

1 **A genome resequencing-based genetic map reveals the recombination**  
2 **landscape of an outbred parasitic nematode in the presence of polyploidy**  
3 **and polyandry**

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## 50 **Abstract**

51 The parasitic nematode *Haemonchus contortus* is an economically and clinically important  
52 pathogen of small ruminants, and a model system for understanding the mechanisms and  
53 evolution of traits such as anthelmintic resistance. Anthelmintic resistance is widespread  
54 and is a major threat to the sustainability of livestock agriculture globally; however, little is  
55 known about the genome architecture and parameters such as recombination that will  
56 ultimately influence the rate at which resistance may evolve and spread. Here we  
57 performed a genetic cross between two divergent strains of *H. contortus*, and subsequently  
58 used whole-genome re-sequencing of a female worm and her brood to identify the  
59 distribution of genome-wide variation that characterises these strains. Using a novel  
60 bioinformatic approach to identify variants that segregate as expected in a pseudo-  
61 testcross, we characterised linkage groups and estimated genetic distances between  
62 markers to generate a chromosome-scale  $F_1$  genetic map composed of 1,618 SNPs. We  
63 exploited this map to reveal the recombination landscape, the first for any parasitic  
64 helminth species, demonstrating extensive variation in recombination rate within and  
65 between chromosomes. Analyses of these data also revealed the extent of polyandry,  
66 whereby at least eight males were found to have contributed to the genetic variation of the  
67 progeny analysed. Triploid offspring were also identified, which we hypothesise are the  
68 result of nondisjunction during female meiosis or polyspermy. These results expand our  
69 knowledge of the genetics of parasitic helminths and the unusual life-history of *H. contortus*,  
70 and will enable more precise characterisation of the evolution and inheritance of genetic  
71 traits such as anthelmintic resistance. This study also demonstrates the feasibility of whole-  
72 genome resequencing data to directly construct a genetic map in a single generation cross  
73 from a non-inbred non-model organism with a complex lifecycle.

## 74 **Author summary**

75 Recombination is a key genetic process, responsible for the generation of novel genotypes  
76 and subsequent phenotypic variation as a result of crossing over between homologous  
77 chromosomes. Populations of strongylid nematodes, such as the gastrointestinal parasites  
78 that infect livestock and humans, are genetically very diverse, but little is known about  
79 patterns of recombination across the genome and how this may contribute to the genetics

80 and evolution of these pathogens. In this study, we performed a genetic cross to quantify  
81 recombination in the barber's pole worm, *Haemonchus contortus*, an important parasite of  
82 sheep and goats. The reproductive traits of this worm make standard genetic crosses  
83 challenging, but by generating whole-genome sequence data from a female worm and her  
84 offspring, we identified genetic variants that act as though they come from a single mating  
85 cross, allowing the use of standard statistical approaches to build a genetic map and explore  
86 the distribution and rates of recombination throughout the genome. A number of genetic  
87 signatures associated with *H. contortus* life history traits were revealed in this analysis: we  
88 extend our understanding of multiple paternity (polyandry) in this species, and provide  
89 evidence and explanation for sporadic increases in chromosome complements (polyploidy)  
90 among the progeny. The resulting genetic map will aid in population genomic studies in  
91 general and enhance ongoing efforts to understand the genetic basis of resistance to the  
92 drugs used to control these worms, as well as for related species that infect humans  
93 throughout the world.

#### 94 **Keywords**

95 F<sub>1</sub> genetic map, genome resequencing, *Haemonchus contortus*, kinship, ploidy, polyandry,  
96 pseudo-testcross, recombination landscape.  
97

## 98 Background

99 Recombination is a key genetic process: the breaking and re-joining of genetic material to  
100 produce novel genotypes and in turn, generate phenotypic variation. In eukaryotes, this is  
101 achieved by crossing-over between homologous chromosomes during the generation of  
102 gametes in meiosis. A common approach to studying recombination is to perform controlled  
103 matings (i.e. genetic crosses) between genetically distinct and inbred parents. The parents  
104 and offspring are then genotyped to construct genetic linkage maps, which aim to order  
105 genes or genetic markers based on the recombination frequency between them. This  
106 approach can also be used to identify regions of the genome underlying phenotypic  
107 variation, and has been widely used for mapping both simple and complex traits in a range  
108 of different organisms [1, 2]. More recently, as whole-genome sequencing data has become  
109 available for many organisms, genetic maps have been used to inform or validate contig  
110 order in genome assemblies [3-7]. Where a contiguous genome assembly is already  
111 available, a linkage map can be used to explore variation in recombination rates throughout  
112 the genome [8] and determine how this has shaped other aspects of genome architecture,  
113 such as the distribution of repeats or the impact of natural selection.

114  
115 Understanding variation in the rate and pattern of recombination is critical, both for  
116 designing and analysing experiments aimed at mapping the genetic basis of phenotypic  
117 traits and in interpreting genetic variation in natural populations. Between species, a  
118 negative relationship between genome size and recombination rate has been described [9].  
119 Within a species, variation in recombination rate is strongly influenced by the sex of the  
120 organism; recombination may not occur in one of the two sexes (typically the heterogametic  
121 sex, i.e. the Haldane-Huxley rule [10]), or, if recombination does occur in both sexes, then  
122 females tend to show a higher recombination rate than males (i.e. heterochiasmy [11]). In  
123 addition, recombination rates have been show to vary considerably within and between  
124 chromosomes, which has been attributed to genomic features including but not limited to  
125 GC content, gene density, gene size, simple repeats, and chromatin state [12-15]. Among  
126 nematodes, recombination is best characterised in the model organism *Caenorhabditis*  
127 *elegans*, where direct comparison of the physical and genetic maps clearly reveals  
128 asymmetrically distributed high and low recombination rate domains in each chromosome,

129 correlated with low and high gene density (and gene expression), respectively [8, 14, 16].  
130 However, the precise local DNA features that mediate these rate changes remain unclear.  
131 Even less is known about recombination in parasitic helminths. Low density genetic maps  
132 are available for only three species, the root knot nematode *Meloidogyne hapla* [5, 6], the  
133 human blood-fluke *Schistosoma mansoni* [17], and the rat gastrointestinal parasite  
134 *Strongyloides ratti* [7], and only discrete regions of recombination variation have been  
135 described in *M. hapla* [5]. Recombination rate variation has been proposed to influence the  
136 distribution of genetic variation, and in turn, evolution of phenotypic traits in *C. elegans* [18-  
137 20]. Therefore, understanding genome-wide recombination variation in parasitic species will  
138 likely be important in predicting the genetic architecture and evolution of important  
139 parasite life history traits, including pathogenicity, response to host immunity and  
140 chemotherapeutic selection.

141  
142 The parasite *Haemonchus contortus* is amongst the most pathogenic of the gastrointestinal  
143 nematodes and exerts significant burdens on animal health and the economic viability of  
144 livestock farming [21]. It is also an emerging model for the biology of parasitic helminths  
145 more widely, particularly for understanding anthelmintic drug action and resistance [22]. In  
146 particular, *H. contortus* is the most genetically tractable of any of the strongylid (clade V)  
147 parasitic nematodes, a large and important group of parasites including key human and  
148 veterinary pathogens. It makes a particularly good model because: (i) it is a sexually  
149 reproducing diploid organism for which the karyotype—five autosomes and XX/XO sex  
150 chromosomes—is well defined [23]; (ii) two published draft genome sequences and  
151 extensive transcriptomic data are available [24-26]; (iii) it is amenable to cryopreservation of  
152 isolates; and (iv) it is one of the few parasitic nematode species in which genetic crosses  
153 have been successfully established [27-33].

154  
155 Anthelmintic drug failure is an important economic and animal health problem, as  
156 anthelmintic resistance is widespread on farms, and populations and isolates resistant to all  
157 major classes of anthelmintics have been described [34-36]. Accordingly, significant  
158 research effort is focused on the development of novel anthelmintics [37] or vaccines [38]  
159 for parasite control. Although research on *H. contortus* has been instrumental in  
160 understanding some of the mechanisms by which resistance arises [34, 39], the genetic

161 basis of resistance remains largely unresolved and is likely complex. For example, while  
162 resistance to benzimidazoles—the class of anthelmintics for which the genetic basis of  
163 resistance is best understood—has been linked clearly to mutations at three sites in the  
164 isotype-1  $\beta$ -tubulin gene [40-42], there is evidence that it is a more complex trait than  
165 previously assumed [2]. In contrast, genome-wide studies of ivermectin response—another  
166 major anthelmintic—in a number of parasitic helminth species support the hypothesis that  
167 this is a quantitative, multigenic trait [43-45]. Therefore, establishing the genomic context in  
168 which drug resistance alleles are inherited using *H. contortus* will help to resolve the  
169 mechanisms by which resistance evolves and spreads in other species of parasitic  
170 nematodes as well.

171

172 The purpose of this study was to produce a genetic map of *H. contortus*, initially in order to  
173 establish an anchored framework for a draft genome under development, and subsequently  
174 to estimate the frequency and distribution of recombination in the genome. To do so, we  
175 performed a cross between two genetically divergent strains of *H. contortus* that differed in  
176 their anthelmintic resistance phenotypes: one that was fully susceptible and one that  
177 showed high levels of resistance to three commonly used anthelmintics [46, 47]. Four  
178 constraints restrict use of *H. contortus* crosses to implement standard classical approaches  
179 for genetic mapping: (i) there is an extremely high level of sequence polymorphism present  
180 both in field and laboratory strains of *H. contortus* [48] (ii) few very highly inbred isolates  
181 are available to use as parents, and so isolates comprise multiple genotypes; (iii) it is  
182 difficult, although not impossible, to perform single parent crosses from inbred lines [49,  
183 50]; and (iv) mating is polyandrous, i.e. multiple males can and will mate with a single  
184 female [51]. We developed a genomic strategy for inferring segregation of single nucleotide  
185 polymorphisms within families by predicting paternal genotypes based on variants present  
186 in a single female and her progeny to construct an  $F_1$  genetic map. We discuss the  
187 implications of recombination, and other novel life history traits identified here, in the  
188 context of generating and maintaining genetic variation in parasite populations, and how  
189 these factors might impact the development and spread of anthelmintic resistance in this  
190 species.

## 191 Results

### 192 Genome sequencing and genetic diversity of a genetic cross between two isolates of *H.*

#### 193 *contortus*

194 A genetic cross was performed between two genetically and phenotypically defined *H.*  
195 *contortus* strains: females were from MHco3(ISE), a serially passaged anthelmintic  
196 susceptible “laboratory” strain that has been well characterised by genomic and  
197 transcriptomic analyses [24, 26], and males were from MHco18(UGA2004), a multi-drug  
198 resistant serially passaged strain originally isolated from the field at the University of  
199 Georgia, USA [46](**Fig 1**). Whole genome sequencing (WGS) was performed on DNA derived  
200 from a single adult MHco3(ISE) female parent and 41 of her F<sub>1</sub> L<sub>3</sub> progeny to achieve a  
201 minimum 30× sequencing coverage per sample (mean sequencing depth: 34.80× ± 16.16  
202 standard deviations (SD)), generating a median yield of 65.97 million reads per sample (**S1**  
203 **Table**). Mapping of the sequencing data was performed using an improved genome  
204 assembly of the MHco3(ISE) isolate described by Laing *et al.* [26], which now consists of five  
205 scaffolds representing the autosomal chromosomes and two scaffolds representing the X  
206 chromosome, for an assembly length of approximately 279 Mb. Sequence depth of the X  
207 chromosome scaffolds relative to the five autosomal scaffolds, together with rates of  
208 heterozygosity on the X chromosome scaffolds, revealed 20 male and 21 female F<sub>1</sub> progeny  
209 in the brood.

210

211

212 **Fig 1. Outline of genetic cross between MHco3(ISE) drug susceptible and**  
213 **MHco18(UGA2004) multi-drug resistant *H. contortus*.** A total of 68 MHco3(ISE) females and  
214 42 MHco18(UGA2004) males (from an infection of 100 individuals of each sex) were  
215 recovered *post mortem*, after which reproductively mature females were incubated *in vitro*  
216 to lay eggs that were subsequently cultured to L<sub>3</sub> stage. These larvae represent the F<sub>1</sub>  
217 generation of the cross.

218

219

220 Approximately 5.3 million single nucleotide polymorphisms (SNPs) that passed stringent  
221 filtering criteria were identified in the autosomal chromosomes (**Fig 2 A; S2 Table**), at a  
222 genome-wide density of 2242 SNPs per 100 kb (**Fig 2 B**), or 1 SNP per 44.6 base pairs (bp). A



223 pseudo-testcross approach was used to generate the  $F_1$  genetic map, which required that  
224 candidate markers: (i) were heterozygous in the female parent; and (ii) segregated in a ratio  
225 statistically indistinguishable from a 1:1 genotype ratio in the  $F_1$  progeny. By using these  
226 criteria, we identified a set of markers that could be analysed using the same statistical  
227 approaches as conventional linkage mapping using a test cross. Analysis of the 730,825  
228 heterozygous SNPs in the female MHco3(ISE) parent demonstrated that the distribution of  
229 variation was not uniform throughout the genome, with a number of long contiguous  
230 regions of homozygosity observed (**Fig 2 C; S1 Fig**). In particular, approximately 27 Mb of the  
231 second half of chromosome IV was largely homozygous, containing about 50% more  
232 homozygous variant sites and about 30% less heterozygous sites compared to the genome-  
233 wide average (**S3 Table**).

234

235 Among the SNPs that were heterozygous in the female parent, 171,876 SNPs segregated at  
236 an approximate 1:1 genotype ratio in the  $F_1$  progeny (**S2 Table**; PT:110 and PT:011). To  
237 avoid including tightly linked SNPs, the 171,876 candidate SNPs were thinned to 1 per  
238 25,000 bp, which resulted in a final candidate list of 5,595 SNPs for analysis in the cross.

239

240

241 **Fig 2. Autosome-wide variant density and candidate genetic map markers identified from**  
242 **the female parent and  $F_1$  progeny.** (A) The five autosomes of *H. contortus*, named based on  
243 synteny with *C. elegans* chromosomes, span 237 Mb. (B) SNP density was calculated in 100  
244 kbp windows, and is presented as the relative variant density of the female parent and all  $F_1$   
245 progeny. (C) Density of heterozygous variants in the female parent. (D) Positions of  
246 candidate pseudo-testcross SNPs that were heterozygous in the female parent and  
247 segregated in a 1:1 genotype ratio in the  $F_1$  progeny. Red annotations in plots (C) and (D)  
248 highlight low density regions, defined as genome-wide mean SNP density minus 3 SD. (E)  
249 Positions of the final set of 1,618 SNPs used in the  $F_1$  genetic map. The plot was produced  
250 using *Circos* [52].

251

252

## 253 **Characterisation of an autosomal F<sub>1</sub> genetic map generated using pseudo-testcross SNP** 254 **markers**

255 Initial analysis of genome-averaged genotype ratios (**S2 Fig**) of the candidate pseudo-  
256 testcross sites in each F<sub>1</sub> individual revealed that most individuals displayed an approximate  
257 50:50 ratio of homozygous:heterozygous genotypes, as expected. However, seven  
258 individuals presented as outliers with an excess of heterozygous genotypes (**S2 Fig A**;  
259 moderate outliers: individuals F1\_12, F1\_30, F1\_40; extreme outliers: individuals F1\_21,  
260 F1\_23, F1\_32, F1\_38). The variant-allele frequency distribution of these individuals (**S3 Fig**)  
261 revealed a skew consistent with a non-diploid complement of chromosomes, with a major  
262 non-reference (relative to the genome assembly) allele frequency peak at approximately  
263 30% and minor peak at 60% frequency. This allele frequency skew was typically found across  
264 all chromosomes within an individual, suggesting that they were not aneuploids. A notable  
265 exception was individual F1\_30 (one of the moderate outliers), where chromosomes I, III,  
266 and V had a distinct allele frequency spectrum consistent with more than two copies of each  
267 chromosome present, relative to chromosomes II and IV, which appeared to be diploid. All  
268 seven of these non-diploid individuals were therefore removed from the pseudo-testcross  
269 analysis (**S2 Fig B, D; n = 34**).

270  
271 A reanalysis of the remaining 34 individuals revealed 217,575 pseudo-testcross SNPs,  
272 129,985 intercross SNPs, and 383,265 SNPs that were heterozygous in the female parent  
273 but did not segregate in a way compatible with analysis as a single-pair mating cross (**Table**  
274 **S2**). Thus, a total of 4,587 pseudo-testcross SNPs (217,575 SNPs thinned to 1 SNP per 25,000  
275 bp) were candidate markers for the map construction using R/QTL (**Fig 2 D**), from which  
276 1,618 SNPs were used in the final genetic map (**Fig 2 E; Table S4**). Recombination plots and  
277 genetic maps for the five autosomes are presented in **Fig 3**, and characteristics of the map  
278 are presented in **Table 1**. The total map distance of the five autosomes was approximately  
279 344.46 cM. The number of markers per chromosome ranged from 215 on chromosome II to  
280 475 on chromosome I, with a mean value of 323.6 markers per chromosome. Significant  
281 gaps in the map correlated with absence, or very low density, of the prerequisite  
282 heterozygous SNPs in the female parent, as described above (**Fig 2 C**). This loss of markers  
283 was most obvious in chromosome IV, where only approximately half of the chromosome is

284 represented in the map, resulting in a map length of 49.21 cM, compared to the average  
 285 map length of other chromosomes of 73.79 cM. The genome-wide recombination rate was  
 286 on average 604.12 ( $\pm$  84.01 SD) kb/cM or 1.68 ( $\pm$  0.25 SD) cM/Mb, which corresponded to  
 287 an overall average number of crossover events per chromosome of 0.69 ( $\pm$  0.12 SD).  
 288 Chromosome IV was again an outlier, with a recombination rate of 2.01 cM/Mb,  
 289 approximately 21% higher than the other four autosomes (1.68 cM/Mb average).

290  
 291

292 **Table 1: Summary characteristics of the F<sub>1</sub> genetic map, including number of markers**  
 293 **used, map length, recombination rate and crossover frequency**

Chromosome	Chromosome length (bp)	Markers used (#)	Genetic map length (cM)	Recombination rate (Kb/cM) <sup>1</sup>	Recombination rate (cM/Mb) <sup>2</sup>	Crossovers per chromosome <sup>3</sup>
I	45778363	475	83.71	546.87	1.83	0.84
II	47384193	215	71.88	660.13	1.51	0.72
III	43564237	363	69.53	626.55	1.60	0.70
IV <sup>4</sup>	51819793	226	49.21	490.85	2.04	0.49 <sup>5</sup>
V	48825595	339	70.13	696.22	1.44	0.70
Total / average	237372181	1618	344.46	604.12	1.68	0.69

294 1. Recombination rate (kb/cM): chromosome length (Kb) / genetic map length  
 295 2. Recombination rate (cM/Mb): genetic map length / (chromosomal length / 10<sup>6</sup>)  
 296 3. Crossovers per chromosome: ( genetic map length / 100 ) / number of chromosomes  
 297 4. The genetic map only spanned ~24 Mb of chromosome IV due to homozygosity in the female parent. As such,  
 298 recombination rates have been calculated for chromosome IV using 24154752 bp (position of the genetic map  
 299 marker closest to the homozygosity region) as the chromosome length.  
 300 5. Likely to be underestimated given only half of the chromosome is present.

301  
 302

303 **Fig 3. Recombination and genetic maps of the five autosomes of *H. contortus*.**

304 Recombination plots depict genotype segregation patterns per F<sub>1</sub> progeny (columns;  
 305 clustered by genetic similarity) of pseudo-testcross markers used in the genetic map (rows).  
 306 Segregating “parental” and “recombinant” haplotypes inherited from the female parent are  
 307 indicated by opposing colour schemes. Genotypes: AA: red; Aa: yellow; aa: white. The  
 308 relationship between SNP position in the recombination map and genetic map position (cM)  
 309 is represented by a connecting grey line; multiple SNPs between which no recombination  
 310 was observed collapse into a single map position in the genetic map (grey ribbon from  
 311 multiple SNPs to a single map marker).

312  
 313

314 Analysis of the X chromosome diversity from the adult female and all progeny revealed  
315 100,016 SNPs in the 23.3 and 18.9 Mb X-linked scaffolds; this frequency (1 SNP per 422 bp)  
316 equates to approximately 10-fold fewer variable sites on the X chromosome relative to the  
317 autosomes. Attempts to generate an X chromosome genetic map were limited by a lack of  
318 prerequisite heterozygous variant sites in the female X chromosome sequences (**Fig S1**). To  
319 explore this further, the diversity of hemizygous genotypes called in the male F<sub>1</sub> progeny,  
320 i.e. genotyped as AA or aa reflecting the haploid X<sup>A</sup> or X<sup>a</sup> allele, respectively, was compared  
321 to genotypes resolved in the female parent (**Fig S4**). Strikingly, male genotypes were entirely  
322 concordant with the female parent, further supporting the lack of segregating genetic  
323 diversity in the female parent diploid X chromosomes. Female F<sub>1</sub> progeny contained both  
324 homozygous and heterozygous sites in their X chromosomes; given the lack of variation in  
325 the female parent, this diversity was entirely inherited from the paternal X chromosome.  
326

#### 327 **Patterns of recombination within autosomal chromosomes of the F<sub>1</sub> progeny**

328 Analysis of recombination rate throughout each chromosome was determined by  
329 comparing physical and genetic distances, which can be visualised in a Marey map [53](**Fig.**  
330 **4**). Recombination rate (**Fig. 4** red line; cM/Mb) was not uniform throughout the  
331 chromosomes, nor was it consistent between chromosomes. Chromosomes I, II and IV  
332 tended to show a pattern of three main recombination rate domains; a reduced  
333 recombination rate domain towards the middle of the chromosome, flanked by domains of  
334 increased recombination rate that extend toward the ends of the chromosomes. This three-  
335 domain pattern was not as clear for chromosomes III and V; chromosome III showed a  
336 greater recombination rate in the first half of the chromosome that decreased throughout  
337 the second half of the chromosome, whereas chromosome V had longer low recombination  
338 rate domains towards the ends of the chromosome arms, and greater recombination rate  
339 towards the middle of the chromosome. It is curious that chromosome IV retained the  
340 three-domain recombination architecture, given that the right arm is largely missing due to  
341 lack of the prerequisite heterozygous sites in this region of the female parent (**Fig 2 C; S1**  
342 **Fig**). Each chromosome also showed evidence of additional low recombination rate domains  
343 at one or both ends of the chromosome in the sub-telomeric regions extending into the  
344 chromosome. Finally, within the elevated recombination rate domains, the recombination

345 rate was not necessarily constant; discrete peaks of high recombination rates were  
346 observed in all chromosomes. However, the relative position of high recombination peaks  
347 was not the same between chromosomes.

348

349 **Fig 4. Analysis of recombination rate variation throughout the genome.** Marey maps were  
350 constructed to show the relationship between the genetic position of each marker (black  
351 point) relative to the physical position of the marker in the genome. Line of best fit was  
352 plotted using default parameters of the *geom\_smooth* function of *ggplot2* in R.  
353 Recombination rates (cM/Mb; red line) were calculated by calculating genetic map distance  
354 in 1 Mb windows throughout the genome from a fitted *loess*-smoothed line of the genetic  
355 map positions.

356

#### 357 **Family structure and kinship among the brood**

358 *H. contortus* is known to be polyandrous [51]. This knowledge, together with the  
359 observation that more than 50% of SNPs did not segregate in either a 1:1 or 1:2:1 genotype  
360 ratio (**Table S2**), suggested that the 41 progeny analysed were sired from more than a single  
361 male parent. An initial analysis of genetic relatedness by principal component analysis (PCA)  
362 of 21,822 autosomal SNPs (complete dataset thinned using a linkage disequilibrium  
363 threshold of 0.5 and minor allele frequency of 0.05) revealed obvious genetic structure, with  
364 at least four (PC 1 vs 2) to as many as six (PC 2 v 3) putative clusters of F<sub>1</sub> progeny (**Fig 5A**),  
365 consistent with the hypothesis that the brood resulted from polyandrous mating.

366

367 To more accurately describe these putative relationships among the progeny, we calculated  
368 kinship coefficients [54], which describe the probability that a given allele in two individuals  
369 is identical by descent (i.e. an allele shared due to recent shared ancestry, as opposed to  
370 identical by state, in which the allele is simply shared by two individuals without common  
371 ancestry), for all pairwise combinations of progeny. Employing all autosomal SNPs ( $n =$   
372 5,323,039 SNPs), this analysis revealed eight clusters of full-sib relationships containing  
373 multiple F<sub>1</sub> progeny (**Fig 5B**). Two individuals, F1\_28 and F1\_45, did not share any pairwise  
374 kinship coefficients consistent with a full-sib relationship with any individual, and hence,  
375 may represent the progeny from additional paternal contributions to the brood. Three  
376 individuals, F1\_21, F1\_23, and F1\_38, seemed to show full-sib relationships with individuals

377 from multiple families via strong kinship associations between themselves and others.  
378 Intriguingly, these were the same individuals identified as outliers with excess  
379 heterozygosity (**S2 Fig**) and that showed a skewed allele frequency distribution (**S3 Fig**)  
380 suggestive of aneuploidy or polyploidy. These autosomal kinship data are further supported  
381 by the observation that X chromosome diversity in the female F<sub>1</sub> progeny, which reflects  
382 paternal X chromosome inheritance in the absence of maternal X chromosome diversity,  
383 clusters the female F<sub>1</sub> progeny into five groups of two or more individuals (**S4 Fig**). Three  
384 unclustered individuals were also identified for the X chromosomes, including individual  
385 F1\_28, which did not share any full-sib relationships in the kinship analysis (**Fig 5B**). These X  
386 chromosome derived clusters are concordant with the full-sib family structure using  
387 autosomal SNPs. Taken together, these data describing the familial relationships among the  
388 F<sub>1</sub> progeny cohort lead us to propose a pedigree consisting of at least eight paternal  
389 contributions (**Fig 5C**).

390

391

392 **Fig 5. Familial relationships determined via analysis of genetic diversity and kinship**  
393 **between full- and half-sibs.** (A) Principal component analysis of parent and progeny genetic  
394 diversity, comparing the top three principal components (PCA). The female parental values  
395 ( $n = 3$ ) are indicated as red points in each plot. (B) Network analysis of kinship coefficients  
396 determined by *KING* [54] and visualised by *Gephi* [55] highlighting full-sib relationships  
397 between progeny. The thickness of the line (edges) represents the kinship coefficient  
398 between individuals (nodes) and is proportionate to the relationship between pairs. (C)  
399 Proposed pedigree of the brood. Full-sib male (blue) and female (pink) progeny are  
400 indicated for each sub-family. Colours used in (B) and (C) represent groups of progeny that  
401 share a common father.

402

## 403 Discussion

404 Our comprehensive genetic characterisation of genome-wide patterns of segregation in  
405 progeny from a brood of parasites revealed extensive variation in recombination rates  
406 across chromosomes, and confirmed previous suggestions of polyandry as the dominant  
407 mating system in *H. contortus* [51]. Moreover, analysis of genetic variation in both  
408 autosomes and the X chromosome identified an extended region of reduced heterozygosity  
409 in the female parent, which could be a genetic consequence of population bottlenecks  
410 during the generation and maintenance of the MHco3(ISE) line. Analysis of allele frequency  
411 spectra also suggested the presence of polyploids among the progeny. The availability of a  
412 largely complete chromosomal scale *H. contortus* genome assembly facilitated such  
413 analyses. Here, we discuss some of the characteristics and challenges associated with the  
414 assembly of a genetic map when homozygous single parent crosses are not available, and  
415 how some of the features of the genetic cross impact on our understanding of *H. contortus*  
416 biology and anthelmintic resistance.

417

## 418 Prediction of genomic structure

419 A small number of linkage maps have been described for free-living nematodes and parasitic  
420 helminths. *H. contortus* was found to have the lowest genome-wide recombination rate  
421 among these helminths, at an average of 604.12 kb/cM throughout the ~280 Mb genome.  
422 However, the relative recombination rate (kb/cM) of *H. contortus* and other nematodes  
423 scales proportionately with genome size, i.e. larger genomes have lower recombination  
424 rates (**Fig S5**). While the recombination rates of some these nematodes are somewhat lower  
425 than predicted by a model describing the relationship between eukaryotic genome size and  
426 recombination rate (**Fig S5**, grey dashed line) [9], they are more consistent with  
427 recombination rates seen among other invertebrates (**Fig S5**, grey points; see  
428 Supplementary Table 1 from Lynch M [9] for invertebrate recombination rate data). The  
429 relationship between genome size and recombination rate is somewhat dependent on the  
430 number of crossovers per chromosome per meiosis; for example, in *C. elegans*, almost  
431 complete crossover interference occurs, such that only a single crossover per pair of  
432 homologous chromosome is observed [56]. In *H. contortus*, some but certainly not complete

433 interference was observed, with an average rate of 0.69 crossovers per chromosome (i.e.  
434 1.38 crossovers per pair of homologous chromosomes). This crossover rate is still  
435 substantially lower than in *S. mansoni*, whereby multiple chiasma per homologous pair have  
436 been observed [57], or in *M. hapla*, whereby recombination between all four chromatids  
437 within a homologous pair has been described [58]. The mechanisms by which this  
438 recombination rate diversity between helminth species is generated are largely unknown;  
439 however, it does provide an insight into the evolutionary potential of these diverse helminth  
440 species.

441

442 To our knowledge, we are the first to report the use of whole genome sequencing to  
443 construct a genetic map of any helminth species. WGS allowed significantly greater  
444 flexibility in choosing high quality variants to be included in the genetic map than other  
445 marker-based approaches such as amplified fragment length or Sanger-sequencing derived  
446 markers, and more recently, higher throughput RADseq and genotype-by-sequencing  
447 approaches, and allowed us to fully exploit the genetic variation in the available progeny.  
448 This was particularly important given that: (i) the progeny were not derived from a cross  
449 between genetically distinct homozygous single male and female parents, as is typical for a  
450 genetic mapping experiment; (ii) the high genetic diversity within isolates meant that a lot  
451 of markers have to be screened and discarded to find “bi-allelic markers” that segregate  
452 appropriately for analysis; and (iii) we did not know how many males would contribute to  
453 the progeny of the cross due to polyandry. As such, we developed a bioinformatics pipeline  
454 to select markers based on the genotype segregation ratio of the progeny (approximate 1:1  
455 genotype ratios: Aa:aa [PT:011] or AA:Aa [PT:110]) and heterozygous sites in the female.  
456 This unusual cross design to account for the biological complexity meant that relatively few  
457 of the sites that differed between parents (pseudo-testcross SNPs represent only 4.09% of  
458 the total SNPs in the brood, and 29.77% of SNPs heterozygous in the female parent, before  
459 deliberate thinning) were usable in the map. A very large panel of traditional markers would  
460 thus have been required even for the relatively small number of progeny analysed here. The  
461 genome-wide resequencing approach that we used would seem to be the only practical way  
462 to generate complete recombination maps in this system. Genome-wide genetic variation  
463 that has been validated as segregating in a Mendelian fashion also provides a valuable  
464 resource for downstream experiments such as: QTL analyses of parasite traits (e.g. drug



465 resistance); using individuals phenotyped *in vitro* using bioassays [59-62]; or as a source of  
466 genome-wide population genetic markers, which typically require low/no linkage  
467 disequilibrium between loci.

468  
469 We initially intended to use the  $F_1$  genetic map to guide improvements of the assembly of  
470 the draft genome for *H. contortus* MHco3(ISE) [26]; while subsequent improvements to the  
471 genome assembly have rendered this unnecessary (unpublished data), the co-linearity of  
472 the genetic and physical maps confirms the accuracy of the current assembly. A number of  
473 features of this dataset would not have been obvious without integrating the genetic map  
474 and physical assembly. The first of these includes the non-uniform distribution of genetic  
475 map markers in the genome. This is most obvious in chromosome IV in which approximately  
476 half of the chromosome is missing from the genetic map, due to a long tract of  
477 homozygosity in the female parent. However, each chromosome contained multiple  
478 megabase-scale gaps that directly corresponded to a deficiency of heterozygosity in the  
479 female parent in these regions. This may reflect the genetic history of this particular strain:  
480 MHco3(ISE) is a laboratory strain that was originally generated by performing 15 rounds of  
481 half-sib matings of an outbred strain [47]; since that time, it has been passaged and  
482 cryopreserved on numerous occasions at an unknown, but likely limited, population size.  
483 Although significant diversity remains in this strain [63], it is probable that population  
484 bottlenecks, increased inbreeding or selection have resulted in discrete regions of the  
485 genome becoming genetically fixed. Secondly, the integration of the genetic map and  
486 contiguous physical genome map allowed us to describe the recombination landscape of  
487 the genome. Although there are similarities in the recombination rate domain structure  
488 with that of *C. elegans* [8, 14], chromosomes III and V have distinct recombination rate  
489 differences compared both to chromosomes I, II and IV of *H. contortus*, and to all  
490 chromosomes of *C. elegans*. The broad-scale distribution is unlikely to be the result of  
491 differential recombination around centromeric sequences, given the similarities in  
492 recombination domain structure with *C. elegans* chromosomes, and that *C. elegans*  
493 chromosomes are holocentric during mitosis [64, 65]. However, it has been proposed that  
494 the low or absent recombination in the chromosome termini may correlate with the  
495 presence of a spindle attachment site that guides segregation of homologous chromosomes  
496 in meiosis [66]. While we have no data to directly test whether *H. contortus* is holocentric,

497 we have identified low recombining chromosome termini consistent with that observed in  
498 *C. elegans*.

499

500 Despite the relatively high marker density used here ( $n = 1,618$ ), many SNPs were  
501 completely linked in seemingly non-recombining regions. Inclusion of a larger number of  
502 progeny would provide additional resolution to more precisely characterise variation in and  
503 transitions between recombination rate domains in each chromosome. Finally, although we  
504 could not generate a genetic map for the X chromosome due to the limited brood size and  
505 the absence of genetic diversity in the female parent, WGS data allowed us to examine  
506 genetic diversity among the female progeny, which highlighted both significant genetic  
507 variation and clustering consistent with shared paternal haplotypes in the autosomes.

508

509

#### 510 **Detection of Polyandry**

511 Technical challenges associated with single male and female mating led us to perform the  
512 genetic cross using 100 immature female MHco3(ISE) and 100 male MHco18(UGA2004)  
513 surgically implanted into the abomasum of a recipient sheep. Analysis of the genetic  
514 diversity among  $F_1$  progeny of a single female revealed discrete groups of progeny; given  
515 that *H. contortus* has been previously described to be polyandrous [51], we hypothesised  
516 that these groups represented the progeny of different male nematodes. In this cross, our  
517 data supports at least eight paternal genotypes contributing to multiple individuals in the  
518 brood ( $n = 41$ ). These data are consistent with the original report of polyandry in *H.*  
519 *contortus*, which described at least 3 to 4 paternal microsatellite-derived genotypes from  
520 the 11 to 17 progeny sampled per single fecund female analysed [51]. Single worm  
521 genotyping of males recovered from the initial genetic cross recipient lamb would provide  
522 further insight into the ancestral relationships among the progeny. The relatively high  
523 frequency of polyandrous pairings would substantially increase the diversity of genotypes  
524 found among the progeny, as more possible pairs of haplotypes would be generated. This  
525 feature of *H. contortus* biology is likely to play a significant role in generating and  
526 maintaining the high levels of genetic diversity characterised in laboratory [63] and field [67,

527 68] isolates of this parasite and is also relevant to other parasitic nematode species where  
528 polyandry has been reported [69-71].  
529

### 530 **Detection of non-diploid patterns of variation**

531 *H. contortus* is a dioecious, sexually reproducing diploid animal. Unexpectedly, we observed  
532 seven of the 41 progeny (17.1%) with an excess of heterozygous genotypes, and with an  
533 allele frequency spectrum that is consistent with a polyploid complement of chromosomes.  
534 Moreover, two distinct patterns of allele frequency spectrum among six of the seven  
535 putative polyploids lead us to hypothesise that these progeny arose by either: (i)  
536 nondisjunction during meiosis 1 of gametogenesis in the female parent; or (ii) polyspermy,  
537 i.e. an egg that has been fertilized by more than one sperm, as a consequence of polyandry  
538 (see **Fig S6** for alternate hypotheses and evidence for the generation of triploid progeny in  
539 the brood). A third hypothesis—nondisjunction during male gametogenesis resulting in  
540 diploid sperm—was excluded; analysis of genotype frequencies among the F<sub>1</sub> progeny at  
541 SNPs at which the female parent was homozygous demonstrated that paternally-derived  
542 alleles from putatively heterozygous sites were segregating independently, resulting in an  
543 approximate 1:1 genotype ratio among all but one individual (**Figure S2C**; the putative  
544 aneuploid F1\_30). This supports the observation that polyploidy was inherited from diploid  
545 gametes derived from the female parent (i.e. nondisjunction), or multiple haploid gametes  
546 from the male parents (i.e. polyspermy).

547

548 Polyploidy has been previously described among nematodes. In *C. elegans*, a range of ploidy  
549 states have been characterised (see Hodgkin J [72] for review of work on natural and  
550 induced tetraploids, triploids and haploids) and is a feature of a cellular organismal growth  
551 into late adulthood due to nuclei endoreplication [73, 74]. However, polyploidy is typically  
552 associated with parthenogenesis in worms (e.g. some *Meloidogyne spp.* [75, 76] and some  
553 *Panagrolaimus spp.* [77]). Polyspermy in worms is thought to be rare, with a single  
554 description in the rodent filarial worm *Acanthocheilonema viteae* [78]; more is understood  
555 in regard to the mechanisms by which polyspermy is prevented [79-81]. However,  
556 polyspermy may be associated with polyandrous mating [82], whereby sexual conflict  
557 among males (at least 8 in the data presented) competing to reproduce with a female likely

558 results in strong selection on male reproductive traits (e.g. sperm count, size and quality),  
559 which increases the likelihood of reproductive success [83]. While this would drive  
560 coevolution of female traits to block polyspermy, it may be that polyspermy is a  
561 consequence of this competition in polyandrous species such as *H. contortus*. Given that  
562 these progeny were sampled at the L<sub>3</sub> stage, we cannot be sure that these individuals would  
563 have developed to adulthood and become reproductively viable. However, a report  
564 describing the karyotype of a single triploid *H. contortus* adult female suggests that they  
565 may be at least developmentally viable [23]. The presence of sporadic polyploidy among the  
566 *H. contortus* F<sub>1</sub> progeny represents a novel finding among parasitic nematodes; further work  
567 is required to determine if triploidy is a feature of *H. contortus* biology and prevalent in the  
568 field, or, is a novel feature of this genetic cross. If the former is true, then it will be  
569 important to be aware of ploidy variation in population genetic studies of *H. contortus*,  
570 particularly if larval stages are sampled.

571

572 A single individual—F1\_30—presented with a variant allele frequency spectrum consistent  
573 with an aneuploid complement of chromosomes. Aneuploidy and other severe  
574 chromosomal abnormalities have been described in experimental hybrid crosses between  
575 *H. contortus* and the related cattle parasite, *Haemonchus placei* [84]; such hybrids have  
576 recently been genetically characterized in the field [85]. Although such chromosomal  
577 abnormalities have not been described in within-species *H. contortus* crosses to date, the  
578 use of whole genome sequencing provides greater resolution over single marker techniques  
579 to detect these chromosome-wide changes, which may have resulted via incompatibility of  
580 rare alleles between the genetically diverse strains used in the cross.

## 581 **Conclusions**

582 In summary, we have undertaken a comprehensive analysis of genetic diversity within a *H.*  
583 *contortus* family derived from an experimental genetic cross. Whole-genome sequencing of  
584 a female and her brood allowed the construction of a F<sub>1</sub> genetic map, despite the  
585 challenging design dictated by the unusual biology and life history of this parasitic helminth.  
586 Development of the genetic map continues to build upon the genetic resources available for  
587 *H. contortus* as an experimentally tractable organism, and provides new insight into the

588 recombination architecture of the genome. These data, together with evidence of polyandry  
589 and polyploidy, highlight the complexities of the underlying biology of *H. contortus*, and  
590 have important implications toward understanding the development and spread of  
591 anthelmintic resistance in this important pathogen of livestock. Clear recombination rate  
592 differences throughout the genome will influence the rate by which a locus correlated (i.e. a  
593 genetic marker linked to resistance), or causally associated (i.e. resistance conferring  
594 mutation) with anthelmintic resistance will evolve within a population, dependent on the  
595 position in the genome that the given locus lies. Incorporating recombination rate  
596 parameters in studies that aim to genetically detect or track the transmission of resistance  
597 will be critical to the utility and interpretation of data derived from such approaches. This  
598 will be particularly the case given the likely multigenic nature of resistance to some, and  
599 perhaps all, anthelmintics.  
600

## 601 **Methods**

### 602 **Construction of the genetic cross and collection of worm samples**

603 A schematic of the experimental genetic cross is outlined in **Fig 1**. Briefly, two parasite naïve  
604 lambs were each infected with ~10,000 infective larvae from one of two ovine-derived *H.*  
605 *contortus* strains, the anthelmintic susceptible MHco3(ISE) [47], or MHco18(UGA2004) [46],  
606 a multi-drug resistant strain that is insensitive to standard manufacturers recommended  
607 dose rates of benzimidazole, imidazothiazole and macrocyclic lactone anthelmintics. At 14  
608 days post infection (DPI), developing sexually immature parasitic stages were recovered  
609 *post mortem*, and the sex of the L<sub>4</sub> stage immature adults was determined by microscopic  
610 examination of gross morphology [86, 87]. A total of 100 MHco3(ISE) female and 100  
611 MHco18(UGA2004) male L<sub>4</sub> (F<sub>0</sub> generation) were surgically transferred into the abomasum  
612 of a donor sheep to allow reproduction that would generate F<sub>1</sub> hybrids between the two  
613 strains. At 28 DPI, 67 MHco3(ISE) females and 42 male MHco18(UGA2004) F<sub>0</sub> from the  
614 recipient sheep were recovered *post mortem*, after which the males were snap frozen in  
615 liquid nitrogen and stored. Sampling was performed at 28 DPI to ensure that all of the  
616 females would have mated, and that they would be mature enough to have more viable  
617 progeny than is thought to be the case in early patency. Individual females were placed into  
618 individual wells of 24-well cluster plates (Sarstedt) containing 1 mL of warm RPMI 1640 cell  
619 culture media containing 1% (v/v) D-glucose, 2 mM glutamine, 100 IU/mL penicillin, 100  
620 mg/mL streptomycin, 125 mg/mL gentamycin, 25 mg/mL amphotericin  
621 B [88] and HEPES (1% v/v) and incubated in 5% CO<sub>2</sub> at 37°C for 48 h to promote egg  
622 shedding. Eggs were transferred at 24 h and 48 h and mixed with fresh helminth egg-free  
623 sheep faeces before being incubated at 24°C for 2 weeks to allow larval development to L<sub>3</sub>.  
624 After this time, a single female parent (F<sub>0</sub>) and a total of 41 F<sub>1</sub> L<sub>3</sub> progeny were individually  
625 stored in preparation for DNA extraction and sequencing library preparation.

626

### 627 **Sample preparation and sequencing**

628 The female parent was dissected on ice to isolate the head and anterior body only (in three  
629 sections, as three technical replicates) to avoid contamination with fertilised eggs present *in*

630 *utero*. The female sections and individual L<sub>3</sub> were transferred into 10 µL of sample lysis  
631 buffer (working solution: 1000 µL Direct PCR Lysis Reagent [Viagen, Los Angeles, USA], 50 µL  
632 1 M DTT, 10 µL 100 mg/ml Proteinase K) in a 96-well plate and allowed to incubate at 60°C  
633 for 2 h followed by 85°C for 45 min. Whole genome amplification (WGA) of each sample  
634 lysate was performed using RepliG amplification. First, 2-5 µL of sample lysate was  
635 combined with 5 µL of 1.3 M Trehalose in a 96-well plate and mixed by gentle tapping,  
636 incubated for 3 min at 95°C, and placed on ice. A 40 µL RepliG amplification mix (29 µL  
637 REPLI-g Reaction Buffer + 1 µL REPLI-g polymerase + 10 µL 1.3 M Trehalose) was added to  
638 each well, and incubated for 16 h at 30°C followed by 10 min at 65°C before being placed on  
639 ice. The WGA DNA was cleaned using Ampure XP beads at a 1.4× bead:DNA reaction ratio,  
640 before being eluted in 50 µL of RNase/DNase-free water and stored at 4°C.

641

642 PCR-free sequencing libraries (mean length of approximately 400 bp) were prepared by  
643 methods previously described [89] and sequenced on an Illumina HiSeq X10, resulting in  
644 approximately  $3.06 \times 10^9$  151-bp paired-end reads (see **S1 Table** for a breakdown of reads  
645 per lane and per sample). Raw sequence data is archived under the ENA study accession  
646 ERPO24253.

647

#### 648 **Mapping and variant analysis**

649 Raw sequence data was mapped to the current unpublished version of the reference  
650 genome for *Haemonchus contortus* (v3.0, available at  
651 [ftp://ngs.sanger.ac.uk/production/pathogens/Haemonchus\\_contortus](ftp://ngs.sanger.ac.uk/production/pathogens/Haemonchus_contortus)) using *Smalt*  
652 (<http://www.sanger.ac.uk/science/tools/smalt-0>) with the mapping parameters “-y 0.8 -i  
653 800”. Data from multiple sequencing lanes for a single sample were merged (*samtools-1.3*  
654 *merge*) and duplicate reads removed (*Picard v2.5.0*;  
655 <https://github.com/broadinstitute/picard>) from the bam files before further processing.  
656 Variants were called using *GATK Unified Genotyper* (v3.3.0)[90]. The raw variant set was  
657 initially filtered to flag variants as low quality if they met the following conditions: quality by  
658 depth (QD) < 2; Fisher’s test of strand bias (FS) > 60; RMS mapping quality (MQ) < 40; rank  
659 sum of alt vs reference mapping quality (MQRankSum) < -12.5; read position rank sum  
660 (ReadPosRankSum) < 8; read depth (DP) < 10. Variants were filtered further using *vcftools*

661 (v0.1.14)[91] to exclude sites with low quality flags, minimise loci with missing data (“--max-  
662 missing 0.8”), exclude indels (“--remove-indels”), exclude SNPs with genotype quality (GQ) <  
663 30, and ensure sites were biallelic (“--min-alleles 2, --max-alleles 2”). A gff file generated  
664 from *RepeatMasker* of the reference genome was also used to filter variants from the vcf  
665 file that were likely associated with repetitive and difficult to map regions.

666

667 Sex determination of the F<sub>1</sub> progeny was performed by measuring: (i) the relative autosome  
668 to X chromosome (characterised and thus named based on synteny with *C. elegans*  
669 autosomes and X chromosome) read depth using *samtools-1.3 bedcov*; and (ii) the relative  
670 heterozygosity of the X chromosome using *vcftools* (v0.1.14) “--het”.

671

## 672 Genetic map construction

673 A “pseudo-testcross” (PT) strategy [92] was employed to generate the genetic map, which  
674 required that each input variant site was: (i) heterozygous in the female parent, and (ii)  
675 segregating in a 1:1 genotype ratio in the F<sub>1</sub> progeny. The segregation pattern of each SNP  
676 was first calculated in the F<sub>1</sub> progeny (with “A: referring to the reference allele and “a” to  
677 the alternative), which resulted in SNPs being placed into one of four categories that best  
678 described the likely genotypes of the parents of the cross for that given SNP: (i) “PT:110”,  
679 i.e. AA×Aa, (ii) “PT:011”, i.e. Aaxaa, (iii) “intercross”, i.e. Aa×Aa, or (iv) SNPs that were  
680 clearly segregating in the brood, but for which the segregation ratio of genotypes in the  
681 progeny did not fit a simple Mendelian segregation pattern that could be generated via  
682 reproduction from a single pair of parents. SNP density was further reduced using *vcftools*  
683 (v0.1.14)[91] --thin as described in the text. The number of filtered SNPs per segregation  
684 group is described in **S2 Table**. Genotypes for autosomal PT:011 and PT:110 SNPs that were  
685 heterozygous in the female parent were imported into *R-3.2.2* [93], after which pairwise  
686 recombination fractions (RF) and logarithm of the odds (LOD) scores were determined for  
687 each chromosome using *R/QTL* [94]. Recombination fractions were converted into map  
688 distance in centimorgans (cM) using the kosambi map function. Variants resulting in  
689 inflation of map distances were identified using *qtl::droponemarker*, and as outliers relative  
690 to surrounding markers via visual inspection of LOD and RF using *qtlcharts::iPlot* [95]. These  
691 aberrant markers were removed in the generation of the final map.



692 A reverse cross design, whereby SNPs were chosen that: (i) segregated in a 1:1  
693 genotype ratio; and (ii) were homozygous in the female parent (and therefore putatively  
694 heterozygous in the male parents) was also performed. Although polyandry prevented a  
695 male-specific genetic map from being constructed (multiple male parents confounded the  
696 calculation of linkage between heterozygous sites), these data were used to determine the  
697 segregation frequency of alleles from the male parents.  
698

### 699 **Recombination landscape**

700 Recombination patterns for each chromosome were visualised first by generating genotype  
701 matrices of pseudo-testcross markers for each chromosome using *vcftools* (v0.1.14) "--012",  
702 followed by plotting using the *gplots::heatmap2* function in R. These maps highlighted  
703 recombination breakpoints, linkage blocks, and regions of excess heterozygosity or reduced  
704 heterozygosity. Recombination rate changes throughout the genome were visualised by  
705 constructing Marey maps, which compare the position of the marker in the genome (base  
706 position in the fasta sequence) to the relative position in the genetic map. A fitted loess  
707 smoothed line of the genetic map positions in 1 Mb windows was performed to calculate  
708 the recombination rate.  
709

### 710 **Kinship analysis**

711 Analysis of genetic relatedness between F<sub>1</sub> progeny was undertaken to characterise  
712 evidence of polyandry and to determine, if present, the impact on the cross analysis.  
713 Principal component analysis (PCA) of genetic distances between the F<sub>1</sub> progeny and female  
714 parent was performed using the *SNPrelate* package in R 3.1.2 [96]. Kinship coefficients were  
715 determined for all pairwise relationships among the F<sub>1</sub> progeny using *KING* [54].  
716 Relationship networks of the pairwise kinship coefficients were visualised using *Gephi* (v  
717 0.9.1; [55]) to highlight full- and half-sib relationships among the F<sub>1</sub> progeny. Layout of the  
718 kinship network graph was determined using the *Force Atlas* parameter, with the nodes (F<sub>1</sub>  
719 individuals) coloured by their proposed kinship group, and the thickness of the edges  
720 proportionate to the kinship coefficient between two F<sub>1</sub> individuals (nodes).  
721

## 722 **List of abbreviations**

723 **cM**: centimorgan; **DPI**: days post infection; **LOD**: logarithm of the odds; **MHco3(ISE)**: inbred  
724 susceptible *H. contortus* strain; **MHco18(UGA2004)**: triple anthelmintic resistant *H.*  
725 *contortus* strain; **PCA**: principle components analysis; **PT**: pseudo-testcross; **QTL**:  
726 quantitative trait loci; **RF**: recombination fraction; **SNP**: single nucleotide polymorphism;  
727 **WGA**: whole genome amplification; **WGS**: whole genome sequencing.

728

## 729 **Declarations**

### 730 **Ethics approval and consent to participate**

731 All experimental procedures described in this manuscript were examined and approved by  
732 the Moredun Research Institute Experiments and Ethics Committee and were conducted  
733 under approved UK Home Office licenses in accordance with the Animals (Scientific  
734 Procedures) Act of 1986. The Home Office licence number is PPL 60/03899 and  
735 experimental code identifier was E46/11.

### 736 **Consent for publication**

737 Not applicable

### 738 **Availability of data and material**

739 The raw sequencing data generated and/or analysed during the current study are available  
740 in the European Nucleotide Archive repository, <http://www.ebi.ac.uk/ena/> under the study  
741 accession number ERP024253. The genome assembly is available at  
742 [ftp://ngs.sanger.ac.uk/production/pathogens/Haemonchus\\_contortus](ftp://ngs.sanger.ac.uk/production/pathogens/Haemonchus_contortus) .

### 743 **Competing interests**

744 The authors declare that they have no competing interests.

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750 **Author's contributions**

751 Conceived the study: ED, RL, AT, JAC, JSG, NDS

752 Undertook the genetic cross: NDS, DJB, AAM

753 Performed the molecular biology: KM, RL

754 Coordinated sequencing: NH

755 Participated in the discussion and interpretation of results: SRD, RL, DJB, CB, UC, JSG, NH,

756 BKM, KM, AAM, AT, AT, MB, ED, JAC, NDS

757 Performed the data analysis: SRD

758 Wrote the first draft of the manuscript: SRD, JAC

759

760 All authors read and critically revised the final manuscript.

761

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768 expert care and assistance with animals.

769

770

771

## 772 Supporting information

773 **S1 Fig. Genome-wide variant density of the female parent.** SNP density is presented as the  
774 number of homozygous reference (AA; panel A), heterozygous (Aa; panel B) and  
775 homozygous variant (aa; panel C) SNPs per 100-kbp. Plots are coloured per chromosome, in  
776 the following order: I (black), II (red), III (green), IV (dark blue), and V (light blue). The X  
777 chromosome has also been included (currently in two scaffolds) as indicated by the purple  
778 and yellow segments.

779

780 **S2 Fig. Genome-wide average pseudo-testcross SNP density in the F<sub>1</sub> progeny.** Pseudo-  
781 testcross markers were chosen based on an approximate segregation ratio of 1:1  
782 homozygous:heterozygous genotypes among the F<sub>1</sub> progeny. Analysis of heterozygous Aa  
783 genotype frequencies (A) of the 41 F<sub>1</sub> progeny revealed a number of individuals presenting  
784 with moderate and extreme heterozygosity. A reanalysis of Aa genotype frequencies after  
785 the outlier individuals were removed (34 individuals remaining) (B) resulted in genotype  
786 frequencies at approximate 1:1 genotype ratio. (C) Comparison of heterozygosity among the  
787 F<sub>1</sub> progeny at SNPs selected that segregate at a 1:1 genotype ratio in the progeny and are  
788 homozygous in the female parent; these sites are therefore putatively heterozygous in the  
789 male parents; i.e. a reverse F<sub>1</sub> cross. In this comparison, only a single F<sub>1</sub> individual – F<sub>1</sub>\_30 –  
790 showed moderate heterozygosity. Individual points are coloured based on deviation from  
791 null expectation (H<sub>0</sub>: 1:1 genotype ratio of homozygous:heterozygous sites) determined by  
792 chi-square analysis ( $\chi^2$ , df=1). Median frequency (solid grey line) and “whiskers” (dashed  
793 grey lines; most extreme point no more than 1.5× the interquartile range) were calculated  
794 using the R function *boxplot.stats*.

795

796 **S3 Fig. Variant allele frequency density plots used to explore ploidy among the F<sub>1</sub> progeny.**  
797 Each plot displays the variant allele frequency of each chromosome (coloured lines) and  
798 genome-wide average (black dotted line).

799

800 **S4 Fig. X chromosome genetic diversity of 41 F<sub>1</sub> progeny and female parent (3 replicate  
801 samples).** SNPs genotyped as hemizygous in male samples were analysed in all samples to  
802 detect segregation of X chromosome variants from the female parent. Female genotypes:  
803 X<sup>A</sup>X<sup>A</sup>: red; X<sup>A</sup>X<sup>a</sup>: yellow; X<sup>a</sup>X<sup>a</sup>: white. Male genotype (hemizygous): X<sup>A</sup>O: red; X<sup>a</sup>O: white.

804

805 **S5 Fig. Relationship between recombination rate (kb/cM) and genome size (Mb).**

806 Recombination rates for helminth species with published genetic maps—*Caenorhabditis*  
807 *elegans* [14], *Haemonchus contortus* (current study), *Meloidogyne hapla* [5, 6], *Pristionchus*  
808 *pacificus* [4], and *Schistosoma mansoni* [17] —were derived from known genome size and  
809 the reported genetic map length. These estimates were compared against a derivation of  
810 the equation presented by Lynch M [9] describing the relationship between recombination  
811 rate and genome size (recombination rate (cM/Mb) = 0.0019×[genome\_size(Mb)]<sup>-0.71</sup>). These  
812 data were converted to kb/cM (1/[cM/Mb]×1000). Based on this equation, recombination

813 rates were estimated for genome sizes between 10 and 1000 Mb (grey dashed line). The  
814 helminth and modelled data were plotted with recombination rates and genome sizes of  
815 invertebrate species presented in Supplementary Table 1 of Lynch M [9].

816

817 **S6 Fig. Alternate hypotheses proposed to explain the segregation of alleles and**  
818 **recombination, and the presence of triploid progeny.** Four hypotheses for the segregation  
819 of genetic variation in gametes produced from the heterozygous female are presented: (1)  
820 normal gametogenesis; (2) nondisjunction in meiosis 1; (3) nondisjunction in meiosis 2; and  
821 (4) polyspermy.

822

823 **S1 Table. Sequencing data used in this study.**

824

825 **S2 Table. Breakdown of genetic variation in the female parent, and proportion of variants**  
826 **in each segregation class.**

827

828 **S3 Table. Genotype concordance between three female parent (replicate) samples.**

829

830 **S4 Table. SNPs used in the final genetic map.**

831

832 **S5 Table. Expected and observed genetic consequences of triploidy via nondisjunction or**  
833 **polyspermy in the cross.** Three alternate hypotheses and data are presented: (1) expected  
834 segregation of pseudo-testcross markers, which were used in the making of the genetic  
835 map; (2) triploidy via nondisjunction; and (3) triploidy via polyspermy.

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Figure 1

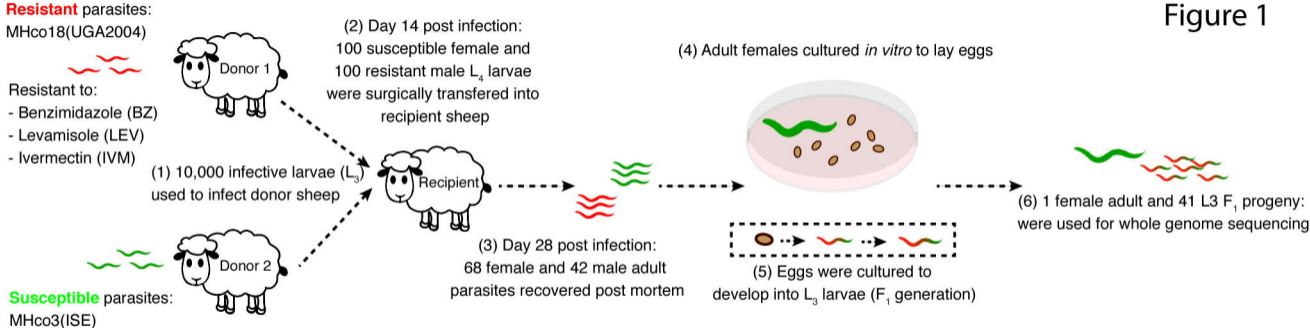


FIGURE 2

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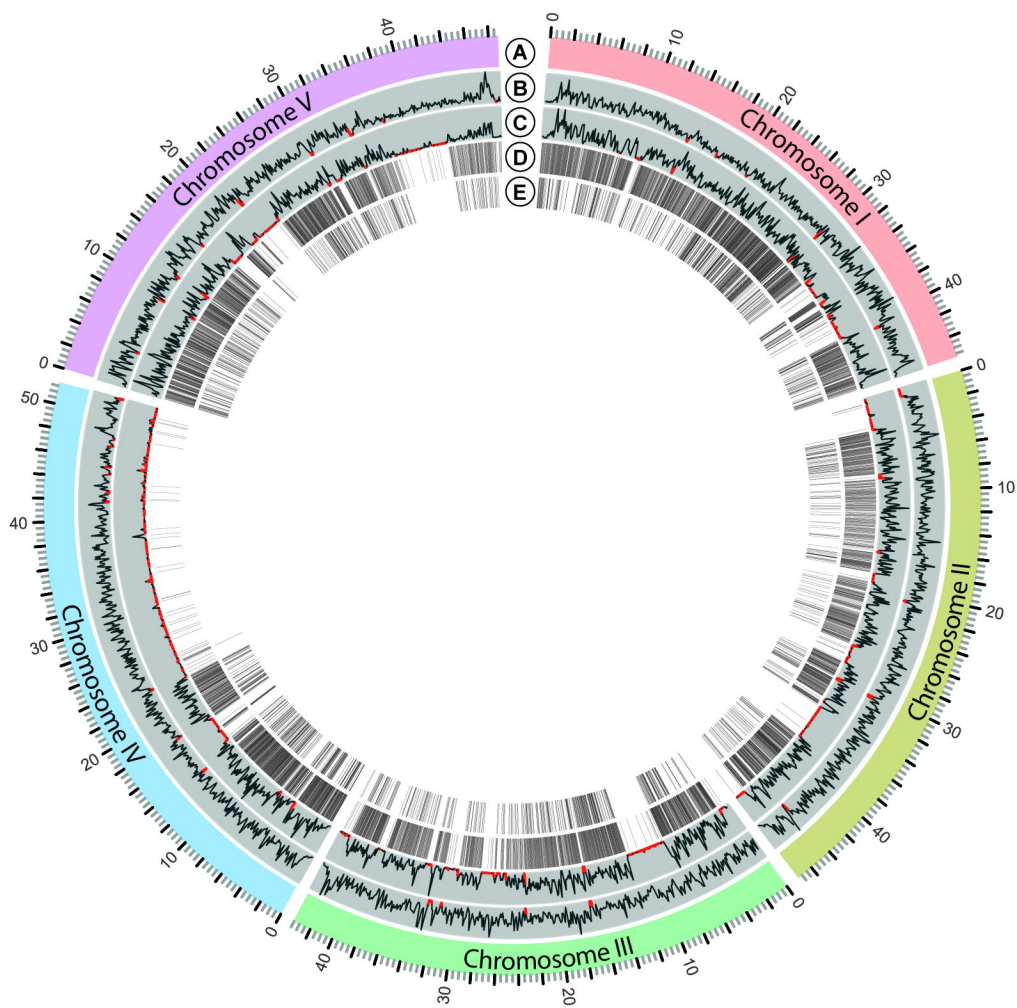
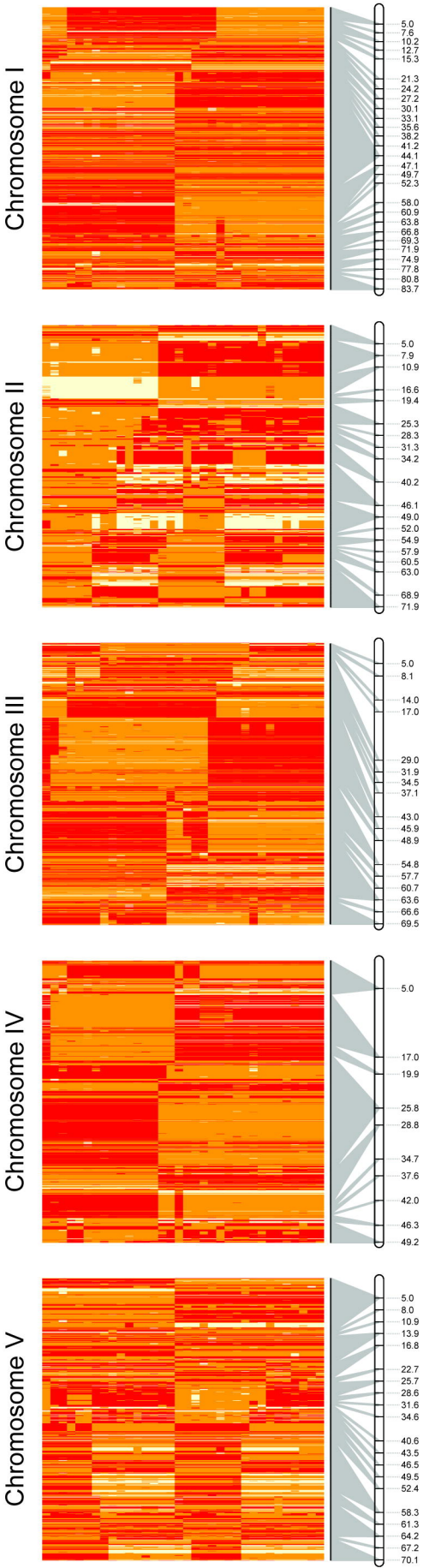


FIGURE 3

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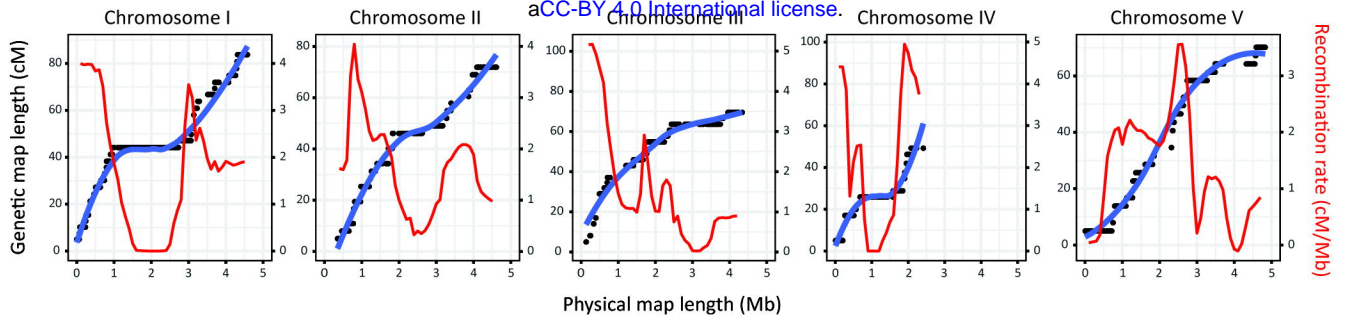


FIGURE 5

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