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A microsatellite marker linkage map of the housefly, *Musca domestica*: evidence for male recombination

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Running title: Linkage map of the housefly

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 were genotyped from three crosses yielding a linkage map consisting of five linkage groups that represent the five autosomes of the housefly. 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 in contrast to most previous studies. The linkage map will add to genetic studies of the housefly.

Introduction

The housefly (*Musca domestica*) is a cosmopolitan species and an important disease vector for livestock and humans (Fotedar *et al.*, 1992). Besides its medical and economic importance, it is also of interest for the evolution of sex determination, as this species harbors several different sex determining systems (Dübendorfer *et al.*, 2002). Even though it has been studied for decades, remarkably little genomic mapping information is available of the housefly and there is a strong call for a genome sequencing project (Gao & Scott, 2006; Scott *et al.*, 2009). 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). These studies have mostly been aimed at localizing sex determining factors, but also at mapping of other genes (Wagoner, 1969; Franco *et al.*, 1982; Denholm *et al.*, 1985; Tomita & Wada, 1989; 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; Hamm & Scott, 2009). There have also been several population genetic studies of houseflies based on mitochondrial sequences (Roehrdanz, 1993; Marquez & Krafsur, 2002, 2003; Cummings & Krafsur, 2005), but we know of only one study that used microsatellite

markers (Krafsur *et al.*, 2005). Here we developed 33 new microsatellite markers to augment the number of molecular markers that can be used in genetic studies.

In the housefly a diverse array of sex determining factors occurs. 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 dominant 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, 1989; Çakir & Kence, 1996; Hamm *et al.*, 2005; Feldmeyer *et al.*, 2008; Kozielska *et al.*, 2008; Hamm & Scott, 2009). In some populations with heterozygous autosomal *M* males, and in all populations with homozygous autosomal *M* males, females carry a dominant female determining factor F^D. F^D is insensitive to suppression by *M*, leading to female development even in the presence of *M* (Dübendorfer *et al.*, 2002, Hediger *et al.*, 2010).

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. 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 this study, we use microsatellite markers to investigate male recombination rates in three populations, one with XY and two with autosomal *M* carrying males.

The aim of this paper is twofold. We present the first genetic linkage map of the housefly using molecular markers. By combining microsatellite markers with traditional visible markers on each of the five autosomes we assign the molecular markers to each of five linkage groups. In addition, we provide further evidence for male recombination in houseflies. We expect that our linkage map will be instrumental for future genome studies, such as revealing the nature of autosomal sex determining factors and for annotation of the housefly genome.

Results

A total of 236 F2 progeny and backcross parents from three crosses (referred to as M2, M3, and MY) were genotyped with 58 microsatellite markers. Of the 33 newly developed microsatellite markers 20 turned out to be informative in at least one of the crosses analyzed. Additionally, seventeen of the previously published 25 microsatellite markers (Endsley *et al.*, 2002; Chakrabarti *et al.*, 2004), plus one marker developed from a GeneBank sequence, were informative in at least one of the crosses (Table 1). A total of 35 microsatellite markers, six frequently used visible mutations plus the trait "sex" were mapped onto five linkage groups, which correspond to the five autosomes of the housefly (Wagoner, 1967). 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 1

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 2). For the

Table 2

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 possible, we did not construct maps separately for females and males 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 distance covered was 165 cM, which is on average 2.3 times the size of the autosomal *M* based 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 (Fig. 1).

Figure 1

The estimated map length for the combined map was 230.9 cM, which is the average of two different methods (see Experimental procedures), 228.9 and 232.9 cM respectively. 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. The total size of the *M. domestica* genome is predicted to be 309-312 Mbp (Gao & Scott, 2006).

Based on 19 marker pairs which were distributed over four autosomes and mapped in both sexes, the average recombination rate was estimated to be 1.92 times higher in females than in males (23% compared to 12%). Single pairwise recombination rates in males between markers with LOD>3 varied between 0-0.29. Average pairwise recombination rates for all mapped markers ranged from 0.04 – 0.28 per autosome (Table 3).

Table 3

Statistical analysis indicated that the full model was not significantly better than the additive model (Appendix Table S1). Further model reduction indicated that removing the

variable "chromosome" from the additive model had no significant effect, however removing the variable "cross" did. Specifically, it appeared that cross M2 showed significantly lower recombination rates than the other two crosses (Appendix Table S2; chromosome IV was not included in the analysis).

Discussion

We present the first genetic linkage map of the housefly, *Musca domestica*, based on molecular 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 five linkage groups representing the five autosomes identified by Wagoner (1967). We did not find any markers linked to either the X or the Y chromosome. Similar to the medfly *Ceratitis capitata* (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). At this point, we do not know the reason for aggregation of microsatellite markers in the housefly linkage map.

The recombination density found in this study is 0.74 cM / Mb (total map size of 229.6 cM estimated in this study divided by 310 Mb according to Gao & Scott (2006)), thus

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, 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 XY males on all autosomes (Table 3). However, 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.

Recombination frequencies in males may reflect the age of the sex-determining mechanism (Ohno, 1967; Rice, 1996; Charlesworth *et al.*, 2005). After a standard chromosome has acquired sex-determining function (so-called neo-sex chromosome), 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 an existing or the emergence of a novel sex-determining gene. The "old" sex chromosome may eventually vanish, if it does not contain essential genes anymore. Spread of recombination suppressors in the genome may eventually lead to genome-wide reduction in crossover frequencies. This process is believed to have general application to organisms with chromosomal sex-determination, and may also act in the housefly where sex-determining factors can be found on different autosomes in different populations, turning these autosomes into neo-sex chromosomes. In this respect the housefly is an interesting study organism for sex-chromosome evolution research, as it

harbors different sex determining mechanisms and male and female heterogametic systems can be compared within a single species.

We hope that this linkage map will serve as starting point for further gene mapping studies in the housefly, such as to identify economically important insecticide resistance genes, to localize and characterize sex determining factors, and to further test hypotheses of sex chromosome evolution. We end with the wish that the linkage map will contribute to the realization of a housefly genome project (Gao & Scott, 2006; Scott *et al*., 2009).

Experimental procedures

Crosses

We studied the segregation of 35 molecular markers in combination with six visible markers in three different housefly crosses. The molecular markers are a subset of 33 newly developed microsatellite markers that we report here, and microsatellite markers that have been published earlier (Endsley *et al.,* 2002; Chakrabarti *et al.,* 2004). For each cross we used a mutant marker strain (named 012345-1) recessive for visible traits on each of the five autosomes (*ali curve* (*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, 1989). 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, 1989; 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, autosomal *M* factors and *M* located 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 generation 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" linkage maps as recombination information will stem exclusively from males. The third cross involved 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 generation, thus brothers and sisters, were mated to create the F2 generation. This cross yielded recombination information for both females and males. 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 construction of 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 Standard Reference Strain and the 012345-1 mutant strain, both obtained from D. Bopp, University of Zürich, Switzerland) 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. Forty-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). Forty-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 forty-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 (Supporting Information Table S2), 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 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 (Supporting Information Table S3).

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 (logarithm of odds) 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 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). Hence, our overall map reflects the ordering of markers, but the relative recombination frequencies differ per strain and sex. The recombination frequencies for all possible marker pairs in each cross were estimated using the LINKMFEX.exe module of the LINKMFEX v2.3 program (R. Danzmann, University of Guelph, http://www.uoguelph.ca/~rdanzman/software/LINKMFEX).

Recombination frequencies were analyzed with logistic models, using the 'glm' procedure in R version 2.9.2 (R Development Core Team, 2009). To correct for

overdispersion the 'family = quasibinomial' option was chosen and F-tests were used to asses statistical significance (Crawley 2005, pp. 256).

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 $(m + 1)/(m - 1)$, 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.

References

Armour, J.A., Neumann, R., Gobert, S. and Jeffreys, A.J. (1994) Isolation of human simple repeat loci by hybridization selection. *Hum Mol Genet* **3**: 565-599.

Benson, G. (1999) Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res* **27**: 573-580.

Çakir, S. and Kence, A. (1996) The distribution of males having XY and XX chromosomes in housefly populations (Diptera: Muscidae) of Turkey. *Genetica* **98**: 205-210.

Chakrabarti, S., Kambhampati, S., Grace, T. and Zurek, L. (2004) Characterization of microsatellite loci in the house fly, *Musca domestica* L. (Diptera: Muscidae). *Mol Ecol* **4**: 728-730.

Chakravarti, A., Lasher, L.K. and Reefer, J.E. (1991) A maximum likelihood method for estimating genome length using genetic linkage data. *Genetics* **128**: 175-182.

Charlesworth, D., Charlesworth, B. and Marais, G. (2005) Steps in the evolution of heteromorphic sex chromosomes. *J Hered* **95**: 118-128.

Crawley, M.J. (2005) Statistics: an introduction using R. John Wiley and Sons, Chirchester, UK.

Cummings, M.A. and Krafsur, E.S. (2005) Spatial diversity in mitochondrial cytochrome c oxidase in house flies. *Med Vet Entomol* **19**: 53-59.

Denholm, I., Franco, M.G., Rubini, P.G. and Vecchi, M. (1983) Identification of a male determinant on the X-chromosome of house-fly (*Musca domestica* L) populations in Southeast England. *Genet Res* **42**: 311-322.

Denholm, I., Franco, M.G., Rubini, P.G. and Vecchi, M. (1985) Geographical variation in house-fly (*Musca domestica* L) sex-determinants within the British Isles. *Genet Res* **47**: 19-27.

Denholm, I., Rubini, P.G., Rovati, C. and Vecchi, M. (1990) Genetic basis of sex determination in two South African strains of house fly (*Musca domestica* L). *S Afr J Sci* **86**: 41-43.

Dübendorfer, A., Hediger, M., Burghardt, G. and Bopp, D. (2002) *Musca domestica*, a window on the evolution of sex determining mechanisms in insects. *Int J Dev Biol* **46**: 75-79.

Endsley, M.A., Baker, M.D. and Krafsur, E.S. (2002) Microsatellite loci in the house fly, *Musca domestica* L. (Diptera : Muscidae). *Mol Ecol* **2**: 72-74.

Feldmeyer, B., Kozielska, M., Kuijper, B., Weissing, F.J., Beukeboom, L.W. and Pen, I. (2008) Climatic variation and the geographical distribution of sex determining mechanisms in the housefly. *Evol Ecol Res* **10**: 797-809.

Fishman, L., Kelly, A.J., Morgan, E. and Willis, J.H. (2001) A genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. *Genetics* **159**: 1701-1716.

Fotedar, R., Banerjee, U., Singh, S., Shriniwas and Verma, A.K. (1992) The housefly (*Musca domestica*) as a carrier of pathogenic microorganisms in a hospital environment. *J Hosp Infect* **20**: 209-215.

Franco, M.G., Rubini, P.G. and Vecchi, M. (1982) Sex determinants and their distribution in various populations of *Musca domestica* L of Western Europe. *Genet Res* **40**: 279-293.

Gao, J. and Scott, J.G. (2006) Use of quantitative real time polymerase chain reaction to estimate the size of the house fly *Musca domestica* genome. *Insect Mol Biol* **15**: 835-837.

Gautschi, B., Tenzer, I., Müller, J.P. and Schmid, B. (2000) Isolation and characterization of microsatellite locie in the bearded vulture (*Gypaetus barbatus*) and cross amplification in three Old World vulture species. *Mol Ecol* **9**: 2193-2195.

Hamm, R.L., Shono, T. and Scott, J.G. (2005) A cline in frequency of autosomal males is not associated with insecticide resistance in house fly (Diptera : Muscidae). *J Econ Entomol* **98**: 171-176.

Hamm, R.L. and Scott, J.G. (2009) A high frequency male determining factor in male *Musca domestica* (Diptera: Muscidae) from Ipswich, Australia. *J Med Entomol* **46**: 169-172.

Hediger, M., Minet, A.D., Niessen, M., Schmidt, R., Hilfiker-Kleiner, D., Cakir, S., Nöthiger, R. and Dübendorfer, A. (1998a) The male-determining activity on the Y chromosome of the housefly (*Musca domestica* L.) consists of separable elements. *Genetics* **150**: 651-661.

Hediger, M., Niessen, M., Müller-Navia, J., Nöthiger, R. and Dübendorfer, A. (1998b) Distribution of heterochromatin on the mitotic chromosomes of *Musca domestica* L. in relation to the activity of male-determining factors. *Chromosoma* **107**: 267-271.

Hediger, M., Henggeler, C., Meier, N., Perez, R., Saccone, G. Bopp, D. (2010) Molecular characterization of the key switch *F* provides a basis for understanding the rapid divergence of the sex-determining pathway in the housefly. *Genetics* **184**: 155-170.

Hiroyoshi, T. (1961) The linkage map of the housefly, *Musca domestica* L. *Genetics* **46**: 1373-1380.

Hiroyoshi, T. (1977) Some new mutants and revised linkage maps of housefly, *Musca domestica* L. *Jap J Gen* **52**: 275-288.

Hiroyoshi, T., Fukumori, Y. and Inoue, H. (1982) Male crossing-over and location of the male-determining factor on the third chromosome in a III^{M}-type strain of the housefly. *Jap J Gen* **57**: 231-239.

International Human Genome Sequencing Consortium (2004) Finishing the euchromatic sequence of the human genome. *Nature* **431**: 931-945.

Kandemir, I., Kandemir, G., Kence, M. and Kence, A. (2006) Map position of phosphoglucomutase (Pgm) locus on autosome IV of house fly (Diptera: Muscidae). *J Econ Entomol* **99**: 2087-2090.

Kosambi, D.D. (1944) The estimation of map distances from recombination values. *Ann Eugen* **12**: 172-175.

Kozaki, T., Shono, T., Tomita, T., Taylor, D. and Kono, Y. (2002) Linkage analysis of an acetylcholinesterase gene in the house fly Musca domestica (Diptera : Muscidae). *J Econ Entomol* **95**: 129-133.

Kozielska, M., Feldmeyer, B., Pen, I., Weissing, F.J. and Beukeboom, L.W. (2008) Are autosomal sex-determining factors of the housefly (*Musca domestica*) spreading north? *Genet Res* **90**: 157-165.

Krafsur, E.S., Cummings, M.A., Endsley, M.A., Marquez, J.G. and Nason, J.D. (2005) Geographic differentiation in the house fly estimated by microsatellite and mitochondrial variation. *J Hered* **96**: 502-512.

La Rota, M., Kantety, R., Yu, J.-K. and Sorrells, M. (2005) Nonrandom distribution and frequencies of genomic and EST-derived microsatellite markers in rice, wheat, and barley. *BMC Genomics* **6**: 23.

Lester, D.S., Crozier, R.H. and Shipp, E. (1979) Recombination in the male housefly, *Musca domestica*. *Experientia* **35**: 175-176.

Marquez, J.G. and Krafsur, E.S. (2002) Gene flow among geographically diverse housefly populations (*Musca domestica* L.): A worldwide survey of mitochondrial diversity. *J Hered* **93**: 254-259.

Marquez, J.G. and Krafsur, E.S. (2003) Mitochondrial diversity evaluated by the single strand conformation polymorphism method in African and North American house flies (*Musca domestica* L.). *Insect Mol Biol* **12**: 99-106.

Milani, R. (1967) The genetics of *Musca domestica* and of other Muscoid flies. In: *In Genetics of Insect Vectors of Disease* (J. W. Wright and R. Pal, eds), pp. 315-369. Elsevier, Amsterdam.

Morgan, T.H. (1914) No crossing over in the male of *Drosophila* of genes in the second and third pairs of chromosomes. *Biol Bull* **26**: 195-204.

Ohno , S. (1967) *Sex Chromosomes and Sex-linked Genes*. Springer, New York, USA.

R Development Core Team (2009) R: A language and environment for statistical computing. R foundation for statistical computing Vienna, Austria, http://www.R-project.org.

Rice, W.R. (1996) Evolution of the Y sex chromosome in animals. *Bioscience* **46**: 331-334

Roehrdanz, R.L.L. (1993) Genome organization and restriction site map of the mitochondrial DNA of the housefly (*Musca domestica*). *Genome* **36**: 367-371.

Rozen, S. and Skaletsky, H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (S. Krawetz and S. Misener, eds), pp. 365-386. Human Press, Totowa, NJ.

Rubini, P.G., Vecchi, M. and Franco, M.G. (1980) Mitotic recombination in *Musca domestica* L. and its influence on mosaicism gynandromorphism and recombination in males. *Genet Res* **35**: 121-130.

Scott, J.G., Liu, N., Kristensen, M. and Clark, A.G. (2009) A case for sequencing the genome of *Musca domestica* (Diptera: Muscidae). *J. Med. Entomol.* **46**: 175-182.

Shimoda, N., Knapik, E.W., Ziniti, J., Sim, C., Yamada, E., Kaplan, S., Jackson, D., de Sauvage, F., Jacob, H. and Fishman, M.C. (1999) Zebrafish genetic map with 2000 microsatellite markers. *Genomics* **58**: 219-232.

Stratikopoulos, E.E., Augustinos, A.A., Petalas, Y.G., Vrahatis, M.N., Mintzas, A., Mathiopoulos, K.D. and Zacharopoulou, A. (2008) An integrated genetic and cytogenetic

map for the Mediterranean fruit fly, *Ceratitis capitata*, based on microsatellite and morphological markers. *Genetica* **133**: 147-157.

Sullivan, R.L. (1961) Linkage and sex limitation of several loci in the housefly. *J Hered* **52**: 282-286.

Tomita, T. and Wada, Y. (1989) Multifactorial sex determination in natural populations of the housefly (*Musca domestica)* in Japan. *Jap J Gen* **64**: 373-382.

Tsukamoto, M., Baba, Y. and Hiraga, S. (1961) Mutations and linkage groups in Japanese strains of the housefly. *Jap J Gen* **36**: 168-174.

Van Ooijen, J.W. and Voorrips, R.E. (2001) JoinMap® 3.0, Software for the calculation of genetic linkage maps. Wageningen, the Netherlands, Plant Research International.

Wagoner, D.E. (1967) Linkage group-karyotype correlation in the house fly determined by cytological analysis of X-ray induced translocations. *Genetics* **57**: 729-739.

Wagoner, D.E. (1969) Presence of male determining factors found on three autosomes in the house fly, *Musca domestica*. *Nature* **223**: 187-188.

Wilfert, L., Gadau, J. and Schmid-Hempel, P. (2007) Variation in genomic recombination rates among animal taxa and the case of social insects. *Heredity* **98**: 189-197.

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Table 1. Overview of informative markers per cross. Prefix Md for *Musca domestica* 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 2. 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, for the M3- and MY-cross linkage group IV was not 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 (%)	70.6	69.0	65.5	79.7
Number of markers	26	18	15	35

Table 3. Average male recombination frequencies for adjacent marker pairs per autosome and cross. Values in parentheses indicate the number of marker pairs

	Autosome				
Cross	I	II	III	IV	V
MY	0.17 (6)	0.28 (2)	0.19 (3)	-	0.18 (2)
M2	0.07 (10)	0.09 (5)	0.04 (4)	0.06 (2)	0.11 (5)
M3	0.14 (4)	0.15 (4)	0.06 (5)	-	0.13 (5)

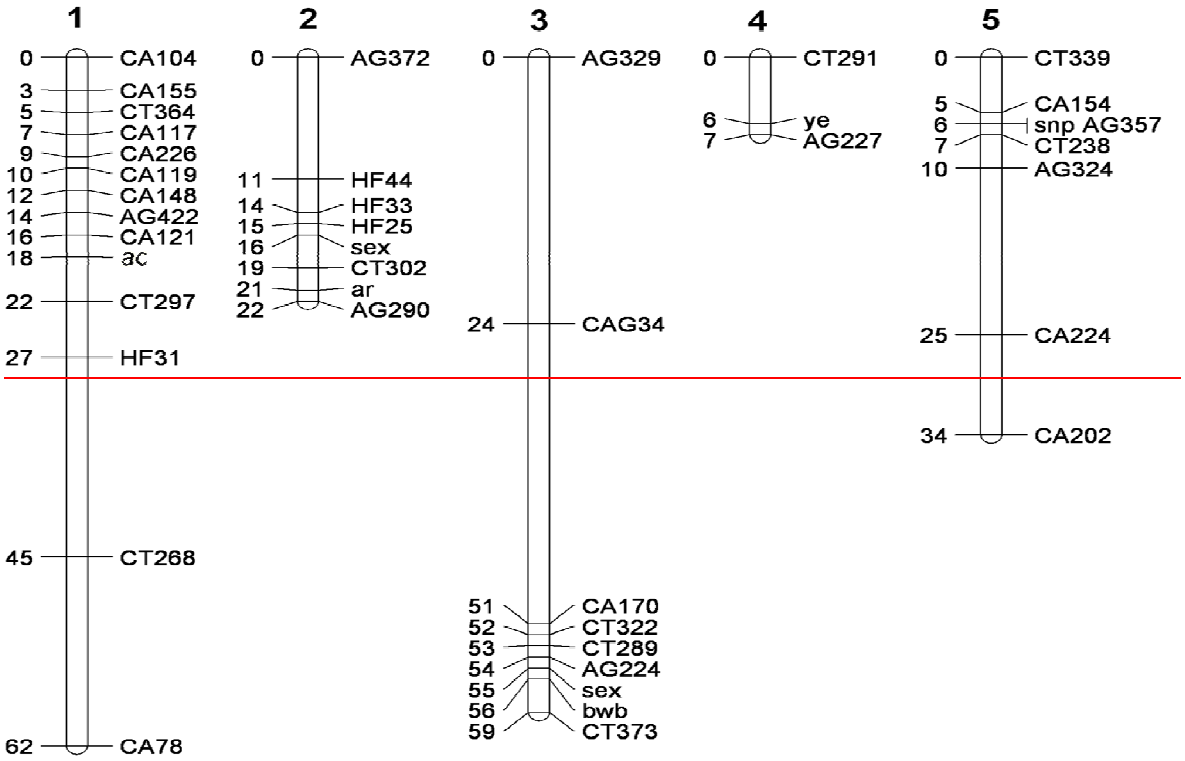

Figure 1. Linkage map of the housefly derived from combining three different mapping populations. Markers are indicated on the right; map distances (in Kosambi cM) on the left of a chromosome. Linkage groups are arranged by chromosome number according to Wagoner (1967). The number of linkage groups corresponds to the number of autosomes, no markers were found on the sex chromosomes. Each chromosome contains one visible marker (*ac, 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, all other markers are microsatellites.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Model reduction using F-tests for comparison between models.

Table S2. GLM testing for significantly deviating recombination frequencies of chromosomes and crosses.

Table S3. Newly developed microsatellite markers with Gene Bank accession numbers, repeat length and annealing temperature.

Table S1. By means of F-tests it was investigated whether removal of a variable from the full model had a significant effect; with recombination frequency as dependent variable. (RDf = residual degrees of freedom; RDev = residual deviance; bold= minimal adequate model)

	RDf	RDev	Df	Dev	F	P
cross* chromosome	49	318.77				
cross + chromosome	51	323.43	-2	-4.66	0.41	0.66
cross + chromosome	51	323.43				
chromosome	53	383.05	-2	-59.62	5.44	0.007
cross + chromosome	51	323.43				
cross	52	323.76	-1	-0.33	0.06	0.81

Table S2. GLM result of the reduced model with recombination frequency as dependent variable and cross as predictor variable. Error distribution was set to quasibinomial to account for overdispersion. Significance is indicated in bold.

Predictor	SE	T	p
crossM2	0.3221	3.279	**0.002**
crossM3	0.2862	1.836	0.072

Table S3. Newly developed microsatellite markers for the housefly. Ta = annealing temperature.

Name	GeneBank 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 GeneBank