *A. vulgare* could depend on expression levels of Z-specific
661 genes, as in the chicken (SMITH *et al.* 2009). Investigating these hypotheses requires searching for
662 Z-specific sequences in the *A. vulgare* genome. While CQ scores resulting from comparisons of ZZ
663 and ZW individuals may not be reliable for this task, as previously discussed, CQ scores obtained
664 by comparing laboratory produced WW individuals (JUCHAULT AND LEGRAND 1972) and ZZ or ZW
665 individuals should be close to zero for Z-specific sequences, similarly to the CQ scores we used to
666 locate W-specific sequences.

667 To conclude, the extremely low molecular divergence of the *A. vulgare* sex chromosomes and their
668 apparently uniform recombination rates allowed us to pinpoint a limited set of regions that could
669 contain the SDR and to identify two potential feminizing genes. Their strict association with the
670 female sex in additional *A. vulgare* lines and their expression levels during sex differentiation will
671 be the focus of future research. An improved reference genome assembly would also lessen the risk
672 that the sex-determining locus was missed due to insufficient sequencing coverage and/or number
673 of informative SNPs, which mostly affect short contigs. By mapping our sequence data on a more
674 contiguous (chromosome-scale) genome assembly and applying our approach on long genomic
675 windows, the SDR of *A. vulgare* should be characterized in its entirety.

676 Data availability

677 The custom code used to perform the data analysis is given in File S2. The genetic data generated in
678 this study are deposited to Genbank under accession numbers [to be deposited].

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688 **Tables**

689 **Table 1**

690 Characteristics of the three *A. vulgare* lines used in this study.

Line/family	WXa/1591	ZM/544	BF/2875
Source location	Helsingør, Denmark	Heraklion, Greece	Nice, France
Collection date	1982	1989	1967
GenBank accession numbers:			
- Father (ZZ)	Xxx	Xxx	Not sequenced
- Mother (ZW)	Xxx	Xxx	Not sequenced
- Pool of 10 sons (ZZ)	Xxx	Xxx	SRR8238987
- Pool of 10 daughters (ZW)	xxx	xxx	SRR8238986

691 Xxx : GenBank accession numbers will be provided at revision stage.

692

693 **Table 2**

694 Annotated genes in contig 20397 containing genomic windows of low chromosome quotient in the
695 three *A. vulgare* lines.

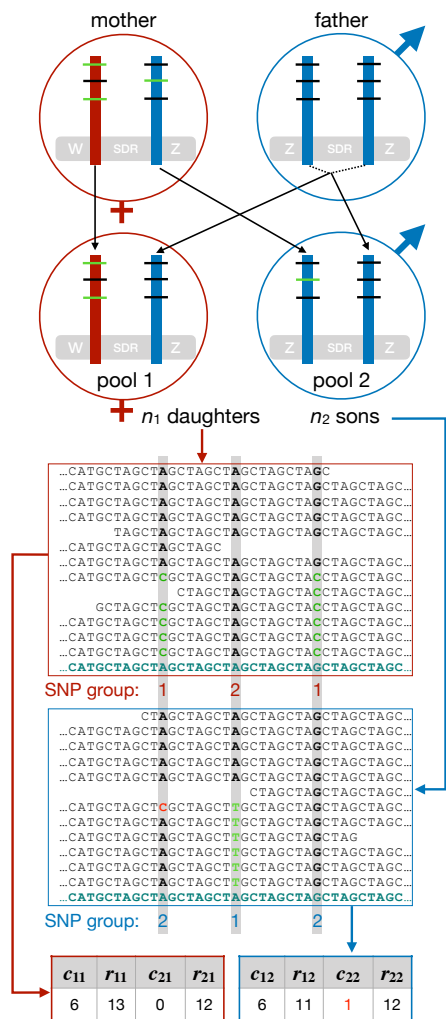
Name	Number of exons	Exon length	Description	Number of paralogs ^a	Highest identity with paralogs ^b
AV_16253	2	204	Hypothetical protein	11	97.0%
AV_16254	2	228	Hypothetical protein	2	98.7%

696 ^a genes that harbor a region of at least 100 bp having a blastn e-value 10^{-4} with the coding

697 sequence of the gene listed in the first column. ^b Identity considers the alignments reported by

698 blastn, not the whole gene lengths.

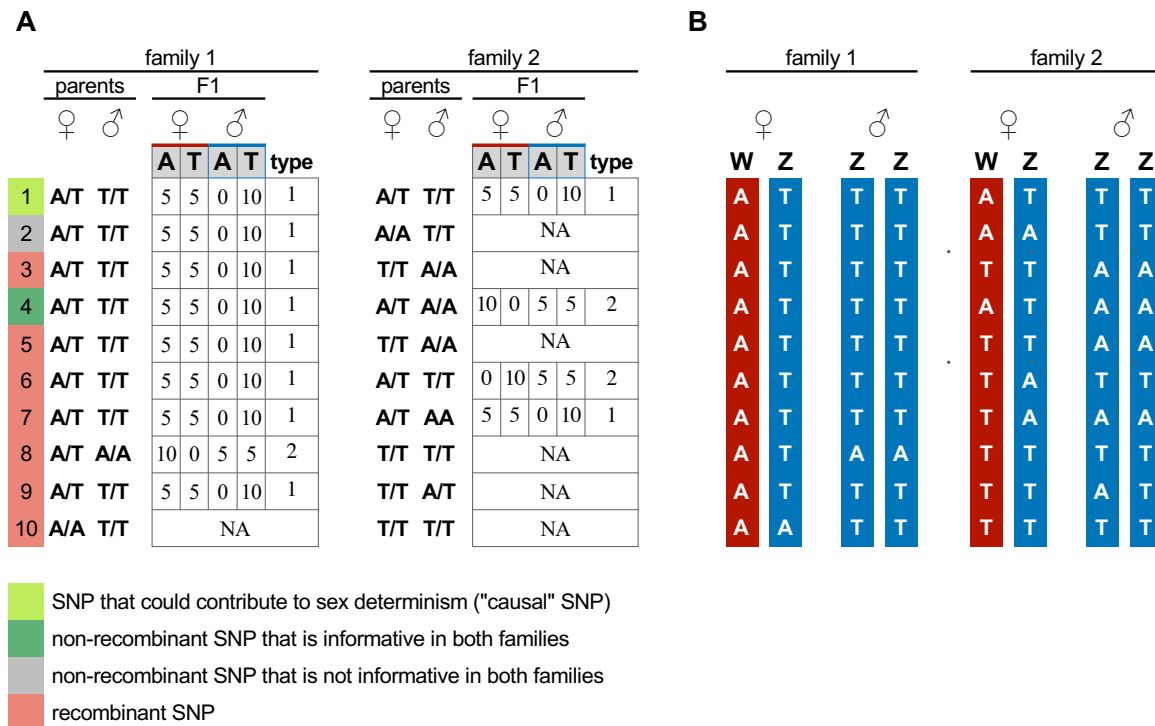
699 Figures



700

701 **Figure 1**

702 Locating the ZW sex determining region (SDR) via a cross where the genomes of several F1
 703 siblings per sex are sequenced in pools and parental genomes sequenced individually. Red/blue rods
 704 represent regions of W/Z chromosomes possessing informative SNPs shown as horizontal
 705 segments. For each SNP, the maternal allele (as defined in the methods section) appears in green
 706 and the paternal allele in black. In this example, no crossing over occurred between the SNPs and
 707 the SDR. The mapping of reads obtained from the pools shows sequences (reads) aligned on the
 708 reference genome (bottom sequence), with three informative SNPs outlined. A sequencing error is
 709 shown in red. Tables at the bottom show the values of the variables used to estimate the frequency
 710 of the focal haplotype (the W-carried haplotype for daughters and the Z-carried haplotype for sons),
 711 based on the mapped reads. See section "Statistical estimation of haplotype frequencies" for the
 712 definition of these variables.

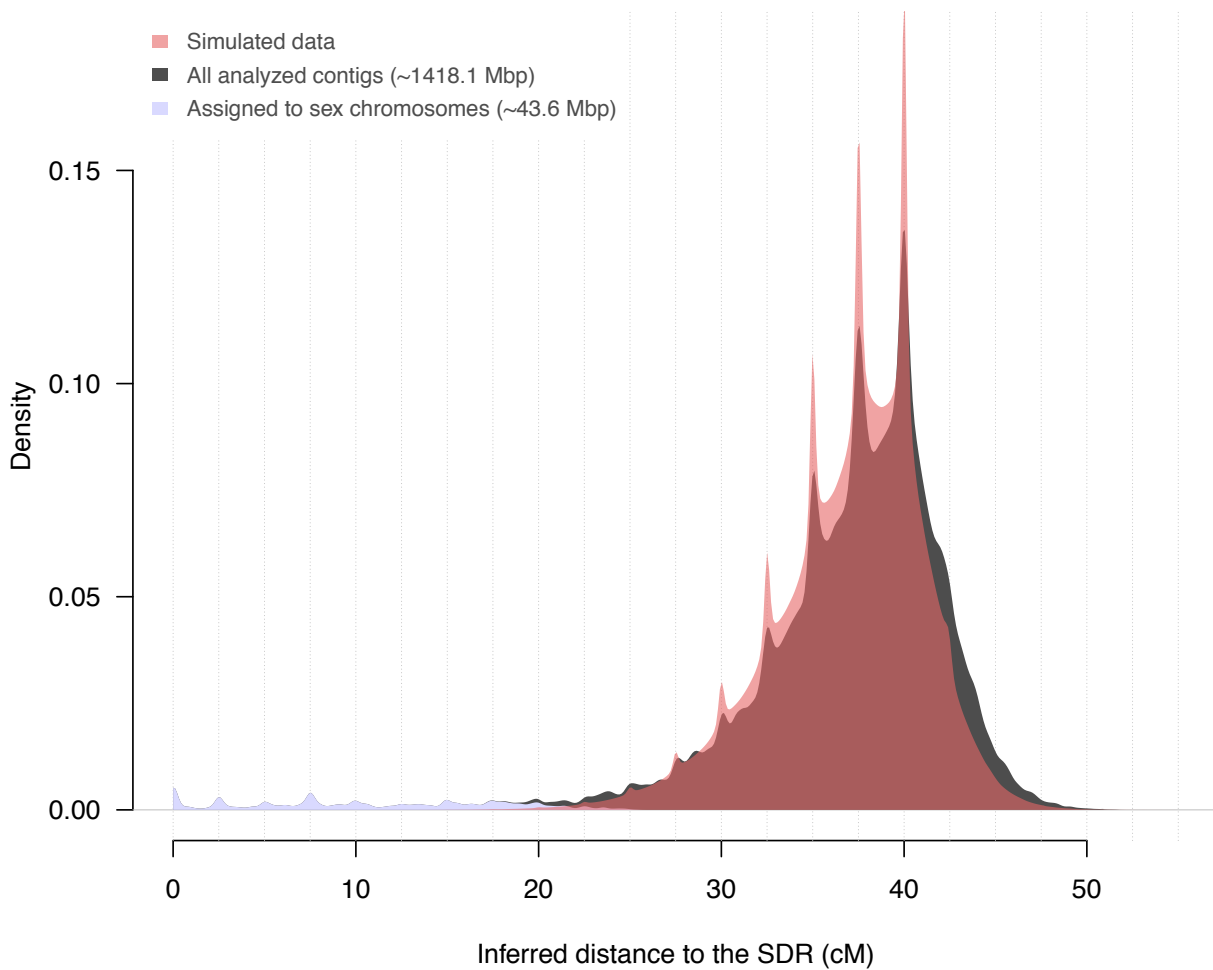


713

714 **Figure 2**

715 Ten hypothetical SNPs at a locus that has not recombined with the SDR during crosses involving
 716 two families. Letters A/T indicate DNA bases (alleles) at the SNPs. A) parental genotypes and data
 717 from F1 pools. Numbers (0, 5, 10) in the F1 tables indicate the number of reads that carry each
 718 allele and are only recorded for informative SNPs (otherwise, "NA" is noted). SNP type is deduced
 719 from allele frequencies in the F1 (see section "General approach"). B) Reconstructed parental
 720 haplotypes shown as vertical rods, using inferred SNP types for heterozygous mothers.
 721 Recombination must have occurred between certain SNPs and the SDR during the divergence of
 722 families, barring homoplasmy in the SNPs. SNP #4 may not have recombined with the SDR, but it is
 723 flanked by two SNPs that must have. Because there is no evidence of recombination between SNP
 724 #4 and these two others (these three SNPs constitute three different haplotypes, not four), they
 725 constitute a single genomic block delineated by the dotted lines.

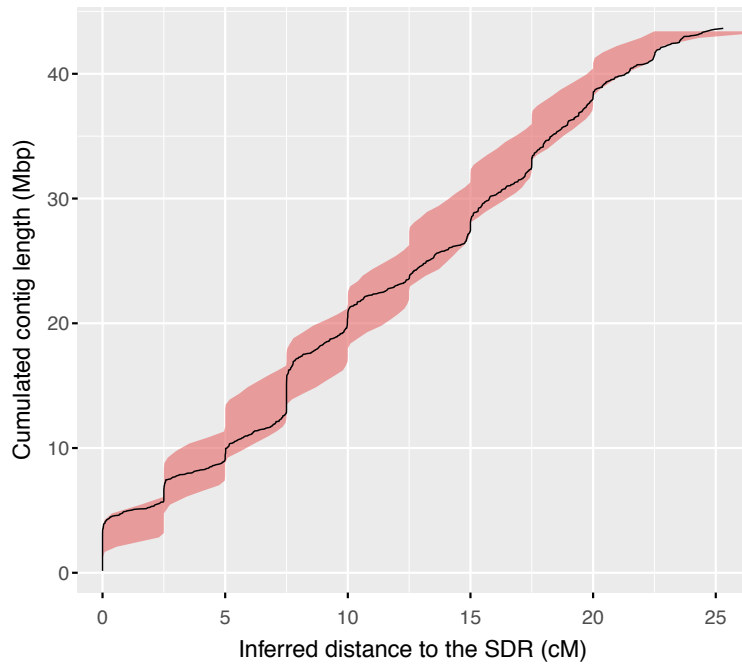
726



727

728 **Figure 3**

729 Distributions of the inferred genetic distance of *A. vulgare* contigs to the SDR for real data and for
 730 simulated data assuming that all contigs locate on autosomes. Genetic distances are inferred from
 731 simulated or observed genetic data from 40 F1 siblings belonging to two families. Vertical dotted
 732 lines represent genetic distances corresponding to integer numbers of recombination events during
 733 the crosses. Distributions only consider contigs for which both families present informative SNPs
 734 (33406 contigs). Contigs whose inferred distance to the SDR was lower than distances yielded by
 735 all simulations were assigned to sex chromosomes and constitute the blue area (see text). The
 736 distribution modes are lower than 50 cM, despite this value being the expectancy for autosomal
 737 contigs, because haplotypes cannot be properly reconstructed for contigs that are distant to the SDR
 738 (Supplementary text).

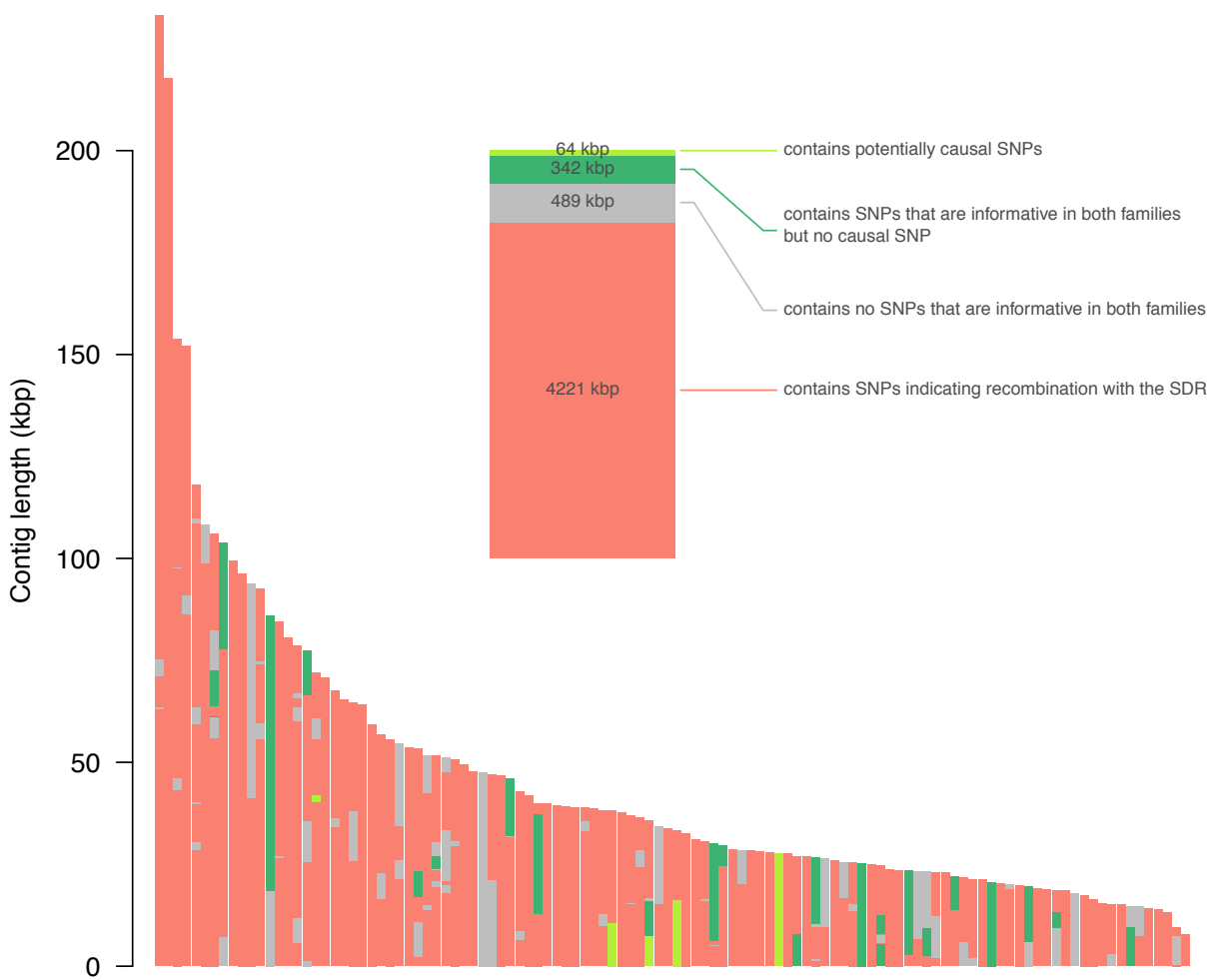


739

740 **Figure 4**

741 Cumulated length of 896 *A. vulgare* contigs that locate below or at a given genetic distance from
742 the sex determining region (SDR). Genetic distances are inferred from genetic data from 40 F1
743 siblings belonging to two families. The black curve represents observed data and the reddish area
744 the 99% confidence intervals obtained from simulated data assuming uniform crossing over rates
745 along sex chromosomes.

746

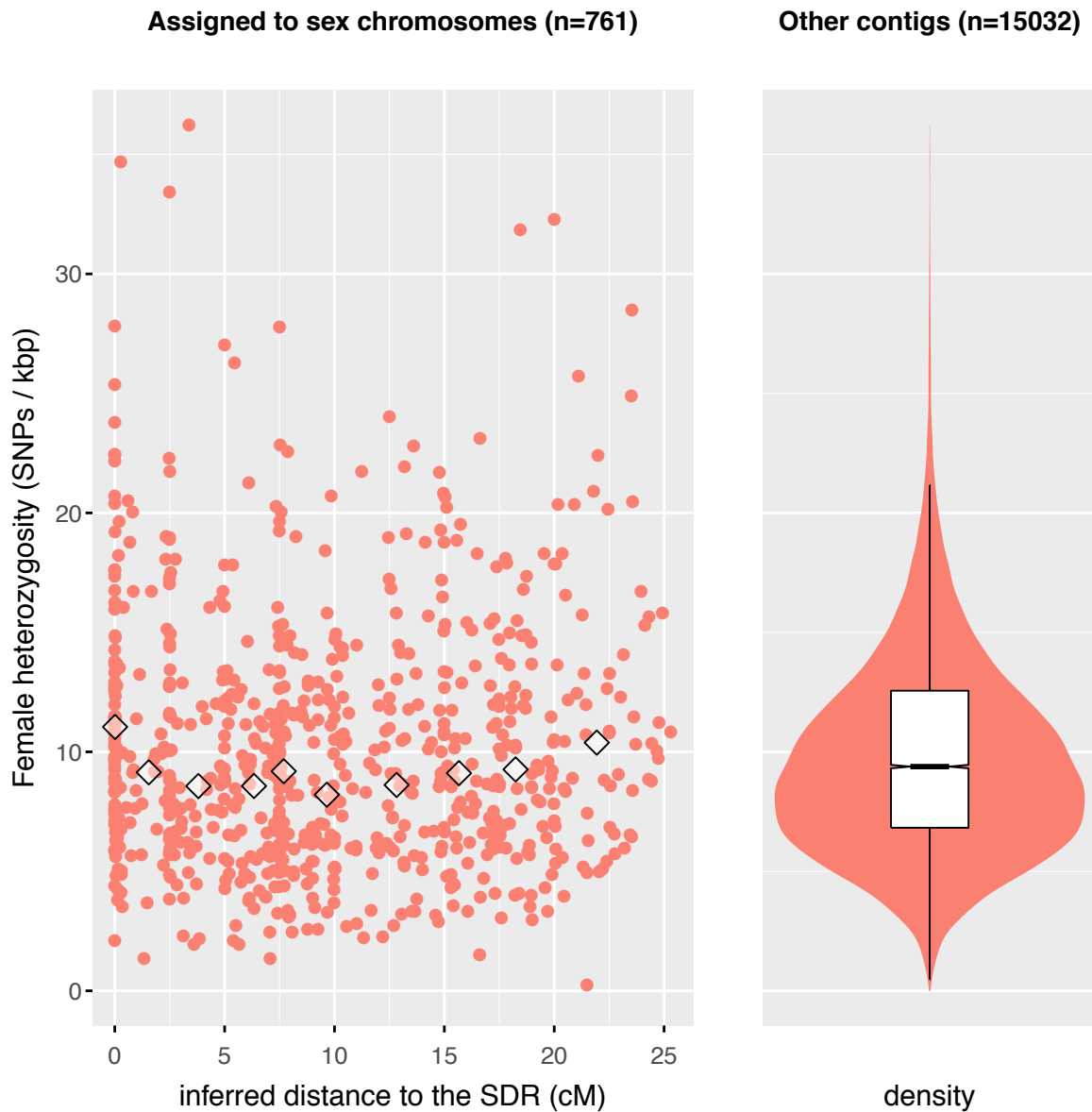


747

748 **Figure 5**

749 The 112 *A. vulgare* contigs that are inferred not to have recombined with the SDR in 40 F1s from
 750 two crosses. Each sectored vertical bar of the larger plot represents a contig. Contigs are ranked
 751 according to their length. Sectors within bars represent the genomic blocks composing contigs (see
 752 section "Localization of genomic regions that may contain the SDR"). Colors represent the SNPs
 753 that genomic blocks carry, using the same color codes as in Figure 2 and in the inset. The inset
 754 shows the total lengths of different categories of genomic blocks according to the SNPs they carry.
 755 Blocks belonging to first three categories (from the top) contain no more than one recombinant SNP
 756 and less than 50% of recombinant SNPs.

757



759

760 **Figure 6**

761 Female heterozygosity as a function of the inferred genetic distance to the SDR for contigs assigned

762 to sex chromosomes (left-hand plot) and its distribution for other contigs (right-hand plot). The

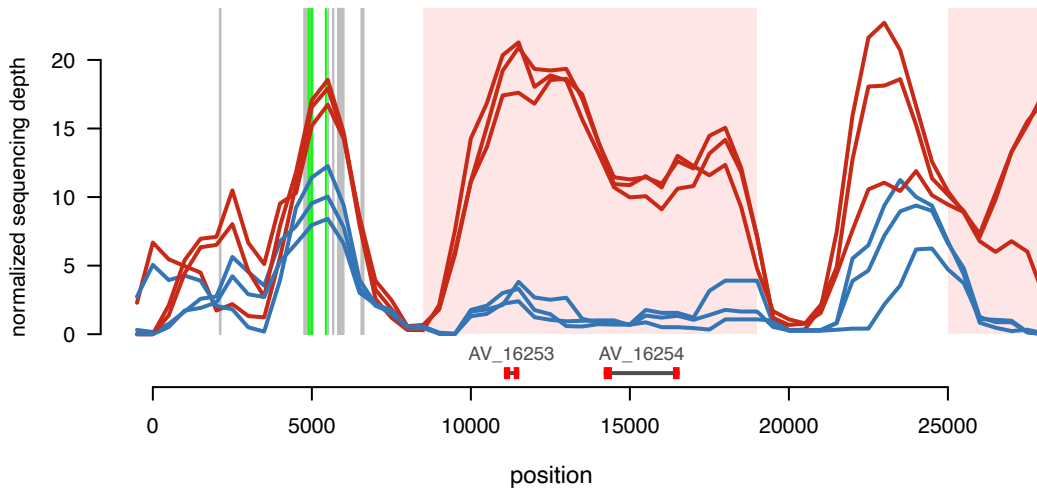
763 diamonds on the left-hand plot represent medians computed for ten classes of genetic distance.

764 Classes are delimited by the deciles and therefore comprise ~70 contigs each. For the density

765 computation (right-hand plot) contigs were weights by the lengths of regions with sufficient

766 sequencing depth to measure heterozygosity (see methods).

767



768

769 **Figure 7**

770 Normalized female (red curves) and male (blue curves) sequencing depths on contig 20397,
 771 presenting low chromosome quotient (CQ), based on sequenced DNA from the progeny of three *A.*
 772 *vulgare* families, constituting 6 pools. Regions of CQ < 0.3 and of female sequencing depth ≥ 5 in
 773 all families are represented as light pink areas. Vertical segments represent informative SNPs. Grey
 774 segments represent SNPs that are variable in a single family. Bright green segments represent SNPs
 775 that are compatible with the control of sex ("causal SNPs", see text). Annotated genes are
 776 represented by horizontal dark grey segments under gene identifiers. Exons are shown as thick red
 777 segments.

778

779 Supplementary text

780 How SNP type inference conditions the estimates

781 SNP type designates the chromosome (Z or W) carrying the allele that is specific to the mother (see
782 section "General approach"). SNP type is inferred by comparing the frequency of this allele
783 between daughters and sons, which permits the reconstruction of maternal haplotypes whose
784 frequencies are estimated in the F1 pools. This inference relies on linkage between a locus and the
785 SDR. In fact, the notion of SNP type is irrelevant with respect to autosomes.

786 To assess how SNP type inference affects the estimated number of recombination events with the
787 SDR, n_{rec} , we performed simulations as described in section "Contig assignment to sex
788 chromosomes and analysis of recombination" (main text). In these simulations, every contig was
789 attributed a genetic distance to the SDR, d , covering every value of the set $\{0 \dots 20/40\}$. n_{rec} was
790 estimated as in equation 3 (main text) and contrasted with the number of recombinants that we
791 simulated (which is the sum of n_{Zi} in daughters and $(n_i - n_{Zi})$ in sons). We also recorded the
792 proportion of SNPs whose type was properly inferred. As a basis of comparison, we performed
793 equivalent simulations in which SNP type was not inferred but taken directly from the simulated
794 SNP type.

795 Results are shown in Figure S1. When the simulated SNP type is used to compute n_{rec} (Figure S1A),
796 n_{rec} provides a good estimate of the actual number of recombinants at all genetic distances to the
797 SDR. When SNP type is inferred (Figure S1B), n_{rec} tends to underestimate the true number of
798 recombinants. The underestimate coincides with errors in SNP type inference (Figure S1C).
799 Expectedly, inference of SNP type does not perform better than a random guess at 20 recombinants
800 (corresponding to 50 cM to the SDR) considering that types 1 and 2 are equipresent in the
801 simulations.

802 Why n_{rec} is underestimated comes from the fact that the "focal haplotype" regroups the maternal
803 alleles of group-1 SNPs, which, given how SNP groups are defined, are more frequent (among
804 reads) in the sex under consideration than in the other. If the two maternal haplotypes are roughly
805 equipresent among sexes of an F1 progeny, as expected for an autosomal locus, our method would
806 create a chimeric focal haplotype gathering the maternal alleles that happen to prevail among reads,
807 and whose inferred frequency will *de facto* be higher than that of real haplotypes. The focal
808 haplotype appearing more frequent in a sex, the number of recombination events with the SDR is
809 underestimated.

810 Exclusion of SNPs that may result from errors

811 For a contig containing many informative SNPs in a pool i , a single SNP should have little
812 influence on $P(f = 0.5 | \mathbf{c}r_i)$, hereafter called p_{05} and representing the posterior probability of
813 absence of recombination with the SDR. In some scenario however, only one or few SNPs can
814 almost nullify p_{05} , which would otherwise be close to 1. This scenario involves an SDR-linked
815 contig for which all SNPs are of group 1 the pool. Due to errors, some "suspicious" SNPs may
816 occur and may be attributed to group 2 if the maternal alleles appear more frequent than in the other
817 pool of the same family. The strong influence that these few group-2 SNPs have on p_{05} arises from
818 the fact that a high value of c_{2i} (leading to c_{2i}/r_{2i} largely exceeding the sequencing error rate ε) is
819 incompatible with $f = 0.5$. On the other hand, $f = 0.5$ involves a high variance in c_{1i} (this variance
820 equals $0.25r_{1i}$ under a binomial distribution). As a result, a single (false) group-2 SNP can easily
821 nullify p_{05} even if hundreds of group-1 SNPs support $f = 0.5$.
822 This risk prompted us to find and exclude suspicious SNPs resulting from errors. To do so, we
823 computed p_{05} on each individual SNP as if it constituted its own haplotype (that is, the read counts
824 c_{1i} , r_{1i} , c_{2i} and r_{2i} only consider the reads covering this SNP, such that r_{1i} or r_{2i} is zero). We reasoned
825 an apparent SNP yielding a value of p_{05} that is much lower than the majority of SNPs of the same
826 contig should result from an error. We excluded these SNPs with the following iterative procedure,
827 which was applied separately to each contig in each pool. We exclude any SNP for which $-\ln(p_{05})$
828 was higher than the mean of this variable added to four times its standard deviation, both computed
829 over on all non-previously excluded SNPs of the contig. We repeat this until no further SNP was
830 excluded. Figure S2 illustrates the approach.

831 Interpretation of the distributions of n_{rec} for real and simulated data

832 The distributions of the inferred distance of contigs to the SDR (Figure 3, main text) show two
833 features that we discuss here.

834 First, the distributions are lower than the expectancy of 50 cM for autosomes. This is due to the fact
835 that n_{rec} underestimates the true number of recombination events at large distances to the SDR (see
836 section "How SNP type inference conditions the estimates").

837 Second, the distribution of the observed genetic distances appears shifted to the right compared to
838 simulations. We suggest that this shift reflects uncontrolled errors. In the absence of error, c_{1i}/r_{1i}
839 and c_{2i}/r_{2i} should negatively correlate, since their respective expectancies are f and $0.5-f$. This is
840 not so if the maternal alleles of a locus represent errors (genotyping error in parents, sequencing
841 errors in F1s etc.) where we expect the ratios to positively correlate, as they represent the error
842 rates. For instance, if the apparent maternal alleles result from errors, c_{1i}/r_{1i} and c_{2i}/r_{2i} ratios may

843 both be low. Similar c_{1i}/r_{1i} and c_{2i}/r_{2i} ratios for a contig result in an inferred value of f closer to
844 0.25, hence increase the inferred distance from the SDR.

845 Evaluating the power of assignment tests to sex chromosomes

846 To evaluate our method of contig assignment to sex chromosomes, we simulated data as described
847 in section "Contig assignment to sex chromosomes and analysis of recombination". We gave every
848 contig a value of d sampled from the set $\{0 \dots 20 / 40\}$ to cover a range of distances to the SDR. As
849 we did for observed data, a contig was assigned to sex chromosomes if the obtained n_{rec} was lower
850 than a low-order quantile of n_{rec} obtained from simulations that used $d = 0.5$. The proportion of
851 contigs assigned to sex chromosomes among those located at <50 cM to the SDR represents the
852 power of the assignment test, i.e., the sensitivity of our approach.

853 To evaluate how the sensitivity of the method depended on its specificity, the order of the
854 aforementioned quantile, which is the risk of false positive or significance level, was allowed to
855 vary in the set $\{0.001, 0.005, 0.01, 0.05\}$. Note that for contig assignment using observed data, the
856 1/1000th quantile was used.

857 We also investigated how the reconstruction of maternal haplotypes, via inference of SNP type,
858 affected the sensitivity of our approach. To this end, we performed a similar set of simulations in
859 which SNP type was not inferred but directly deduced from the chromosome carrying the maternal
860 allele, which is set during the simulation (see main text). In this case, reconstructed haplotypes
861 correspond to simulated haplotypes.

862 Figure S3A shows how the ability to assign contigs to sex chromosomes depends on the distance
863 from the SDR, the significance level and on whether SNP type was inferred. At the 0.001
864 significance level and when SNP type is inferred (as it was the case for observed data), sensitivity
865 falls under 60% at 20 cM to the SDR.

866 Figure S3B shows the proportion of contigs assigned to sex chromosomes among those locating at
867 or below a certain distance to the SDR, assuming uniform crossing over rates along sex
868 chromosomes. For instance, $\sim 86\%$ of contigs locating at ≤ 20 cM to the SDR are assigned to sex
869 chromosomes at the 0.001 risk.

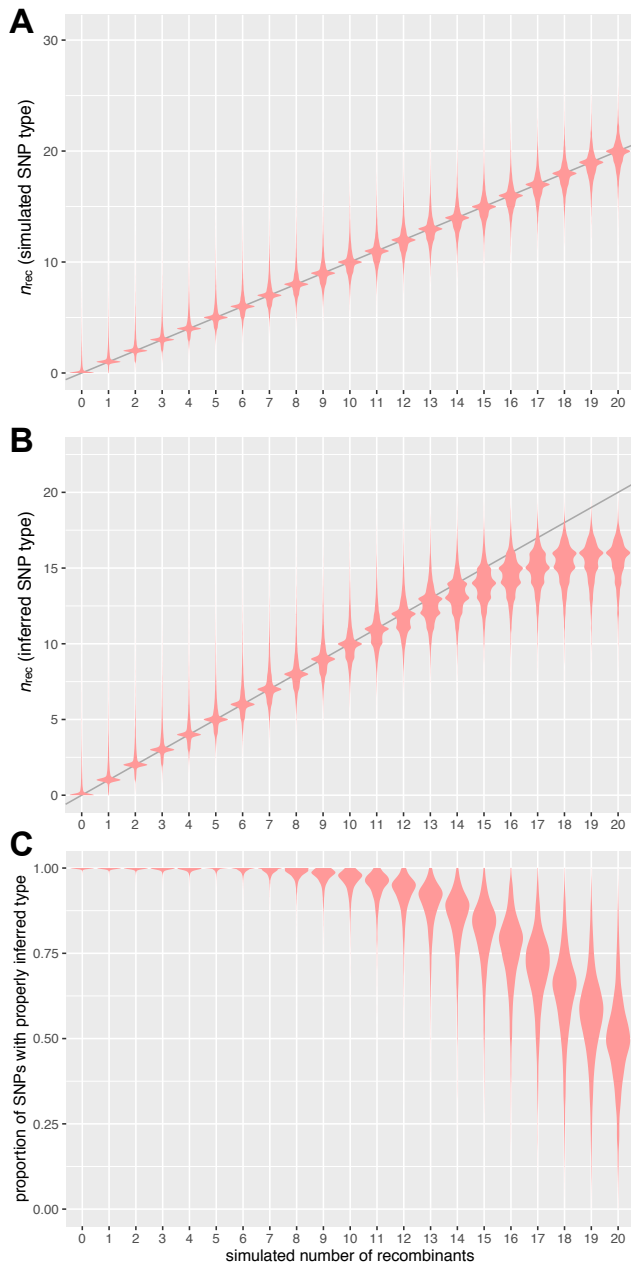
870 The reduction of sensitivity due to lack of knowledge on SNP type (represented by the distance
871 between plain and dotted lines on the Y axis) is higher at intermediate genetic distances (Figure
872 S3A). This is due to the fact that SNP types are properly inferred for contigs that locate close to the
873 SDR (see section "How SNP type inference conditions the estimates"). At high genetic distances,
874 knowing the true SNP types provides little benefit as the high number of crossing overs make
875 distant contigs similar to autosomal contigs.

876 When it comes to the number of contigs properly assigned among those locating at a certain
877 distance or closer to the SDR (Figure S3B), not knowing SNP type incurs a loss of sensitivity of
878 eight percentage points at most (~72% vs. ~64% sensitivity at 32.5 cM).

879 [Delineation of genomic blocks based on parental genotypes](#)

880 The "genomic blocks" (see section "Localization of genomic regions that may contain the SDR")
881 are contig regions within which no SNP shows evidence for recombination with another. Each
882 block is considered as a unit when it comes to the possible location of the SDR. To delineate
883 blocks, we proceeded as follows. We first generate every possible pairs of SNPs in a contig, using
884 those we refer to as "selected SNPs". We then check whether a pair of SNP constitutes four
885 different haplotypes among parents, in which case, recombination must have occurred between the
886 SNPs. To generate these two-SNP haplotypes, we use knowledge of the chromosomes (either the W
887 or Z) carrying the alleles of informative SNPs for heterozygous mothers (this knowledge is
888 permitted by inference of SNP type, see main text). We do not reconstruct the haplotypes of a father
889 that is heterozygous at both SNPs, as we do not know the chromosomes carrying the SNP alleles.
890 We then scan the SNP pairs to delineate the longest continuous regions containing no pair of
891 recombinant SNPs. These regions often overlap (Figure S5A). We removed overlaps by iteratively
892 shortening the shortest region that overlapped with any other (Figure S5B).

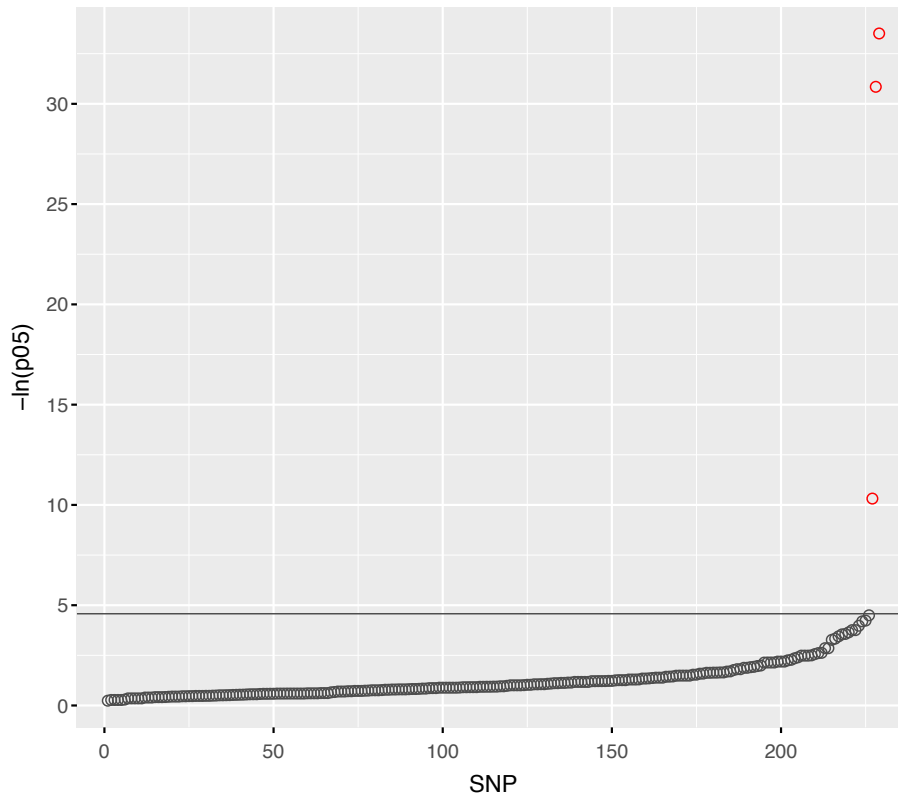
893



895

896 **Figure S1**

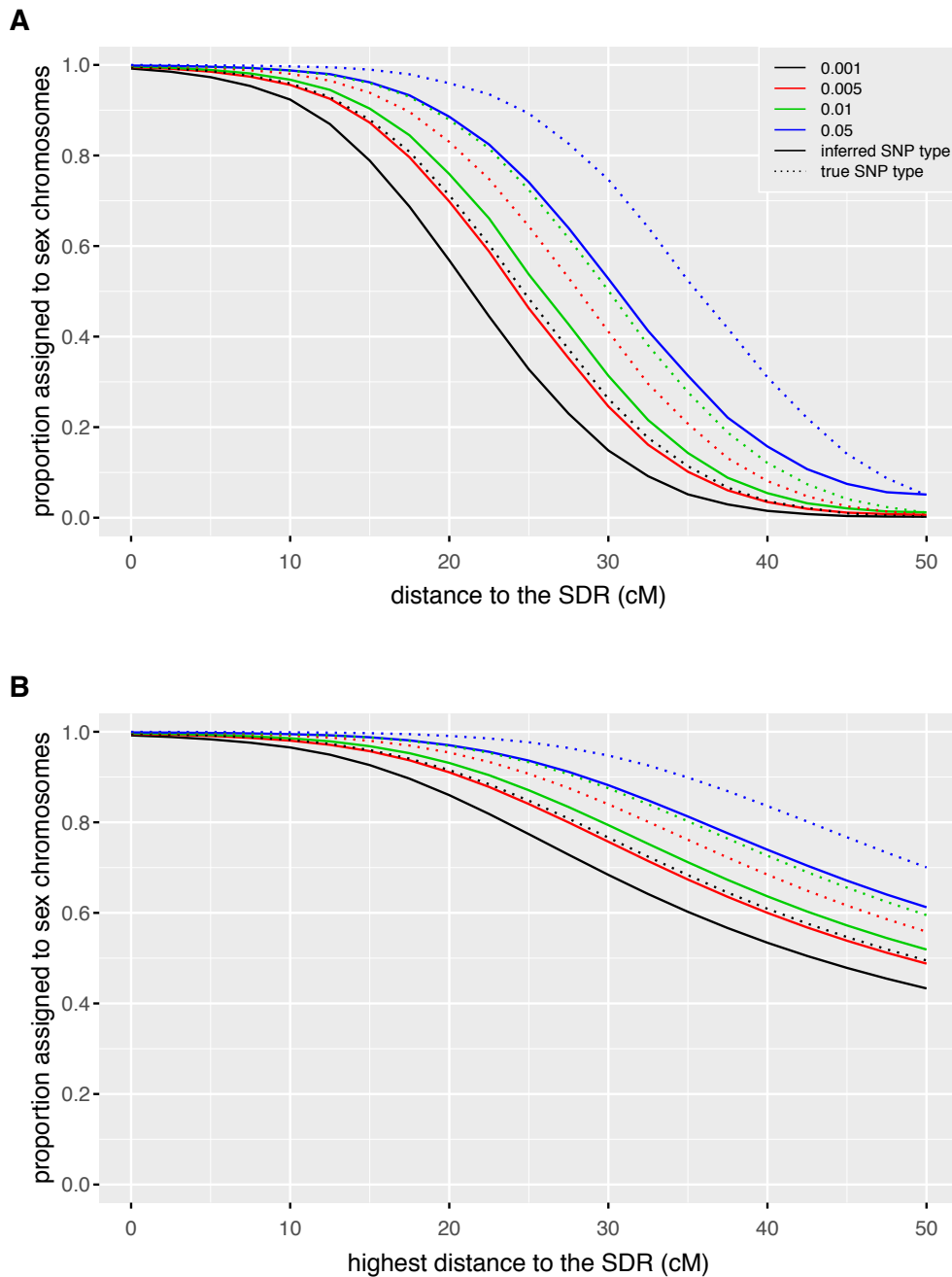
897 Results of simulations assessing the accuracy of the estimate of the number of recombinants with
 898 the SDR. The data points constituting the distributions are contigs possessing informative SNPs in
 899 both families. In A), the number of recombinants between the SDR and a contig was estimated
 900 without inference of SNP type, but using knowledge of the simulated SNP type. In B), the estimates
 901 rely on the inference of SNP type. Panel C) shows the proportion of SNPs whose type was properly
 902 inferred, combining both families.



903

904 **Figure S2**

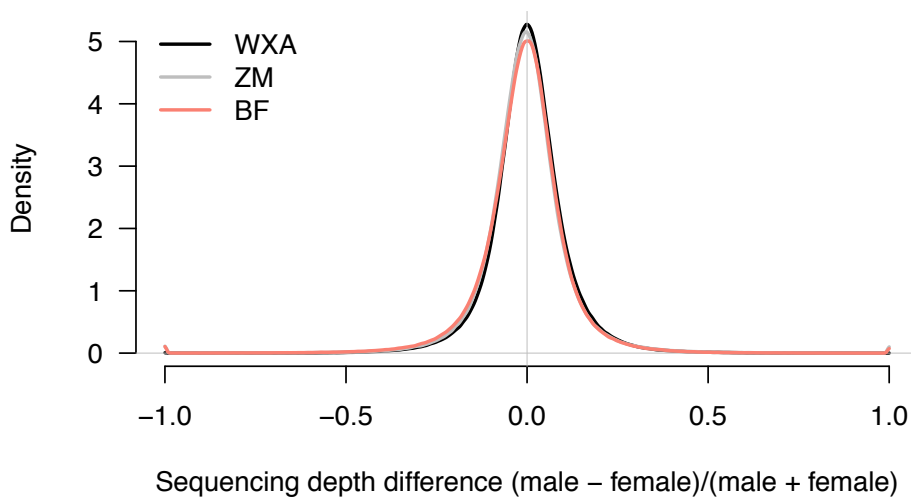
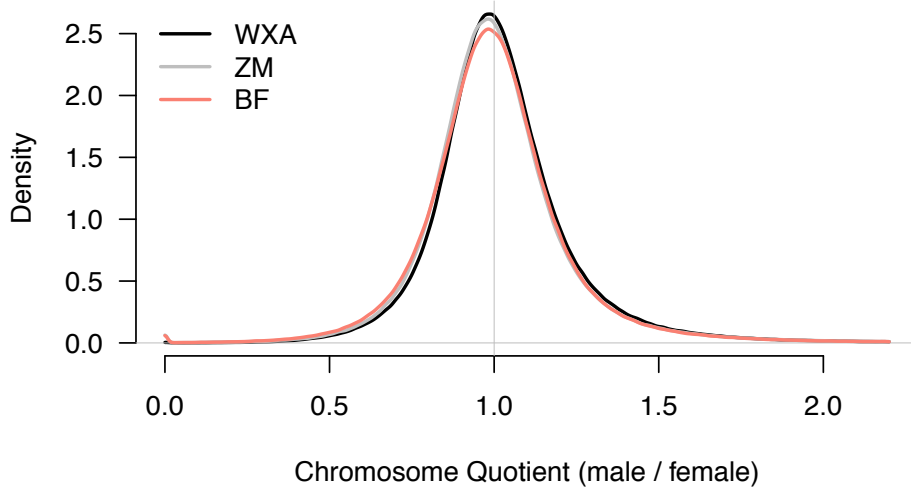
905 $-\ln(p_{05})$ (the probability that $f = 0.5$) for individual SNPs of contig 28198 in
 906 daughters from the WXA family. SNPs are ordered by increasing $-\ln(p_{05})$. Group-1 SNPs are
 907 shown as dark grey points, group-2 SNPs as red points. The horizontal line represents the threshold
 908 above which SNPs are excluded. Excluding the three group-2 SNPs result in $p_{05} \approx 0.99$ for the
 909 whole contig. Not excluding these SNPs results in $p_{05} \approx 0$.



910

911 **Figure S3**

912 Power of the assignment of contigs to sex chromosomes. A) Proportion of contigs assigned to sex
 913 chromosomes as a function of the simulated genetic distance between contigs and the sex-
 914 determining region (parameter d , here expressed in cM). B) Proportion of contigs assigned to sex
 915 chromosomes among contigs whose simulated genetic distance equals or is lower than the value of
 916 the X axis, assuming that genetic distance to the SDR varies linearly with the number of contigs. In
 917 both panels, colors represent the different significance levels used, and line styles indicate whether
 918 SNP types were inferred or directly taken from values set during simulations (see supplementary
 919 text).

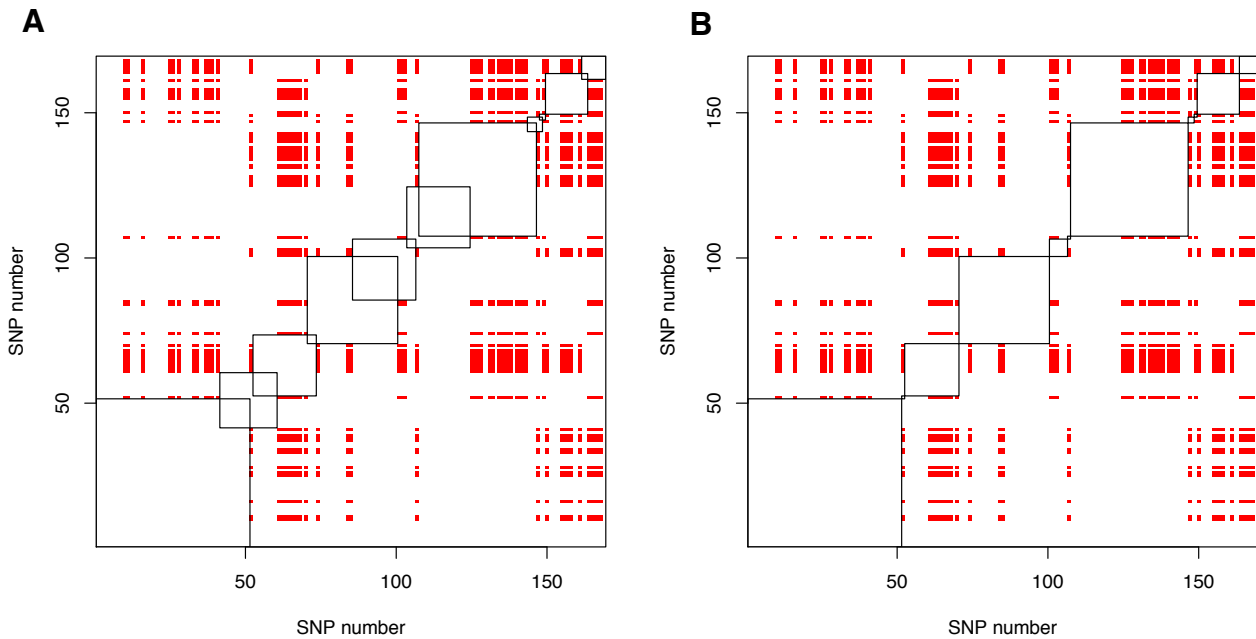


920

921 **Figure S4**

922 Comparison of normalized sequencing depths between sons and daughters within 3 families of *A.*
 923 *vulgare*, whose names are shown in the plot legends. Sequencing depths are averages computed for
 924 2kpb genomic windows sliding by 500pb. Windows of mean sequencing depth < 3 (summed within
 925 a family) are excluded to reduce noise. The Chromosome Quotient (top) presumably has a mode < 1
 926 due to the right skewness of the variable, which varies from zero to infinity. This does not indicate
 927 that normalized sequencing depths is typically lower in males. The variable used in the bottom plot
 928 does not have this issue as it ranges from -1 to 1. Its mode is ~0 and extreme values are very rare,
 929 indicating that most genomic windows have similar sequences between sexes.

930



931

932 **Figure S5**

933 Delineation of genomic blocks in contig 42878, which contains 169 SNPs used to infer
934 recombination with the SDR. In each image, a row/column represents a SNP according to its
935 relative position in the contig. Each "pixel" is therefore a pair of SNPs. A red pixel denotes
936 evidence for recombination between the SNPs of a pair. The ascending diagonal can be viewed as
937 the 169 SNPs. Squares represents genomic blocks (see text). In A), the initial blocks are
938 overlapping. In B), the smallest blocks were iteratively shortened to avoid overlaps. The three
939 shortest blocks are removed afterwards as they contain only one selected SNP each.

940 [Supplementary files](#)

941 **File S1**

942 This excel file contains information about each contig analyzed. For each family, the posteriori
943 probability of the focal haplotype frequency f being 0.5 is given, together with the posterior
944 probability of the most likely haplotype frequency, and the read counts. Other columns include the
945 contig name, its length, its inferred genetic distance to the SDR (n_{rec}), whether it is assigned to sex
946 chromosomes, and the female heterozygosity.

947

948 **File S2**

949 Compressed archive of the custom code used in this study.

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951

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