Genetic architecture of individual meiotic crossover rate and distribution in a large Atlantic Salmon (*Salmo salar)* breeding population.

Cathrine Brekke[1,2], Susan E. Johnston[1], Tim M. Knutsen[3], Peer Berg[2]

[1]Institute of Ecology and Evolution, School of Biology, University of Edinburgh, Edinburgh, EH9 3FL, United Kingdom

[2]Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Post Box 5003, 1433, Ås, Norway

[3]AquaGen AS, Post Box 1240, 7462, Trondheim, Norway

*Corresponding author: cathrine.brekke@ed.ac.uk

Abstract

Meiotic recombination through chromosomal crossovers ensures proper segregation of homologous chromosomes in meiosis, while also breaking down linkage disequilibrium and shuffling alleles at loci located on the same chromosome. Rates of recombination can vary between species, but also between and within individuals, sex and chromosomes within species. Indeed, the Atlantic salmon genome is known to have clear sex differences in recombination with female biased heterochiasmy and markedly different landscapes of crossovers between males and females. However, little is known about the genetic control of these patterns and how this differs at the individual level. Here, we investigate genetic variation in individual measures of recombination in >5000 large full-sib families of a Norwegian Atlantic salmon breeding population with high-density SNP genotypes. We show that females had 1.6× higher crossover counts (CC) than males, with autosomal linkage maps

spanning a total of 2174 cM and 1483 cM in males and females, respectively. However, females had 8× higher intra-chromosomal genetic shuffling ($\bar{r}$) than males, which is explained by an extreme telomeric bias of male crossovers. CC was heritable in females (h^2 = 0.11) and males (h^2 = 0.10), and $\bar{r}$ was also heritable in both sex but with a lower heritability in females (h^2 = 0.06) than in males (h^2 = 0.11). Inter-sex genetic correlations for CC and $\bar{r}$ were close to zero, suggesting that rates and distribution of crossovers are genetically distinct traits in males and females, and that there is a potential for independent genetic change in both sexes in the Atlantic Salmon. Together, these findings give novel insights into recombination and sex-differences in salmonids and contribute to a better understanding of the genetic architecture underlying recombination in eukaryotes more broadly.

Author Summary

When germ cells are produced during meiosis, the chromosomes are reorganized and combined in new ways through meiotic crossovers. This contributes to new genetic variation from one generation to the next. The rate of crossovers can vary largely within and between species and will often occur in distinct hotspots throughout the genome. The Atlantic salmon is known to have distinct differences between males and females both in the number and positioning of crossovers. In male meiosis, crossovers occur only in the very ends of the chromosomes, which is an extreme pattern also compared to other species. In this study we looked at variation in distribution and number of crossovers between individuals and find that both traits vary and are heritable in both sex but are genetically distinct traits in males and females indicating that male and female crossover distribution can evolve independently within the population. We also find that the position and number of crossovers that occur during meiosis in Atlantic salmon is likely affected by many genes. These findings contribute

to the understanding of how crossover rates and distribution evolve in Atlantic salmon in particular and how they may evolve between and within species in general.

Introduction

Meiotic recombination is a fundamental part of sexual reproduction, where chromosomal crossing-over between the maternal and paternal chromosomes during synapsis in the early prophase of meiosis, leads to novel combinations of alleles in the gametes transmitted to the next generation. It is of large interest in studies of both wild and domestic species, as it breaks up linkage between loci located on the same chromosome and in turn affects the speed and degree of responses to selection (1,2). Recombination also has a mechanistic role in the proper segregation of chromosomes during meiosis; a lack of crossovers can lead to harmful outcomes, such as aneuploidy (i.e. the incorrect number of chromosomes in gametes); (3–6), whereas high rates of recombination can be associated with increased mutation rates at crossover sites (7). Yet, there is large variation in recombination rates within and between species, populations, sexes, individuals, and chromosomes across sexually reproducing eukaryotes (8,9), suggesting a combination of mechanistic and evolutionary processes in driving this variation.

Studies of individual crossover counts in mammals and birds often show that it is variable and heritable, with a conserved, oligogenic architecture (10–18). Genes including *RNF212*, *REC8, SPO11* and *RNF212B* are repeatedly associated with recombination rate in mammal and bird studies (19–24) whereas the locus *PRDM9* has been identified as a gene that determines recombination hotspot positioning in mammals (25). Furthermore, most species studied show sex differences in the degree and direction of recombination rates and landscapes (known as heterochiasmy) and underlying genetic architectures often differ

73 between the sexes, through sex-limited or sex-differential effects of associated loci (9,12–
74 17,21,26,27)). The causes and consequences of this sexual dimorphism has been of interest
75 for decades, with arguments often centring around sex differences in the fitness consequences
76 of preserving beneficial linked alleles ("haplotypes") or generating novel haplotypes that
77 increase gamete and/or offspring fitness (28–31). However, understanding the broader scale
78 evolution of heterochiasmy remains to be fully understood, due to a lack of suitable empirical
79 data to test hypotheses (30,32–36). Notably, these previous comparative studies have mainly
80 focussed on differences in linkage map lengths and/or crossover counts, yet the evolutionary
81 arguments above centre around the preservation/generation of haplotypic variation. However,
82 theoretical work has shown that the rate of allelic shuffling (i.e. the uncoupling of linked
83 allelic variation on chromosomes) is influenced by crossover positioning, where a crossover
84 on the middle of a chromosome will lead to higher rates of allelic shuffling than a crossover
85 on distal regions (37). Therefore, studies of heterochiasmy should consider not only sex-
86 differences in crossover counts, but also in rates of allelic shuffling within chromosomes, to
87 better disentangle processes related to chromosome disjunction and genetic linkage.
88
89 The Salmonidae family (salmon, char, trout, whitefish and grayling) share an ancestor that
90 underwent a whole genome duplication (WGD) event some 50-100 million years ago (38).
91 Studies in salmonids find that chromosome arms that still show high sequence similarity
92 exchange genetic material during meiosis in a quadrivalent formation (39), which appears to
93 be almost exclusive to male meiosis (40,41), and therefore may lead to different
94 recombination patterns between the sexes. Indeed, studies in Atlantic salmon (*Salmo salar*)
95 have shown extreme and distinct differences in recombination landscapes between males and
96 females (42,43). An early linkage analysis in Atlantic salmon with 442 markers showed little
97 to no recombination in male linkage groups, implying that female recombination was 5 times

98 higher than that of males (44). However, as marker densities increased (to 5650 markers), it
99 was discovered that males do recombine with an overall rate relatively close to females in
100 most chromosomes, but almost exclusively in the telomeric regions (42), a pattern which
101 could not be picked up by lower-density arrays. These stark differences in landscape indicate
102 the presence of large sex-differences in allelic shuffling between linked loci in these species,
103 but the causes and consequences of variation in meiotic crossovers remain unknown. In this
104 study, we investigated individual-, sex- and population-level variation in meiotic crossover
105 rates and landscapes in a large breeding population of Atlantic salmon. We use data from
106 more than 5000 full sib families with genotypes on ~35,000 SNP markers to: (a) construct
107 sex-specific linkage maps; (b) quantify individual crossover counts and rates of intra-
108 chromosomal allelic shuffling; (c) determine their heritabilities and genetic architectures
109 within each sex; and (d) investigate cross-sex genetic correlations.
110

111 ## Results

112 **Linkage mapping.**

113 The sex-specific linkage maps spanned a total of 2173.80 cM in females and 1482.96 cM in
114 males, with the female map 1.47 times than in males (Table 1, Fig 1). In agreement with Lien
115 et al. (2011), the biggest sex differences were on chromosomes 2, 8 and 17, where the female
116 maps were 5.34, 28.13 and 16.09 times longer than the male maps, respectively. In these
117 three chromosomes, we detected very few crossover events in males, with male maps only 2-
118 20 cM long. Obligate crossing-over results in a minimum predicted map length of 50cM;
119 therefore, we assume that we are unable to pick up all crossovers in males on their
120 chromosomes, perhaps either due to lower marker coverage in the telomeric regions on these
121 chromosomes or due to multivalent pairing and crossing over with a different pair of
122 chromosomes (39). For the remaining chromosomes, the genetic map length is 1 to 2.1 times

123 longer in females. Chromosome-level results from the linkage mapping for all chromosomes

124 are provided in Table 1, and Marey maps showing the relationship between the physical and

125 genetic length of the chromosomes are shown in Fig 1. The full linkage map can be found in

126

127 **Table 1. Summary of linkage mapping results by sex and chromosome**

Chr	Number of markers	Chr length (Mb)	Male cM	Female cM	Female/male ratio	Male rate (cM/Mb)	Female rate (cM/Mb)
ssa1	2828	174.50	101.15	130.32	1.29	0.58	0.75
ssa2	749	95.48	19.54	104.45	5.34	0.20	1.09
ssa3	1417	105.78	59.92	107.26	1.79	0.57	1.01
ssa4	1485	90.54	56.88	101.11	1.78	0.63	1.12
ssa5	1246	92.79	62.60	103.35	1.65	0.67	1.11
ssa6	1255	96.06	54.88	115.71	2.11	0.57	1.20
ssa7	852	68.86	67.55	101.37	1.50	0.98	1.47
ssa8	300	28.86	2.01	56.40	28.13	0.07	1.95
ssa9	2333	161.28	55.68	97.65	1.75	0.35	0.61
ssa10	1881	125.88	56.54	80.23	1.42	0.45	0.64
ssa11	1392	111.87	50.92	78.75	1.55	0.46	0.70
ssa12	1485	101.68	55.15	79.27	1.44	0.54	0.78
ssa13	1611	114.42	60.33	76.21	1.26	0.53	0.67
ssa14	1557	101.98	60.94	69.79	1.15	0.60	0.68
ssa15	1524	110.67	55.84	70.27	1.26	0.50	0.63
ssa16	1302	96.49	48.83	64.02	1.31	0.51	0.66
ssa17	681	87.49	3.79	60.96	16.09	0.04	0.70
ssa18	1067	84.08	45.04	63.04	1.40	0.54	0.75
ssa19	1157	88.11	52.57	59.48	1.13	0.60	0.68
ssa20	1331	96.85	53.81	61.61	1.14	0.56	0.64
ssa21	962	59.82	49.88	54.37	1.09	0.83	0.91
ssa22	1122	63.82	52.31	56.56	1.08	0.82	0.89
ssa23	949	52.46	55.52	55.48	1.00	1.06	1.06
ssa24	925	49.35	52.75	54.53	1.03	1.07	1.10
ssa25	888	54.39	50.67	54.06	1.07	0.93	0.99
ssa26	593	55.99	48.91	53.05	1.08	0.87	0.95
ssa27	825	45.31	48.75	53.57	1.10	1.08	1.18
ssa28	606	41.47	48.55	56.60	1.17	1.17	1.36
ssa29	709	43.05	51.66	54.34	1.05	1.20	1.26
Total	35032	2499.33	1482.96	2173.80	1.47	0.59	0.87

128 Mb is the physical length of the chromosomes in megabases. cM is the estimated genetic
129 length of the chromosomes in centiMorgan. Male and female rate is the recombination rate in

cM/Mb. Total is the the total lengths and rates of the 29 chromosomes. Physical length of the chromosomes are from reference genome Ssal_v3.1(GenBank accession GCA_905237065.1)

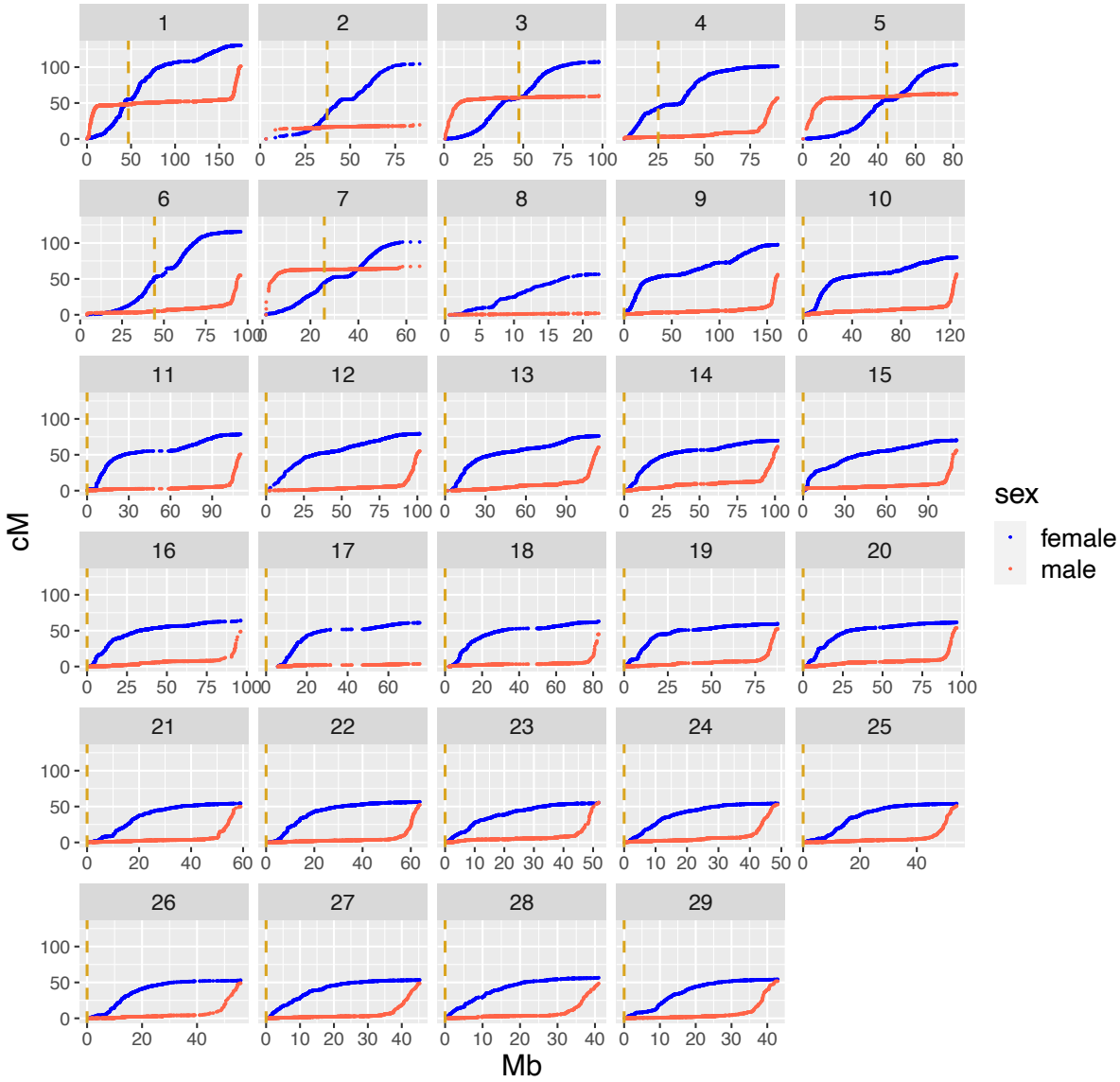
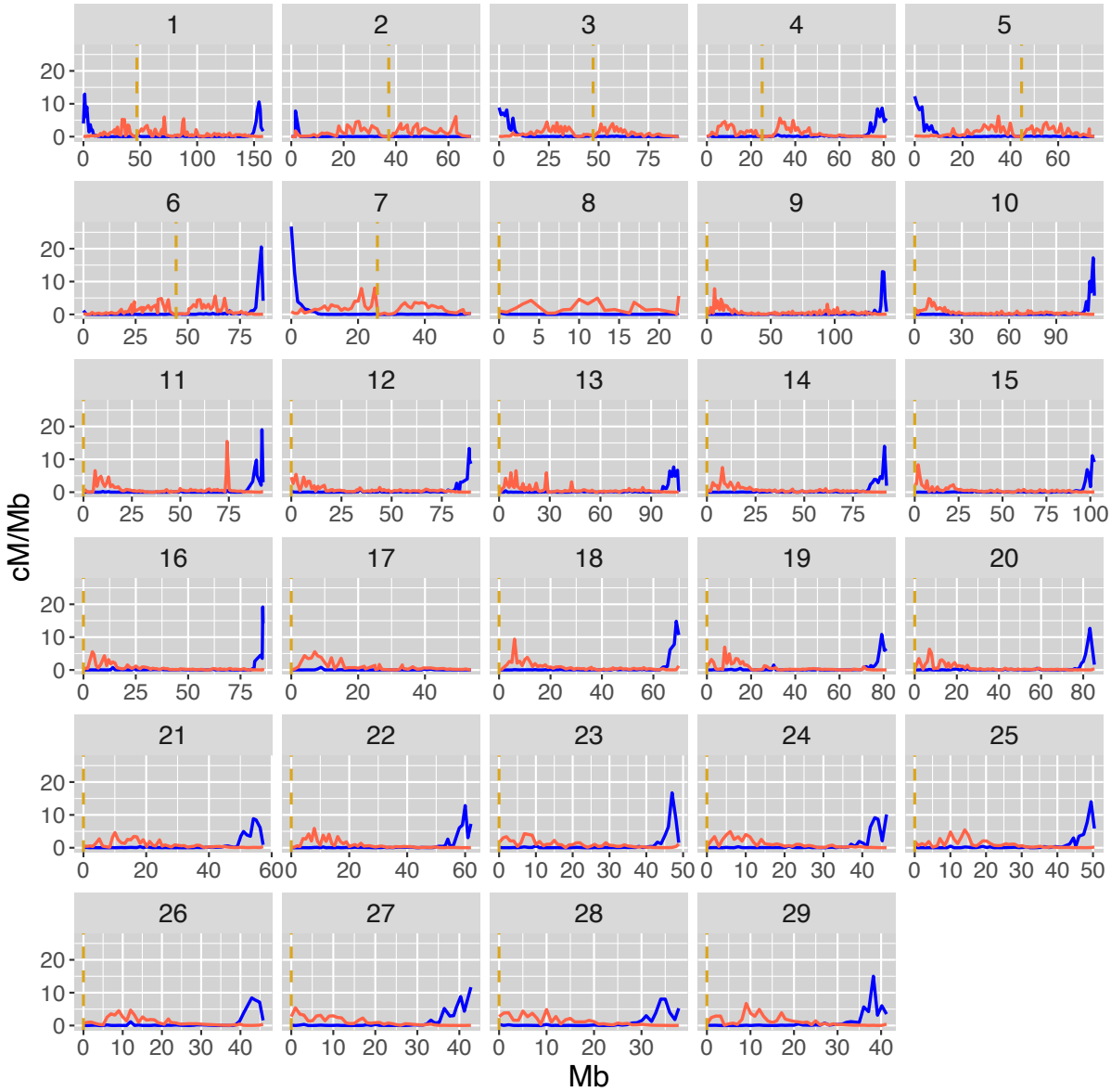


Fig 1. Male and female Marey maps for the 29 Atlantic salmon chromosomes. The physical position in Mb is plotted against the genetic position in cM for the SNP markers within each linkage group. Female positions in red and male in blue. The dashed vertical lines in yellow are the centromere positions from the reference genome Ssal_v3.1 (GenBank accession GCA_905237065.1).

Fine-scale recombination rates along the genome

Patterns of recombination rates across the genome were strikingly different between males and females, in agreement with previous studies (42,43). Male recombination rates were

144 highly elevated in the sub-telomeric regions (e.g. up to ~10Mb from telomeres) and almost
145 non-existent in the rest of the genome (Fig 2). Conversely, female recombination rates were
146 elevated in peri-centromeric regions and reduced in telomeric regions (Fig 2). In immediate
147 proximity to the centromere, both male and female recombination rates are close to zero in
148 most chromosomes, indicating that the centromere itself has a suppressive effect on
149 recombination (Fig 2).
150



151
152
153 **Fig 2. Fine-scale sex-specific recombination rate along the 29 Atlantic salmon**
154 **chromosomes.**

The recombination rate in cM/Mb within each 1Mb bin for males in blue and females in red. The dashed vertical lines in yellow are the centromere positions from the reference genome Ssal_v3.1 (GenBank accession GCA_905237065.1).

Individual recombination rates

The total numbers of observations (i.e. individual gametes) were 287,063 from 1,580 unique females, and 287,111 from 889 unique males. Crossover count (CC) was approximately normally-distributed in both males and females (Fig 3), with a mean of 19.6 (± 3.7 SD) in females and 12.1 (± 2.9 SD) in males (Table 2). Intra-chromosomal genetic shuffling ($\bar{r}$) was normally distributed in males and females (Fig 4), with a mean of 8.06×10^{-3} (± 1.87×10^{-3} SD) in females and 1.01×10^{-3} (± 0.56×10^{-3} SD) in males, representing 8-fold higher levels of shuffling in females (Table 2).

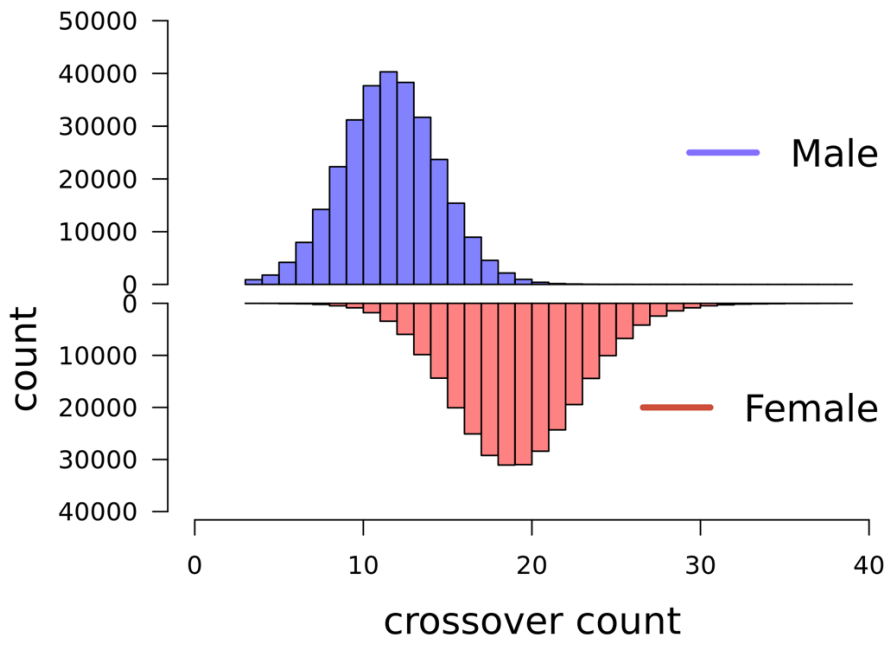


Fig 3. Distribution of crossover count (CC).
for paternal gametes in blue (top) and maternal gametes in red (bottom).

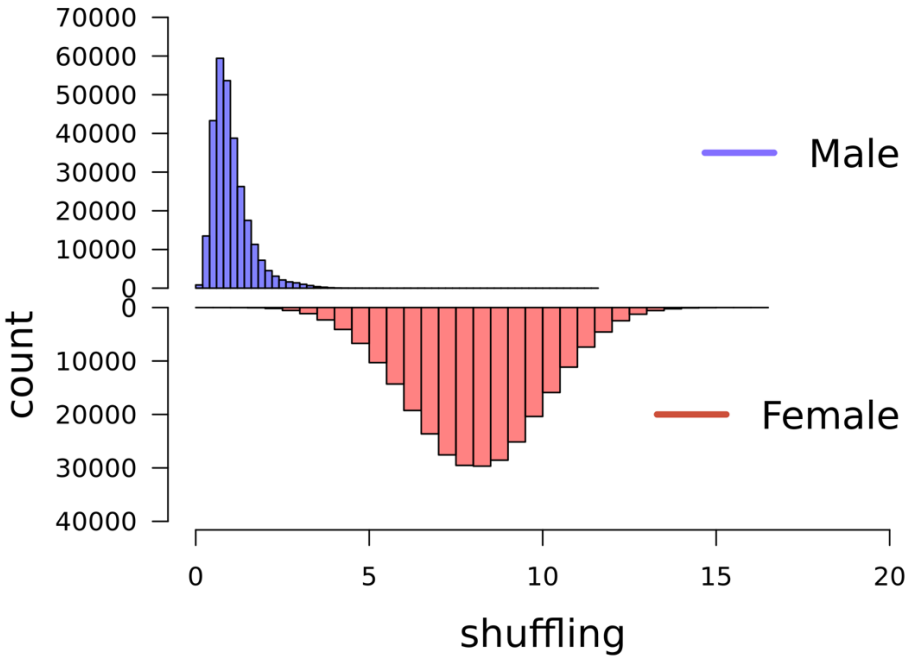


Fig 4. Distribution of intrachromosomal genetic shuffling $\bar{r}$.
Maternal gametes in red and paternal gametes in blue.

Genetic variation in measures of recombination

The heritability (h^2) for CC was 0.11 (SE = 0.01) in females and 0.10 (SE = 0.02) in males. Genetic shuffling ($\bar{r}$) was also significantly heritable in both sex, but lower in females (h^2 = 0.06, SE = 0.01) than in males (h^2 = 0.11, SE = 0.03). Results from the variance component estimations for both traits is presented in table 2. The genetic correlations between CC and $\bar{r}$ was 0.86 (0.01) in females, but only 0.42 (0.05) in males. The corresponding phenotypic correlations were 0.68 (0.00) for females and 0.43 (0.00) for females, i.e. phenotypic correlation between CC and $\bar{r}$ was lower than the genetic correlations for both sex. The genetic correlations between male and female CC and $\bar{r}$ were 0.10 (0.06) and -0.10 (0.06), respectively (Table 3).

Table 2. Results from variance component estimation of CC and $\bar{r}$.

Trait	sex	N_{FIDs}	N_{obs}	mean (sd)	h^2(SE)	V_p	V_e
CC	female	1580	287 063	19.6 (3.7)	0.11 (0.01)	14.2	11.7
CC	male	889	287 111	12.1 (2.9)	0.10 (0.02)	8.9	6.9
$\bar{r}$	female	1580	287 063	8.06 (1.87) e10^{-3}	0.06 (0.01)	3.8 e10^{-3}	3.1 e10^{-3}
$\bar{r}$	male	889	287 111	1.01 (0.56) e10^{-3}	0.11 (0.03)	0.4 e10^{-3}	0.2 e10^{-3}

N_{FIDs} are the total number of FIDs (with repeated observations), N_{obs} is the total number of observations (meiosis) for each sex. Mean is the mean CC or $\bar{r}$ with standard deviations in parenthesis. h^2 is the heritability estimate with standard errors in parenthesis. Effect f is the estimated effect of inbreeding (f) for each trait and sex.

Table 3. Genetic and phenotypic correlations.

	Male shuffling	Female shuffling	Male CC	Female CC
Male shuffling		-	0.426 (0.003)	-
Female shuffling	-0.104 (0.060)		-	0.679 (0.002)
Male CC	0.646 (0.022)	-		-
Female CC	-	0.892 (0.008)	0.102 (0.060)	

In the lower triangle are genetic correlations between males and females for both traits with standard errors in parenthesis. Phenotypic Pearson's correlations between the two traits for each sex is in the upper triangle.

Genome wide association studies.

Genome wide association studies did not identify any significant quantitative trait loci for either crossover count or shuffling in males or females. In the GWA analysis for female CC and shuffling, there were one and three markers, respectively, that reached the significance threshold, but they were not supported by other markers in the same region and there were no clear candidate genes close to any of these markers (Fig 5 and 6). The top ten SNP markers for each GWAS are reported in Supplementary Table 1.

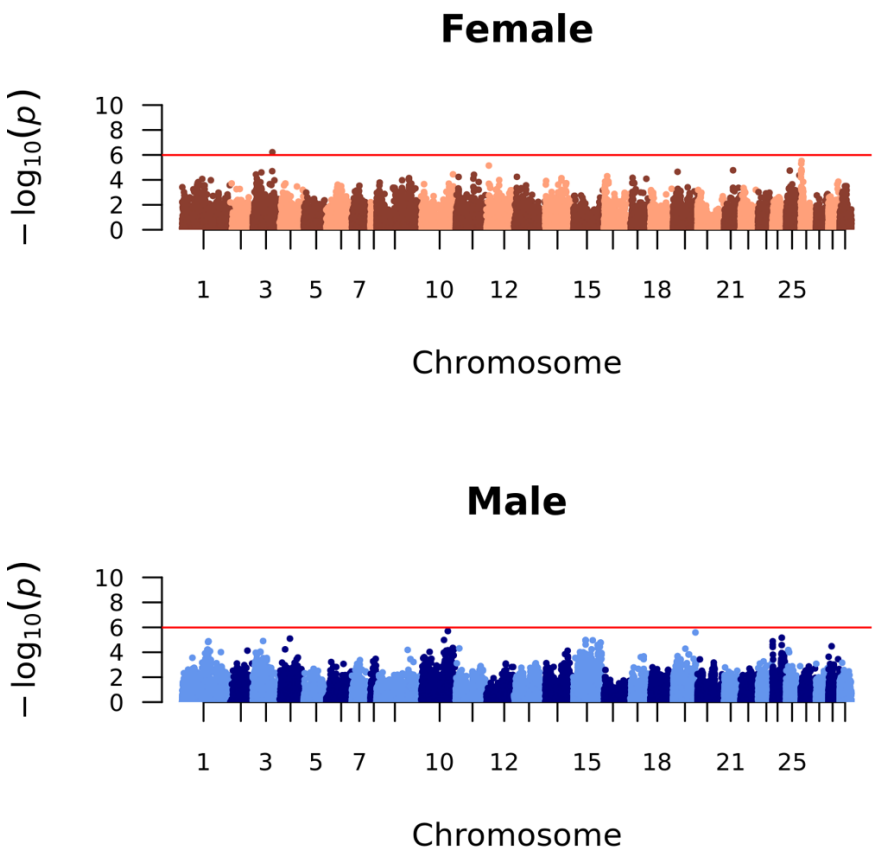


Fig 5. Manhattan plots of genome-wide association between markers and crossover count (CC). The red line is the genome-wide significance threshold using a Bonferroni correction. The Y axis is the negative logarithm of the p-values, and the x-axis is the physical positions of the markers with alternating colours for chromosomes 1-29 for males (A) and females (B).

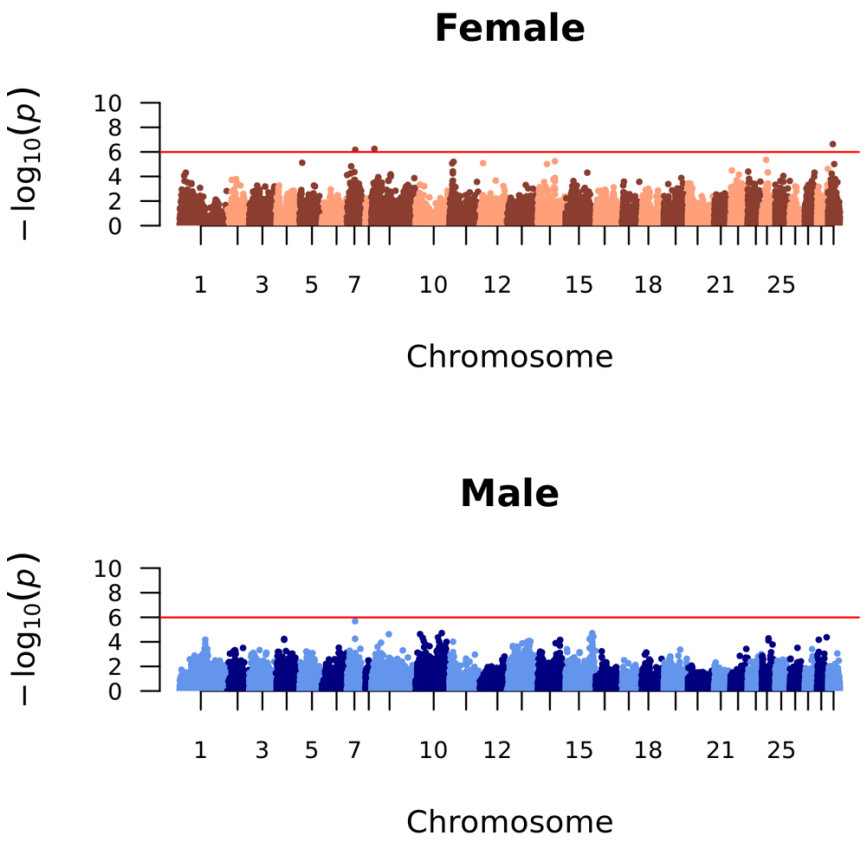


Fig 6. Manhattan plots of genome-wide association between markers and intrachromosomal shuffling ($\bar{r}$).
The red line is the genome-wide significance threshold = 0.05/Number of markers per analysis. The Y axis is the negative logarithm of the p-values, and the x-axis is the physical positions of the markers with alternating colours for chromosomes 1-29 for males (A) and females (B).

Discussion

In this study, we confirm substantial sex-differences in genome-wide recombination rates and landscapes in Atlantic salmon, as previously reported by Lien et al (42) and Gonen et al. (43). Our study is the first to investigate this variation at the individual level, both in terms of crossover count (CC) and the degree to which alleles are shuffled on chromosomes to create new haplotypes ($\bar{r}$). We showed that CC is heritable in both males and females, but with a

244 low inter-sex genetic correlation, suggesting that the genetic architecture of CC is sex
245 specific. Similarly, $\bar{r}$ is heritable in females, but not in males. Our findings show that whilst
246 females have ~1.6 times more crossovers than males, they have eight times more genetic
247 shuffling of alleles from one generation to the next, due to the extreme sex differences in
248 crossover locations. We do not find any quantitative trait loci for either trait or sex,
249 suggesting that the genetic variation in the two measures of recombination is polygenic. Here,
250 we explore the results in more detail and discuss how the findings may be relevant in the
251 breeding work on Atlantic Salmon, as well as how they contribute to the understanding of
252 variation in rates and patterns of recombination in general.
253
254 **Extreme sex-differences in crossover landscapes.**
255 The sex-specific linkage map showed a female-biased heterochiasmy in salmon, but more
256 striking was the large differences in the distribution of crossovers along the chromosomes.
257 Male crossovers occured strictly in the telomeric regions, while female crossovers tend to
258 occur closer to the centromeres, leaving regions in the middle of the acrocentric
259 chromosomes (ssa8 to ssa29) with very low rates in both males and females (Fig 2). The total
260 length in cM of the male and female linkage maps was shorter than previously published
261 maps (42,43). This may be due to more accurate inference of physical marker positions
262 relative to the assembled genome, rather than *de novo* inference of marker loci based on
263 genetic linkage; incorrectly placed markers can often lead to inflation in map lengths. Also,
264 the number of families and individuals used to create the linkage maps in this study is much
265 higher than in previous published maps, with our full-sib family structures well suited for
266 linkage mapping. Nevertheless, the relative difference between the sexes was similar to both
267 studies, with females showing ~1.38 and ~ 1.5 times longer maps than males in Lien et al.
268 (2011) and Gonen et al. (2014), respectively.

269

270 On the chromosomes with the largest sex differences in total genetic length (ssa2, ssa8 and
271 ssa17), the male maps had little to no recombination in one or both telomeric regions of the
272 chromosome (Fig 1). These regions coincide with regions reported in the paper by Lien et al
273 (45) to have blocks of > 90% sequence similarity with blocks on other chromosomes.
274 Therefore, a potential explanation for the lack of crossovers between homologs in these
275 regions is that these chromosomes are experiencing delayed rediploidization and are forming
276 quadrivalents during meiosis (45). Cytological studies in different salmonid species find that
277 multivalent pairing happens between the chromosomes with high sequence similarity and that
278 the phenomena occur primarily in males (39,41). The high sequence similarity and tetrasomic
279 inheritance makes these areas difficult to map, and these regions are indeed characterised by
280 low marker density in our dataset. This means that there might be crossovers occurring in
281 these regions that we are unable to pick up. In addition, if there is homeologue pairing in
282 these regions, recombination events occurring between the two homeologue chromosomes
283 may lead to an underestimation of genome-wide recombination rates in male Atlantic salmon;
284 removing these three chromosomes reduces the sex difference in map lengths from 1.47 times
285 higher in females to 1.33 times higher. Mechanistic explanations for the extreme difference in
286 crossover distribution in males and females remain unresolved. One compelling avenue for
287 further study in Salmonids, is that in other autotetraploid species such as *Arabidopsis*
288 *arenosa*, recombination rates are reduced, and crossover interference appears to increase (46–
289 48). Therefore, increased homeologue pairing in males may benefit from reduced
290 recombination combined with telomeric crossing-over in order to mitigate issues arising from
291 quadrivalent formation and/or mispairing of chromosomes during the crossover process.
292

293 Considering the evolutionary consequences of our findings, male recombination at the very
294 end of the chromosomes leaves the haplotypes almost intact, which may be beneficial in a
295 successful male to preserve their advantageous allelic combinations, particularly if males
296 experience stronger diploid and/or haploid selection (28,31). However, the same could be
297 argued for other species that do not share the same recombination patterns, yet may have
298 stronger differences in selection between the sexes (e.g. with stronger sexual dimorphism
299 and/or differential investment in gametes and offspring; (33,49). However, we cannot rule out
300 similar importance of purely mechanistic suggestions, such as difference in timing of meiosis,
301 chromatin structure and synaptonemal complex length between males and females resulting
302 in sex-specific regions accessible for the recombination machinery at the time of meiosis or
303 differences in the strength of crossover interference (49,50).
304
305 **Genetic variation in individual measures of recombination**
306 To the best of our knowledge, these are the first results on genetic variation for individual
307 measures of recombination in Atlantic salmon. Heritability for CC is moderate, 0.10 in males
308 and 0.11 in females. This is consistent with estimates in other vertebrate species, which range
309 from around 0.05 – 0.18 in pigs (16,51), sheep (12,13), cattle (15,21,52) and red deer (20), to
310 as high as 0.41 and 0.46 in some *Drosophila* strains (17) and mouse lines (10), respectively.
311 The genetic correlation between male and female CC in our study was very low at 0.10
312 (0.06). Despite the relatively large confidence interval, this estimate indicates that different
313 loci affect CC in males and females. Intra-chromosomal shuffling $\bar{r}$ was also significantly
314 heritable in females ($h^2 = 0.06$) and males ($h^2 = 0.11$). Considering the genetic correlation
315 between CC and $\bar{r}$ within each sex, we showed that this correlation was substantially lower in
316 males than in females, 0.43 and 0.68 respectively.
317

318 We did not find any QTLs in either sex for either trait in the genome-wide association
319 analysis, suggesting that these traits are polygenic in Atlantic salmon. This is in contrast to
320 studies of individual recombination rates in mammals, where most studies point to a
321 conserved oligogenic architecture of the trait (13,14,16,22). However, we cannot rule out
322 that our dataset has reduced power to detect loci with a modest effect on recombination.
323 Compared to other studies in livestock (51) and human (27), the number of FIDs in our study
324 are lower, impacting our ability to detect significant trait loci.
325
326 **What are the implications for breeding?**
327 Our results suggest that there is the genetic potential to increase or decrease the amount of
328 allelic shuffling and/or crossover counts within Atlantic salmon, and that this can be achieved
329 independently in males and females, due to low genetic correlations between the sexes.
330 However, the relatively low heritabilities and polygenic nature of these traits suggest that
331 genetic change may be slow because the many small effect loci can be pleiotropic and/or
332 linked with genes affecting other traits prioritised in selection, and that a corresponding
333 phenotypic change will be modest (53). Previous focus on the relevance of recombination for
334 animal breeding has been local or genome-wide rates of crossovers (53–55). In our study, the
335 striking difference between male and female shuffling in Atlantic salmon demonstrates the
336 critical importance of crossover for shuffling and creation of novel haplotypes that may have
337 previously been overlooked. There are considerable differences in the probability of shuffling
338 between linked loci in male and female meiosis, with males transmitting haplotypes to
339 offspring that are relatively unchanged. However, we must also consider that each offspring
340 will always inherit exactly one paternal and one maternal gamete and in the subsequent
341 generations, alleles will segregate independently of which sex they were transmitted from in
342 previous generations, so the population level effect of the conserved haplotypes in male

343 salmon may be limited. Furthermore, little is known about the potential biological and fitness
344 consequences of altering rates or distribution of crossovers. The mechanisms leading to the
345 observed variation in shuffling are likely associated with the mechanisms that control
346 crossover location and rate. For example, if low rates of crossovers in both males and females
347 in chromosome centres is associated with chromosome fusions following the whole genome
348 duplication, attempting to select for crossover location closer to chromosome centres may
349 cause reduction in fertility (56). Similarly, despite some evidence for a potential to increase
350 genetic gain with higher recombination rates (53), selecting for higher recombination rates is
351 not likely to be beneficial or even possible with crossover interference. The negative
352 consequences of extensive recombination rates remain to be understood, although higher
353 mutagenic load is likely (14), combined with the fact that there seems to be an upper limit for
354 number of crossovers per chromosome shared among species across a broad selection of taxa
355 (8),
356
357 **Conclusions and future directions.**
358 In conclusion, this study shows that there is genetic variation in genome-wide rates and
359 distribution of recombination in Atlantic salmon. Consistent with previous studies, Atlantic
360 salmon show extreme levels of heterochiasmy, especially in the distribution of crossover
361 events along the chromosomes. We show that the genetic correlation between male and
362 female rates and distribution of crossovers is very low, suggesting that they behave and may
363 be altered as different traits in males and females. Future studies should aim to include the
364 multivalent pairing in male meiosis to get the full picture of male crossover rates and
365 distribution. Overall, these findings provide a basis to better understand the causes and
366 consequences of recombination rate variation in general, and the genetic architecture of the
367 trait in Atlantic salmon specifically.

Methods

Atlantic salmon genetic dataset.

Genotypes from a total of 375,381 individuals were available from the Norwegian AquaGen Atlantic Salmon (*Salmo salar*) breeding population for this study. The breeding work in this population started in 1970 and it has founders from 41 Norwegian rivers. The individuals in this dataset were born between year 2008 and 2020. Individuals were genotyped on two different customized SNP arrays, Affymetrix custom 49K and Affymetrix custom 70K. Genotype calls were generated using the Thermo Fisher Best Practices Genotyping Analysis Workflow. Markers in the categories PolyHighResolution and NoMinorHom were kept for further analysis, i.e SNPs with well-seperated genotype clusters and two or more alleles in the genotype calls, and where one cluster is homozygous, and one is heterozygous for biallelic SNPs and only one homozygous cluster and one or more heterozygous cluster appear for multiallelic SNPs (57). Finally, only markers common to both SNP arrays were kept for further analysis. resulting in a set of 35,033 markers and a total genotyping call rate of 0.997 in the final dataset. The physical positions of the SNPs were determined based on the Atlantic salmon reference genome assembly Ssal_v3.1 (GenBank accession GCA_905237065.1). This data set is referred to hereafter as the 35K dataset. All focal individuals (see section three generation full-sib families below) were also imputed to the 49.087 SNP markers on the customized Affymetrix 49K chip; this dataset was used for the genome wide association analysis only.

Three generation full-sib families.

The pedigree was ordered into full-sib families as follows: for each unique sire – dam mating pair, hereafter referred to as the focal individuals or FIDs, we constructed a three-generation

family that included their offspring and potentially genotyped parents (see Fig 7 for illustration of the family structure). This enables phasing of the gametes transmitted from the FIDs to the offspring, in turn identifying the crossover positions that occurred during meiosis in the FIDs. Therefore, recombination phenotypes (i.e. crossover count and intra-chromosomal shuffling) are assigned to the FIDs. An FID can be in several families if the individual is mated with several individuals in the pedigree, or as an offspring or grandparent, but our study design means that each meiosis was only counted once. The 35K set had a total of 5568 unique full-sib families with number of offspring ranging from 1 to 537. Because the number of full-sibs were high in most of the families, genotyped grandparents were not vital for proper phasing of the offspring gametes and therefore not set as a strict criteria for inclusion.

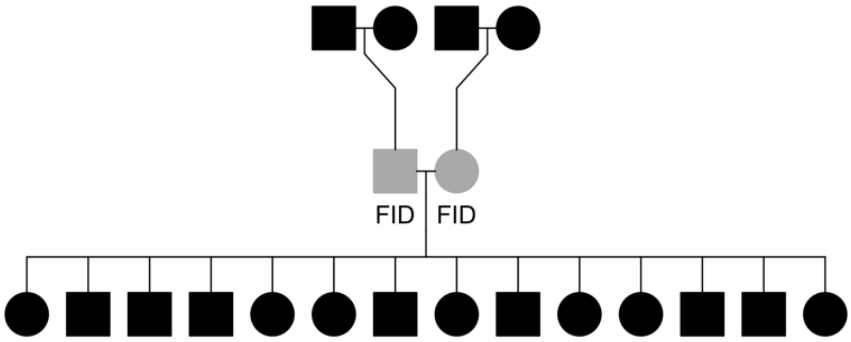


Fig 7. Illustration of the full sib family structures.
This structure is used to phase gametes that are transmitted from focal individuals (FIDs, in grey) to their offspring. In cases where parents in the FIDs are known, they are included to improve phasing accuracy.

415 **Linkage mapping.**

416 Linkage mapping was conducted in LepMap3 (58) assuming marker orders were the same as
417 on the Atlantic salmon genome and that each chromosome constituted a linkage group. The
418 *filtering2* module was run as suggested for multi-family datasets with a *datatolerance* = 0.01
419 to filter markers based on segregation distortion. The *separatechromosomes2* module was run
420 within linkage group and markers that were not assigned to the main group (LOD score < 5)
421 were excluded, as suggested in species where chromosome-level assemblies and marker
422 positions are well established. The *ordermarkers2* module was run with the option to evaluate
423 the given marker order, i.e. to calculate the centimorgan (cM) positions using the Haldane
424 mapping function option. A total of 35,032 markers across 29 chromosomes were included in
425 the final linkage map. These data were then used to calculate approximate fine-scale
426 recombination rates across the genome. SNPs were assigned to bins of 1Mb based on their
427 genomic positions, and the cM/Mb rate was measured as the difference in cM divided by the
428 difference in Mb between the first and last SNP marker within each bin. Finally, it should be
429 noted that Atlantic salmon do not have distinct sex chromosomes but have an autosomal sex-
430 determining region on Ssa3 (59).

431

432 **Estimation of individual recombination rates.**

433 Crossover count (CC) was determined for each gamete transmitted from an FID to their
434 offspring from gamete-phased output of the *orderMarkers2* module in LepMap3, and
435 assigned to the FID in which the meiosis took place. Intra-chromosomal genetic shuffling, $\bar{r}$,
436 was calculated as the probability that a randomly chosen pair of loci on the same
437 chromosome was unpaired during gamete production (in meiosis) following the method
438 suggested by Veller et al. (37):

439

$$\mathbb{E}[\bar{r}] = \sum_{k=1}^{n} 2p_k(1 - p_k)\, L_k^2$$

where k is the chromosome number 1-29, p is the proportion of paternally inherited alleles, $1-p$ is the proportion of maternally inherited alleles, and L is the length of the chromosome as a fraction of the total length of the genome. This method determines $\bar{r}$ for each gamete and is assigned as a phenotype of the FID in which meiosis took place.

Genetic variation in measures of recombination.

Variance components for individual CC and $\bar{r}$ were estimated in DMU v6 (60) with a repeatability model using the Restricted Maximum Likelihood (REML) and average information (AI) algorithm. The model was:

$$\mathbf{Y = sex + id1 + id2 + b_2 * het + e}$$

where Y is the response variable (CC or $\bar{r}$), **sex** is the fixed effect of sex, **id1** is the random additive genetic effect of the FID (N= 2,469) with a covariance matrix proportional to the genomic relationship matrix, **id2** is the random effect of the FIDs permanent environment (i.e. environmental effects that are constant across repeated measures on an FID), **het** is the individual inbreeding coefficient (method-of-moments F) calculated with the *--het* function in PLINK v1.9 (61), $\mathbf{b_2}$ is the regression of CC or $\bar{r}$ on **het** of the FID, and **e** is the residual effect. The narrow-sense heritability (h^2) was defined as the proportion of phenotypic variance explained by the additive genetic effect **id1** (i.e. the estimated additive genetic variance divided by the sum of variances estimated for all random effects) and was estimated separately for each sex. Repeatability was measured as the sum of the genetic variance and

464 permanent environment variance divided by the sum of all variances estimated for all random
465 effects.
466
467 **Genome wide association studies (GWAS).**
468 We conducted GWAS for CC and $\bar{r}$ using the 49K SNP dataset using a mixed linear model-
469 based analysis in GCTA version 1.93.2a_beta (62), where the chromosome that the focal SNP
470 is located on is left out of the genomic relationship matrix as implemented in the *--mlma-loco*
471 module. The model was structured as follows:
472
473 $$\bar{y}_n = a + bx + g + e$$
474
475 Where $\bar{\boldsymbol{y}}_{\boldsymbol{n}}$ is the mean CC or $\bar{r}$ for *n* observations on the individual, *a* is the trait mean, *b* is
476 the fixed additive effect of the SNP tested for association and *x* is the SNP genotypes 0, 1 and
477 2 for the homozygote, heterozygote and opposite homozygote respectively, *g* is a vector of
478 random effects assumed to ~N(0,Gσ_a^2) where G is the genomic relatedness matrix calculated
479 with SNP markers on all the autosomes except the chromosome of the SNP currently tested
480 for association and σ_a^2 is the genetic variance, and *e* is the residual term ~N(0,Iσ_e^2). To
481 account for *n* multiple observations per FID, $\bar{y}_n$ was weighted based on the number of
482 observations, and trait and sex-specific heritabilities (h^2) and repeatabilities (t) estimated
483 following the method from Garrick et al., (63). The significance threshold for association was
484 determined using a Bonferroni correction at α = 0.05, determined as P = 0.05/49,087 = 1.02
485 $\times 10^{-6}$.
486
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686
687 **Table S1. Linkage map.** Male and female genetic positions in cM for all SNP markers in the
688 35K dataset. See additional file.
689
690 **Table S2. Top 10 SNPs from GWAS on shuffling and crossover count in both sex**

Trait	Sex	Chr	SNP name	bp	Freq	b	SE	p
CC	Male	10	Affx-87165681	97158781	0.216	0.936	0.197	2.00E-06
		19	Affx-986688163	80841039	0.209	-0.844	0.179	2.58E-06
		24	Affx-517031085	33571077	0.286	0.778	0.173	6.79E-06
		4	Affx-87621822	40639301	0.177	0.890	0.199	8.01E-06
		10	Affx-86913301	83219166	0.176	0.850	0.193	1.04E-05
		15	Affx-87001560	44530526	0.517	0.651	0.148	1.09E-05
		15	Affx-87481996	44534054	0.517	0.651	0.148	1.09E-05
		15	Affx-87658641	44541415	0.483	-0.651	0.148	1.09E-05
		15	Affx-98990447	44543717	0.483	-0.651	0.148	1.09E-05
		15	Affx-517023952	70320171	0.407	-0.656	0.149	1.10E-05
CC	Female	3	Affx-87260986	71736893	0.286	-0.643	0.129	6.00E-07
		26	Affx-517021201	3373590	0.327	-0.567	0.121	3.01E-06
		26	Affx-87324563	2403259	0.221	0.662	0.145	5.06E-06
		12	Affx-93396222	10860972	0.349	-0.538	0.120	7.09E-06
		26	Affx-86915404	2971226	0.450	0.498	0.115	1.52E-05
		21	Affx-86977045	32739741	0.192	-0.626	0.146	1.69E-05
		25	Affx-86916185	14361093	0.181	0.628	0.147	1.81E-05
		26	Affx-87723009	2274470	0.285	0.582	0.136	1.85E-05
		26	Affx-87775881	2569233	0.267	-0.552	0.129	1.91E-05
		3	Affx-87597243	71065500	0.498	-0.496	0.116	2.01E-05
Shuffling	Male	7	Affx-87702070	30082074	0.177	0.253	0.053	2.08E-06
		15	Affx-87438278	97920722	0.201	0.208	0.049	1.94E-05
		10	Affx-87814965	96995476	0.399	0.180	0.042	1.94E-05
		10	Affx-87169893	17900600	0.567	-0.179	0.042	2.34E-05
		9	Affx-87137995	63788953	0.370	0.197	0.047	2.37E-05
		15	Affx-87191200	95892936	0.239	0.194	0.047	3.65E-05
		15	Affx-87285851	101670539	0.192	0.215	0.052	3.70E-05
		15	Affx-87285853	101671231	0.192	0.215	0.052	3.70E-05
		28	Affx-87026689	35657626	0.198	0.218	0.053	4.24E-05
		15	Affx-87320647	97937595	0.315	0.176	0.043	4.27E-05
Shuffling	Female	29	Affx-87275877	17198796	0.200	0.252	0.049	2.32E-07
		9	Affx-87805131	11090861	0.539	-0.198	0.040	5.58E-07
		7	Affx-87197793	30511902	0.196	-0.256	0.051	6.68E-07
		24	Affx-87658020	15513356	0.339	-0.182	0.040	4.46E-06
		14	Affx-87347934	62926154	0.440	-0.175	0.039	5.71E-06
		11	Affx-87566793	14623343	0.219	0.209	0.046	6.33E-06
		5	Affx-87225703	10195400	0.290	0.193	0.043	7.59E-06

12	Affx-87008423	14255690	0.218	0.214	0.048	8.24E-06
11	Affx-86997783	9768960	0.491	0.178	0.040	8.65E-06
14	Affx-87808468	33998757	0.132	-0.242	0.055	9.73E-06

bp is the basepair position of the SNP marker, **Freq** is the allele frequency of the effect allele, **b** is the the effect estimate and **SE** the subsequent standard error of the effect estimate, **p** is the p value.

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Competing interests

TMK is employed in Aquagen. All authors declare that the results are presented in full and as such present no conflict of interest. The other authors declare that they have no competing interests for this study.