COVER SHEET

TITLE: Transmission Ratio Distortion in the Hybrid Offspring of Three Mouse Subspecies

AUTHOR'S NAME: Jennifer Margaret Wagner

MAJOR: Genetics / Microbiology

DEPARTMENT: Genetics

MENTOR: Bret Payseur, PhD

DEPARTMENT: Genetics

MENTOR(2): n/a

DEPARTMENT(2): n/a

YEAR: 2010

(The following statement must be included if you want your paper included in the library's electronic repository.)

ABSTRACT

Transmission Ratio Distortion in the Hybrid Offspring of Three Mouse Subspecies

A crucial component of understanding the origin of new species is understanding how reproductive isolation evolves between divergent populations The relative importance of Dobzhansky-Muller incompatibilities as compared to meiotic drivers in the origin of post-zygotic isolation therefore represents an important question in evolutionary biology. This study begins answering that question by identifying regions of significant transmission ratio distortion (TRD) in the F2 progeny of reciprocal intercrosses conducted between three wild-derived inbred mouse (*Mus musculus*) strains: WSB/EiJ, PWD/PhJ, and CAST/EiJ. Analysis of genotype data at 212 autosomal single nucleotide polymorphisms (SNPs) from across the genome revealed eleven significantly distorted regions in a WSB/EiJ X PWD/PhJ reciprocal intercross, of which seven were sex-specific, and eight significantly distorted regions in a WSB/EiJ X CAST/EiJ reciprocal intercross, of which all were sex-specific. Increased levels of TRD observed in the WSB/EiJ X PWD/PhJ reciprocal intercross as compared to the WSB/EiJ X CAST/EiJ reciprocal intercross suggest greater functional divergence of *M. m. musculus* than *M. m. casteneus* from *M. m. domesticus*. Additionally, the observation that the majority of TRD is sex-specific indicates that the sex chromosomes are important in establishing post-zygotic reproductive isolation. Based on the TRD found, as well as the sex ratios observed in each cross, it seems likely that Dobzhansky-Muller incompatibilities play a more important role than meiotic drivers in the origin of post-zygotic isolation in house mice.

Jennifer Wagner / Genetics, Microbiology
Author Name/Major

Jennifer Wagner
Author Signature

05/14/10
Date

Bret Payseur / Genetics
Mentor Name/Department

Bret Payseur
Mentor Signature

Transmission Ratio Distortion in the Hybrid Offspring of Three Mouse Subspecies

Senior Honors Thesis

Jennifer Margaret Wagner

Mentor:
Bret A. Payseur, Ph.D.

Department of Genetics, University of Wisconsin Madison

May 15, 2010

Abstract

A crucial component of understanding the origin of new species is understanding how reproductive isolation evolves between divergent populations The relative importance of Dobzhansky-Muller incompatibilities as compared to meiotic drivers in the origin of post-zygotic isolation therefore represents an important question in evolutionary biology. This study begins answering that question by identifying regions of significant transmission ratio distortion (TRD) in the F2 progeny of reciprocal intercrosses conducted between three wild-derived inbred mouse (*Mus musculus*) strains: WSB/EiJ, PWD/PhJ, and CAST/EiJ. Analysis of genotype data at 212 autosomal single nucleotide polymorphisms (SNPs) from across the genome revealed eleven significantly distorted regions in a WSB/EiJ X PWD/PhJ reciprocal intercross, of which seven were sex-specific, and eight significantly distorted regions in a WSB/EiJ X CAST/EiJ reciprocal intercross, of which all were sex-specific. Increased levels of TRD observed in the WSB/EiJ X PWD/PhJ reciprocal intercross as compared to the WSB/EiJ X CAST/EiJ reciprocal intercross suggest greater functional divergence of *M. m. musculus* than *M. m. casteneus* from *M. m. domesticus*. Additionally, the observation that the majority of TRD is sex-specific indicates that the sex chromosomes are important in establishing post-zygotic reproductive isolation. Based on the TRD found, as well as the sex ratios observed in each cross, it seems likely that Dobzhansky-Muller incompatibilities play a more important role than meiotic drivers in the origin of post-zygotic isolation in house mice.

Introduction

A fundamental question underlying the origin of new species is how reproductive isolation evolves between divergent populations. Reproductive isolation can result from pre-zygotic mechanisms including habitat, temporal, and behavioral isolation, as well as anatomical and gametic incompatibility; however, of particular interest in understanding reproductive isolation, especially in its beginning stages, are post-zygotic mechanisms, such as reduced hybrid viability, reduced hybrid fertility, and hybrid breakdown. Once viewed as inconsistent with Darwin's theory of natural selection due to the idea that natural selection should not permit the evolution of unfit offspring, the existence of hybrid dysfunction, and its link to reproductive isolation, have traditionally been explained by invoking the Dobzhansky-Muller model (Figure 1) (Fitzpatrick, 2008; Willis, 2009). Dobzhansky (1936) and Muller (1940) demonstrated independently that hybrid dysfunction could occur, and subsequently, contribute to speciation, if the dysfunction was a byproduct of selective pressures acting on isolated populations. Under this model, different genetic changes arise and become fixed in populations that have become isolated from one another (Dobzhansky, 1936; Muller 1940). Though these genetic changes provide increased levels of Darwinian fitness (or are selectively neutral) on the genetic background of their respective host populations, the Dobzhansky-Muller model predicts that some subset of the changes occurring in each isolated population are incompatible with changes that have occurred in other populations (Dobzhansky, 1936; Muller 1940; Fitzpatrick, 2008; Willis, 2009). When those incompatible changes are combined in hybrids, the hybrids are dysfunctional (Dobzhansky, 1936; Muller 1940). An alternative explanation of hybrid dysfunction is the theory of meiotic drive, which

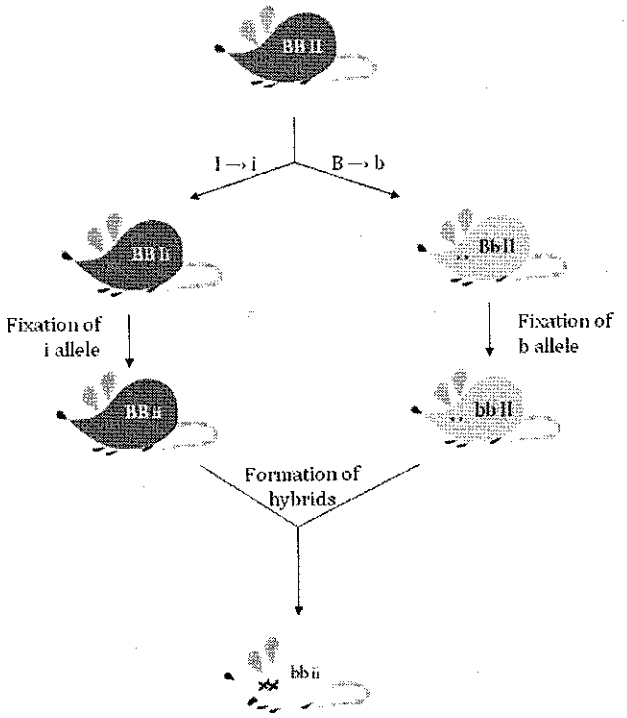


Figure 1. Dobzhansky-Muller model diagram. Dobzhansky (1936) and Muller (1940) independently proposed that genetic changes arising in two isolated populations, which increase the Darwinian fitness of the individuals in those populations, may be incompatible with one another when combined on a hybrid background, and thereby, lead to hybrid dysfunction. The simplest formalization of this model is diagrammed to the left. A black mouse, homozygous at one locus for the B allele, and at another locus for the I allele, splits into two isolated daughter populations. In one population, a mutation at the I locus gives rise to a new allele, i, that gives the mice pink ears. In the other population, a mutation arises at the B locus, giving rise to a new allele, b, that causes gray coat color in the mice. These two new mutations are advantagous in their respective populations, and as a result, eventually go to fixation. However, when the pink-ear and gray populations come back together and mate, their hybrids suffer dysfunction due to the incompatibility of the newly-arisen i and b alleles.

maintains that hybrid incompatibilities can evolve as a byproduct of intragenomic conflict (Willis, 2009). Mendel's law of segregation holds that during each generation in sexually reproducing organisms, each pair of homologous chromosomes segregates during meiosis such that each allele at each locus has an equal probability of transmission from the parent to the offspring (de Villena and Sapienza, 2001). However, if, through mutation, one allele acquires the ability to manipulate the normal Mendelian mechanism of inheritance such that it has a transmission advantage over other alleles at that locus, the allele, called a "driver", could invade a population, even if it negatively impacts an individual's Darwinian fitness (Willis, 2009). Under this model, alleles, called "suppressors", which arise following the origin of the driver allele, and are capable of quelling its negative effects, would have a selective advantage and, like the driver allele before it, would also invade the population (Frank, 1991; Willis, 2009). If continued, this interplay between newly-arising driver and suppressor loci could prevent the

accumulation of the negative impacts arising drivers would have on Darwinian fitness, and ultimately, prevent the species extinction that would eventually ensue if the negative effects of the driver loci were left unchecked (Willis, 2009). If, however, these driver and suppressor loci became decoupled in hybrids of two diverged populations, hybrid dysfunction could result from the unmasking of the drivers' negative effects (Frank, 1991; Willis, 2009).

While the Dobzhansky-Muller model and the theory of meiotic drive provide differing explanations for the mechanisms by which post-zygotic reproductive isolation evolves, both predict that hybrids between divergent populations could show transmission ratio distortion, meaning that Mendel's laws of segregation will be violated (Hall and Willis, 2005). Under the Dobzhansky-Muller model, this distortion will result from the inviability of particular hybrid offspring, who carry incompatible combinations of the genetic changes that arose in their isolated parent populations (Hall and Willis, 2005). In contrast, under the theory of meiotic drive, transmission ratio distortion will occur due to the ability of drivers to manipulate gamete production to their advantage – the mechanism that originally allowed the driver to invade its host population will cause that same driver to be over-represented in the hybrid offspring (Hall and Willis, 2005). As increased levels of genomic divergence between two populations have been observed to correlate with larger numbers of distorted loci in their hybrids, the occurrence of transmission ratio distortion and its association with the genetic divergence of isolated populations has been well established (Hall and Willis, 2005). In addition, through a number of studies on organisms such as Asian rice (*Oryza sativa*), the monkey flower (*Mimulus guttatus*), the fruit fly (*Drosophila melanogaster*), and the house mouse, the

involvement of both Dobzhansky-Muller incompatibilities and meiotic drivers in transmission ratio distortion has been clearly demonstrated (de Villena, *et al.*, 2000; Harushima, *et al.*, 2001; Tao and Hartl, 2003; Fishman and Willis, 2005; Yang, *et al.*, 2004, Phadnis and Orr, 2009). The relative contributions of Dobzhansky-Muller incompatibilities and meiotic drivers to the occurrence of transmission ratio distortion, and, consequently, to reproductive isolation, however, is not well understood. One study of transmission ratio distortion, conducted on a cross between two rice varieties, showed that approximately 45% of the transmission ratio distortion observed across the genome in the F2 progeny was due to meiotic drive, while Dobzhansky-Muller incompatibilities accounted for the other 55% (Harushima, *et al.*, 2001). Other studies examining the relative importance of these two models for how transmission ratio distortion occurs, particularly studies of populations currently undergoing speciation are lacking. As a result of the intimate connection transmission ratio distortion is predicted to have with the processes underlying hybrid dysfunction, and thereby, the origin of species, a key step in beginning to understand speciation will be to examine transmission ratio distortion in the hybrid offspring of those populations that are currently undergoing speciation, and establish the underlying biological mechanisms by which that transmission ratio distortion is occurring.

This study identifies transmission ratio distortion in the hybrid offspring of intercrosses conducted between wild-derived inbred strains of three house mouse (*Mus musculus*) groups: *Mus musculus domesticus*, *Mus musculus musculus*, and *Mus musculus castaneus*. These three groups, which are thought to have originated in a region located in the Middle East called the Fertile Crescent, began diverging from one another

approximately 500,000 years ago (Geraldes, *et al.*, 2008; Silver, 1995). Today, the three groups can be distinguished from one another morphologically and molecularly. In addition, the groups occupy different regions of the world, with *M. m. musculus* inhabiting Eastern Europe and northern Asia, *M. m. castaneus* inhabiting Southeast Asia and Indonesia, and *M. m. domesticus* inhabiting the Americas, Western Europe, Africa, and Australia (Silver, 1995). While these groups are mostly non-overlapping in their ranges, observations of the levels of genetic exchange that exist where they do meet has led to an ongoing debate over whether these groups represent separate species, or whether they are simply subspecies within a single all-encompassing house mouse species (Silver, 1995). Specifically, where *M. m. musculus* and *M. m. domesticus* meet in the center of Europe, selection against their hybrids has effectively prevented the genetic exchange of most regions of the genome between these two groups, and led to a stably-maintained 20 km wide hybrid zone that separates *M. m. musculus* to the east from *M. m. domesticus* to the west (Silver, 1995). In contrast, where *M. m. musculus* and *M. m. castaneus* meet in Japan, the two groups have mixed so completely that, until the advent of molecular DNA analysis methods, their hybrids were considered a separate group of mice (*Mus musculus molossinus*) (Silver, 1995). As evidenced by the question of whether these groups should be considered as belonging to the same or different species, the three groups are currently in the early stages of speciation, making the house mouse an excellent system in which to study transmission ratio distortion, and, in turn, post-zygotic mechanisms of reproductive isolation (Silver, 1995).

In this study, the genomic regions experiencing transmission ratio distortion in the F2 progeny of crosses between the wild-derived inbred strains WSB/EiJ (derived from *M.*

m. domesticus), PWD/PhJ (derived from *M. m. musculus*), and CAST/EiJ (derived from *M. m. castaneus*) are identified. The levels of distortion found in the F2 progeny of a reciprocal intercross between WSB/EiJ and PWD/PhJ are compared to the levels of distortion found in the F2 progeny of a reciprocal intercross between WSB/EiJ and PWD/PhJ in an effort to better understand the speciation history of the three mouse groups. Additionally, to gain insight into the contribution of the sex chromosomes to post-zygotic reproductive isolation, differences in the levels of transmission ratio distortion found in F2 males as compared to F2 females are assessed in each reciprocal intercross. A crucial starting point for understanding the mechanisms that are producing transmission ratio distortion, and consequently, dysfunction in the hybrids of these three mouse subspecies, this study represents a key step toward uncovering the processes governing the development of post-zygotic reproductive isolation and speciation.

Methods

Generation of Mice

F2 mapping populations were generated from WSB/EiJ, PWD/PhJ, and CAST/EiJ mice as part of a larger Quantitative Trait Locus (QTL) mapping study aimed at uncovering the genetic basis of male hybrid sterility. WSB/EiJ mice were reciprocally crossed to both PWD/PhJ mice and CAST/EiJ mice to generate four classes of F1 individuals, which were then crossed to generate four classes of F2 individuals, as shown in Figures 2 and 3. All mice used for this study were housed in the University of Wisconsin Madison Genetics and Biotechnology Animal Facility in accordance with institutional animal care and use regulations. Mice were reared using the standard husbandry procedures of their housing facility. In total, 555 F2 mice were generated from the WSB/EiJ female X PWD/PhJ male intercross, 13 F2 mice were generated from the

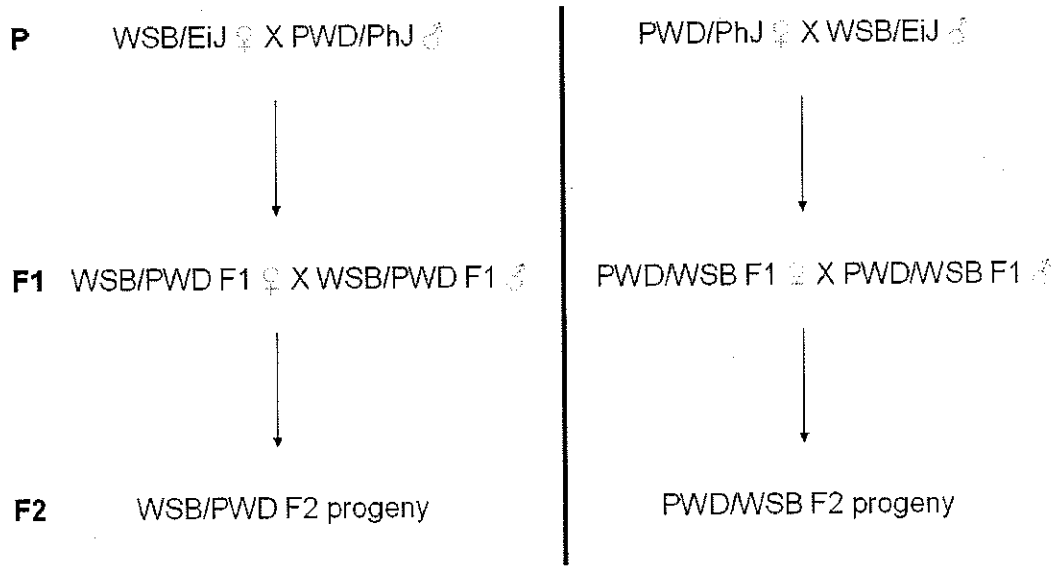


Figure 2. Diagram of the reciprocal intercross conducted between WSB/EiJ and PWD/PhJ mice. WSB/EiJ females were bred with PWD/PhJ males to produce an F1 population that was, in turn, crossed to produce 555 F2 progeny. Separately, PWD/PhJ females were bred with WSB/EiJ males to produce an F1 population that was also, in turn, crossed to produce 13 F2 progeny.

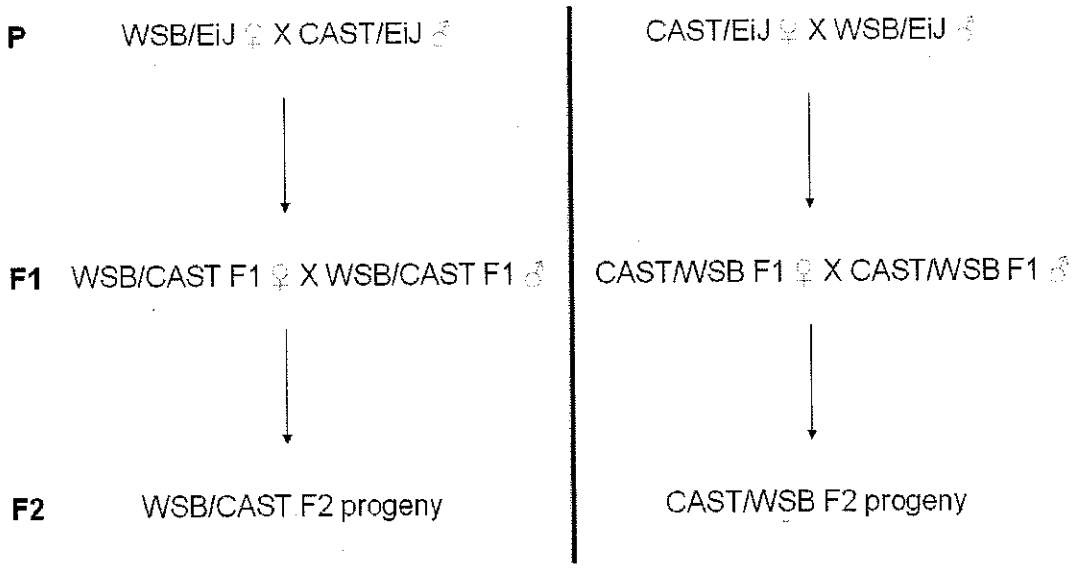


Figure 2. Diagram of the reciprocal intercross conducted between WSB/EiJ and CAST/EiJ mice. WSB/EiJ females were bred with CAST/EiJ males to produce an F1 population that was, in turn, crossed to produce 168 F2 progeny. Separately, CAST/EiJ females were bred with WSB/EiJ males to produce an F1 population that was also, in turn, crossed to produce 437 F2 progeny.

PWD/PhJ female X WSB/EiJ male intercross, 168 F2 mice were generated from the WSB/EiJ female X CAST/EiJ male intercross, and 437 F2 mice were generated from the CAST/EiJ female X WSB/EiJ male intercross (Michael White, unpublished data). For the purposes of this study, the F2 progeny from the WSB/EiJ female X PWD/PhJ male and PWD/PhJ female X WSB/EiJ male intercrosses were pooled into one large reciprocal intercross. Likewise, the WSB/EiJ female X CAST/EiJ male and CAST/EiJ female X WSB/EiJ male intercrosses were also pooled.

Mouse Genotyping

All mice were sacrificed at approximately age 70 days. From each mouse, the liver was dissected out and then flash frozen in liquid nitrogen to -80 °C. Using the Wizard Genomic DNA Purification Kit from Promega, whole genomic DNA for each

mouse was extracted from finely chopped liver with the Animal Tissue (Mouse Brain and Liver) protocol, as specified by Miller *et al.* (1988).

From each cross, two parental, four F1, and all F2 mice were genotyped using Sequenom's iPLEX Gold system at 329 single nucleotide polymorphisms (SNPs) spaced at roughly 5.5 centiMorgan intervals across the euchromatic genome (Tim Wiltshire and Brian Steffy, unpublished data). Identical SNP markers were used for all crosses. Markers for which an error in genotyping occurred in any of the parental or F1 generation mice were removed from the data set. Analyses of transmission ratio distortion in the F2 generation focused on 212 autosomal SNPs.

Identification of Regions Experiencing Transmission Ratio Distortion

Six data groups were analyzed for transmission ratio distortion: all the F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross, only the female F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross, only the male F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross, all the F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross, only the female F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross, and only the male F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross. For each data group, R/qtl (Broman, *et al.*, 2008), a freely available package for the statistical programming environment R (R Development Core Team, 2008), was used to determine the number of F2 progeny in each genotypic class (WSB/EiJ homozygote, PWD/PhJ or CAST/EiJ homozygote, or heterozygote), as well as the number of individuals for which genotype information was missing, at each SNP in the genome. Additionally, from the data set including all F2 progeny of the cross, R/qtl

was used to calculate the genetic linkage map, in centiMorgans, for the WSB/EiJ and PWD/PhJ reciprocal intercross as well as the WSB/EiJ and CAST/EiJ reciprocal intercross. The genetic map estimated from the F2s in each reciprocal intercross was used to position the SNP markers in all data groups for that cross. From this data, the statistical programming environment R could be used to measure, for each SNP in the genome having at least two other SNPs within 20 centiMorgans distal to it, the median WSB/EiJ homozygote to PWD/PhJ or CAST/EiJ homozygote ratio, the median PWD/PhJ or CAST/EiJ homozygote to WSB/EiJ homozygote ratio, the median WSB/EiJ homozygote to heterozygote ratio, the median PWD/PhJ or CAST/EiJ homozygote ratio to heterozygote ratio, the median heterozygote to WSB/EiJ homozygote ratio, and the median heterozygote to PWD/PhJ or CAST/EiJ homozygote ratio for the group of SNPs in that same 20 centiMorgan window (R Development Core Team, 2008). These ratios are hereafter referred to for the WSB/EiJ and PWD/PhJ reciprocal intercross as the DD:MM, MM:DD, DD:DM, MM:DM, DM:DD, DM:MM ratios, respectively, while for the WSB/EiJ and CAST/EiJ reciprocal intercross, these ratios are referred to as the DD:CC, CC:DD, DD:DC, CC:DC, DC:DD, DC:CC ratios, respectively, (D stands for a *M. m. domesticus* allele, C stands for a *M. m. castaneus* allele, and M stands for a *M. m. musculus* allele). The "sliding window" statistics were collected, as described above, for all six data groups.

After collecting statistics on the genotypes observed at each SNP marker, the statistical programming environment R was used to simulate a null distribution for each sliding window statistic in each data group. First, a permutation was performed in which the genotype frequencies at each SNP in the observed data set were detached from their

respective SNP marker positions, and randomly assigned, without replacement, to those same SNP marker positions. Next, sliding window statistics were collected as described above for this permuted data set. Finally, for each category of sliding window statistic, the smallest value of that statistic observed in the permuted data set was recorded. These three steps were repeated 10,000 X's to generate each null distribution of each sliding window statistic in each data group. Then, from each of the null distributions generated, the 10^{th} percentile value was calculated. Any sliding window from the observed data set that contained a sliding window statistic that was smaller than the 10th percentile value calculated from the corresponding null distribution for that window statistic was concluded to be experiencing a significant level of transmission ratio distortion.

Results

Genetic Linkage Maps

The autosomal linkage map for the WSB/EiJ and PWD/PhJ reciprocal intercross totaled 1445.4 centiMorgans, with an average distance of 7.5 centiMorgans between SNP markers on the same chromosome. In comparison, the autosomal linkage map for the WSB/EiJ and CAST/EiJ reciprocal intercross was 1572.6 centiMorgans in total length, with an average of inter-SNP distance of 8.1 centiMorgans. The positions on each map of the SNP markers used for analysis are shown in Figures 4 and 5.

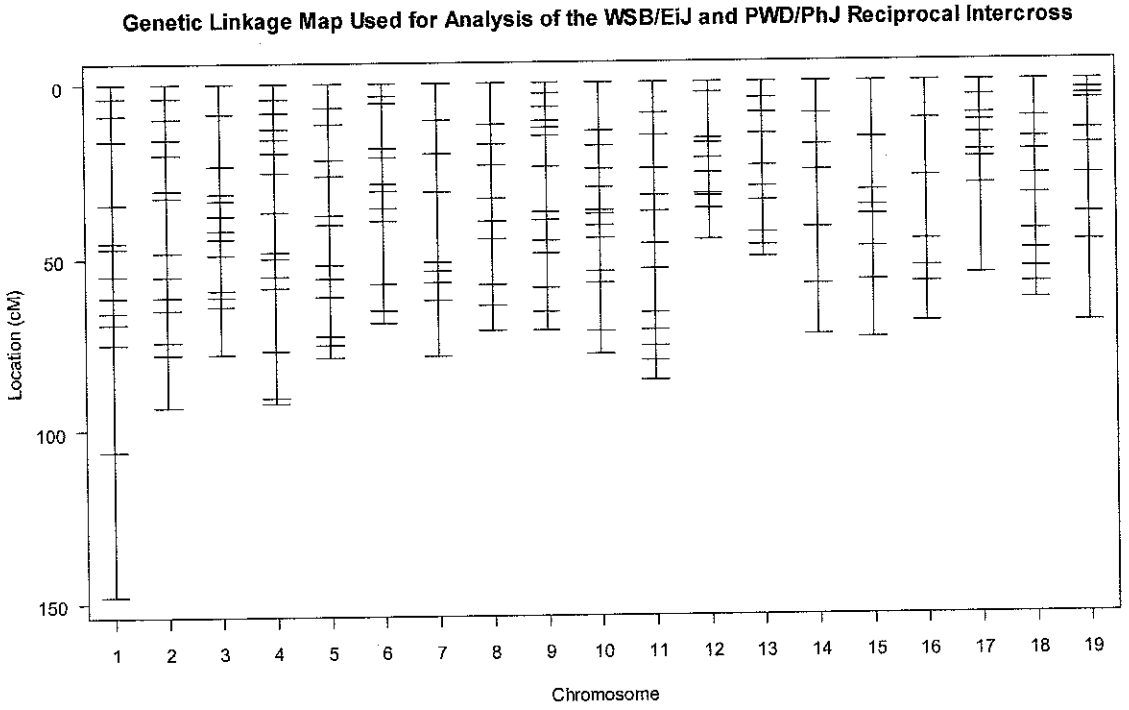


Figure 4. The genetic linkage map used for analysis of the WSB/EiJ and PWD/PhJ reciprocal intercross. Using the est.map function in R/qtl (Broman, *et al.*, 2008) and the genotypes from all F2s in the WSB/EiJ and PWD/PhJ reciprocal intercross, the position, in centiMorgans, of each of the 212 SNPs being analyzed in that cross for transmission ratio distortion was determined. In the plot above, the 19 vertical lines represent each of the 19 mouse autosomes, while the horizontal bars represent the centiMorgan positions of each of the SNPs.

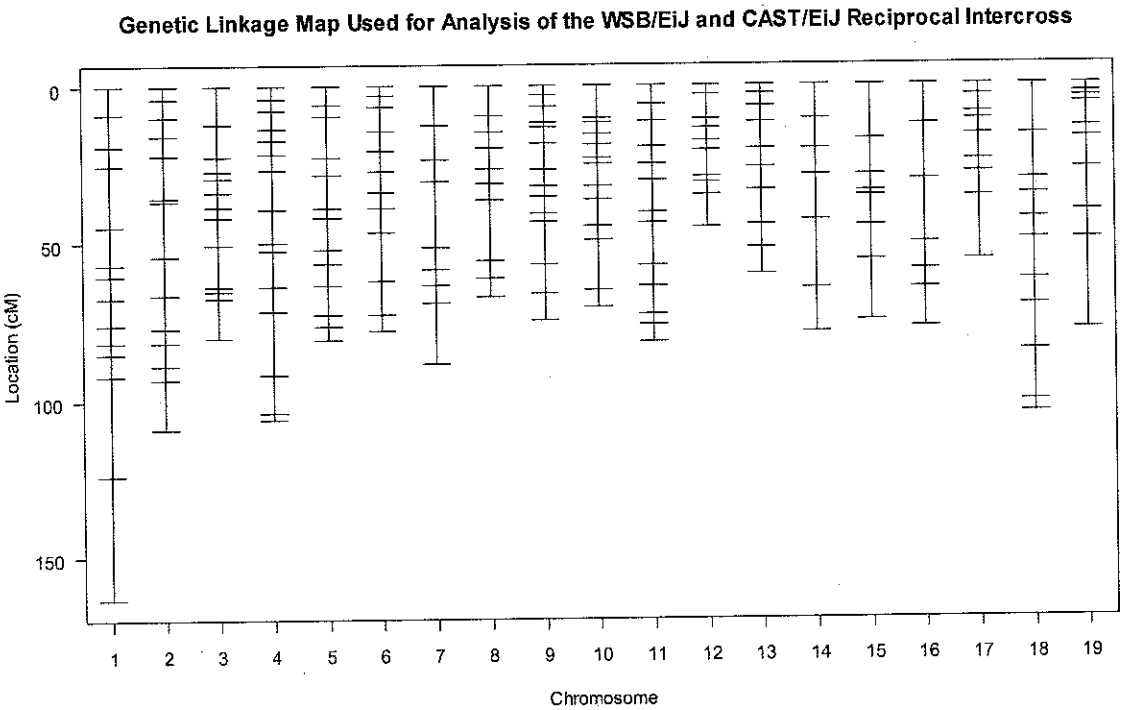


Figure 5. The genetic linkage map used for analysis of the WSB/EiJ and CAST/EiJ reciprocal intercross. Using the est.map function in R/qtl (Broman, *et al.*, 2008) and the genotypes from all F2s in the WSB/EiJ and CAST/EiJ reciprocal intercross, the position, in centiMorgans, of each of the 212 SNPs being analyzed in that cross for transmission ratio distortion was determined. In the plot above, the 19 vertical lines represent each of the 19 mouse autosomes, while the horizontal bars represent the centiMorgan positions of each of the SNPs.

Transmission Ratio Distortion in the WSB/EiJ and PWD/PhJ reciprocal intercross

In QTL studies, markers that exhibit non-Mendelian patterns of inheritance are often assumed to reflect genotyping errors. To identify markers having biased genotype frequencies due to underlying biological mechanisms, rather than as a result of genotyping error, clusters of markers experiencing transmission ratio distortion skewed in the same direction were identified with a genomic sliding window analysis. Using this method, three regions of transmission ratio distortion were found to exist for progeny from the WSB/EiJ and PWD/PhJ reciprocal intercross: a region from 4 – 50

centiMorgans on Chromosome 4 in which there are significantly more WSB/EiJ homozygotes than PWD/PhJ homozygotes; a region from 45 – 58 centiMorgans on Chromosome 10 in which the ratio of heterozygotes to PWD/PhJ homozygotes is significantly greater than 2:1; and a region from 5 – 38 centiMorgans on Chromosome 19 in which there are significantly more PWD/PhJ homozygotes than WSB/EiJ homozygotes. Additionally, when the F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross were split by sex into two separate groups, there were five regions in the female F2 progeny and six regions in the male F2 progeny found to be experiencing transmission ratio distortion. For the females, those regions included a region from 77 – 92 centiMorgans on Chromosome 4 in which the ratio of heterozygotes to either homozygotic class was significantly greater than 2:1; a region from 0 – 21 centiMorgans on Chromosome 6 in which the ratio of PWD/PhJ homozygotes to heterozygotes was significantly greater than 1:2; the previously identified region from 41 – 58 centiMorgans on Chromosome 10 in which the ratio of heterozygotes to either homozygotic class is significantly greater than 2:1; a region from 9 – 24 centiMorgans on Chromosome 13 in which there are significantly more WSB/EiJ homozygotes than PWD/PhJ homozygotes; and a region from 35 – 38 centiMorgans on Chromosome 15 in which there are significantly more WSB/EiJ homozygotes than PWD/PhJ homozygotes. The regions of transmission ratio distortion found in male F2 progeny from the WSB/EiJ and PWD/PhJ reciprocal intercross included a region from 34 – 66 centiMorgans on Chromosome 1 in which the ratio of heterozygotes to either homozygotic class is significantly greater than 2:1; the previously identified region from 16 – 92 centiMorgans on Chromosome 4 in which there are significantly more WSB/EiJ homozygotes than PWD/PhJ homozygotes; a

region from 0 – 18 centiMorgans on Chromosome 8 in which the ratio of heterozygotes to WSB/EiJ homozygotes is significantly greater than 2:1; a region from 49 – 71 centiMorgans on Chromosome 9 in which there is a significant bias towards PWD/PhJ homozygotes over the other two genotypic classes; a region from 36 – 48 centiMorgans on Chromosome 15 in which the ratio of heterozygotes to WSB/EiJ homozygotes is significantly greater than 2:1; and the previously-identified region from 5 – 38 centiMorgans on Chromosome 19 in which there are significantly more PWD/PhJ homozygotes than WSB/EiJ homozygotes. A list of the markers found to be experiencing transmission ratio distortion, including the genotype frequencies observed at those markers, can be found in Tables 1 – 3. Additionally, plots of the genotype frequencies observed at all markers for all F2 progeny, only female F2 progeny, and only male F2 progeny from the WSB/EiJ and PWD/PhJ reciprocal intercross are shown in Figures 6 – 8, respectively.

Table 1. Genotype counts observed at each of the SNP markers determined to be experiencing significant transmission ratio distortion in all F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross.

Chromosome	Location (cM)	Missing Individuals	WSB/EiJ homozygotes	Heterozygotes	PWD/PhJ homozygotes	Type of bias	Sliding window test p-value	Chi-Square Test p-value
5	5/38	24	269	379	242	3	8/: 5F.13	2/93F.12
5	9/27	32	278	362	242	3	8/: 5F.13	2/37F.13
5	23/92	7	275	378	244	3	5/84F.13	9/2: F.13
5	27/12	7	282	375	23:	3	4/81F.13	2/4: F.13
5	2: /97	8	272	394	22:	3	2/77F.13	5/43F.13
5	36/49	6	273	397	228	—	—	3/77F.13
5	47/83	263	218	321	212	6	: /56F.13	: /24F.12
5	59/61	23	265	3: 4	222	3-6	4/62F.13	2/91F.13
5	61/25	9	271	399	225	3	8/51F.13	2/: 6F.13
21	55/95	9	253	413	229	6	7/92F.13	8/58F.13
21	65/42	9	254	412	229	—	—	8/: 3F.13
21	68/74	9	242	419	234	—	—	7/78F.13
2:	6/68	24	232	3: 1	257	2	5/65F.13	3/14F.12
2:	25/45	24	231	389	26:	2	3/48F.13	7/62F.13
2:	29/75	22	229	391	272	2	3/48F.13	4/77F.13
2:	38/22	: 1	84	379	24:	2	8/11F.13	5/48F.17
2:	49/54	24	225	393	272	—	—	2/92F.13

A sliding window analysis, which identified clusters of SNP markers all experiencing genotype skews in the same direction, detected significant transmission ratio distortion in all F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross at the SNP marker positions listed in the table above. The first column of the table gives the chromosome on which the significantly skewed SNP marker is located, while the second column provides the centiMorgan position of the SNP, as calculated from a linkage analysis conducted in R/qtl on the SNP genotype data from all F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross (Broman, *et al.*, 2008). The third column gives the number of F2 individuals for which genotyping at that SNP failed. The fourth, fifth, and sixth columns provide the number of F2 individuals that were genotyped at the significantly distorted marker as homozygotic for the WSB/EiJ allele, heterozygotic, and homozygotic for the PWD/PhJ allele, respectively. If the marker was the first marker in a window found by the sliding window analyses to be significantly distorted, the seventh column indicates the direction(s) in which the sliding window analysis identified the significant genotype bias (1 = more PWD/PhJ homozygotes than WSB/EiJ homozygotes; 2 = more WSB/EiJ homozygotes than PWD/PhJ homozygotes; 3 = more than one PWD/PhJ homozygote to every two heterozygotes; 4 = more than one WSB/EiJ homozygote to every two heterozygotes; 5 = more than two heterozygotes to every one PWD/PhJ homozygote; 6 = more than two heterozygotes to every one WSB/EiJ homozygotes; – = the marker was not the first marker in a significantly distorted window). Likewise, for each marker that was the first marker in a window found by the sliding window analyses to be significantly distorted, the eighth column gives the p-value corresponding to that

significant sliding test statistic. If more than one type of bias was found for a sliding window, column eight displays the more significant p-value generated from that window. Finally, the ninth column provides the p-value obtained for each SNP marker from a Chi-Square test of Mendelian inheritance patterns (1:2:1 WSB/EiJ homozygote: heterozygote: PWD/PhJ homozygote) that was conducted in R/qtl (Broman, *et al.*, 2008).

Table 2. Genotype counts observed at each of the SNP markers determined to be experiencing significant transmission ratio distortion in the female F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross.

Chromosome	Location (cM)	Missing individuals	WSB/EiJ homozygotes	heterozygotes	PWD/PhJ homozygotes	Type of bias	Sliding window test p-value	Chi-Square Test p-value
5	88/12	22	65	241	56	6	5/91F.14	9/72F.13
5	: 1/77	2	62	251	59	—	—	3/97F.13
5	: 3/23	2	63	252	57	—	—	2/91F.13
7	1/11	2	74	212	86	4	4/4: F.13	4/23F.13
7	4/75	8	72	212	82	4	8/57F.13	9/39F.13
7	6/84	8	69	211	86	2	6/91F.13	3/91F.13
7	29/84	33	56	212	83	—	—	2/: 7F.13
7	32/47	3	62	224	85	—	—	9/11F.13
21	52/36	5	66	241	62	6	5/68F.13	3/87F.12
21	55/95	6	63	246	59	6	4/: 4F.13	7/9: F.13
21	65/42	6	64	245	59	—	—	9/97F.13
21	68/74	4	57	253	5:	—	—	: /22F.14
24	: /11	21	72	229	62	3	8/19F.13	6/: : F.12
24	25/: 1	4	76	237	57	—	—	2/47F.12
24	35/43	4	74	23:	56	—	—	2/12F.12
26	46/78	5	75	236	58	3	5/93F.13	2/: 5F.12
26	49/56	4	79	231	5:	—	—	3/25F.12

A sliding window analysis, which identified clusters of SNP markers all experiencing genotype skews in the same direction, detected significant transmission ratio distortion in the female F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross at the SNP marker positions listed in the table above. The first column of the table gives the chromosome on which the significantly skewed SNP marker is located, while the second column provides the centiMorgan position of the SNP, as calculated from a linkage analysis conducted in R/qtl on the SNP genotype data from all F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross (Broman, *et al.*, 2008). The third column gives the number of female F2 individuals for which genotyping at that SNP failed. The fourth, fifth, and sixth columns provide the number of female F2 individuals that were genotyped at the significantly distorted marker as homozygotic for the WSB/EiJ allele, heterozygotic, and homozygotic for the PWD/PhJ allele, respectively. If the marker was the first marker in a window found by the sliding window analyses to be significantly distorted, the seventh column indicates the direction(s) in which the sliding window analysis identified the significant genotype bias (1 = more PWD/PhJ homozygotes than WSB/EiJ homozygotes; 2 = more WSB/EiJ homozygotes than PWD/PhJ homozygotes; 3

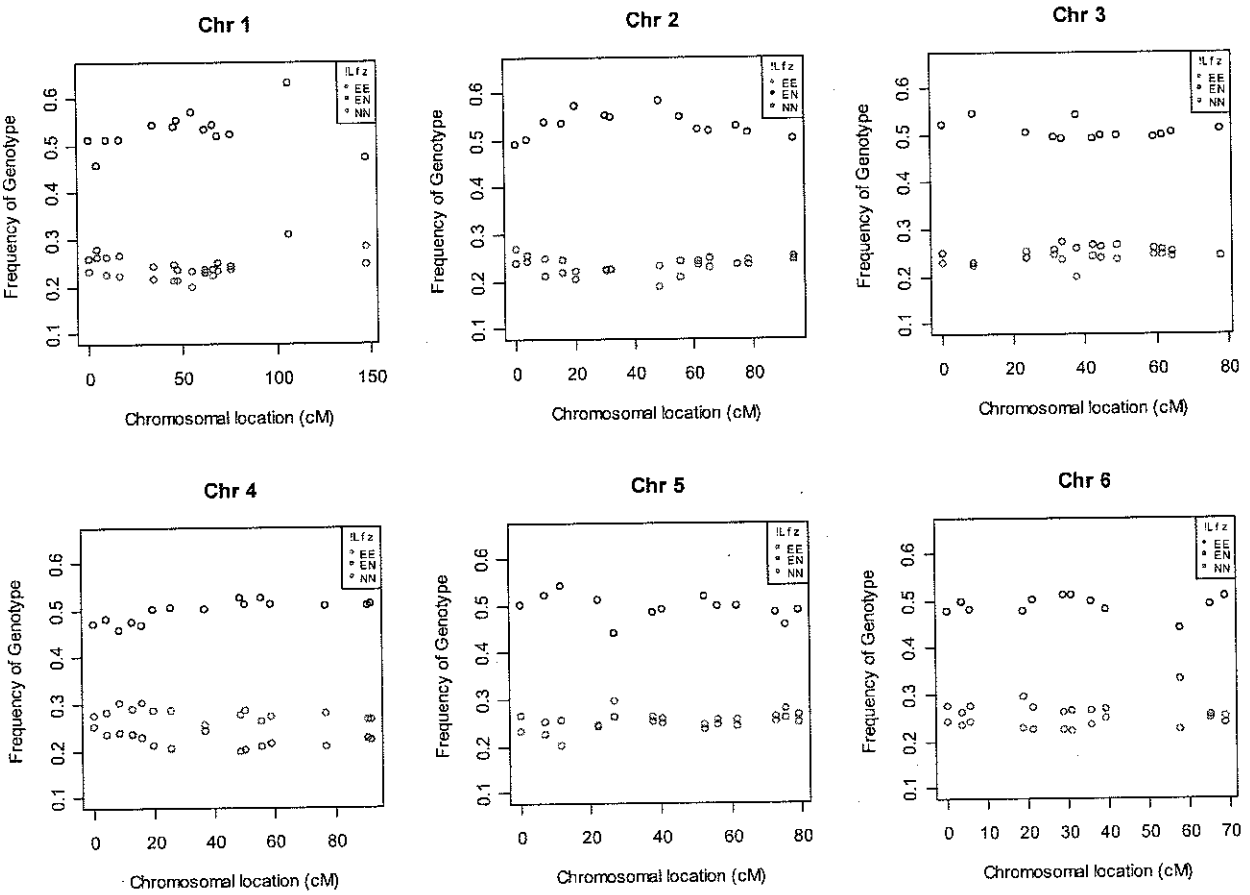
= more than one PWD/PhJ homozygote to every two heterozygotes; 4 = more than one WSB/EiJ homozygote to every two heterozygotes; 5 = more than two heterozygotes to every one PWD/PhJ homozygote; 6 = more than two heterozygotes to every one WSB/EiJ homozygotes; – = the marker was not the first marker in a significantly distorted window). Likewise, for each marker that was the first marker in a window found by the sliding window analyses to be significantly distorted, the eighth column gives the p-value corresponding to that significant sliding test statistic. If more than one type of bias was found for a sliding window, column eight displays the more significant p-value generated from that window. Finally, the ninth column provides the p-value obtained for each SNP marker from a Chi-Square test of Mendelian inheritance patterns (1:2:1 WSB/EiJ homozygote: heterozygote: PWD/PhJ homozygote) that was conducted in R/qtl (Broman, *et al.*, 2008).

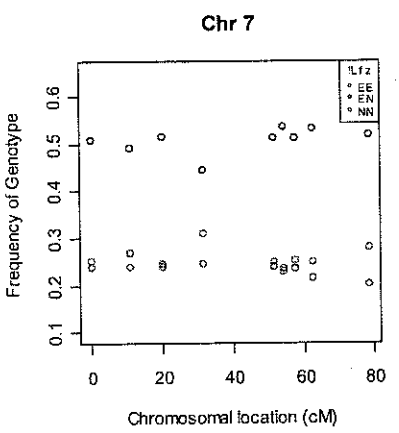
Table 3. Genotype counts observed at each of the SNP markers determined to be experiencing significant transmission ratio distortion in the male F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross.

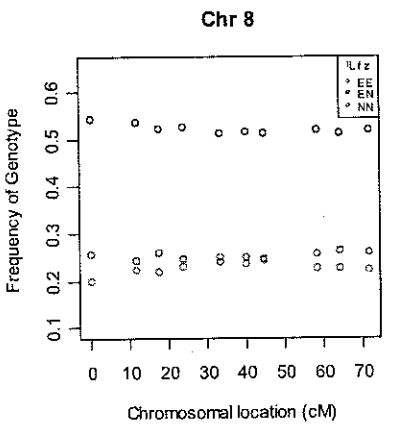
Chromosome	Location (cM)	Missing Individuals	WSB/EiJ homozygotes	heterozygotes	PWD/PhJ homozygotes	Type of bias	Sliding window test p-value	Chi-Square Test p-value
2	45/62	7	83	291	83	6	3/: 1F.14	2/46F.12
2	56/52	8	79	299	78	6	6/11F.15	2/3: F.13
2	58/21	:	78	2: 4	72	6	6/11F.15	2/35F.14
2	65/: 4	5	74	313	72	6	8/81F.14	9/86F.16
2	72/55	29	81	296	68	—	—	3/76F.14
2	76/7:	8	78	2: 1	77	—	—	7/63F.14
5	27/12	3	212	266	83	3	3/62F.13	5/81F.13
5	2: /97	5	: 7	279	73	3	4/: 5F.13	3/58F.13
5	36/49	1	: 6	283	74	—	—	4/45F.13
5	47/83	95	76	234	69	—	—	9/2: F.12
5	59/61	:	: 2	276	76	3	4/59F.13	2/18F.12
5	61/25	5	: 7	274	78	3	3/56F.13	8/69F.13
5	66/4:	6	98	286	74	—	—	7/61F.13
5	69/69	9	: 3	277	75	—	—	8/61F.13
5	88/12	29	: 9	257	79	5	7/66F.13	3/: 5F.13
5	: 1/77	8	: 9	257	8:	—	—	8/4: F.13
5	: 3/23	22	: 8	255	89	—	—	8/26F.13
9	1/11	42	66	282	84	7	: /86F.13	2/65F.13
9	22/: 3	7	75	294	88	—	—	4/: 1F.13
9	28/72	5	74	289	96	—	—	6/81F.13
:	5: /35	:	82	272	9:	2	9/89F.13	4/75F.12
:	6: /23	6	83	264	211	2	9/89F.13	6/25F.13
:	76/: 2	6	76	275	: 7	—	—	6/24F.13
:	82/54	25	77	267	: 5	—	—	9/27F.13
26	46/78	23	67	285	99	7	7/99F.13	: /81F.14
26	49/56	8	71	294	91	—	—	2/77F.13
26	58/85	36	81	284	73	—	—	6/26F.13
2:	6/68	9	78	285	92	2	4/: : F.13	2/: 1F.12
2:	25/45	9	76	272	: 7	2	2/31F.13	6/17F.13
2:	29/75	8	76	273	: 7	2	2/31F.13	6/21F.13
2:	38/22	61	47	274	92	2	7/23F.13	2/76F.16
2:	49/54	22	6:	276	: 6	—	—	2/53F.13

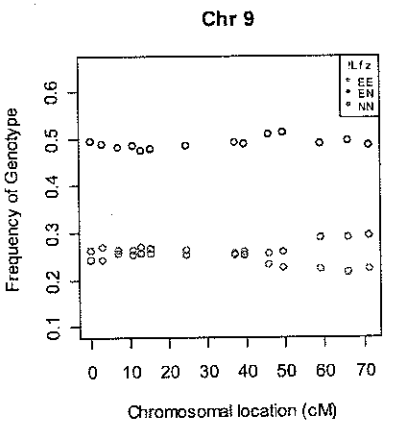
A sliding window analysis, which identified clusters of SNP markers all experiencing genotype skews in the same direction, detected significant transmission ratio distortion in the male F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross at the SNP marker positions listed in the table above. The first column of the table gives the chromosome on which the significantly skewed SNP marker is located, while the second column provides the centiMorgan position of the SNP, as calculated from a linkage analysis conducted in R/qtl on the SNP genotype data from all F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross (Broman, *et al.*, 2008). The third column

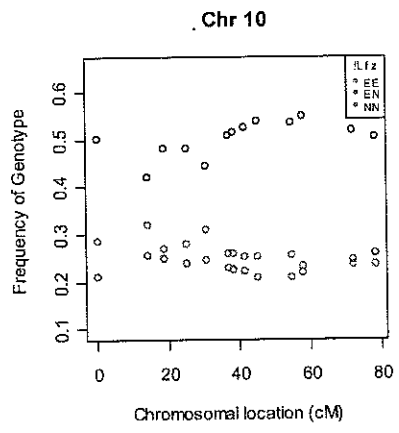
gives the number of male F2 individuals for which genotyping at that SNP failed. The fourth, fifth, and sixth columns provide the number of male F2 individuals that were genotyped at the significantly distorted marker as homozygotic for the WSB/EiJ allele, heterozygotic, and homozygotic for the PWD/PhJ allele, respectively. If the marker was the first marker in a window found by the sliding window analyses to be significantly distorted, the seventh column indicates the direction(s) in which the sliding window analysis identified the significant genotype bias (1 = more PWD/PhJ homozygotes than WSB/EiJ homozygotes; 2 = more WSB/EiJ homozygotes than PWD/PhJ homozygotes; 3 = more than one PWD/PhJ homozygote to every two heterozygotes; 4 = more than one WSB/EiJ homozygote to every two heterozygotes; 5 = more than two heterozygotes to every one PWD/PhJ homozygote; 6 = more than two heterozygotes to every one WSB/EiJ homozygotes; – = the marker was not the first marker in a significantly distorted window). Likewise, for each marker that was the first marker in a window found by the sliding window analyses to be significantly distorted, the eighth column gives the p-value corresponding to that significant sliding test statistic. If more than one type of bias was found for a sliding window, column eight displays the more significant p-value generated from that window. Finally, the ninth column provides the p-value obtained for each SNP marker from a Chi-Square test of Mendelian inheritance patterns (1:2:1 WSB/EiJ homozygote: heterozygote: PWD/PhJ homozygote) that was conducted in R/qtl (Broman, *et al.*, 2008).

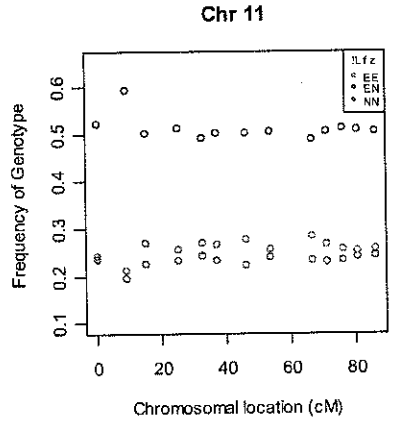


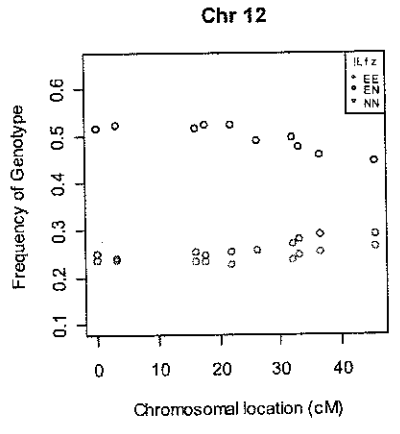

Chr 7
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

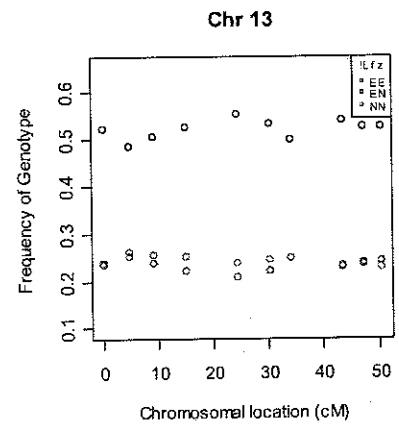

Chr 8
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

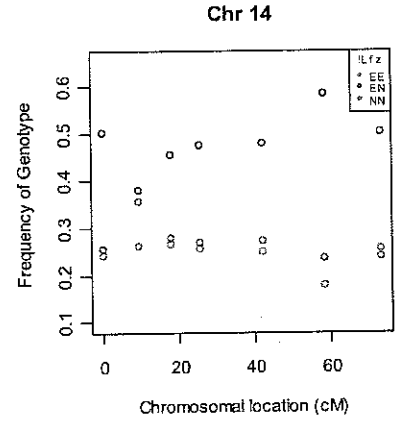

Chr 9
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

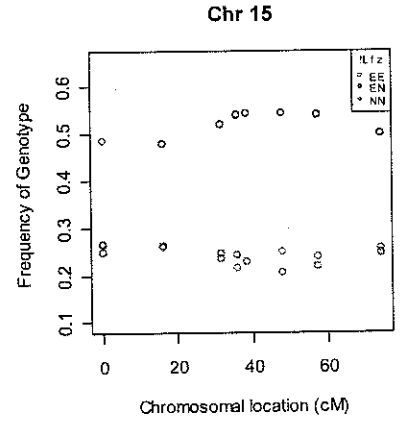

Chr 10
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 11
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 12
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 13
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 14
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 15
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

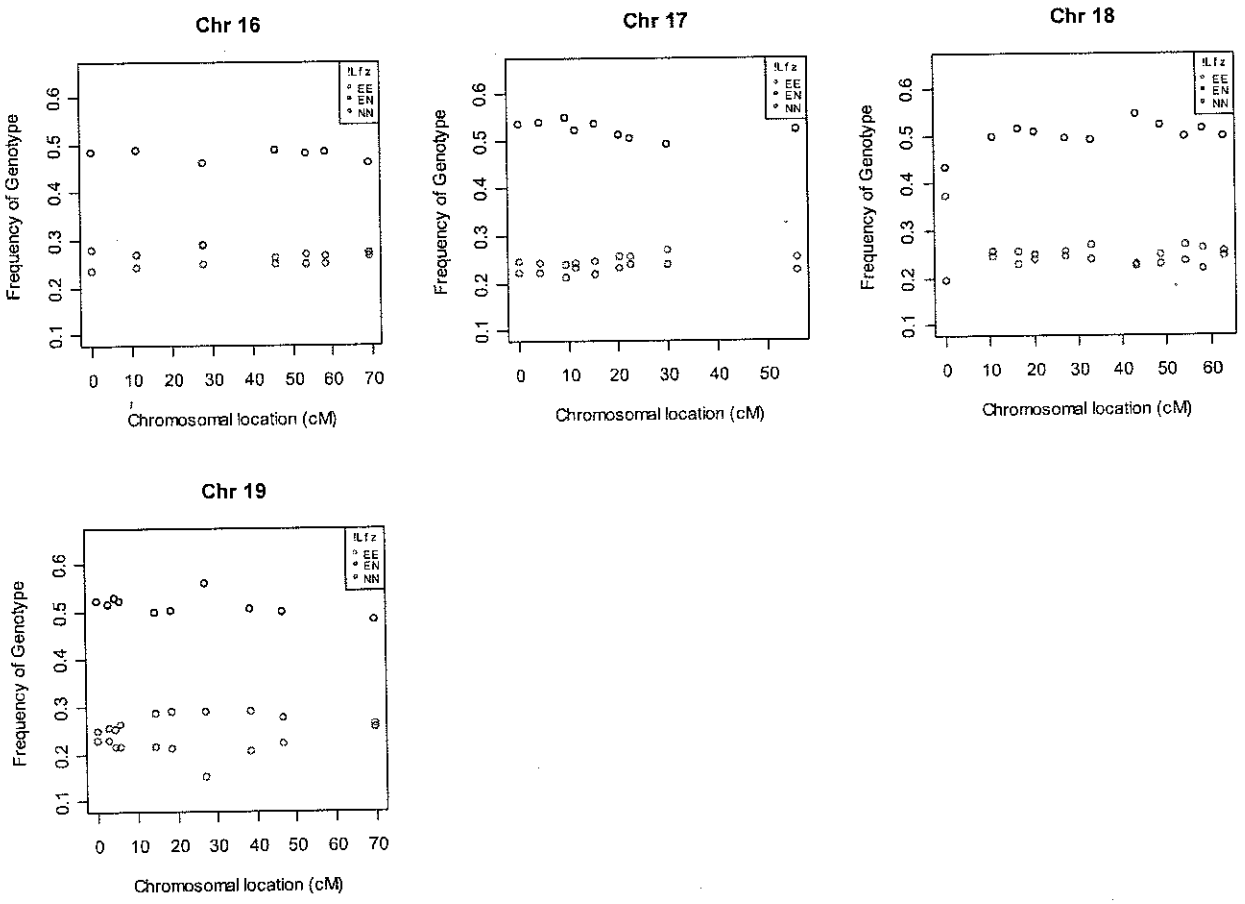
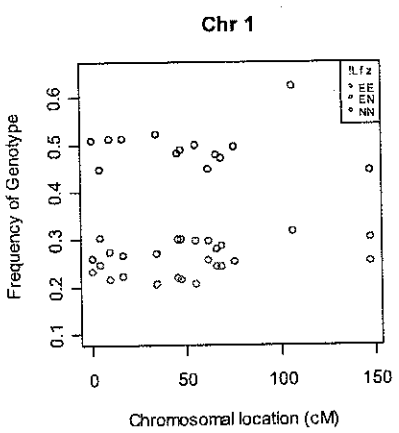
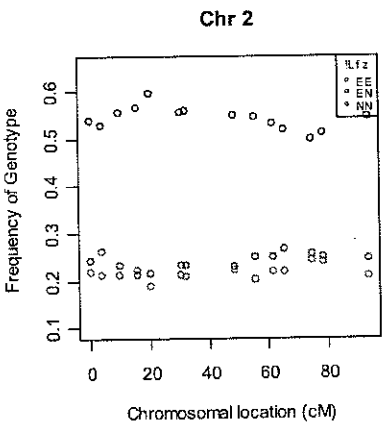
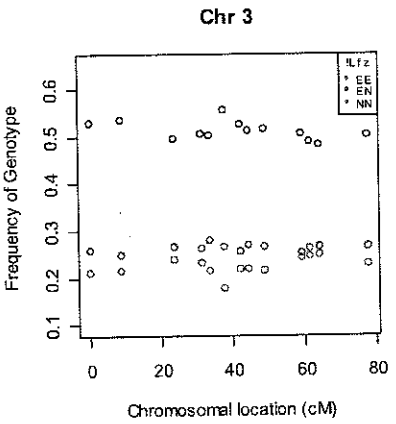
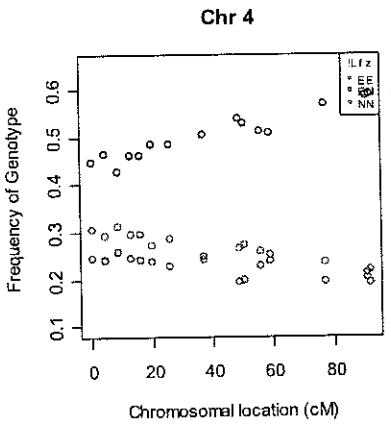


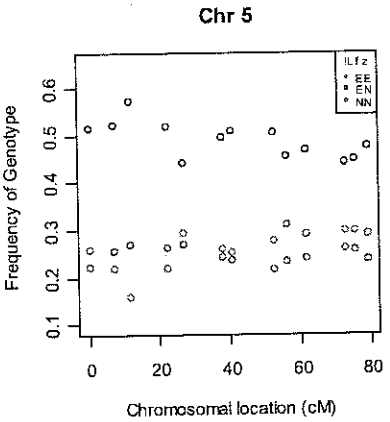
Figure 6. Plots, by chromosome, of the genotype frequencies observed at each SNP marker for all F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross. Genotype frequencies (calculated by dividing the total number of individuals in each individual genotypic class by the total number of individuals genotyped at the SNP) were plotted along the genetic linkage map calculated for the cross. All mouse chromosomes are acrocentric, so the left end of each chromosome plot corresponds to the chromosome end proximal to the centromere. Green circles represent the frequency of the WSB/EiJ homozygotic class, pink circles represent the frequency of the PWD/PhJ homozygotic class, and blue circles represent the frequency of the heterozygotic class. Expected genotype frequencies for these three classes are 0.25, 0.25, and 0.50, respectively.

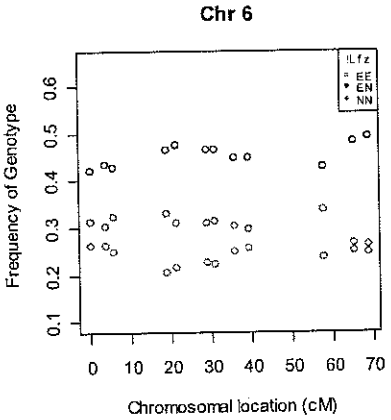

Chr 1
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

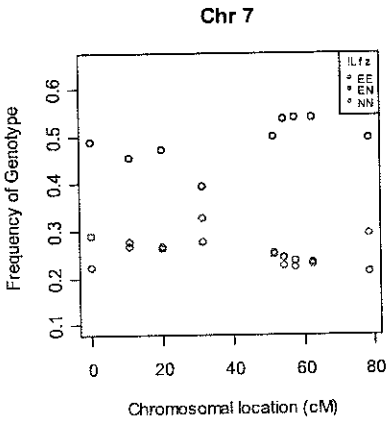

Chr 2
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

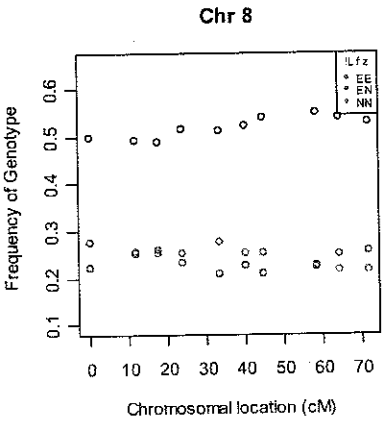

Chr 3
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

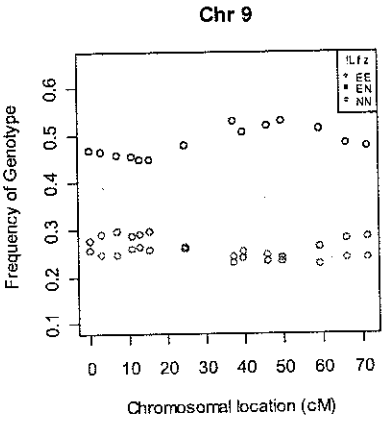

Chr 4
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN

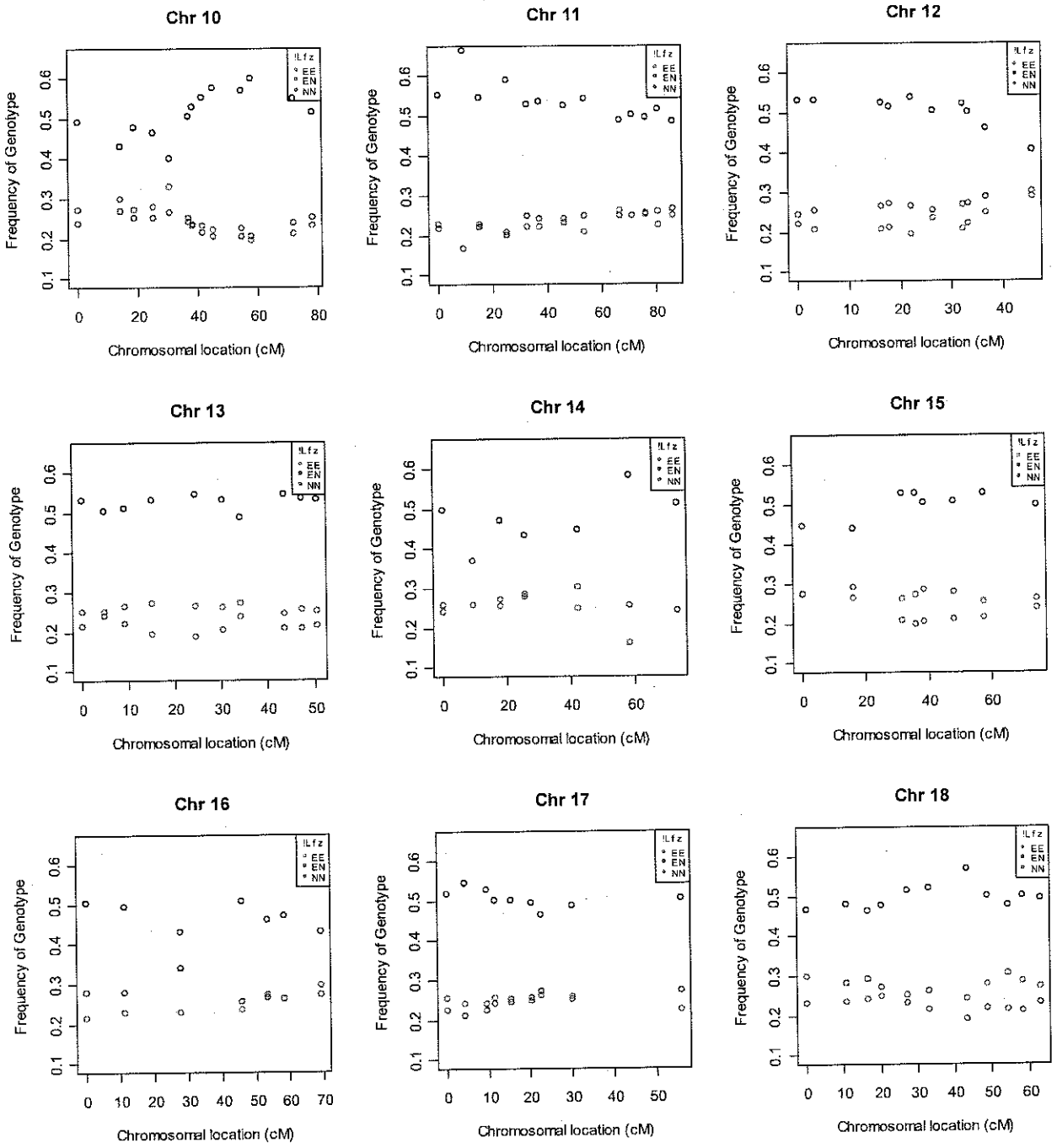

Chr 5
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 6
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 7
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 8
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 9
Frequency of Genotype
Chromosomal location (cM)
EE
EN
NN


Chr 10
Chr 11
Chr 12
Chr 13
Chr 14
Chr 15
Chr 16
Chr 17
Chr 18
Frequency of Genotype
Chromosomal location (cM)
!Lfz
EE
EN
NN

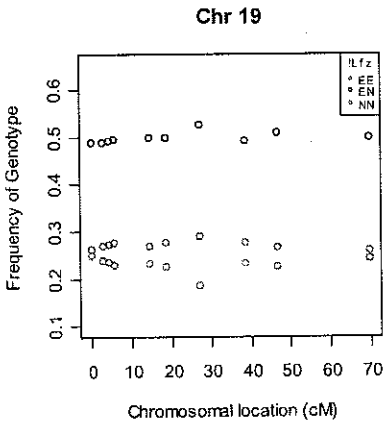
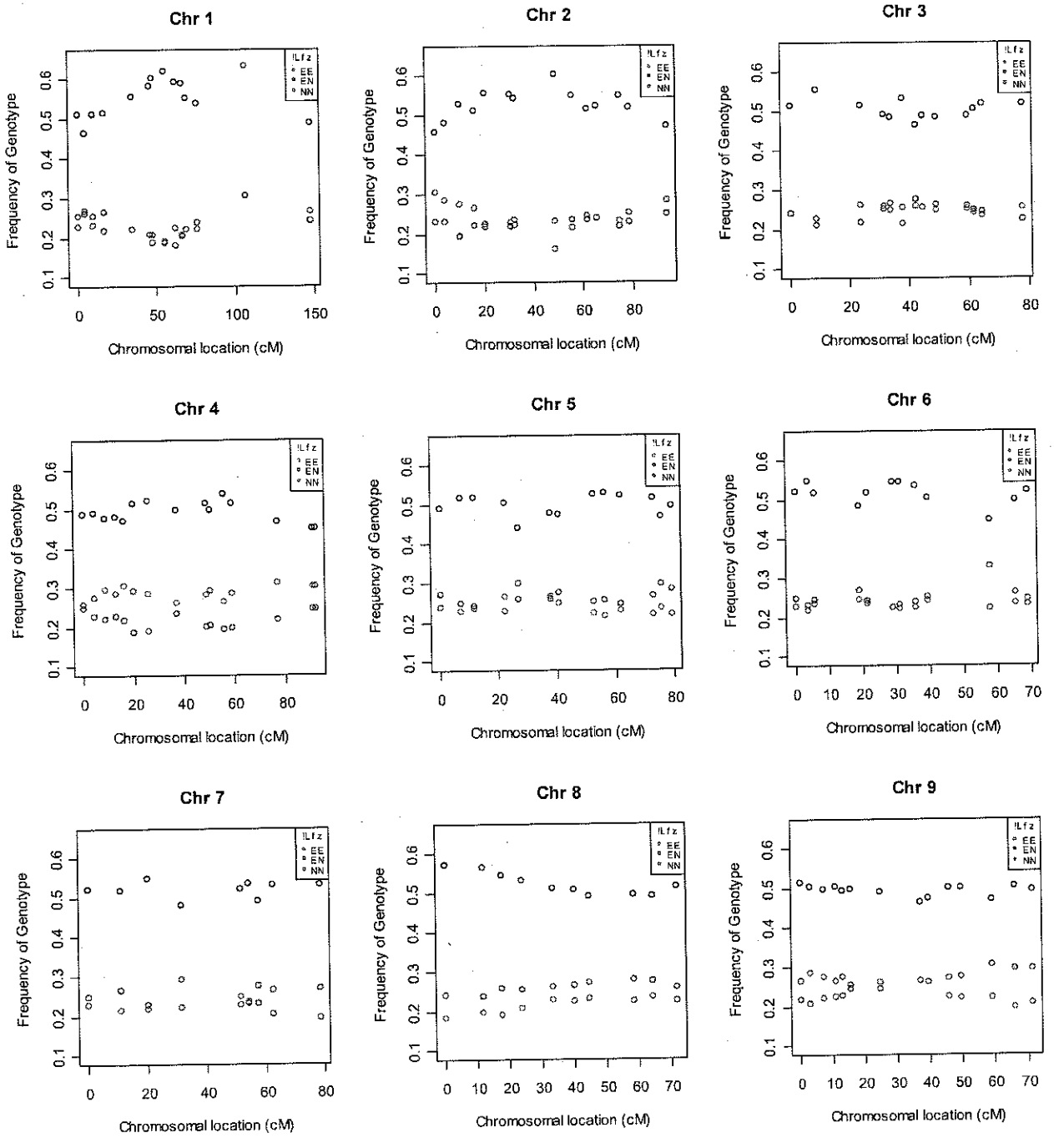
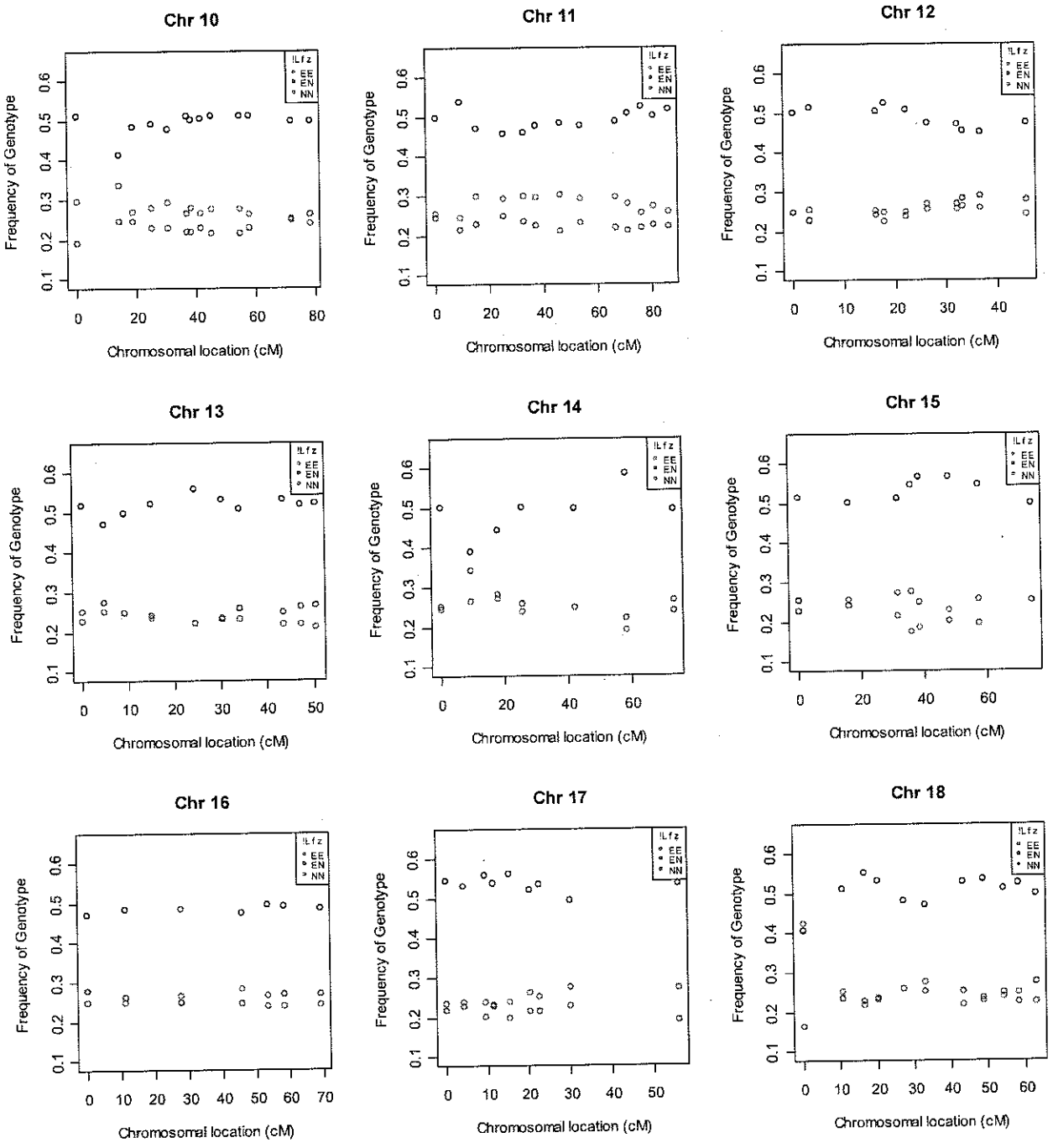


Figure 7. Plots, by chromosome, of the genotype frequencies observed at each SNP marker for the female F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross. Genotype frequencies (calculated by dividing the total number of individuals in each individual genotypic class by the total number of individuals genotyped at the SNP) were plotted along the genetic linkage map calculated for the cross. All mouse chromosomes are acrocentric, so the left end of each chromosome plot corresponds to the chromosome end proximal to the centromere. Green circles represent the frequency of the WSB/EiJ homozygotic class, pink circles represent the frequency of the PWD/PhJ homozygotic class, and blue circles represent the frequency of the heterozygotic class. Expected genotype frequencies for these three classes are 0.25, 0.25, and 0.50, respectively.


Chr 1
Chr 2
Chr 3
Chr 4
Chr 5
Chr 6
Chr 7
Chr 8
Chr 9
Frequency of Genotype
Chromosomal location (cM)
lLfz
EE
EN
NN


Chr 10
Chr 11
Chr 12
Chr 13
Chr 14
Chr 15
Chr 16
Chr 17
Chr 18
Frequency of Genotype
Chromosomal location (cM)
lLfz
EE
EN
NN

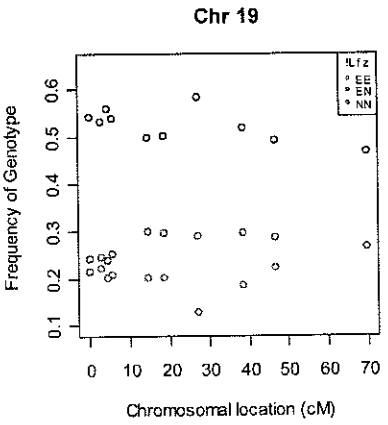


Figure 8. Plots, by chromosome, of the genotype frequencies observed at each SNP marker for the male F2 progeny of the WSB/EiJ and PWD/PhJ reciprocal intercross. Genotype frequencies (calculated by dividing the total number of individuals in each individual genotypic class by the total number of individuals genotyped at the SNP) were plotted along the genetic linkage map calculated for the cross. All mouse chromosomes are acrocentric, so the left end of each chromosome plot corresponds to the chromosome end proximal to the centromere. Green circles represent the frequency of the WSB/EiJ homozygotic class, pink circles represent the frequency of the PWD/PhJ homozygotic class, and blue circles represent the frequency of the heterozygotic class. Expected genotype frequencies for these three classes are 0.25, 0.25, and 0.50, respectively.

Transmission Ratio Distortion in the WSB/EiJ and CAST/EiJ reciprocal intercross

Using the sliding window method to identify clusters of SNP markers all experiencing genotype skews in the same direction did not reveal any regions of significant transmission ratio distortion for all F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross. However, when the F2 progeny were split by sex and the same sliding window analysis was conducted, five regions of significant transmission ratio distortion were found in the females and three regions were found in the males. The regions identified in the females included a region from 10 – 54 centiMorgans on Chromosome 2 in which the ratio of heterozygotes to WSB/EiJ homozygotes is

significantly greater than 2:1; a region from 0 – 22 centiMorgans on Chromosome 3 in which there are significantly more CAST/EiJ homozygotes than WSB/EiJ homozygotes; a region from 26 – 64 centiMorgans on Chromosome 4 in which there are significantly more WSB/EiJ homozygotes than CAST/EiJ homozygotes; a region from 0 – 15 centiMorgans on Chromosome 10 in which there are significantly more WSB/EiJ homozygotes than CAST/EiJ homozygotes; and a region from 3 – 23 centiMorgans on Chromosome 17 in which the ratio of heterozygotes to CAST/EiJ homozygotes is significantly greater than 2:1. In the male F2 progeny, significant transmission ratio was found for a region from 0 – 11 centiMorgans on Chromosome 17 in which there are significantly more CAST/EiJ homozygotes than WSB/EiJ homozygotes; a region from 42 – 62 centiMorgans on Chromosome 18 in which there are significantly more WSB/EiJ homozygotes than CAST/EiJ homozygotes; and a region from 84 – 104 centiMorgans on Chromosome 18 in which the ratio of heterozygotes to CAST/EiJ homozygotes is significantly greater than 2:1. A list of the markers found to be experiencing transmission ratio distortion in the female and male F2 progeny, including the genotype frequencies observed at those markers, can be found in Tables 4 – 5. Additionally, plots of the genotype frequencies observed at all markers for all F2 progeny, only female F2 progeny, and only male F2 progeny from the WSB/EiJ and CAST/EiJ reciprocal intercross are shown in Figures 9 – 11, respectively.

Table 4. Genotype counts observed at each of the SNP markers determined to be experiencing significant transmission ratio distortion in the female F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross.

Chromosome	Location (cM)	Missing Individuals	WSB/EiJ homozygotes	Heterozygotes	CAST/EiJ homozygotes	Type of bias	Sliding window test p-value	Chi-Square Test p-value
3	21/18	6	53	261	74	2-7	4/45F.13	4/46F.14
3	26/91	7	56	257	74	7	4/95F.13	2/74F.13
3	33/19	5	59	247	83	7	9/49F.13	7/4: F.13
3	46/66	34	55	247	68	7	9/49F.13	4/81F.13
3	47/67	9	58	261	66	—	—	9/13F.14
3	65/55	56	54	238	56	—	—	3/97F.13
4	1/11	32	57	22:	85	—	—	4/86F.13
4	23/33	25	67	225	87	4	: /: 9F.13	2/13F.12
4	33/2:	25	72	219	88	—	—	6/78F.13
5	37/73	21	85	238	5:	—	—	8/: 6F.13
5	4: /32	73	56	: 6	69	3	4/: : F.13	4/73F.12
5	61/13	3	92	238	61	3	4/: : F.13	3/45F.13
5	63/64	3	92	237	62	—	—	3/96F.13
5	74/99	32	9:	: 7	65	—	—	6/96F.16
21	1/11	4:	78	227	49	3-5	8/71F.14	2/7: F.13
21	21/21	7	7:	251	56	3	: /61F.13	3/85F.13
21	22/94	7	85	247	55	3	4/29F.13	2/64F.13
21	26/42	:	87	241	56	—	—	2/96F.13
28	4/23	6	75	251	62	5	2/21F.13	2/62F.12
28	9/82	22	71	251	5:	5	2/21F.13	9/: 4F.13
28	21/: 1	8	75	251	5:	5	2/21F.13	: /84F.13
28	26/98	:	75	252	57	—	—	5/17F.13
28	34/92	4	74	257	59	—	—	4/95F.13

A sliding window analysis, which identified clusters of SNP markers all experiencing genotype skews in the same direction, detected significant transmission ratio distortion in the female F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross at the SNP marker positions listed in the table above. The first column of the table gives the chromosome on which the significantly skewed SNP marker is located, while the second column provides the centiMorgan position of the SNP, as calculated from a linkage analysis conducted in R/qtl on the SNP genotype data from all F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross (Broman, *et al.*, 2008). The third column gives the number of female F2 individuals for which genotyping at that SNP failed. The fourth, fifth, and sixth columns provide the number of female F2 individuals that were genotyped at the significantly distorted marker as homozygotic for the WSB/EiJ allele, heterozygotic, and homozygotic for the CAST/EiJ allele, respectively. If the marker was the first marker in a window found by the sliding window analyses to be significantly distorted, the seventh column indicates the direction(s) in which the sliding window analysis identified the significant genotype bias (1 = more CAST/EiJ homozygotes than WSB/EiJ homozygotes; 2 = more WSB/EiJ homozygotes than CAST/EiJ homozygotes; 3

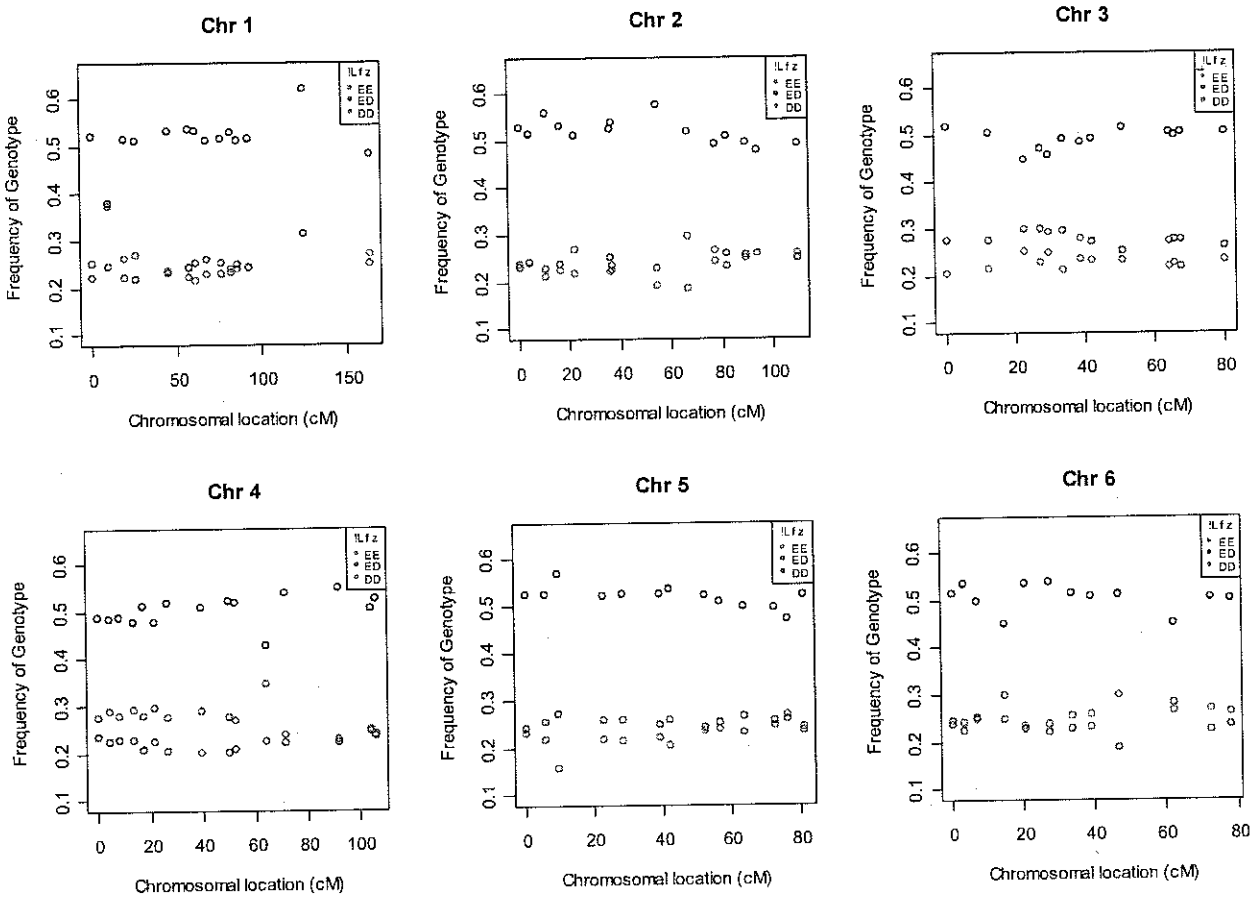
= more than one CAST/EiJ homozygote to every two heterozygotes; 4 = more than one WSB/EiJ homozygote to every two heterozygotes; 5 = more than two heterozygotes to every one CAST/EiJ homozygote; 6 = more than two heterozygotes to every one WSB/EiJ homozygotes; – = the marker was not the first marker in a significantly distorted window). Likewise, for each marker that was the first marker in a window found by the sliding window analyses to be significantly distorted, the eighth column gives the p-value corresponding to that significant sliding test statistic. If more than one type of bias was found for a sliding window, column eight displays the more significant p-value generated from that window. Finally, the ninth column provides the p-value obtained for each SNP marker from a Chi-Square test of Mendelian inheritance patterns (1:2:1 WSB/EiJ homozygote: heterozygote: CAST/EiJ homozygote) that was conducted in R/qtl (Broman, *et al.*, 2008).

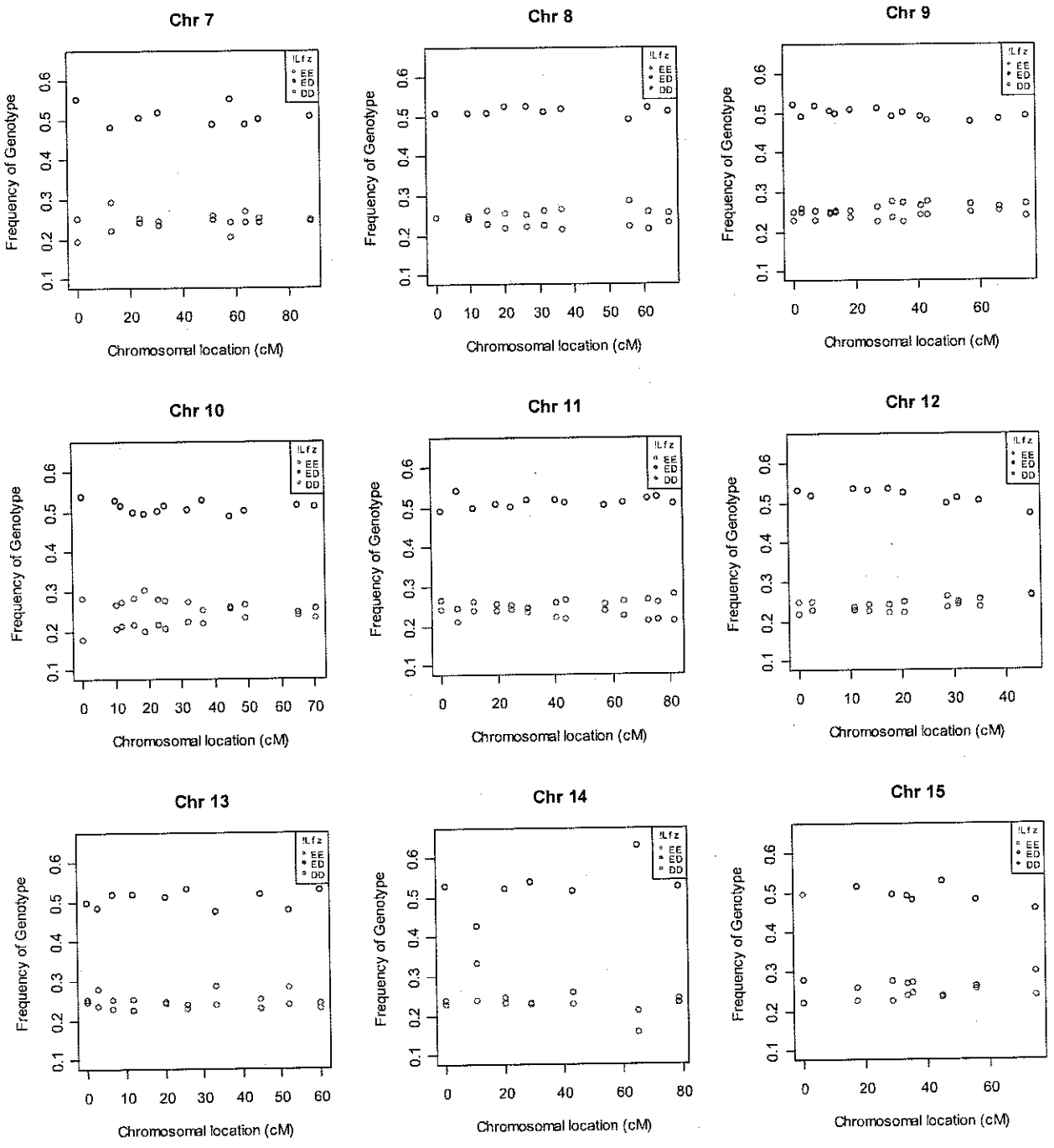
Table 5. Genotype counts observed at each of the SNP markers determined to be experiencing significant transmission ratio distortion in the male F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross.

Chromosome	Location (cM)	Missing Individuals	WSB/EiJ homozygotes	heterozygotes	CAST/EiJ homozygotes	Type of bias	Sliding window test p-value	Chi-Square Test p-value
28	1/11	24	79	279	:7	2	8/49F.13	:/31F.13
28	4/23	22	81	274	212	2	7/79F.13	6/22F.13
28	9/82	34	76	272	:7	—	—	6/17F.13
28	21/: 1	9	79	279	212	—	—	4/: 5F.13
29	53/56	:	: 1	289	79	6	2/: 5F.13	2/42F.12
29	5: /37	6	211	288	74	—	—	2/45F.13
29	73/29	22	: 4	284	79	—	—	2/35F.12
29	95/47	:	262	233	74	3	4/77F.13	4/43F.27
29	211/63	2:	: 3	286	6:	—	—	2/57F.13
29	215/23	23	: :	281	75	—	—	3/46F.13

A sliding window analysis, which identified clusters of SNP markers all experiencing genotype skews in the same direction, detected significant transmission ratio distortion in the male F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross at the SNP marker positions listed in the table above. The first column of the table gives the chromosome on which the significantly skewed SNP marker is located, while the second column provides the centiMorgan position of the SNP, as calculated from a linkage analysis conducted in R/qtl on the SNP genotype data from all F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross (Broman, *et al.*, 2008). The third column gives the number of male F2 individuals for which genotyping at that SNP failed. The fourth, fifth, and sixth columns provide the number of male F2 individuals that were genotyped at the significantly distorted marker as homozygotic for the WSB/EiJ allele, heterozygotic, and homozygotic for the CAST/EiJ allele, respectively. If the marker was the first marker in a window found by the sliding window analyses to be significantly distorted, the seventh column indicates the direction(s) in which the sliding window analysis identified the significant genotype bias (1 = more CAST/EiJ homozygotes than

WSB/EiJ homozygotes; 2 = more WSB/EiJ homozygotes than CAST/EiJ homozygotes; 3 = more than one CAST/EiJ homozygote to every two heterozygotes; 4 = more than one WSB/EiJ homozygote to every two heterozygotes; 5 = more than two heterozygotes to every one CAST/EiJ homozygote; 6 = more than two heterozygotes to every one WSB/EiJ homozygotes; – = the marker was not the first marker in a significantly distorted window). Likewise, for each marker that was the first marker in a window found by the sliding window analyses to be significantly distorted, the eighth column gives the p-value corresponding to that significant sliding test statistic. If more than one type of bias was found for a sliding window, column eight displays the more significant p-value generated from that window. Finally, the ninth column provides the p-value obtained for each SNP marker from a Chi-Square test of Mendelian inheritance patterns (1:2:1 WSB/EiJ homozygote: heterozygote: CAST/EiJ homozygote) that was conducted in R/qtl (Broman, *et al.*, 2008).




Chr 7
Chr 8
Chr 9
Chr 10
Chr 11
Chr 12
Chr 13
Chr 14
Chr 15
Frequency of Genotype
Chromosomal location (cM)
lLfz
EE
ED
DD

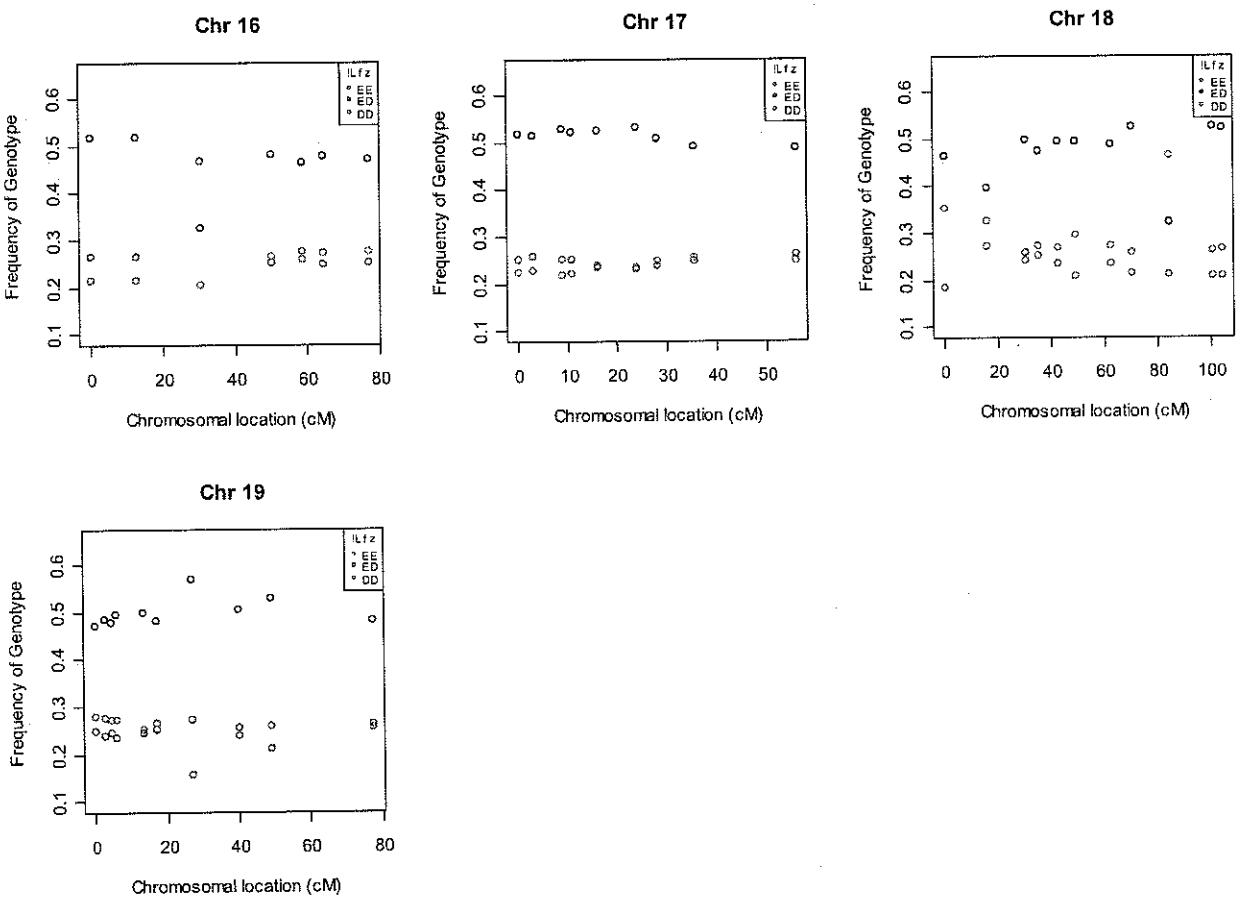
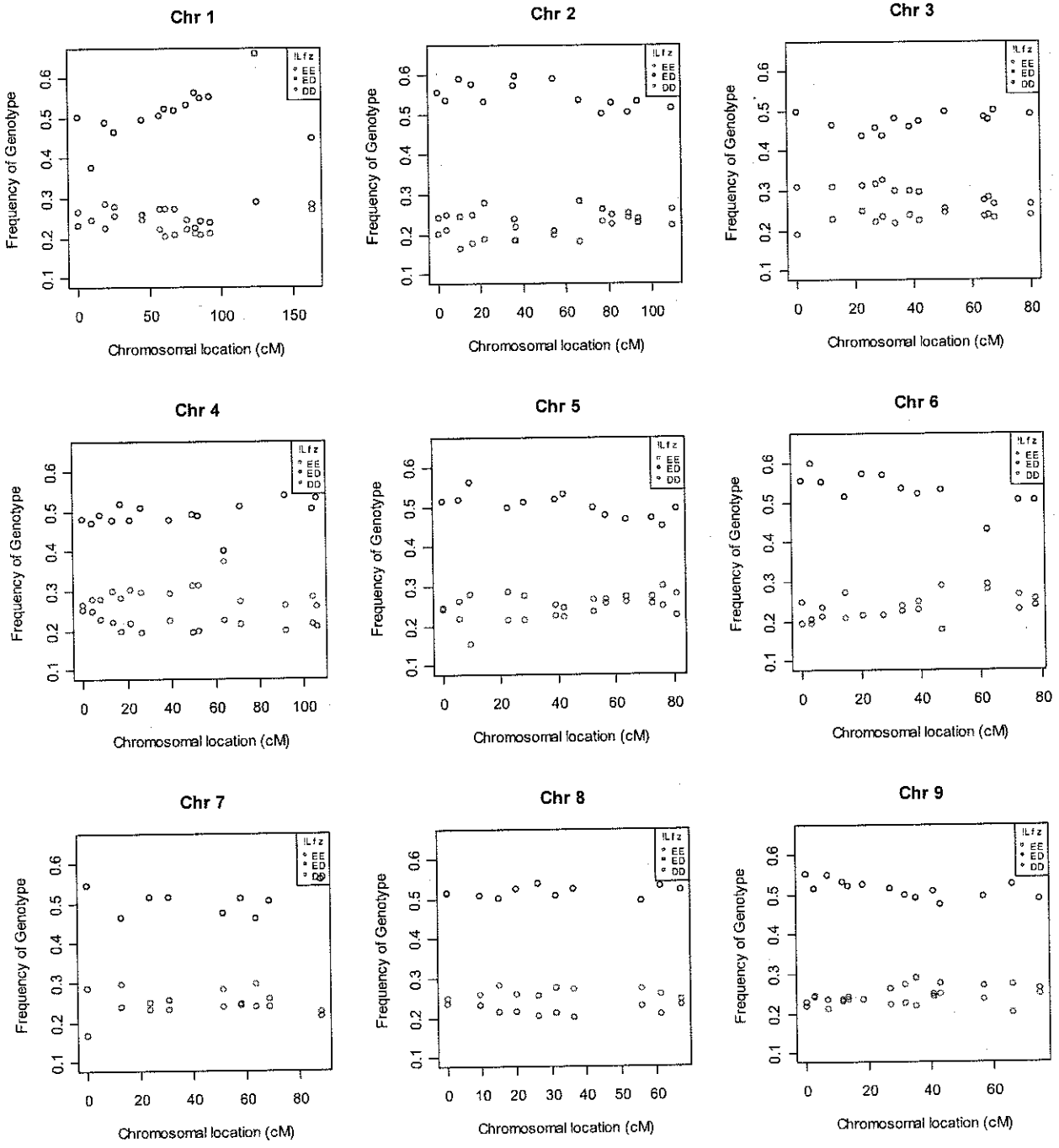
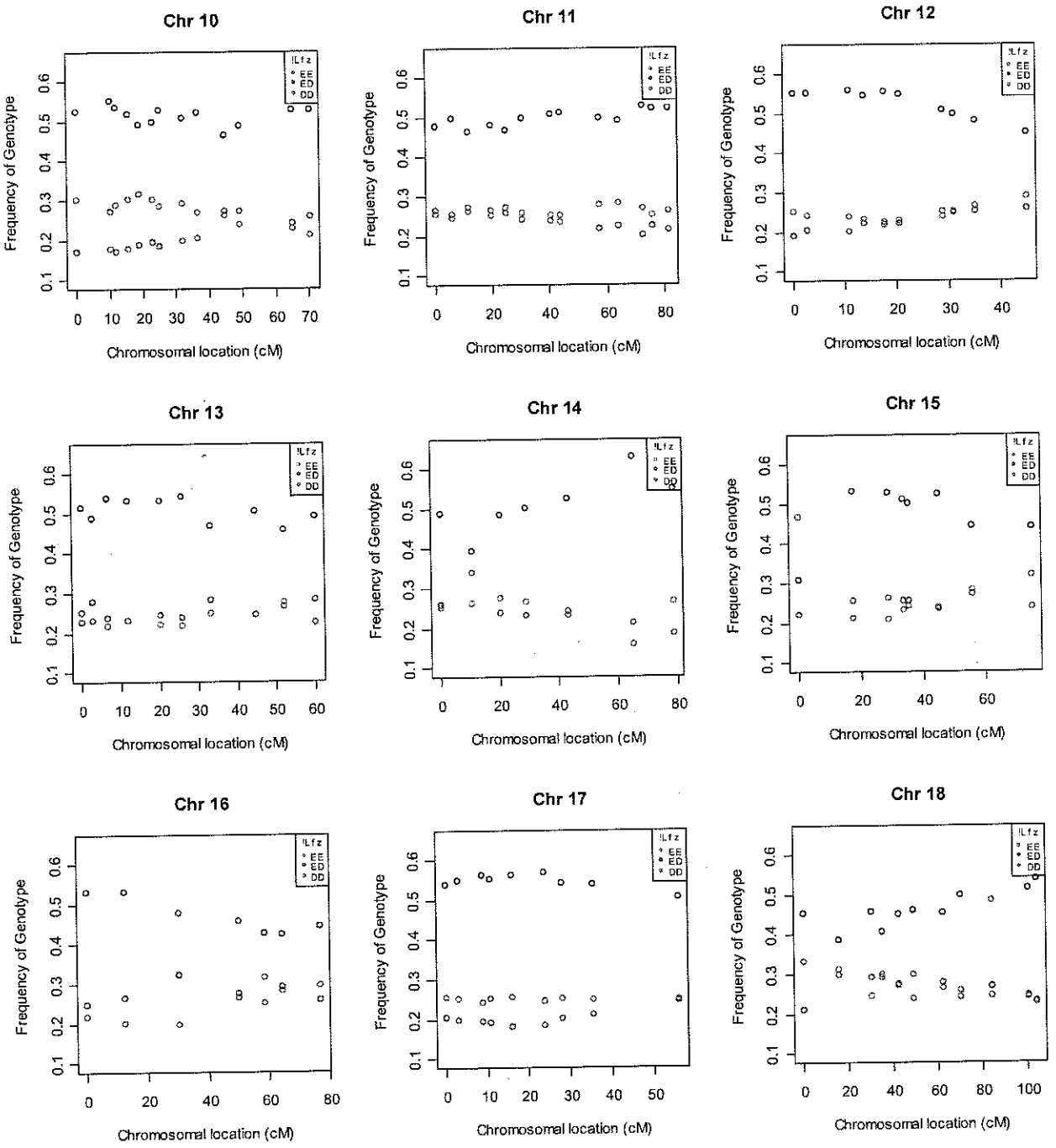


Figure 9. Plots, by chromosome, of the genotype frequencies observed at each SNP marker for all F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross. Genotype frequencies (calculated by dividing the total number of individuals in each individual genotypic class by the total number of individuals genotyped at the SNP) were plotted along the genetic linkage map calculated for the cross. All mouse chromosomes are acrocentric, so the left end of each chromosome plot corresponds to the chromosome end proximal to the centromere. Green circles represent the frequency of the WSB/EiJ homozygotic class, pink circles represent the frequency of the CAST/EiJ homozygotic class, and blue circles represent the frequency of the heterozygotic class. Expected genotype frequencies for these three classes are 0.25, 0.25, and 0.50, respectively.


Chr 1
Chr 2
Chr 3
Chr 4
Chr 5
Chr 6
Chr 7
Chr 8
Chr 9
Frequency of Genotype
Chromosomal location (cM)
ILfz
EE
ED
DD


Chr 10
Chr 11
Chr 12
Chr 13
Chr 14
Chr 15
Chr 16
Chr 17
Chr 18
Frequency of Genotype
Chromosomal location (cM)
lLfz
EE
ED
DD

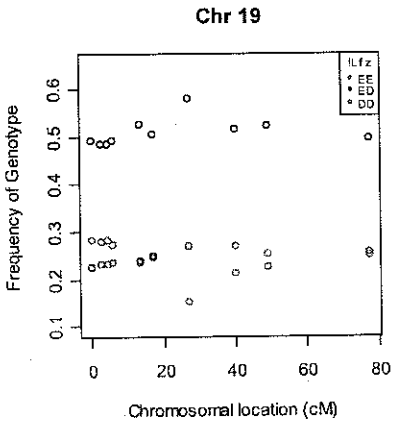
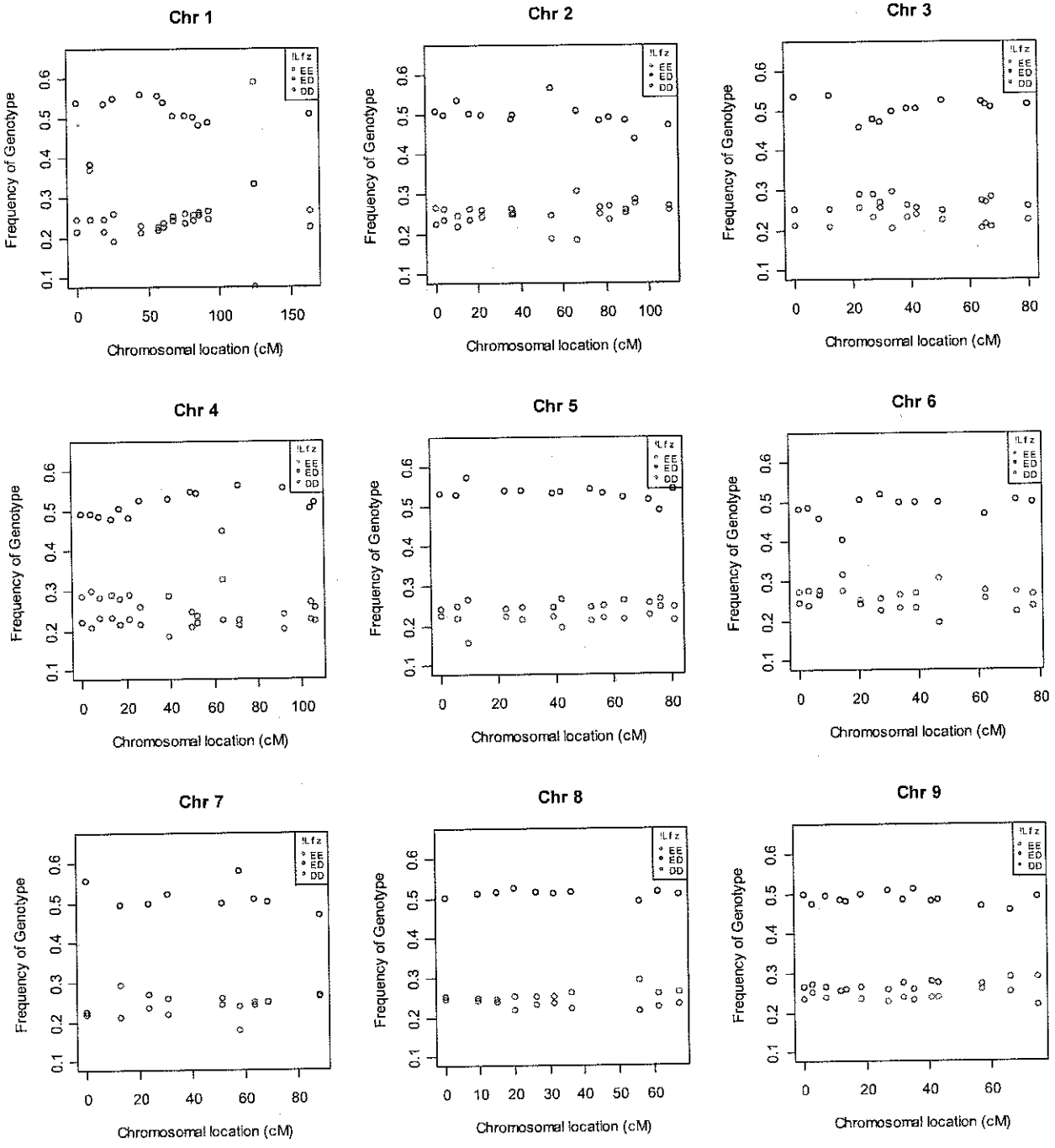
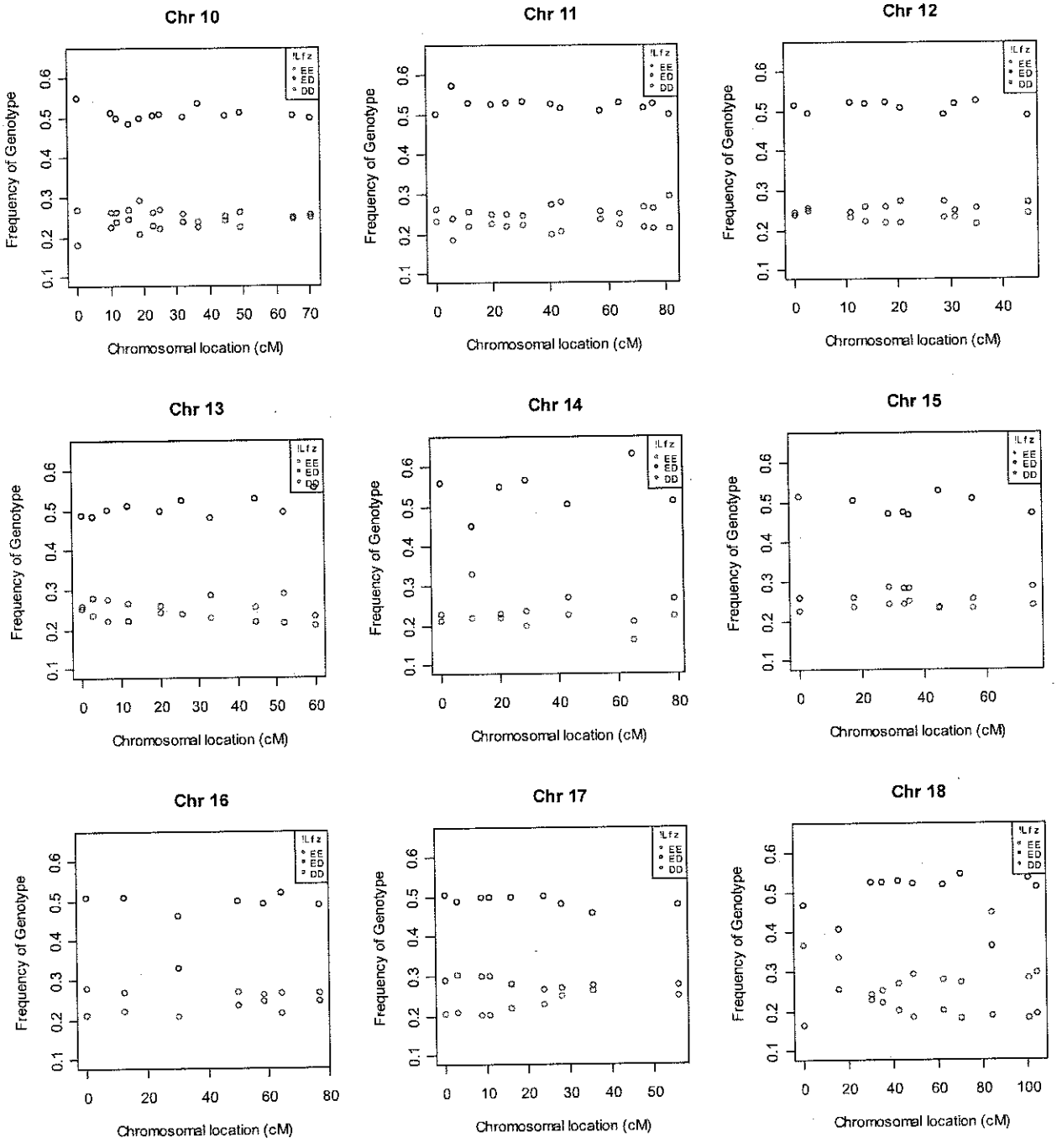


Figure 10. Plots, by chromosome, of the genotype frequencies observed at each SNP marker for the female F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross. Genotype frequencies (calculated by dividing the total number of individuals in each individual genotypic class by the total number of individuals genotyped at the SNP) were plotted along the genetic linkage map calculated for the cross. All mouse chromosomes are acrocentric, so the left end of each chromosome plot corresponds to the chromosome end proximal to the centromere. Green circles represent the frequency of the WSB/EiJ homozygotic class, pink circles represent the frequency of the CAST/EiJ homozygotic class, and blue circles represent the frequency of the heterozygotic class. Expected genotype frequencies for these three classes are 0.25, 0.25, and 0.50, respectively.


Chr 1
Chr 2
Chr 3
Chr 4
Chr 5
Chr 6
Chr 7
Chr 8
Chr 9
Frequency of Genotype
Chromosomal location (cM)
lLfz
EE
ED
DD


Chr 10
Chr 11
Chr 12
Chr 13
Chr 14
Chr 15
Chr 16
Chr 17
Chr 18
Frequency of Genotype
Chromosomal location (cM)
lLfz
EE
ED
DD

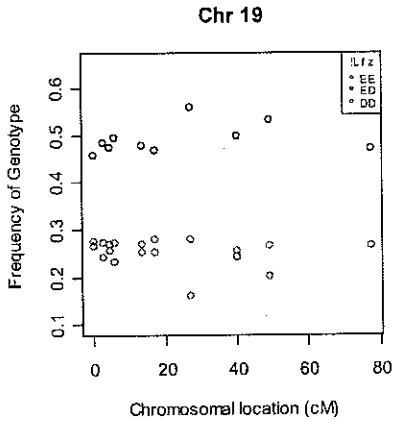


Figure 11. Plots, by chromosome, of the genotype frequencies observed at each SNP marker for the male F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross. Genotype frequencies (calculated by dividing the total number of individuals in each individual genotypic class by the total number of individuals genotyped at the SNP) were plotted along the genetic linkage map calculated for the cross. All mouse chromosomes are acrocentric, so the left end of each chromosome plot corresponds to the chromosome end proximal to the centromere. Green circles represent the frequency of the WSB/EiJ homozygotic class, pink circles represent the frequency of the CAST/EiJ homozygotic class, and blue circles represent the frequency of the heterozygotic class. Expected genotype frequencies for these three classes are 0.25, 0.25, and 0.50, respectively.

Discussion

As expected from observations in the wild that hybrids of the three house mouse groups *M. m. domesticus*,*M. m. musculus*, and *M. m. castaneus* do not survive nor reproduce as well as their parents, significant levels of transmission ratio distortion were detected in the F2 progeny of reciprocal intercrosses between wild-derived inbred strains of these three groups (Silver, 1995). While it could be argued that the transmission ratio distortion observed in this study is not due to underlying biological mechanisms at play, but rather to the ascertainment bias of the SNPs used, this explanation seems highly unlikely. The SNPs genotyped in this study were ascertained from the sequence of the classical inbred mouse strain C57BL/6, which is > 90% *M. m. domesticus* (Yang, *et al.*, 2007), and therefore, ascertainment bias would be predicted to cause genotyping errors more frequently for PWD/PhJ and CAST/EiJ SNP alleles than for WSB/EiJ SNP alleles. Thus, if the transmission ratio distortion observed was an artifact of ascertainment bias, it would be expected that that the distortion would consistently bias the genotypes in favor of WSB/EiJ alleles and that this bias would affect all regions of the genome relatively equally. Neither of these predicted effects of ascertainment bias is observed in this study: transmission ratio distortion is limited to distinct regions of the genome, and the observed genotype skews favor the three genotypic classes (the WSB/EiJ homozygotic class, the PWD/PhJ or CAST/EiJ homozygotic class, and the heterozygotic class) with relatively equal frequencies.

Interestingly, while a study of fine-scale phylogenetic discordance across the house mouse genome showed the strongest support for a phylogenetic tree of the three mouse groups with *M. m. musculus* and *M. m. castaneus* sister to one another (White, *et*

al., 2009), suggesting that these two groups are diverged from *M. m. domesticus* at the same time, more transmission ratio distortion was observed in the WSB/EiJ and PWD/EiJ reciprocal intercross than in the reciprocal intercross between WSB/CAST/EiJ. This difference in transmission distortion might reflect less gene flow between *M. m. musculus* and *M. m. domesticus* (as compared to *M. m. castaneus* and *M. m. domesticus*) or faster evolution of the *M. m. musculus* lineage (as compared to *M. m. castaneus* lineage). Reduced gene flow between *M. m. musculus* and *M. m. domesticus* would increase opportunities for independent evolution of both Dobzhanksy-Muller incompatibilities and meiotic drivers. Alternatively, because it would cause a faster accrual in the *M. m. musculus* lineage of the genetic changes that lead to Dobzhansky-Muller incompatibilities and the origin of new meiotic drivers and suppressors, more rapid evolution of the *M. m. musculus* lineage as compared to *M. m. castaneus* lineage could also generate higher levels of transmission ratio distortion in the WSB/EiJ X PWD/PhJ reciprocal intercross. As increased genetic drift would be expected to result in more rapid evolution, and genetic drift acts more strongly in smaller populations, the findings of previous studies that the effective population size is smaller in *M. m. musculus* than it is in *M. m. castaneus*, at 60,000 – 120,000 individuals for *M. m. musculus* and 200,000 – 400,000 individuals *M. m. castaneus*, provides some support for this second explanation (Geraldes, *et al.*, 2008). However, the possibility of increased isolation between *M. m. musculus* and *M. m. domesticus* as compared to *M. m. castaneus* and *M. m. domesticus* cannot be ruled out.

In comparing the transmission ratio distortion observed in the WSB/EiJ and PWD/EiJ reciprocal intercross to that observed in the WSB/EiJ and CAST/EiJ reciprocal

intercross, it is interesting to note that only one region, located on Chromosome 4, is found in common in the two crosses. While the sliding window test showed that the bias found for this region in favor of the WSB/EiJ allele as compared to either the PWD/PhJ or the CAST/EiJ allele was only significant in three of the data sets examined, including all the F2 progeny of the WSB/EiJ and PWD/EiJ reciprocal intercross, only the female F2 progeny of the WSB/EiJ and PWD/EiJ reciprocal intercross, and only the male F2 progeny of the WSB/EiJ and CAST/EiJ reciprocal intercross, visual inspection of the genotype frequencies shown in the Chromosome 4 plots in Figures 6 – 11 reveals that all six data groups examined in this study do exhibit the skew. One possible explanation for this common region of transmission ratio distortion may be that there is a Dobzhansky-Muller incompatibility between an allele found elsewhere in the genome that is specific to WSB/EiJ and an allele on Chromosome 4 that is common to both PWD/PhJ and CAST/EiJ. An alternative explanation of the common bias on Chromosome 4 toward the WSB/EiJ homozygotic class is that the distortion is being caused by a meiotic driver that arose in the *M. m. domesticus* lineage and has become uncoupled from its suppressor in the F1 hybrids. Such uncoupling, which could occur in male F1s if the suppressor of this driver was located on the X Chromosome or in both sexes if the suppressor was incompletely dominant, would lead to an increased frequency of transmission of the WSB/EiJ allele to the F2 generation, at the expense of either the PWD/PhJ or the CAST/EiJ allele. While the lack of a third reciprocal intercross between PWD/PhJ and CAST/EiJ prevents identification of potential *M. m. musculus* or *M. m. castaneus* meiotic drivers using similar reasoning, the detection of only one possible *M. m. domesticus* driver in this study suggests that Dobzhansky-Muller incompatibilities likely play a more

important role in the initial establishment of post-zygotic reproductive isolation, than do meiotic drivers.

In addition to comparing transmission ratio distortion between the two reciprocal intercrosses, transmission ratio distortion was also compared between the two sexes within each cross. In both reciprocal intercrosses, several regions of sex-specific transmission ratio distortion were identified, suggesting that the observed regions of transmission ratio distortion might be interacting with the sex chromosomes. This importance of the sex chromosomes in transmission ratio distortion, and thereby, in hybrid dysfunction and post-zygotic reproductive isolation, is unsurprising for a few reasons. First, the effective population sizes of the X and Y Chromosomes are three-quarters and one-quarter, respectively, of the effective population size of the autosomes, making it more likely *a priori* that the sex chromosomes would experience faster lineage sorting, and therefore, greater differentiation between groups than the autosomes (Geraldes, *et al.*, 2008). Second, previous studies have shown that, as would be expected, the sex chromosomes are, in fact, more differentiated than the autosomes between mouse groups (Geraldes, *et al.*, 2008). Finally, several previous studies have revealed a role of the X chromosome in hybrid male sterility, a form of post-zygotic reproductive isolation (Oka, *et al.*, 2004; Storchova, *et al.*, 2004; Good, *et al.*, 2008).

Interesting new insights into the relative importance of Dobzhansky-Muller incompatibilities and meiotic drivers in causing transmission ratio distortion can be gained by considering, in combination with the transmission ratio distortion observed in this study, data collected on the sex ratios of the F2 progeny in each reciprocal cross. Based on this sex ratio data, meiotic drive seems unlikely to be responsible for the

observed sex-specific transmission ratio distortion. For example, one possibility is that there is a meiotic driver located in the transmission ratio distortion region that is interacting with a suppressor on one (but not the other) of the sex chromosomes to generate the observed sex-specific transmission ratio distortion. This explanation is unlikely because it also requires that either X-carrying or Y-carrying sperm would consistently have a transmission advantage even on the native genetic background of the driver, and therefore that there would be sex ratio bias in the driver's host population. None of the three mouse groups used in this study show significant sex ratio biases. Alternatively, the observed transmission ratio distortion could be due to a meiotic driver located on one of the sex chromosomes that interacts with a suppressor located in the transmission ratio distortion region. Though more probable than the first explanation for the observed sex-specific transmission ratio distortion, this explanation also seems unlikely because a driver on one of the sex chromosomes that became uncoupled from its suppressor on an autosome, as would happen during the production of hybrid F1 gametes, would cause sex ratio distortion of the hybrid F2 generation. While there was severe sex ratio distortion ($p < 0.01$) in one direction of the WSB/EiJ X PWD/PhJ reciprocal intercross, this cross direction only contributed 13 individuals to the 564 individuals that were genotyped for the reciprocal intercross, and is unlikely to have made a contribution to the data set that was large enough to cause a detectable transmission ratio distortion skew (Michael White and John Hvala, unpublished data). In the WSB/EiJ X CAST/EiJ reciprocal cross, transmission ratio distortion that was coupled to a sex ratio skew may have been slightly more detectable, due to the presence of a moderately significant ($p < 0.10$) male skew in one direction of the cross (Michael White and John Hvala,

unpublished data). However, though this skewed direction, which made up 168 individuals of the 605 individuals genotyped in total for the cross, contributed more to the cross data set than did the skewed direction in the WSB/EiJ X PWD/PhJ reciprocal intercross, the mildness of the sex ratio bias also makes it unlikely in the WSB/EiJ X CAST/EiJ reciprocal intercross that there is a meiotic driver on the sex chromosomes interacting strongly enough with a suppressor on the autosomes that it would be detected as a transmission ratio distortion region in this study. A third possible explanation that involves meiotic drive causing the observed sex-specific transmission ratio distortion is that a driver/suppressor interaction exists between the pseudo-autosomal region of the sex chromosomes and the observed regions of transmission ratio distortion. Like the first two meiotic drive possibilities, this explanation seems unlikely because a driver located in the pseudo-autosomal region that was interacting with a suppressor located in the observed region of transmission ratio distortion, or vice versus, would cause a sex ratio skew in the F2 progeny in each direction of each reciprocal intercross. While sex ratio distortion is observed in one direction of both crosses, the other direction shows no evidence of significant sex ratio skew (Michael White and John Hvala, unpublished data). Instead, while meiotic drive cannot be ruled out completely, it seems most likely that the observed sex-specific transmission ratio distortion is due to Dobzhansky-Muller incompatibilities. As the majority of the transmission ratio distortion observed in both reciprocal intercrosses conducted in this study is sex-specific, and therefore, most likely to be caused by Dobzhansky-Muller incompatibilities, the findings of this study suggest a much more important role for Dobzhansky-Muller incompatibilities than for meiotic

drive in establishing hybrid dysfunction, and thereby, post-zygotic reproductive isolation, during early speciation.

While this study provides evidence for a larger relative contribution of Dobzhansky-Muller incompatibilities than meiotic drive to causing post-zygotic reproductive isolation in recently diverged populations, future studies will be needed to confirm these findings. In particular, studying transmission ratio distortion in the progeny of backcrosses of the F1's that were generated in this study to their parents could be used to subdivide the regions of transmission ratio distortion into those regions occurring due to meiotic drive in females, those occurring due to meiotic drive in males, and those that were not occurring in a sex-specific manner – i.e. regions distorted either due to meiotic drive in both sexes or as a result of Dobzhansky-Muller incompatibilities. Genotyping of the gametes of the F1s could also provide insight into which distorted regions were being caused by sex-specific meiotic drive. Additionally, future studies of transmission ratio distortion in a reciprocal intercross between PWD/PhJ and CAST/EiJ can be used to confirm findings made in this study, as well as gain new insights into the speciation history of the three mouse groups *M. m. domesticus*, *M. m. musculus*, and *M. m. castaneus*.

Acknowledgements

Thank to you to Bret Payseur for providing me with the opportunity to work on this project as well as for sharing his time, knowledge, support, guidance, and encouragement with me throughout its duration. Thank you also to Michael White for always willingly offering his advice and assistance, and especially, for generating all the mice used in this study; Beth Dumont for her assistance with R; Tim Wiltshire and Brian Steffy at the University of North Carolina Chapel Hill for generating the SNP genotype data used; and the entire Payseur lab, including Bret, Mike, and Beth, as well as Peicheng Jing, Ryan Haasl, John Hvala, Melissa Gray, Leslie Turner, Maria Stubbings, and Erin O'Flanagan for the insight and suggestions they provided during lab meetings. This work was supported by the Cargill-Benevenga Research Scholarship.

References

Broman KW, Wu H, with ideas from Churchill G, Sen S, and contributions from Yandell B. 2008. qtl: Tools for analyzing QTL experiments. R package version 1.09-43. Available from <http://www.rqtl.org>.

de Villena FPM, de la Casa-Esperón E, Briscoe TL, Sapienza C. 2000. A genetic test to determine the origin of maternal transmission ratio distortion: Meiotic drive at the mouse Om locus. Genetics 154(1):333-342.

de Villena FPM and Sapienza C. 2001. Nonrandom segregation during meiosis: the unfairness of females. Mammalian Genome 12: 331-339.

Dobzhansky, T. 1936. Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics 21: 113–135.

Fishman L and Willis JH. 2005. A novel meiotic drive locus almost completely distorts segregation in Mimulus (monkeyflower) hybrids. Genetics 169(1): 347-353.

Fitzpatrick BM. 2008. Dobzhansky-Muller model of hybrid dysfunction supported by poor burst-speed performance in hybrid tiger salamanders. Journal of Evolutionary Biology 21(1): 342-351.

Frank SA. 1991. Divergence of meiotic drive-suppression systems as an explanation for sex-biased hybrid sterility and inviability. Evolution 45(2):262-267.

Geraldes A, Basset P, Gibson B, Smith KL, Harr B, Yu HT, Bulatova N, Ziv Y, Nachman MW. 2008. Inferring the history of speciation in house mice from autosomal, X-linked, Y-linked, and mitochondrial genes. Molecular Ecology 17(24): 5349-5363.

Good JM, Handel MA, Nachman MW (2008) Asymmetry and polymorphism of hybrid male sterility during the early stages of speciation in house mice. Evolution 62: 50-65.

Hall MC, Willis JH. 2005. Transmission ratio distortion in intraspecific hybrids of *Mimulus guttatus*: Implications for genomic divergence. Genetics 170(1):375-386.

Harushima Y, Nakagahra M, Yano M, Sasaki T, and Kurata N. 2001. A genome-wide survey of reproductive barriers in an intraspecific hybrid. Genetics 159(2): 883-892.

Miller SA, Dykes DD, and Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl. Acids Res.* 16:1215.

Muller, H.J. 1940. Bearing of the Drosophila work on systematics. In: The New Systematics (J. S. Huxley, ed.), pp. 185–268. Clarendon Press, Oxford.

Oka A, Mita A, Sakurai-Yamatani N, Yamamoto H, Takagi N, Takano-Shimizu T, Toshimori K, Moriwaki K, Shiroishi T. 2004. Hybrid breakdown caused by substitution of the X chromosome between to two mouse subspecies. Genetics. 166: 913-924.

Phadnis N, Orr HA. 2009. A single gene causes both male sterility and segregation distortion in *Drosophila* hybrids. Science 323(5912):376-379. BioEssays 22(12):1085-1094.

R Development Core Team. 2008. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available from <http://www.R-project.org>.

Silver LM. 1995. Mouse genetics. Concepts and applications. New York: Oxford University Press.

Storchova R, Gregorova S, Buckiova D, Kyselova V, Divina P, Forejt J. 2004. Genetic analysis of X-linked hybrid sterility in the house mouse. *Mammalian Genome.* 15:515-524.

Tao Y and Hartl DL. 2003. Genetic dissection of hybrid incompatibilities between Drosophila simulans and D-mauritiana. III. Heterogeneous accumulation of hybrid incompatibilities, degree of dominance, and implications for Haldane's rule. Evolution 57(11): 2580-2598.

White MA. 2010. Reciprocal intercrosses between WSB/EiJ and PWD/PhJ and between WSB/EiJ and CAST/EiJ. Unpublished raw data. Contact: mawhite1@wisc.edu

White MA, Ane C, Dewey CN, Larget B, and Payseur BA. 2009. Fine-Scale Phylogenetic Discordance across the House Mouse Genome. PLoS Genetics 5(11): e1000729.

Willis JH. 2009. Origin of Species in Overdrive. Science 323(5912): 350-351.

Wiltshire, T and Steffy B. 2009. SNP genotypes data from crosses conducted by Michael White. Unpublished raw data. Contact: timw@unc.edu

Yang H, Bell TA, Churchill GA, and de Villena FPM. 2007. On the subspecific origin of the laboratory mouse. Nature Genetics 39(9): 110-1107.

Yang YY, Lin FJ, Chang HY. 2004. Sex ratio distortion in hybrids of *Drosophila albomicans* and *D-nasuta* [Abstact]. Zoological Studies [cited 2009 Feb 6] 43(3):622-628. Available from: ISI Web of Knowledge [v4.5] database.