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Recombination rate variation in mice from an isolated island

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Abstract

Recombination rate is a heritable trait that varies among individuals. Despite the major impact of recombination rate on patterns of genetic diversity and the efficacy of selection, natural variation in this phenotype remains poorly characterized. We present a comparison of genetic maps, sampling 1,212 meioses, from a unique population of wild house mice (*Mus musculus domesticus*) that recently colonized remote Gough Island. Crosses to a mainland reference strain (WSB/EiJ) reveal pervasive variation in recombination rate among Gough Island mice, including sub-chromosomal intervals spanning up to 28% of the genome. In spite of this high level of polymorphism, the genome-wide recombination rate does not significantly vary. In general, we find that recombination rate varies more when measured in smaller genomic intervals. Using the current standard genetic map of the laboratory mouse to polarize intervals with divergent recombination rates, we infer that the majority of evolutionary change occurred in one of the two tested lines of Gough Island mice. Our results confirm that natural populations harbor a high level of recombination rate polymorphism and highlight the disparities in recombination rate evolution across genomic scales.

Keywords

recombination; recombination rate evolution; linkage map; island evolution

Introduction

Recombination ensures the fidelity of chromosome pairing and segregation during meiosis (Tsubouchi and Roeder 2003; Hassold 2007; Lao et al. 2013). Recombination also shapes evolution by shuffling alleles across genetic backgrounds, increasing the efficacy of selection and muddling a population's genealogical history (Felsenstein 1974; Otto and Lenormand 2002; Griffiths and Marjoram 1996; Schierup and Hein 2000). Despite

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Data Accessibility

The genotypes of mice used in this study are available from the QTL Archive at the Jackson Laboratory, <http://phenome.jax.org/db/q?rtm=projects/projdet&reqprojid=539>. Sequences of the four parental GI mice are available under NCBI Bioproject Accession: PRJNA352398.

recombination's conserved functional roles, its rate varies among individuals, within and between species (True et al. 1996; Graffelman et al. 2007; Coop et al. 2008; Dumont and Payseur 2011a; Smukowski and Noor 2011; Comeron et al. 2012). Recombination rate shows resemblance among relatives (Kong et al. 2004; Coop et al. 2008; Fledel-Alon et al. 2011), maintains strain differences in a common environment (Koehler et al. 2002; Dumont and Payseur 2011b; Baier et al. 2014), and responds to artificial selection (Chinnici 1971; Kidwell and Kidwell 1976; Charlesworth and Charlesworth 1985), revealing a genetic component to individual variation. Multiple loci, including specific genes, that confer recombination rate differences have been identified (Kong et al. 2008; Murdoch et al. 2010; Dumont and Payseur 2011b; Parvanov et al. 2010; Sandor et al. 2012; Ma et al. 2015; Johnston et al. 2016; Hunter et al. 2016).

Patterns of individual variation in recombination rate depend on the genomic scale of measurement. Cytological techniques, which visualize and enumerate crossovers in meiocytes (Anderson et al. 1999; Koehler et al. 2002; Baier et al. 2014), offer direct estimates of the genome-wide recombination rate. At this scale, individual recombination rates are constrained by the requirement that each chromosome (or chromosome arm) has at least one crossover for proper chromosome segregation (Mather 1936; Villena and Sapienza 2001; Fledel-Alon et al. 2009). The connection between recombination rate and karyotype is perhaps most visible in the elevated recombination rates of bird species with genomes that are rich in micro-chromosomes (Burt et al. 2002; Groenen et al. 2009; Backstrom et al. 2010). Beyond this karyotypic pattern, the genome-wide recombination rate shows a phylogenetic signal across mammals (Dumont and Payseur 2008; Segura et al. 2013; Froehlich et al. 2015), suggesting that evolution proceeds at a slow to moderate pace.

Genetic linkage maps reveal heterogeneity in recombination rate on the chromosomal scale. Along a chromosome, recombination is often suppressed near the centromere and elevated in distal regions (Beadle 1932; Mahtani and Willard 1998; Lynn et al. 2004; Talbert and Henikoff 2010). Sex appears to be a major determinant of these stereotypical patterns: elevated recombination near telomeres predominates in males from a variety of species (Broman et al. 1998; Kochakpour and Moens 2008; Giraut et al. 2011; Liu et al. 2014; Ma et al. 2015; Smeds et al. 2016). Several genomic parameters correlate with recombination rate across the genome, including gene density, GC content, nucleotide diversity, repetitive elements, chromosome position, and certain sequence motifs (Begun and Aquadro 1992; Eyre-Walker 1993; Fullerton et al. 2001; Rizzon et al. 2002; Kong et al. 2002; Jensen-Seaman et al. 2004; Winckler et al. 2005; de Massy 2013). A subset of megabase-sized intervals also exhibit significant differences in recombination rate among individuals (Broman et al. 1998; Kong et al. 2002; Dumont et al. 2011; Smukowski and Noor 2011; Comeron et al. 2012).

On the finer scale, recombination rates across the genome can span orders of magnitude. In humans, mice, and yeast, crossovers are concentrated in kilobase-sized regions termed "recombination hotspots" (Gerton et al. 2000; Jeffreys et al. 2001; Paigen et al. 2008). Sperm genotyping demonstrates variation in the intensity and position of recombination hotspots among men (Cullen et al. 2002; Neumann and Jeffreys 2006; Tiemann-Boege et al. 2006; Webb et al. 2008). Comparisons of linkage disequilibrium suggest rapid evolution of

hotspots among human populations (McVean et al. 2004; Evans and Cardon 2005) and other primates (Ptak et al. 2005; Winckler et al. 2005; Brunshwig et al. 2012), but evolutionary stasis among finches (Singhal et al. 2015).

The collection of techniques for quantifying recombination rate currently forms a disjointed picture of how recombination evolves. Estimating recombination rate from the transmission of markers among relatives offers key advantages for characterizing recombination rate evolution. In contrast to other approaches, genetic linkage maps measure current recombination rates across the entire genome and traverse the many genomic scales on which recombination rate varies. High-resolution genetic maps exist for a growing number of species (Kong et al. 2002; Cox et al. 2009; Rockman and Kruglyak 2009; Comeron et al. 2012; Kawakami et al. 2014). Despite this progress, statistical comparisons among genetic maps with the purpose of quantifying recombination rate evolution remain rare (Poissant et al. 2010; Dumont et al. 2011; Comeron et al. 2012; Ross et al. 2015). Genomic scans for specific intervals that show heritable variation in recombination rate among individuals from a single natural population are even less common (but see Comeron et al. 2012).

Recombination has been studied more extensively in laboratory mice (*Mus musculus*) than in any other vertebrate, combining dense genetic maps (Shifman et al. 2006; Cox et al. 2009; Liu et al. 2011) with mechanistic studies (Holloway et al. 2008; Paigen and Petkov 2010; Bolcun-Filas and Schimenti 2012; Cole et al. 2014; Hunter et al. 2015). This work provides a rich context for understanding the evolution of recombination in wild mice. At the same time, distinct histories and environments raise the prospect that recombination experiences contrasting evolutionary dynamics in wild mice and laboratory mice. Although studies of human pedigrees have detected individual differences in recombination rate (Broman et al. 1998; Kong et al. 2002), mice provide additional advantages as a model system. First, genetic and non-genetic contributions to recombination rate can be separated – a challenging task in humans – by raising mice in a common environment. This possibility is important in light of the evidence that recombination rate is shaped by an array of environmental factors (Bomblies et al. 2015). Second, inbred mice can be used to generate large recombinant families, providing statistical power to identify recombination rate differences among strains in sub-chromosomal intervals.

Here we take advantage of an unusual population of wild mice to understand how recombination rate evolves in nature. Gough Island is a remote, South Atlantic island that was colonized by house mice, probably only a few hundred generations ago (Gray et al. 2014). By comparing genetic maps, we find substantial variation in the recombination rate on multiple genomic scales between two lineages of mice from this isolated island. In contrast to most previous examinations of recombination rate variation, we identify specific genomic intervals with heritable differences in rate within a single natural population.

Materials and Methods

Mice, crosses, and genotyping

Intercrosses involved mice derived from Gough Island and the wild-derived inbred strain WSB/EiJ (Jackson Laboratory, Bar Harbor, ME), which is descended from mainland North

America. Gough Island is a remote volcanic island in the South Atlantic Ocean, part of a protected wildlife refuge that has been designated a UNESCO World Heritage Site. Mice from Gough Island (GI) were live-trapped and transported to the Charmany Instructional Facility at the University of Wisconsin – Madison. A breeding colony consisting of 25 mature females and 21 mature males was established. All mice were housed in microisolator cages separated by sex in a temperature-controlled room (68–72°) set to a 12-hour light/dark cycle. Food (Teklad 6% fat mouse/rat diet; Harlan Laboratories, Madison, WI) and water were provided *ad libitum*, corn cobs ground to 1/8th inch were provided for bedding (Waldschmidt and Sons, Madison, WI), and irradiated sunflower seeds (Harlan Laboratories) and nesting material were provided weekly for enrichment. For identification, pups were toe tattooed with sterile lancets and tattoo paste starting at 1 week, while ears were punched at weaning (3–4 weeks) to identify adults. Additional details on the transport, housing, and establishment of the breeding colony of GI mice can be found in Gray et al. (2015).

We began the intercrosses with a full-sib male and female chosen from each of two partially inbred lines in our breeding colony of GI mice, denoted Line A and Line B. These lines were created from four generations of full-sib mating among the laboratory-born offspring of wild GI mice. The four mice, one male and one female from each of Line A and Line B, were each crossed with WSB/EiJ to generate four independent sets of F₁s and four F₂ intercrosses (Figure 1). We generated 497 F₂ mice from the Line A cross and 877 F₂s mice from the Line B cross, for a total of 1374 F₂s. Among these mice, 419 from the Line A cross and 793 from the Line B cross were incorporated into subsequent analyses after removing mice that were not genotyped or contained obvious genotyping errors.

Mice were genotyped on the Mega Mouse Universal Genotyping Array (MegaMUGA; Morgan et al. 2016), an Illumina Infinium array containing 77,808 markers. The vast majority of markers on the array are single nucleotide polymorphisms (SNPs), though a few structural and transgenic markers are also present. Markers cover the autosomes, sex chromosomes, and mitochondria with an average spacing of ~33 kb across the genome. Liver tissue from F₂ individuals, as well as controls, was sent to GeneSeek (NeoGene, Lincoln, Nebraska) for DNA extraction and genotyping. Samples were divided among 16 96-well plates and several steps were taken to identify and control for errors. WSB samples were placed on each plate to account for plate extraction effects, parental GI samples were replicated across plates, and the sample from the first well of each plate was replicated across plates. Markers with high rates of missing data were omitted, as were mice with high rates of missing genotypes. We removed a small number of individuals that showed many Mendelian inconsistencies or mismatched genotypic and phenotypic sex.

Construction and comparison of genetic maps

To construct and compare genetic maps, we selected a subset of 11,833 informative SNPs from our genotype data. These SNPs were invariant among the GI mice we genotyped and displayed the segregation patterns expected for a standard F₂ intercross between inbred lines. Distances between informative markers and the total number of crossovers recorded for each chromosome are summarized in Supplemental Table 2. Mapping distances for each cross were estimated from these SNPs using the Lander-Green hidden Markov model

(Lander and Green 1987) implemented in the R/qtl est.map function (Broman and Sen 2009). We assumed a genotyping error rate of 0.2% and converted recombination fractions to map distances with the Carter-Falconer map function (Carter and Falconer 1951). To determine thresholds for statistical significance in map length comparisons, we permuted line labels across mice and reconstructed genetic maps. We performed 2000 permutations, retaining the numbers of mice labeled as Line A or Line B in each permutation. The map length of a chromosome was determined to be significantly different between GI lines if the observed difference was at least as extreme as 5% of differences for that chromosome among permutations. We used the same approach to test for significant differences in overall map length.

To compare recombination rates among GI lines on a finer scale, we examined map lengths in sliding windows across the genome. Windows spanned 10 Mb, 25 Mb, and 50 Mb with a step size of 250 kb. We calculated a p-value for the observed difference in map length of each window as the proportion of replicates that produced a difference greater than or equal to the observed value. Each window had a respective set of 2000 permutations from which we calculated the p-value. To control for false positives arising from multiple testing in these overlapping windows, we used a false discovery rate approach (Storey and Tibshirani 2002). We calculated a q-value for each window using the full set of p-values across all windows and rejected the null hypothesis of no difference in map length at $q < 0.1$. The distributions of p-values across windows were not independent, but our use of an empirical null should be conservative (Leek and Storey 2011). We combined overlapping windows showing significant differences to generate a final list of divergent intervals. We checked the performance of the multiple testing correction by looking for windows with significantly different map lengths in an additional set of 100 permuted maps. After controlling for false discovery using our larger set of 2000 permutations, each set of 100 permuted maps had an average of less than 10 significant windows at the $q < 0.1$ level. This is the number of significant windows we should expect in the absence of true differences in recombination rate. Finally, it is possible that the difference in sample size between the two GI line crosses could contribute to observed variation in recombination rate. To account for this effect, we repeated the search for significantly different windows with an equivalent number of mice from the two lines. Specifically, we randomly selected a subset of 419 F2 individuals from Line B to match the number in Line A, constructed maps from this subset, and created 1,000 sets of permuted maps from this subset to calculate p-values.

To provide perspective for the differences in recombination rate between GI lines, we conducted additional comparisons with the mouse reference genetic map (downloaded from <http://cgd.jax.org/mousemapconverter/>). The mouse reference genetic map features data from a heterogeneous stock (HS) mouse population derived from crosses involving eight classically inbred strains (Shifman et al. 2006; Cox et al. 2009). The HS map, which sampled 3,546 meioses, was constructed from genotypes at 10,195 SNPs. For comparison to GI maps, we converted the HS map positions from the Kosambi map function to the Carter-Falconer map function, and anchored the proximal markers of the GI maps using interpolated recombination rates, following the practice in Cox et al. (2009). We re-estimated recombination rates for the HS map in sliding windows using the approach as

described above. We computed correlations in recombination rate by comparing the same windows in the HS and GI maps.

Genomic features, sequencing, and motif search

To identify potential determinants of recombination rate variation, we calculated correlations between genomic features known to be associated with recombination and differences in recombination rate among GI lines. Positions of genomic features were downloaded from the UCSC annotation database for NCBI Build 37/mm9. For chromatin state, annotations by ChromHMM (Ernst and Kellis 2012) on mouse testis – the only annotated germline tissue – were downloaded from the Mouse Encode Consortium (Mouse ENCODE Consortium 2012; Yue et al. 2014). To evaluate the influence of evolutionary breakpoint regions (Farré et al. 2013; Ullastres et al. 2014), we identified 364 such regions in a synteny analysis between the mouse and human reference genomes with *Cassis* (Lemaitre et al 2008; Baudet et al. 2010).

To minimize autocorrelation, we estimated recombination rate in non-overlapping windows for these analyses, again sized at 10 Mb, 25 Mb, and 50 Mb. We also compared recombination rate differences among GI lines to rates estimated in corresponding non-overlapping windows for the HS map.

The PRDM9 protein is a major determinant of crossover position in the mouse genome (Baudat et al. 2010; Parvanov et al. 2010; Grey et al. 2011). We used new genome sequences to test the prediction that windows with divergent recombination rates are enriched for polymorphic binding sites for PRDM9. We sequenced the genomes of the four GI parents of our intercrosses at an average 10X coverage using 100 bp paired-end reads on an Illumina HiSeq 2500. Reads were mapped to the C57BL/6 mouse reference genome using the Burrows-Wheeler Aligner (Li and Durbin 2009) and SNPs were called with SAMtools 1.0 (Li et al. 2009). Using these sequences, we identified fixed differences between GI Lines A and B and constructed a 50 bp window centered on each fixed difference. We used this set of windows to query against the consensus PRDM9 binding motif using its position weight matrix (Brick et al. 2012) and FIMO from the MEME Suite of bioinformatics tools (Grant et al. 2011). Additionally, we performed a signed test in intervals with divergent recombination rates to determine whether the number of variable motifs correlated with the direction of divergence. Finally, we used PCR primers fl1500U20, mZPrdm9-R1, Meis284L23, and mZPrdm9-F1 (Buard et al. 2014) to amplify the last exon of PRDM9 in the GI lines. We ran the PCR products on 1% agarose gels to determine whether their sizes matched alleles known to confer recombination rate differences within *M. m. domesticus* (Buard et al. 2014).

Results

We documented recombination rate variation between two lines of Gough Island (GI) mice (Lines A and B) by comparing genetic maps built from two sets of F₂ intercrosses with a common reference strain (WSB/EiJ). The genome-wide recombination rate, represented by total map length, was similar, at 1347 cM (Line A) and 1340 cM (Line B). Despite similarity in the genome-wide rate, five chromosomes (2, 5, 8, 9, and 18) showed statistically significant differences in map length (Table 1). Notably, chromosome 8 was 16% longer in

Line B. This disparity among five chromosomes represents a significant departure from the expectation that no difference exists between the two GI Lines ($P < 0.002$; Fisher's combined p -value test).

To measure variation in recombination rate on a sub-chromosomal scale, we tiled the genome with overlapping windows of fixed physical size and compared map lengths across pairs of corresponding windows for the two lines. We repeated the analysis with three window sizes – 50 Mb, 25 Mb, and 10 Mb – to capture differences in recombination rate on multiple scales. We found 500 (50 Mb windows), 483 (25 Mb), and 165 (10 Mb) windows with significant recombination rate differences. These numbers exceed null expectations generated by analyses of permuted datasets (see Methods). On all three scales, windows that exhibited significantly different map lengths were typically adjacent to other significant windows (Figure 2). We combined significant windows that overlapped into discrete intervals for further analysis. Fourteen chromosomes harbored intervals with divergent recombination rate on at least one scale (Table 2); these intervals often displayed divergence across multiple window sizes (Figure 2). It is notable that no divergent intervals were identified on the X chromosome. Similar comparisons of recombination rate made between strains from different subspecies (*Mus musculus castaneus* and *Mus musculus musculus*) identified a disproportionately large number of divergent intervals on the X chromosome (Dumont et al. 2011).

The magnitude of variation in recombination rate was inversely correlated with interval size ($p < 5 \times 10^{-5}$). The greatest differences in recombination rate, as percentages of the mean (109% on chromosome 1) and as absolute differences (0.61 vs 0.96 cM/Mb on chromosome 18), were visible in the 10 Mb window analysis. In many cases, intervals discovered while scanning with smaller windows localized differences found in scans with larger windows. For example, among the differences in map length found on chromosome 15, which were identified at all three scales, the majority localized to a single 12.5 Mb interval.

Patterns of variation in recombination rate from these sub-chromosomal intervals generally matched patterns at the chromosome and genome level. Chromosomes with significantly different map lengths (Table 1) contained intervals with consistent differences. Total differences in map length, as a sum of the divergence in all intervals, were not statistically significant on any window size – mirroring the absence of a significant difference in the whole genome recombination rate. These observations suggest that coarse variation in recombination rate is due to the clustering of differences at finer scales. Chromosomes 8 and 9 showed a contrasting pattern: most of the differences in recombination rate on these chromosomes were only significant when surveyed at the 50 Mb or whole chromosome level. However, differences in statistical power are a challenge when comparing rates across chromosomes. Longer chromosomes have many fewer crossovers per Mb, reducing our ability to detect differences at finer scales.

Comparison to the standard mouse genetic map

Variation in the genetic maps described thus far represents an estimate of recombination rate polymorphism between two GI mice. To place this variation in a broader perspective, we compared the two genetic maps from crossing GI mice to WSB/EiJ with the standard

genetic map of the laboratory mouse (the HS map). On the genome-wide level, the GI maps, at 1388 cM (averaged and converted to match the HS analysis), were shorter than the 1457 cM of the HS map. Local recombination rates in both GI lines were highly correlated with those in the HS (Table 3). Overall, the GI maps showed higher correlation with each other than with the HS map, with the degree of correlation decreasing as resolution increased.

We further investigated the relationship between the GI maps and HS map by focusing on windows for which recombination was significantly divergent between GI lines. Following the pattern observed in the comparison between GI lines, rate differences between the GI and HS maps were more pronounced in smaller windows (Table 2). By assuming that the two GI lines are more closely related to each other than either is to the collection of strains that comprise the HS (*i.e.* HS is the outgroup), we could polarize the changes in recombination rate in the GI lines. We observed a consistent bias: most GI-divergent intervals showed greater rate differences between Line A and the HS than between Line B and the HS (paired t-test, intervals from windows 50 Mb: $p = 0.093$, 25 Mb: $p = 0.004$, 10 Mb: $p = 0.037$) (Table 2). This bias was also seen in the collection of significantly different sliding windows, for which Line A rates showed substantially greater dispersion from HS rates (Levene's test, $p < 1 \times 10^{-15}$ for all three window sizes). Figure 3 illustrates this for 25 Mb windows; the greater squared residual deviation of rates from Line A relative to HS can be seen in the third panel. We repeated this analysis using a random subset of individuals from Line B, matching the number of individuals in Line A to account for the possibility that the smaller sample size in Line A was responsible for its higher divergence from HS. This power-matched comparison detected 71% as many significant windows as did the analysis with the full sample, but these significant windows continued to show higher dispersion from HS in Line A vs. Line B (Levene's test, $p < 5 \times 10^{-5}$ for all three window sizes). This pattern suggests that evolution in Line A was disproportionately responsible for differences between GI lines. For example, the 109% difference between GI lines in a region of chromosome 1 mostly reflects an evolutionary reduction in recombination rate in Line A.

Correlates of recombination rate polymorphism

Within-genome variation in recombination rate has been associated with a variety of genomic characteristics, including GC content, classes of repetitive elements, chromatin structure, gene density, evolutionary breakpoint regions, and distance from the centromere (Fullerton et al. 2001; Rizzon et al. 2002; Jensen-Seaman et al. 2004; Freudenberg et al. 2009; Paigen et al. 2008; Paape et al. 2012; Farré et al. 2013; Kawakami et al. 2014; Liu et al. 2014). We examined whether these genomic features were also associated with recombination rate differences among individuals, *i.e.* between the two lines of GI mice. We estimated recombination rates in non-overlapping windows of 10 Mb, 25 Mb, and 50 Mb in the two GI lines and calculated the difference ($\text{Rate}_{\text{LineA}} - \text{Rate}_{\text{LineB}}$) between corresponding windows. Among the genomic features we evaluated, only the number of long terminal repeats (LTRs) was significantly correlated ($p < 0.05$) with the difference in recombination rate among GI lines at all three scales (Table 4). At the intermediate 25 Mb scale, we also detected a significant correlation between gene density and recombination rate difference. Structural rearrangements are one of the genomic features highly correlated with recombination rate variation that is absent from this analysis. The difficulty of detecting

small rearrangements means that we cannot rule out the role they may be playing in recombination rate polymorphism among GI lines.

To determine whether regions of high or low recombination are enriched for recombination rate divergence, we computed correlations between the relative recombination rate differences among GI lines, $| \text{Rate}_{\text{LineA}} - \text{Rate}_{\text{LineB}} | / \text{Mean Rate}$, and recombination rate in HS mice (in non-overlapping windows). We observed a significant negative correlation ($p < 0.05$) at the 10 Mb and 50 Mb scale (Table 4), corresponding to higher relative variation in recombination rates in regions that experience less recombination.

PRDM9 and motifs

A portion of the kilobase-scale variation in recombination rate observed in mice is explained by specific sequence motifs (Winckler et al. 2005; Myers et al. 2008; Steiner et al. 2009). One of the best-studied motifs is the 13-bp consensus sequence bound by PRDM9, a H3K4 trimethyltransferase with a zinc finger DNA-binding domain (Hayashi et al. 2005) that regulates the location and intensity of recombination hotspots (Myers et al. 2010; Baudat et al. 2010; Parvanov et al. 2010). Variation in recombination at this fine scale depends on both allelic variation in PRDM9 – found in different subspecies and strains of mice (Baudat et al. 2010; Kono et al. 2014; Smagulova et al. 2016) – and polymorphisms in or near DNA binding sites (Baker et al. 2015). We investigated whether these determinants of hotspot variation relate to recombination rate variation between GI lines. First, we found that the two GI lines do not harbor allelic differences in PRDM9 that have been previously associated with variation in recombination rate. Next, we tested another straightforward hypothesis: the difference in the number of predicted PRDM9 binding sites among GI lines is correlated with the difference in recombination rate across genomic intervals; Stevison et al. 2016 tested a similar hypothesis across different species. Using whole-genome sequences from the parents of both crosses, we counted predicted PRDM9 binding sites in intervals of divergent recombination, and compared these values between the GI lines (see Methods). We found no evidence for an association of PRDM9 binding site number with either the direction or intensity of variation in recombination rate (Supplemental Table 1). While we lack the resolution to detect among-line differences in recombination rate at the kb scale, our results argue against an important contribution of PRDM9 to differences at the 10 Mb scale.

Discussion

Our results reveal substantial polymorphism in the recombination rate within an isolated island population. Because the mice for this study were raised in a common environment, we interpret this polymorphism as being caused by genetic variation. Up to 28% of the genome is included within sub-chromosomal intervals that show divergent rates among Gough Island mice, and five chromosomes exhibit different map lengths. In contrast, the total genome-wide rate does not significantly vary. Two hypotheses could explain this disparity among genomic scales. First, stabilizing selection could maintain a genome-wide recombination rate even as variation accumulates at the sub-chromosomal level. This type of selection would mirror the molecular homeostasis that buffers against perturbations to the total crossover number in individual cells (Martini et al. 2006; Rosu et al. 2011; Cole et al. 2012;

Lao et al. 2013). The meiotic requirement of at least one crossover per chromosome (or per chromosome arm) places a lower limit on the genome-wide recombination rate. However, our discovery of polymorphism in the chromosomal recombination rate in the absence of significant differences for total number of crossovers suggests that factors beyond proper disjunction constrain the evolution of the genome-wide rate. Indeed, experiments promoting an excess number of crossovers, with mutants that disable crossover-suppressing proteins, indicate a controlled upper bound that is independent of a biochemical maximum (Youds et al. 2010; De Muys et al. 2012; Crismani et al. 2012). Other evidence for direct or indirect selection on overall crossover number includes the observation that mothers with higher genome-wide recombination rates are more fecund (Kong et al. 2004; Stefansson et al. 2005; Fledel-Alon et al. 2009). Perhaps recombination rate at the sub-chromosomal level has little or no effect on fitness, thereby allowing polymorphism to accumulate. An alternative explanation for the disparity in variation among genomic scales is that selective constraints on recombination rate do not operate on the genome-wide rate. If the lower limit, set by meiotic requirements, is truly dictated by karyotype, the focus of selection should fall on recombination rates at finer (chromosomal) scales. Under this scenario, evolutionary changes to recombination rate on finer scales since the common ancestor of the two GI lines have incidentally balanced such that the overall rate remains unchanged. In this model, the genome-wide rate between these lines would presumably begin to diverge after more time passes.

We discovered that on the sub-chromosomal scale, recombination rate differences between GI lines and HS mice are asymmetric, with Line A showing more divergence. Perhaps this bias indicates a closer phylogenetic relationship between Line B and the HS mice. This seems unlikely given the history of the HS as an amalgam of classical inbred strains and the geographically singular origin of the GI mice. Alternatively, this bias might reflect the random sampling of recombination polymorphism from the GI population. Perhaps a small number of mutations modifying recombination rate are segregating in the population. Further genetic studies will be required to test this hypothesis.

Our identification of genomic correlates of recombination rate polymorphism provides additional clues into the dynamics of recombination rate evolution. Relative differences in recombination rate between GI lines were negatively correlated with the rate in the HS mice. This suggests that recombinationally cold regions are more likely to experience greater relative changes in recombination rate. We also found LTR density to be negatively correlated with differences in recombination rate between GI lines, suggesting that the evolution of recombination rate may be slower in these regions. LTR density has been strongly associated with regions of lower recombination in other organisms (Rizzon et al. 2002; Groenen et al. 2009), and LTR retrotransposons have been shown to influence double-strand break formation in meiosis (Sasaki et al. 2013).

A similar study of recombination rate variation in house mice (Dumont et al. 2011) provides an unusual opportunity to compare the evolutionary tempo of recombination rate on two timescales. Dumont et al. (2011) statistically compared genetic maps from representatives of two house mouse subspecies, *M. m. musculus* and *M. m. castaneus*. Like us, these authors constructed genetic maps from F₂ intercrosses to the WSB/EiJ reference strain, using sample

sizes (580 and 554 F₂s in the two crosses) similar to ours. Thirty-one divergent windows were detected, differing on average by 4.5 cM between the *M. m. musculus* and *M. m. castaneus* maps. Divergent windows between the two GI lines are fewer and show smaller differences in recombination rate, consistent with the more recent divergence of these lines compared to *M. m. musculus* and *M. m. castaneus*. Eight of the divergent intervals we identified overlap with genomic windows that significantly differ between strains of *M. m. musculus* and *M. m. castaneus*. This is approximately consistent with the amount of overlap expected by chance given the size of the divergent intervals. However, the absence of divergent intervals on the X chromosome in the GI lines is notable given their disproportionate localization to this chromosome in the intersubspecific comparison. Our experimental design has reduced power to detect differences on the X chromosome, since the X does not recombine in F₁ males, but this is an issue shared with the previous study.

We temper our conclusions about recombination rate evolution by noting a few challenges with our experimental design. First, F₂ genetic maps reveal average recombination rates in F₁ females and F₁ males. In mice, sex is a key determinant of recombination rate on multiple genomic scales (Lynn et al. 2005; Paigen et al. 2008; Cox et al. 2009; Liu et al. 2014), raising the prospect that females and males could differ in patterns of polymorphism. Our study is blind to this axis of variation. Second, although we detected differences among GI lines on the Mb scale, many more meioses would be required to find variation on the scale of recombination hotspots. A parallel issue is our inability to detect minor structural changes in the mouse genome. Some differences in mapping distance between GI lines could reflect inversions and other structural rearrangements segregating within the GI population. Third, the statistical power to detect differences in recombination rate depends on the total number of crossovers observed. Contrasting patterns across chromosomes, like the absence of differences at finer scales on some chromosomes, may be due to these differences in power. Finally, our interpretation of genetic map differences as polymorphism among GI lines assumes that crossing to the common reference strain (WSB/EiJ) had similar effects on recombination for both lines. Although there is no obvious reason to question this assumption, it is important to remember that the recombination we profiled occurred in F₁s and not in the GI lines. Immunocytology suggests that the genome-wide recombination rate is higher in GI mice than in WSB/EiJ (Dumont and Payseur 2011a).

The observation of substantial differences in recombination rate among a few mice drawn from an isolated population suggests that genetic variation in recombination rate is likely to be pervasive among wild house mice. The balance of evolutionary factors that govern recombination rate in nature remains unclear. A coherent model for the evolution of recombination rate will need to integrate and explain patterns of variation found on each genomic scale.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cross Design
Partially inbred lines of Gough Island mice were crossed with the wild-derived inbred strain WSB/EiJ. Four independent F₂ intercrosses were generated from these crosses.

Figure 2. Recombination rate differences between two lines of Gough Island Mice

Differences in recombination rate between two lines of Gough Island mice visualized as a gradient along chromosomes. Intensity of gradient from yellow to purple indicates greater relative recombination rate in Line A and Line B respectively. Plot markers with whiskers show center and range of 10, 25, and 50 Mb windows where recombination rates differ significantly (10% FDR).

Figure 3. Comparisons of recombination rates in Gough Island lines and the heterogeneous stock (HS)

A comparison of recombination rates using 25 Mb sliding windows in (A) the HS mice and Gough Line A, and (B) the HS mice and Gough Line B. Each point represents a single 25 Mb window with respective recombination rates along the x and y-axis. Windows that were significantly different between Gough Island Line A and Line B are highlighted in bold on these plots. (C) Squared difference in recombination rate between each Gough Island line and HS for these significantly different windows, showing substantially more dispersion from HS in Line A.

Table 1

Genetic map lengths for each chromosome in two lines of Gough Island mice.

Chr	Line A (cM)	Line B (cM)	Diff. (cM)	% diff.	p-value
1	94.35	92.01	2.34	2.5%	0.444
2	89.18	96.15	-6.97	-7.5%	0.019 *
3	72.90	73.39	-0.49	-0.7%	0.897
4	76.83	76.40	0.43	0.6%	0.852
5	77.88	84.09	-6.21	-7.7%	0.041 *
6	70.61	69.21	1.40	2.0%	0.627
7	75.89	78.64	-2.75	-3.6%	0.338
8	60.10	70.24	-10.14	-15.6%	0.001 *
9	59.85	65.24	-5.39	-8.6%	0.032 *
10	67.17	67.73	-0.56	-0.8%	0.839
11	81.01	76.22	4.79	6.1%	0.092
12	58.00	57.27	0.73	1.3%	0.744
13	55.21	56.25	-1.04	-1.9%	0.675
14	60.29	57.96	2.33	3.9%	0.340
15	52.18	54.82	-2.64	-4.9%	0.291
16	54.22	52.83	1.39	2.6%	0.509
17	55.12	53.17	1.95	3.6%	0.427
18	56.52	50.59	5.93	11.1%	0.006 *
19	51.99	49.39	2.60	5.1%	0.231
X	70.25	65.64	4.60	6.8%	0.221
Total	1339.55	1347.24	-7.69	-0.6%	0.562

Asterisks highlight chromosomes with significantly different map lengths at the $p < 0.05$ level. P-values are calculated from two-tailed tests using 2000 permutations. Percent difference calculated as $(\text{LengthLineA} - \text{LengthLineB}) / \text{Mean}$.

Table 2

Regions of divergent recombination between Gough Island lines

50 Mb windows									
Physical position (Mb)					% diff. with:				
chr	begin	end	size	Line A rate (cM/Mb)	Line B rate (cM/Mb)	% diff.	HS rate (cM/Mb)	Line A	Line B
1	68.7	156.9	88.25	0.291	0.354	-20%	0.365	-20%	-3%
1	143.7	193.7	50	0.717	0.617	15%	0.692	4%	-11%
2	3.3	56.1	52.75	0.500	0.582	-15%	0.563	-11%	3%
5	3.4	69.9	66.5	0.426	0.535	-23%	0.527	-19%	2%
6	95.4	148.4	53	0.692	0.587	16%	0.638	9%	-8%
7	69.9	134.4	64.5	0.408	0.502	-21%	0.542	-25%	-7%
8	5.1	98.6	93.5	0.370	0.460	-22%	0.493	-25%	-7%
9	27.8	77.8	50	0.480	0.574	-18%	0.583	-18%	-2%
11	31.5	92.3	60.75	0.620	0.527	16%	0.631	-2%	-17%
15	19.1	89.4	70.25	0.430	0.497	-14%	0.523	-18%	-5%
17	39.3	94.6	55.25	0.814	0.711	14%	0.754	8%	-6%
18	22.2	88.5	66.25	0.748	0.638	16%	0.704	6%	-9%
				Total:	Total:	0.8%	Total:	-2.9%	-2.0%

25 Mb windows									
Physical position (Mb)					% diff. with:				
chr	begin	end	size	Line A rate (cM/Mb)	Line B rate (cM/Mb)	% diff.	HS rate (cM/Mb)	Line A	Line B
1	3.9	30.2	26.3	0.603	0.456	28%	0.370	63%	23%
1	81.9	130.4	48.5	0.179	0.291	-48%	0.294	-39%	-1%
1	166.4	191.9	25.5	0.929	0.743	22%	0.940	-1%	-21%
2	5.8	44.6	38.8	0.456	0.607	-28%	0.611	-25%	-1%
2	139.1	171.3	32.3	0.557	0.732	-27%	0.793	-30%	-8%
4	58.5	84.3	25.8	0.432	0.310	33%	0.295	47%	5%
5	3.4	49.2	45.8	0.366	0.543	-39%	0.532	-31%	2%
6	122.2	148.9	26.8	0.892	0.740	19%	0.790	13%	-6%
7	97.9	129.9	32.0	0.294	0.406	-32%	0.503	-42%	-19%
8	5.1	32.4	27.3	0.410	0.537	-27%	0.594	-31%	-10%

25 Mb windows											
Physical position (Mb)				Line A rate (cM/Mb)		Line B rate (cM/Mb)		% diff.		% diff. with:	
chr	begin	end	size	Line A rate (cM/Mb)	Line B rate (cM/Mb)	Line B rate (cM/Mb)	HS rate (cM/Mb)	% diff.	HS rate (cM/Mb)	Line A	Line B
11	29.0	67.3	38.3	0.718	0.599	0.599	0.645	18%	0.645	11%	-7%
14	98.9	124.7	25.8	0.762	0.597	0.597	0.678	24%	0.678	12%	-12%
15	41.1	81.1	40.0	0.408	0.554	0.554	0.556	-30%	0.556	-27%	0%
17	7.8	42.6	34.8	0.251	0.365	0.365	0.429	-37%	0.429	-41%	-15%
17	66.1	94.1	28.0	1.063	0.878	0.878	0.908	19%	0.908	17%	-3%
18	38.2	64.5	26.3	0.708	0.543	0.543	0.661	26%	0.661	7%	-18%
18	54.2	86.5	32.3	1.013	0.818	0.818	0.907	21%	0.907	12%	-10%
					Total:			0.1%	Total:	-1.8%	-1.7%
10 Mb windows											
Physical position (Mb)				Line A rate (cM/Mb)		Line B rate (cM/Mb)		% diff.		% diff. with:	
chr	begin	end	size	Line A rate (cM/Mb)	Line B rate (cM/Mb)	Line B rate (cM/Mb)	HS rate (cM/Mb)	% diff.	HS rate (cM/Mb)	Line A	Line B
1	97.9	123.2	25.3	0.064	0.218	0.218	0.203	-109%	0.203	-68%	7%
2	148.8	159.6	10.8	0.259	0.487	0.487	0.611	-61%	0.611	-58%	-20%
3	102.2	114.2	12.0	0.205	0.386	0.386	0.356	-61%	0.356	-43%	8%
4	73.5	84.3	10.8	0.502	0.272	0.272	0.351	59%	0.351	43%	-23%
5	11.2	30.9	19.8	0.336	0.606	0.606	0.652	-57%	0.652	-48%	-7%
7	97.9	114.9	17.0	0.172	0.277	0.277	0.370	-47%	0.370	-54%	-25%
8	18.9	28.9	10.0	0.203	0.395	0.395	0.588	-64%	0.588	-65%	-33%
11	45.5	60.0	14.5	0.754	0.476	0.476	0.719	45%	0.719	5%	-34%
14	108.4	119.4	11.0	0.686	0.385	0.385	0.568	56%	0.568	21%	-32%
15	48.6	61.1	12.5	0.340	0.584	0.584	0.571	-53%	0.571	-41%	2%
18	53.5	64.2	10.8	0.958	0.605	0.605	0.756	45%	0.756	27%	-20%
					Total:			0.5%	Total:	-1.5%	-1.0%

Recombination rate was estimated in 10 Mb, 25 Mb, and 50 Mb sliding windows across the genome. Windows with significantly divergent recombination between Gough Island lines were identified by permutation. Tables above show significant regions constructed from overlapping windows for each window size and their percent difference from the mean rate. The recombination rate in these genomic regions as measured in the heterogeneous stock (HS) is also shown for comparison. Total percentages are calculated from sums across intervals for a given window size.

Correlation of recombination rates between Gough Island lines and heterogeneous stock (HS)

Table 3

Correlation between	50 Mb		25 Mb		10 Mb	
	p	95% CI	p	95% CI	p	95% CI
Line A and Line B	0.909	0.905 – 0.914	0.913	0.909 – 0.916	0.882	0.877 – 0.886
Line A and HS	0.897	0.893 – 0.901	0.855	0.850 – 0.860	0.764	0.755 – 0.772
Line B and HS	0.925	0.922 – 0.927	0.891	0.887 – 0.895	0.798	0.791 – 0.805

Pearson correlation coefficients calculated for corresponding 10, 25, and 50 Mb sliding windows across the genome with respective 95% confidence intervals. Correlation coefficients were highly significant for all comparisons.

Table 4

Correlation with variation in recombination rate between Gough Island lines

Genomic feature	50 Mb		25 Mb		10 Mb	
	ρ	p-value	ρ	p-value	ρ	p-value
GC content	0.019	0.82	-0.049	0.49	-0.053	0.44
CpG Islands	-0.116	0.53	-0.055	0.63	-0.100	0.14
Gene Density	-0.010	0.90	-0.168	0.02	-0.112	0.10
Chromatin State	-	0.11	-	0.99	-	0.99
LINE	0.046	0.57	0.094	0.18	0.068	0.32
LTR	-0.222	<0.01	-0.172	0.01	-0.141	0.04
Satellite	-0.041	0.62	0.000	0.99	0.000	0.99
Breakpoint Regions	0.014	0.94	-0.046	0.68	-0.096	0.16
Distance to Centromere	0.037	0.64	0.084	0.23	0.032	0.64
Recombination Rate	-0.308	0.03	-0.142	0.15	-0.233	<0.01

Correlation of recombination rate differences between Gough Island lines in 10 Mb, 25 Mb, and 50 Mb non-overlapping windows with selected genomic features. Pearson correlation coefficient and p-value presented for GC content, CpG islands, gene density, long interspersed elements (LINE), long terminal repeats (LTR), and satellite DNA from mouse reference annotations. For chromatin state, the most prevalent state in each window was used as a categorical variable and p-values reported are from an analysis of variance. Evolutionary breakpoint regions were identified by an analysis of synteny between human and mouse reference genomes using Cassis (see Methods). For correlation to recombination rate itself, absolute differences in rate between the Gough lines divided by their mean rate were compared to rates from the HS map.