The effect of temperature on sex determination

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CHAPTER 8

A microsatellite linkage map of the housefly, *Musca domestica*; implications for recombination rate and sex chromosome evolution

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Abstract

We present the first molecular marker linkage map for *Musca domestica* containing 35 microsatellite plus six visible markers. We report the development of 33 new microsatellite markers of which 19 are included in the linkage map. 236 F2 individuals from three mapping crosses were genotyped. Linkage maps of each cross were combined into a single composite map consisting of five linkage groups representing the five autosomes of the housefly. None of the markers mapped to either the X or the Y chromosome. The map covers a total of 229.6 cM with an average marker spacing of 4.4 cM spanning approximately 80.2% of the genome. We found up to 29% recombination in male houseflies confirming previous studies. Recombination frequencies on autosomes depended on the presence/absence of the male determining factor *M*, as predicted for early stage sex chromosomes. The linkage map will aid further genome studies, in particular those aimed at revealing the nature and location of the housefly autosomal sex determining factors and testing hypotheses of sex chromosome evolution.

Introduction

The housefly (*Musca domestica*) is a cosmopolitan species and an important disease vector for cattle and humans (Fotedar *et al.,* 1992). It is also of interest for the evolution of sex determination, as this species harbors several different sex determining systems (Dübendorfer *et al.,* 2002). Despite its medical and economic importance, and even though it has been studied for decades, remarkably little genomic mapping information is available of the housefly. Thus far, linkage studies in the housefly are constrained to back crosses with mutants carrying visible mutations (Hiroyoshi, 1961; Tsukamoto *et al.,* 1961; Wagoner, 1967; Hiroyoshi, 1977), and have mostly been used to localize sex determining factors, but also for mapping of other genes (Wagoner, 1969; Franco *et al.,* 1982; Denholm *et al.,* 1985; Tomita & Wada, 1989b; Denholm *et al.,* 1990; Çakir & Kence, 1996; Kozaki *et al.,* 2002; Hamm *et al.,* 2005; Kandemir *et al.,* 2006; Feldmeyer *et al.,* 2008; Kozielska *et al.,* 2008). Whereas several studies on mitochondrial genes, as well as population genetic studies based on mitochondrial sequences are available (Roehrdanz, 1993; Marquez & Krafsur, 2002; 2003; Cummings & Krafsur, 2005), we know of only one population genetic study where microsatellite markers have been used (Krafsur *et al.,* 2005). With the advancement of molecular marker techniques, a first microsatellite linkage map of the housefly can now be constructed.

Up to date, the overall consensus among housefly researchers has been that there is little or no recombination in male houseflies (Rubini *et al.,* 1980), similar to *Drosophila* where male recombination is completely absent (Morgan, 1914). Hiroyoshi (1961) found no recombination at all, whereas Sullivan (1961) and Milani (1967) observed some recombination in mutant strains with visible mutations, suggesting that recombination in males might be population dependent (Milani, 1967). In a later study, Lester *et al.* (1979) reported up to 31% male recombination in an Australian housefly strain. Rubini *et al.* (1980), however, attributed the rare occurrence of recombinants of heterozygous males and the appearance of mosaics to mitotic recombination. Few years later, Hiroyoshi *et al.* (1982) also found male recombination in low frequency in several Japanese populations. One aspect that all these studies on male recombination have in common, as also noted by Hiroyoshi *et al.* (1982), is that they investigated populations with autosomal sex determining factors. In the

housefly a diverse array of sex determining factors exist. In so called "standard" populations females are XX and males are XY (Dübendorfer *et al.,* 2002). All individuals are homozygous for the female determining factor (*F*) on chromosome IV. Males additionally possess the male determining factor (*M*) on the Y chromosome which suppresses *F* and leads to male development (Hediger *et al.,* 1998a). In some populations individuals are homozygous for *M* on an autosome and sometimes males carry multiple *M* factors on different autosomes (Franco *et al.,* 1982; Tomita & Wada, 1989b; Çakir & Kence, 1996; Hamm *et al.,* 2005; Feldmeyer *et al.,* 2008; Kozielska *et al.,* 2008). In most populations with autosomal *M*, in particular in populations with homozygous autosomal *M* males, females carry a dominant female determining factor F^D, which is insensitive to suppression by *M*, leading to female development even in the presence of *M* (Dübendorfer *et al.,* 2002). IN this study, we investigate recombination rates in three populations, one with XY males and two with autosomal *M* carrying males.

The evolution of sex chromosomes is thought to follow a certain pattern (Charlesworth *et al.,* 2005). After a so-called neo-sex chromosome has acquired sex determining function, recombination will gradually reduce along the chromosome starting from the sex chromosome locus. Due to lack of recombination the sex determining chromosome will gradually degrade. At some point another gene on a different chromosome might take over sex determining function either by transposition of the sex determining gene or evolution of a novel sex determining gene. The "old" sex chromosome may eventually vanish, if it does not contain essential genes anymore. This process is believed to have general application to many organisms with chromosomal sex determination and may also act in the housefly where *M* factors can be found on different autosomes in different populations, turning these autosomes into neo sex chromosomes. With our data we can compare recombination frequencies between autosomes with and without sex determining factors.

Hence, the aims of this paper are threefold. We present the first genetic linkage map of the housefly using molecular markers. By combining microsatellites with traditional visible markers on each of the five autosomes we directly assign the molecular markers and linkage groups to a particular chromosome. In addition, we provide further evidence for male recombination

in houseflies and we consider chromosomal recombination frequency in the context of sex chromosome evolution theory. We expect that our linkage map will be instrumental for future genome studies, such as revealing the nature of autosomal sex determining factors, for annotation of the housefly genome, and for further testing hypotheses of sex chromosome evolution.

Experimental procedures

Crosses

We studied the segregation of 35 microsatellites in combination with six visible markers in three different crosses. The microsatellites are a combination of the newly developed microsatellites that we report here, and microsatellites that have been published earlier (Endsley *et al.,* 2002; Chakrabarti *et al.,* 2004). For each cross we used a mutant marker strain (012345-1) recessive for visible traits on each of the five autosomes (*ali curly* (*ac*) on linkage group 1; *aristapedia* (*ar*) on 2; *brown body* (*bwb*) on 3; *yellow eyes* (*ye*) on 4; *snip wings* (*snp*) on 5) (see Tomita & Wada, 1989b). This strain has been used by several authors to determine the position of the male determining factor *M* in natural populations by back crossing wild type males with mutant females (Tomita & Wada, 1989b; Feldmeyer *et al.,* 2008; Kozielska *et al.,* 2008). Since the visible mutations have been cytologically assigned to chromosomes (Wagoner, 1967) we can directly associate the markers with linkage groups and chromosomes.

The wild type males in our crosses came from populations which contained both autosomal *M* factors and *M* on the Y chromosome. We individually crossed wild type males to mutant females. F1 male offspring were backcrossed to mutant females. Because of sex-linked inheritance of the phenotype, the F2 reveals the location of the *M* factor (for more details see Denholm *et al.,* 1983). For the linkage analysis we chose the strain FVG, collected in Faverges, France (2004), with autosomal *M* on chromosome II (this cross will be called M2-cross) and the strain WAD, collected in Warden, South Africa (2005), with autosomal *M* on chromosome III (this cross will be called M3-cross). Since females from the mutant strain are homozygous at almost all loci, these two crosses result in “male only” maps as recombination information will stem exclusively from males. The third cross was constructed by mating a female from the strain UML, collected in Umhlali, South Africa (2005), to an XY male

of the mutant strain (MY-cross). Males and females of the resulting F1, thus brothers and sisters, were mated to create the F2 generation. This cross resulted in recombination information for both female and male. We genotyped 58 offspring of the M2-cross, 98 offspring of the M3-cross and 80 offspring of the MY-cross, resulting in an overall number of 236 individuals for the combined linkage map.

Microsatellite development and genotyping

Genomic DNA of male houseflies was collected from four different strains; two laboratory strains (WHO, World Health Organization and the 012345-1 mutant strain, both obtained from Zürich) and two wild caught strains (FVG, Faverges, France and MID, Midlaren, The Netherlands). Males of these strains carried the Y chromosome. DNA was extracted using a standard proteinase K/salt-chloroform protocol and pooled for all stains.

An enriched library was made by Ecogenics GmbH (Zürich, Switzerland) from size selected genomic DNA ligated into SAULA/SAULB-linker (Armour *et al.,* 1994) and enriched by magnetic bead selection with biotin-labelled (GA) 13 and (TAC) 8 oligonucleotide repeats (Gautschi *et al.,* 2000). Of 951 recombinant colonies screened, 271 gave a positive signal after hybridization. Plasmids from 192 positive clones were sequenced of which 168 yielded microsatellite sequences. Fourty-three out of the 168 sequences were duplicates leaving 125 sequences that were analyzed with the software Tandem Repeat Finder (Benson, 1999) to identify the repeat motif, length and position of the repeat sequence. The microsatellite motives were tandem repeats of either CT (52%) or AG (48%). Primers were designed using the software PRIMER3 (Rozen & Skaletsky, 2000). Fourty-three sequences (34%) were either too small or the repeat flanking region was too small for primer design, leaving 82 sequences for which primers could be designed.

A total of six individuals (three females and three males from three different strains) were initially screened for marker amplification and polymorphism on a 5% agarose gel. Thirty-eight primer pairs failed to amplify or gave dubious amplification patterns and were discarded for further analysis. From the remaining fourty-four markers the forward primers were labeled with a fluorescent dye (FAM, HEX or NED). PCR reactions were performed in 1X

PCR buffer magnesium free (Promega) with 2.5 mM $MgCl_2$, 0.2mM dNTPs (Roche), 0.2µM of each primer, 0.4 units of Taq polymerase (Promega) and approximately 5ng of template DNA. The PCR profile was 1 cycle of 15 min at 95°C followed by 25 cycles of 30 sec at 94°C, 90 sec at the primer specific annealing temperature (Table 8.1), 60 sec at 72°C, and a final cycle of 10 min 72°C. Reactions were carried out in an Eppendorf mastercycler gradient machine. PCR products were analyzed on an ABI 3730 automatic sequencer with ROX-500 as size standard. The size of the fragments was calculated using GeneMapper 4.0 software (Applied Biosystems).

Of the 44 loci tested, eleven turned out to be monomorphic or gave unreliable results and 33 were polymorphic and suitable for use (Table 8.1). The nomenclature for the microsatellites is equivalent to Endsley *et al.* (2002), with Md referring to *M. domestica*, followed by the repeat type and the microsatellite sequence number. Additionally we developed one more microsatellite marker from available microsatellite sequences in GeneBank (Table 8.1).

Linkage analysis

We constructed a linkage map for each of the three crosses separately using JoinMap 3.0 (Van Ooijen & Voorrips, 2001). We used the population type code "CP" in JoinMap to allow for heterozygous and homozygous diploid parents and assigned genotype codes for each locus depending on the segregation type (for details see the JoinMap manual). All markers were tested for significant deviation from Mendelian segregation by χ^2 analysis ($p < 0.01$). Markers that deviated significantly from Mendelian expectations were included in linkage groups if their presence did not alter the order established without them. Marker placement was determined using a minimum LOD score threshold of 4.0. The Kosambi mapping function that incorporates the possibility of crossover interference was used to convert recombination frequencies into map distances (Kosambi 1944). After establishing separate linkage maps per cross we joined the linkage maps by using the "combine groups for map integration" command of JoinMap for groups that had enough overlapping markers and linkage was

Table 8.1: Newly developed microsatellite markers for the housefly, *M. domestica*. T_a = annealing temperature.

Name	GenBank Accession	Repeat length	Primer sequence		T_a
			Forward	Reverse	
MdCT220	FJ231915	13	TGCTGTTGTGACCTCGACTC	AAATGAAAAATTCCGCCAAG	56
MdCT222	FJ231914	44	GGCAATGACCTCTTGACCTT	AAACTCATAGCCTGCGTTCG	56
MdAG224	FJ231912	18	ACTGCCCTTCTCCACTTCCT	TTTGACCGAAGGTATGACCA	56
MdAG227	FJ231910	23	TATTGCAGCTCCCCCATAAG	TGGTCAATGGTTTCAGGTCA	56
MdAG228	FJ231909	15	CTCCAACCAGCCACCATATC	TTTTGGGTTCACGAGAGAGG	56
MdCT238	FJ231905	19	TGCAATGGAAAGACAACAGG	GTGGCGTTGATTTTCCTGAC	58
MdCT268	FJ231922	13	CTTCATCAGACCCACAATTTCA	TTAGCAAACGCCAACATCTG	56
MdCT289	FJ231930	16	TCGGCATATGAACGATTTGA	CGGTGACCCGCTACTCTTTA	58
MdCT297	FJ231934	22	AGACAAAGTTTCCAAGTGAGAATATG	TAGAGCGTTGCTCGCTTACA	56
MdAG290	FJ231931	13	CGACTGATTGTCAGCATGGA	CCATCTGCAAAAAGAACAATACA	56
MdCT291	FJ231932	22	CATCCGTCGGTTCATTCATT	ATGCAATCTTCTCGGCTCAC	56
MdCT302	FJ231937	22	AGTTTTCTCCGGCAGTCGT	GTCCAGTGTCACCAAATCCA	56
MdCT322	FJ231943	19	AACAATTTATGCCGGCTCAG	TCTTCAGGTCCTCTGCAACC	58
MdAG324	FJ231944	14	TTCCCATGAAAAATGTCAGC	CCACTCATTCTGGTACCTCCA	56
MdAG328	FJ231945	15	GTGGGGTGTGCACAAGAAG	CCCGTGTAGAAAGTGTGCAA	56
MdAG329	FJ231946	18	CTGCAATGATGTGAGGTTGG	AACAATTTATGCCGGCTCAG	60
MdCT339	FJ231949	15	GGCGCACACTCTACATAGCA	GAGCGTTTGAGAGCTTAGCA	56
MdAG357	FJ231952	31	TCGTAAGACTGGCGAAAAGAA	AGACTCCTCGGTCATCAAAAA	56
MdCT364	FJ231955	16	CACCCGTGTAGAAAGTGTGC	GGGGTGTGCACAAGAAGAAG	56
MdAG372	FJ231960	19	GTCCGACTTCTGGTCGAAAG	CATTTTCCGCTTCTGCTTGT	60
MdCT373	FJ231961	15	CGGATGGTGAGAATTGTTTTC	CAAGGGAGCTGAGAGAAACG	56
MdAG422	FJ231976	21	TAGAGCGTTGCTCGCTTACA	CTAGACAAAGTTTCCAAGTGAGAAT	56
MdCT234	FJ231907	20	GCTACAAACGGAATGACGA	TCGCGATCCTGGAAAATTAG	56
MdCT243	FJ231903	17	CGGTGGCAGATAAACTTCCT	CAGAAAATGAGCAGTGGTCAAA	58
MdAG247	FJ231900	12	CCTCCCACAAATGAATGGTC	ATTTTGAAGAAAGCCGCTCA	56
MdCT269	FJ231923	16	CGATGTAGAAGCTGGCTGTG	GCCTGCCTTCAGCTCTTCTA	58
MdCT276	FJ231926	17	TTCAAGGCGACTACTGCAAA	ACGACGTTTCGGTCTTGTCT	56
MdAG318	FJ231941	23	ATGAGCGTTTTGGATGTTCC	TTTCCGTTTAGATCGCATCC	56
MdCT319	FJ231942	15	GCGATTTCCGTCTCTCAGTC	TGGGTATGTCTCGCTTCCTT	56
MdAG336	FJ231948	20	ACAAACTGCTGGACAACGAA	GAACTTACACCGCAACAGCA	56
MdAG341	FJ231951	24	TGCCACAGAAGCATAAGAGG	TAGGCGCGAAGGGACTAATA	56
MdCT399	FJ231969	19	TTCGTATTCCAAAATCGGTTC	TTTTATCGGTTGGTGTCGTG	56
MdCT413	FJ231973	21	TCTTTCGCTCTCTCTCTCTCTAAAA	ACAAACCCAACCCTGAGAGA	56
MdCAG78[1]	AF380993	24	GCAAGGTGAAAAAGGTCCAG	CGGGAGYAGCATCCATTTTC	56

[1]sequence previously published in GenBank

sufficient. This was not possible for linkage group (=chromosome) IV as only the M2-cross yielded more than two linked markers on this group.

We note that the conventional way of constructing a linkage map is to analyze both sexes separately when recombination frequencies differ. As the number of linked markers to construct a "female only" map was too small and the number of linked markers increased by including female recombination information we included both sexes in one map (for the MY- and the combined map). To obtain pairwise recombination frequencies for all markers per linkage group, and also separate for each sex (MY-cross) we used the LINKMFEX.exe module of the LINKMFEX v2.3 program (R. Danzmann, University of Guelph, http://www.uoguelph.ca/~rdanzman/software/LINKMFEX). To determine whether recombination frequencies of autosomes carrying sex determining factors differ from autosomes without them, we performed a Wilcoxon rank sum test in R (R Development Core Team, 2006) by introducing a variable coding for autosomes with *M* or *F* (1) versus autosomes without (0). Since the number of similar marker pairs between the crosses is small, as well as the overall number of markers on certain linkage groups, we compared the average recombination frequency of all possible marker pairs per chromosome and cross.

Map length and coverage

Two approaches were used to estimate the map length of *M. domestica*: 1) G_{e1}: to compensate for the two chromosome ends beyond the outer most marker of the linkage group 2s (s = average spacing of the linkage map) were added to the length of each group (Fishman *et al.,* 2001); 2) G_{e2}: each linkage group was multiplied by the factor $(m + 1)/(m\text{-}1)$, where m is the number of markers in each linkage group, irrespective of markers mapping to the same location. The estimated map length is the sum of the revised length of all linkage groups (Chakravarti *et al.,* 1991). The final estimated map length (G_e) is the average of the two estimated map lengths. The observed map length was calculated as the length of the framework map (G_{of}). Map coverage then was calculated as G_{of} / G_e.

Results

A total of 236 F2 progeny and backcross parents from three crosses (referred to as M2, M3, and MY) were genotyped for 58 microsatellite markers. Of the 33 newly developed microsatellite markers 20 turned out to be informative in at least one of the crosses analyzed. Seventeen of the previously published 25 microsatellite markers (Endsley *et al.,* 2002; Chakrabarti *et al.,* 2004), plus one marker developed from a GenBank sequence, were informative in at least one of the crosses (Table 8.2). A total of 35 microsatellite markers, six visible mutations plus "sex" as seventh visible trait mapped into five linkage groups, which correspond to the five autosomes of the housefly. None of the microsatellite markers mapped to the X or the Y- chromosome. Three markers, MdCT222, MdAG228 and MdCA06, did not map to any of the linkage groups.

Table 8.2: Overview of informative markers per cross. Prefix Md has been omitted. Underlined markers were analyzed in more than one cross. Markers in parentheses did not map to any of the linkage groups. Earlier published markers are described in Endsley *et al.* (2002) and Chakrabarti *et al.* (2004).

Cross	No.offspring	Polymorphic markers	
		Newly developed	Earlier published
M2	58	CT238, CT291, CT289, CT297, CT322, CT339, CT364, CT373, AG224, AG227,AG290,AG324, AG422 (AG228, AG357)	CA104, CA117, CA119, CA121, CA148, CA154, CA155, CA224, CA226, HF25, HF31, HF33, HF44 (CAG34)
M3	98	CT238, CT289, CT297, CT302, CT339, CT364, CT373, AG224, AG324, AG328, AG357, AG372, AG422 (AG227, CT291, AG329, CT222)	CA104, CA154, CA170, HF33, HF44
MY	80	CT268, CT291, CT297, CT302, AG329, AG422, CAG78 (CT322, AG224, AG290)	CA104, CA117, CA170, CA202, CA224, CAG34, HF31, HF44 (CA06)

Table 8.3: Observed and estimated map lengths and coverage for each of the three crosses separately and the combined linkage map. Values are based on all five autosomes for the M2-cross and the combined map, but for the M3- and MY-cross no linkage group IV was available.

	M2	M3	MY	Combined
Observed map length (cM)	78	64	165	184
Estimated genome length (cM)	110.5	92.8	252.12	230.91
Coverage (%)	70.59	68.97	65.45	79.68
Number of markers	26	18	15	35

For the M2-cross (where males carry the *M* factor on autosome II) five linkage groups were found, representing all five autosomes ranging in size from 6-34 cM and consisting of 3-11 markers per group. The total linkage distance covered by these markers was 78 cM with an average spacing of 3.0 cM between markers for the whole framework map (Table 8.3). For the M3-cross (*M* factor on autosome III) linkage groups for autosomes I-III and V were found, ranging in size from 3-30 cM and consisting of 5-6 markers per group. The total map size was 64 cM with an average spacing of 3.2 cM between markers. The M2 and M3-crosses yielded recombination frequencies for males only, since the females are homozygous for almost all markers (see Experimental procedures for details). Although potentially possible, we did not construct maps separately for each sex in the MY-cross, because the number of markers per linkage group in females was mostly too small. For the MY-cross we found linkage groups for autosomes I-III and V, ranging in size from 12-62 cM and consisting of 3-8 markers per group. The total linkage distance covered was 165 cM, which is on average 2.3 times the size of the autosomal *M* maps, and with an average marker spacing of 9.2 cM. After joining the three maps, the combined map consisted of five linkage groups ranging in size from 7-62 cM, containing 3-14 markers per group and a total map size of 184 cM with an average spacing of 4.5 cM between markers (Figure 8.2).

The estimated map length for the combined map was 230.9 cM, which is the average of the two methods (see Experimental procedures), respectively 228.9 and 232.9 cM (Table 8.3). The combined map covers about 79.7% of the genome, calculated as the observed length of 184 cM divided by the estimated length of 230.9 cM.

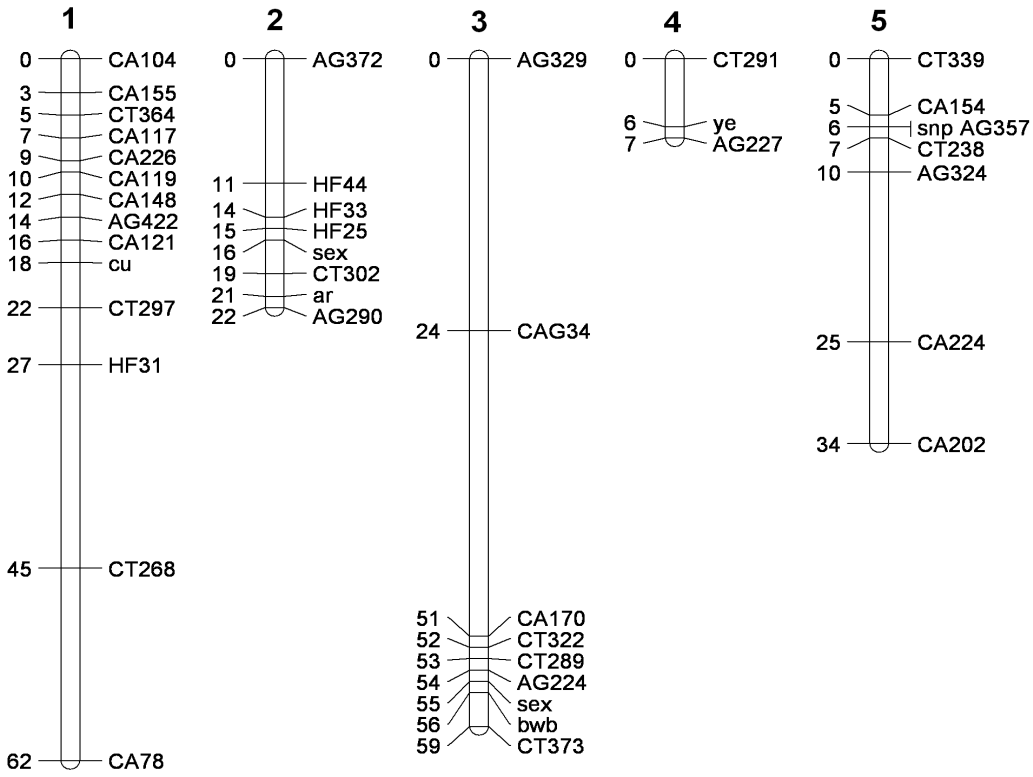

Figure 8.1: Linkage map of the housefly, from combining three different mapping populations. Markers are indicated on the right; map distances (in Kosambi cM) on the left of a chromosome. Linkage groups are arranged by chromosome number according to Wagoner (1967). The number of linkage groups corresponds to the number of autosomes, no markers were found on the sex chromosomes. Each chromosome contains one visible marker (*cu, ar, bwb, ye, snp*, see Experimental procedures), the marker "sex" occurs twice as it was once mapped with a strain that contained *M* on the second and once with a strain that contained *M* on the third autosome, and all other markers are microsatellites.

Based on 19 marker pairs distributed over four autosomes and mapped in both sexes, the average female recombination rate was estimated to be 1.8 times higher than in males (23% compared to 12%). Single pairwise recombination rates in males between markers with LOD>3 varied between 0-29%. Average pairwise recombination rates for all mapped markers ranged from 0.06 – 0.23 per autosome (Table 8.4). Autosomes carrying one of the sex determining factors (*M* or *F*) show significantly reduced recombination frequency compared to autosomes without sex determining factors (Wilcoxon rank sum test, W = 26299, $p<0.0001$), consistent with predictions of sex chromosome evolution.

Table 8.4: Average male recombination frequencies for all possible pairwise marker combinations per autosome for each cross. Values in parentheses indicate the number of marker pairs. Average recombination frequencies on chromosomes containing a sex determining factor is significantly reduced, indicated in bold (Wilcoxon rank sum test, W = 26299, $p<0.0001$).

Cross	Autosome				
	I	II	III	IV	V
MY	0.16 (21)	0.22 (3)	0.23 (6)	-	0.21 (3)
M2	0.17 (45)	**0.10** (15)	0.19 (15)	**0.08** (3)	0.21 (21)
M3	0.18 (10)	0.18 (10)	**0.06** (15)	-	0.19 (15)

Discussion

We present the first genetic linkage map of the housefly, *Musca domestica*, using microsatellite markers. With the help of visible markers that had previously been assigned to the five autosomes we were able to place 35 microsatellite markers on to five linkage groups representing the five autosomes. We did not find any markers linked to either the X or the Y chromosome, which is most likely due to technical reasons. Similar to the medfly (Stratikopoulos *et al.,* 2008), the X and Y chromosome of the housefly consist mainly of heterochromatin (Hediger *et al.,* 1998b). Heterochromatic regions are known to be refractory to cloning and sequencing strategies (International Human Genome Sequencing Consortium, 2004), which would explain their absence in our library.

The distribution of microsatellite loci along the linkage map appears to be non-random. In all five linkage groups we find clusters of markers towards one end of the linkage group. Non-random distribution of microsatellite markers along linkage groups has also been observed in for example rice, zebrafish and the medfly (Shimoda *et al.,* 1999; La Rota *et al.,* 2005; Stratikopoulos *et al.,* 2008). In rice the accumulation of microsatellites in certain regions of the genome is correlated with gene-rich regions (La Rota *et al.,* 2005), but in zebrafish it was attributed to the accumulation of CA/GT sequences in these chromosomal regions (Shimoda *et al.,* 1999).

The recombination density found in this study is quite low with 0.8 cM / Mb (total map size of 229.6 cM estimated in this study divided by 295 Mb according to Gao & Scott (2006), but is comparable to other Dipteran insects where recombination densities range between 0.1-3.1 cM / Mb (reviewed and discussed in Wilfert *et al.,* 2007).

Studies on housefly male recombination have found varying results, ranging from no recombination (Hiroyoshi, 1961; Rubini *et al.,* 1980) up to 31% (Lester *et al.,* 1979). With our crosses we confirm the occurrence of recombination in males as we find recombination frequencies up to 29%, thus supporting the claim of Lester *et al.* (1979) to revise the assumption of recombination absence in male houseflies. We did not only find recombination in crosses with autosomal *M* males but also in the MY-cross on all autosomes (Table 8.4). It is not possible to discern whether this is due to crossing two different strains, i.e. two unrelated genomes disrupt recombination suppression in males, or whether recombination actually occurs widespread in "standard" XY populations.

The occurrence of male determining factors on different autosomes in various populations make the housefly a suitable organism for studying sex chromosome evolution. All hitherto studies of male recombination have in common that they investigated populations with autosomal *M*. From studies investigating frequencies of sex determining factors we know that F^D and autosomal *M* often co-occur in populations (Tomita & Wada, 1989b; Kozielska *et al.,* 2008, Feldmeyer *et al.,* 2008). This has two important implications for the evolution of recombination suppression as envisaged in the hypothesis of Charlesworth *et al.,* (2005). First, autosomal *M* chromosomes can recombine in females with F^D which makes it harder for any male recombination suppressor

to settle on the *M* carrying autosome. Second, F^D carrying females are the heterogametic sex and one would rather expect females to evolve reduced recombination rates. Charlesworth *et al.* (2005) predict that one should be able to detect differences in recombination frequency between differentially advanced sex chromosomes. We have indications that the recombination frequency is reduced on chromosomes with sex determining function as both *M* carrying autosomes II and III, and the *F* carrying autosome IV were found to have reduced recombination. Our analysis is still limited in resolution since we can only compare recombination frequencies of a limited number of shared markers between the crosses. In addition, we use all possible pairwise combinations instead of only adjacent marker pairs which violates independence of used recombination rates. Ideally one should compare recombination frequencies of autosomes with and without *M*, which may allow to (1) estimate the relative time that has passed since the autosome has acquired sex determining function, assuming that the lower the recombination frequency, the longer *M* is located on the autosome; (2) investigate whether similar changes take place on different chromosomes with sex determining function; (3) study sex chromosome evolution in a male versus female heterogametic system.

We hope that this linkage map will serve as starting point for further gene mapping studies such as identifying economically important insecticide resistance genes, locating and characterizing sex determining factors, and to further test hypotheses of sex chromosome evolution. Finally we express the wish that the map will increase the chance of a future housefly genome project (Gao & Scott, 2006).

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