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Sex chromosome evolution in the house fly

Li, Xuan

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Chapter

I

**General introduction and
thesis overview**

Sexual reproduction is a long-existing phenomenon in eukaryotes. Through meiosis and karyogamy, sexual reproduction allows for genetic recombination within a species and can bring out the hidden, but advantageous genetic variants that facilitate organisms to adapt to the environment. Except for some unicellular organisms which produce isogamic gametes, most sexually reproducing species have female and male gametes that differ in size and shape. The female and male function can be combined within an individual (hermaphroditism) or occur as separate sexes (gonochorism). Sexual dimorphism is established by regulatory hierarchies that determine which path of sexual development a zygote should take. The process of sex determination has received significant research attention in various animals such as mammals, birds, fish and insects. Many studies have shown that the mechanisms of sex determination vary widely among species (Marin and Baker 1998; Zarkower 2001; Kaiser and Bachtrog 2010; Nakamura 2010). Such differences can exist at various levels. In both invertebrate and vertebrate sex determination can differ at the level of the triggering cues, chromosome composition or even the key genes. Why do so many sex determination systems exist? What are the evolutionary forces that drive this variation? One way to gain more insight into how sex determination mechanisms can change is to identify the chromosomes and genes involved and compare their genetic and genomic regulation.

Diversity in sex determination systems

Hayes (1998) defined “sex determination” as the “mechanisms that direct sex (gonadal) differentiation”. Sex determination will lead to sexual differentiation, i.e. sex-specific behaviors and morphologies. The end-products of sex differentiation can be female and male individuals, which is the case for almost all sexually reproducing higher eukaryotes, or result in hermaphrodites, for example, abundant in mollusks and plants (Dellaporta and Calderon-Urrea 1993; Heller 1993). However, there is much variation in the initial signals that trigger sex differentiation, as well as in the gene pathways that regulate these developmental processes. A traditional distinction is between genetic sex determination (GSD) and environmental sex determination (ESD) to categorize the sex determination systems that are triggered by either environmental or genetic cues. GSD refers to the situation that the sex of a species is decided by the inherited genetic material. For example, in birds and mammals, sex determining genes inherited from parents are responsible for the differentiation of the sexual fates of embryos (Goodfellow and Lovell-Badge 1993; Smith and Sinclair

2004). ESD describes the situation that sexual differentiation is influenced by environmental factors, for instance, the temperature in reptiles (Devlin and Nagahama 2002). However, as more and more evidence is accumulating of the co-existence of both systems in one species, ESD and GSD may be viewed as just the two ends of a continuum of sex determination systems (Sarre *et al.* 2004; Beukeboom and Perrin 2014).

In many vertebrate species, dimorphic sex chromosomes and pivotal sex determining genes are involved in sex determination in the zygote. In mammals, males are heterogametic, which means that individuals with an X and a Y chromosome will develop into males and XX individuals into females. Many genes are involved in the sex determination process in mammals (XX/XY system). Graves and Peichel (2010) provided an overview of pathways with several pivotal genes that are indispensable for testis or ovary development in mammals, including *Sf1*, *Sry*, *Sox9*, *Fgf9*, *Dmrt1*, *Rsop1*, *Wnt4*, *β -catenin*. In birds, the female is heterozygous for sex chromosomes. When the combination of sex chromosomes is homogametic ZZ, the individual becomes male, when it is heterogametic ZW it becomes female (ZW/ZZ system). Like in mammals, *Dmrt1* and *Sox9* play important roles in testis development in birds (Smith *et al.* 1997; Nakabayashi *et al.* 1998; Raymond *et al.* 1999; Smith *et al.* 1999; Kim *et al.* 2003; Murdock and Wibbels 2003).

Fishes are one of the largest vertebrate groups including nearly 30000 species (Helfman *et al.* 2009). There are various sex determining systems ranging from ESD to GSD. In species with GSD, sex can be determined by either type of sex chromosome combination, i.e. both the XY type and ZW type (Magurran and Garcia 2000). Sex determination in amphibians is mainly by GSD, but ESD also occurs. Morphologically distinguishable sex chromosomes are not commonly observed, but male heterogamety (XY) and female heterogamety (ZW) have been reported from the Urodela and Anura.

Most invertebrates have GSD. There are species with distinguishable sex chromosomes, for example, *Drosophila melanogaster* and *Caenorhabditis elegans* (XX/XY). In these two species, sex is determined by the X chromosome to autosome (X:A) ratio (Hodgkin 1992) or the X chromosome dose (Erickson and Quintero 2007), rather than a dominant male-determining gene of the Y. ZW/ZZ systems also exist in invertebrates like Lepidoptera. In silkworm (*Bombyx mori*) a dominant feminizing factor *Fem*, a female-specific piRNA, is located on the W chromosome which makes

individuals with ZW genotype develop towards females (Suzuki 2010; Kiuchi *et al.* 2014). Genetic sex determination can also occur without sex chromosomes in invertebrates. In hymenopteran insects, such as bees, ants and wasps, unfertilized eggs develop as haploid males, and fertilized eggs form diploid females. There also exist additional, more peculiar, forms of GSD, such as monogeny, where all offspring of a particular individual female are either exclusively male or exclusively female (Beukeboom and Perrin 2014; Blackmon *et al.* 2017).

Sex chromosome evolution

The evolution of sex chromosomes is an important topic in evolutionary biology. Following Muller's (1918) original proposal, it is now widely accepted that heterogeneous sex chromosomes, like the XY chromosomes and the ZW chromosomes, originate from a homologous pair of autosomes. Several different stages in the evolution of sex chromosomes are distinguished (Bachtrog *et al.* 2014; Beukeboom and Perrin 2014; Schenkel and Beukeboom 2016). The process starts with a pair of homologous autosomes, of which one gains sex determining function by acquiring a sex determining gene through, for example, mutation or translocation of an existing gene (Charlesworth *et al.* 2005; Carvalho *et al.* 2009; Pease and Hahn 2012). Such a chromosome is referred to as proto-sex chromosome because the sex determining function was recently gained. Next, the chromosomal region around the sex determining gene undergoes recombination arrest which gradually extends over the whole chromosome. The reason for recombination arrest of sex chromosomes like Y and W is that sex determining genes are favored as a whole linkage group by selection (Beukeboom and Perrin 2014). Any crossing over within the sex determining region would likely cause improper sexual differentiation or intersexuality, as it can lead to sex determining associated genes separating to different chromosomes and causing deleterious effects in the opposite sex, which will be selected against (Rice 1987). As time goes by, deleterious recessive mutations start to accumulate on the minor chromosome (Y or W) leading to progressive degeneration as we nowadays observe in many Y and W chromosomes. Meanwhile, the major chromosome (X or Z) keeps recombining in the homozygous sex and its integrity is maintained. Although this hypothesis of sex chromosome evolution is universally accepted, it has hardly been tested in different species. One of the reasons is a lack of suitable systems.

Sex determination systems are typically invariable within species. Polymorphic

systems in which multiple different sex-determining chromosomes co-occur are less prevalent. They are, however, of special interest because they allow for testing of the evolutionary theory of sex chromosomes. In several species, polymorphic sex chromosomes have been reported, such as octoploid strawberries (*Fragaria*) (Tennesen *et al.* 2018), Atlantic salmon (*Salmo salar*) (Kijas *et al.* 2018), Japanese threespine stickleback (*Gasterosteus aculeatus*) (Yoshida *et al.* 2014) and house fly (*Musca domestica*) (Dübendorfer *et al.* 2002; Hamm *et al.* 2015; Sharma 2018). In addition, sex chromosome turnover events take place during evolution. Such events can be mediated by sex chromosome-autosome fusion (Yoshida *et al.* 2014) or translocation of the sex determining region in the genome (Sharma *et al.* 2017; Kijas *et al.* 2018; Tennesen *et al.* 2018). Turnovers that change the genomic location of the sex determining region may be important drivers of sexual dimorphism and speciation. However, little is known about the forces and mechanisms that cause such a phenomenon and its evolutionary consequences.

Insect sex determination pathways

Insect sex determination pathways consist of a series of genes that regulate each other in a hierarchical fashion. Studies on a limited number of orders, such as Diptera, Hymenoptera and Lepidoptera, have revealed that the downstream cascade of the regulatory networks in the studied species are quite similar and conservative. In all the studies species, the *doublesex* (*dsx*) gene plays a binary switch role on the crossroad of sexual fate (Beukeboom and Perrin 2014; Bopp *et al.* 2014). The RNA of *dsx* has two sex-specific splicing forms (*dsx^M* in males and *dsx^F* in females) which direct individuals to develop towards either male or female. In most cases, such splicing activity is controlled by a gene named *transformer* (*tra*), whose RNA also has sex-specific splicing forms. *Tra* is a key element in the sex determination pathway in many insects, although exceptions such as lepidopteran species exist (Traut *et al.* 2007; Verhulst *et al.* 2010b).

At the upstream part of the regulatory cascade resides sex determiners that are referred to as the primary signal. In *D. melanogaster*, the dose of X-linked signal elements regulates the expression of the gene *sex lethal* (*sxl*) (Erickson and Quintero 2007). When there are two doses of the X chromosome, *sxl* is activated and positively regulates female sexual differentiation through its action on *tra*. In some other dipterans, dominant male determiners instead of the X:A ratio play a key role in sex differentiation (Saccone *et al.* 2002; Hamm *et al.* 2015; Sharma *et al.* 2017;

Meccariello *et al.* 2019). The male determiner in house fly is the gene *Musca domestica male determiner (Mdmd)*, whereas in medfly, *Ceratitis capitata*, it is the gene *Maleness-on-the-Y (MoY)* (Sharma *et al.* 2017; Meccariello *et al.* 2019). In honeybee *Apis mellifera*, sex is determined by the *complementary sex determiner (csd)* gene. When *csd* is hemizygous or homozygous, individuals develop into males, whereas heterozygosity of the *csd* locus leads to female development (Beye *et al.* 2003; Hasselmann *et al.* 2008). In the parasitoid wasp *Nasonia vitripennis*, a maternally imprinted gene *wasp overruler of masculinization (wom)* is silenced in unfertilized eggs resulting in male development (Verhulst *et al.* 2010a; Zou *et al.* 2020). In fertilized eggs, paternally inherited *wom* functions and directs female development (Zou *et al.* 2020). Some other identified sex determining genes are *Nix* and *Yob* in the mosquitoes *Aedes aegypti* and *Anopheles gambiae* (Hall *et al.* 2015; Krzywinska *et al.* 2016). These genes are non-homologous, illustrating that primary signals are highly variable and species-specific.

House fly sex determination

The house fly (*Musca domestica*) is a dipteran insect with a worldwide distribution. It is a vector of many diseases including enteric fever, cholera, shigellosis and salmonellosis (Greenberg 1971; Bidawid *et al.* 1978; Harwood and James 1979; Echeverria *et al.* 1983). It also has economic value as a potential protein source for livestock. House fly larvae and pupae are for example used as feed in the poultry breeding industry. They can convert waste materials into useful high-quality food supplements and reduce the pollution caused by organic waste (Calvert *et al.* 1969).

The house fly is of particular interest for research on sex determination. The orthologs of *tra (Mdtra)* and *dsx (Mdtsx)* are also the key genes in the sex determination pathway of the house fly (Hediger *et al.* 2004; Hediger *et al.* 2010). *Mdtra* acts as a binary switch that directs female development by having its RNA splicing into either female specific isoform (*Mdtra^F*) or male specific isoform (*Mdtra^M*). In female individuals, *Mdtra* is expressed in the maternal germline. Maternal *Mdtra* and *Mdtra2* mRNA are fed forward into zygotic expression of functional *Mdtra*. Zygotic MdTRA, along with MdTRA2 starts a self-regulatory loop that keeps *Mdtra* mRNA splicing to the functional female isoform, that will promote the splicing of *Mdtsx* mRNA into its female-specific isoform (Bopp 2010; Hediger *et al.* 2010). As a consequence, female morphological and behavioral differentiation will be initiated. However, in the presence of a dominant male-determining locus (*M*), it

stops the activation of the *Mdtra* auto-regulation loop by inhibiting the splicing of *Mdtra* RNA into a functional female isoform in developing male zygotes. As a result, without the functional TRA, the product of *Mddsx* will splice into the male-specific isoform, which will lead to male differentiation (Dübendorfer *et al.* 2002; Hamm *et al.* 2015). Interestingly, there is a scenario, where individuals with an *M* locus can develop into females if the dominant female-determining factor *Mdtra^D* is present. *Mdtra^D* is a mutant allele of *Mdtra* and its splicing product by default is always the female isoform regardless of whether the maternal provision of *Mdtra* and *Mdtra2* mRNA is available. Thus, *Mdtra^D* is insensitive to the presence of the *M* locus and sets *Mddsx* into the female mode of expression, resulting in female development.

The house fly possesses 5 pairs of autosomes (autosome I-V) and one pair of morphologically different (the Y being shorter than the X) XY sex chromosomes (Stevens 1908; Metz 1916; Wagoner 1967). The *M* locus has been found on the Y chromosome (*M^Y*) and the *Mdtra* gene is on chromosome IV (McDonald *et al.* 1978; Hediger *et al.* 1998). The Y chromosome contains two male determining regions that locate on the short arm and long arm respectively (Hediger *et al.* 1998). Yet, *M* loci can also be present on any of the five autosomes (*M^{I-V}*, collectively known as *M^A*), or even the X chromosome (*M^X*) (Franco *et al.* 1982; Denholm *et al.* 1983; Kozielska *et al.* 2008; Hamm *et al.* 2015). For example, in central and southern Italian house fly populations, genetic tests demonstrated that the *M* locus is commonly found on autosome II or III rather than the Y chromosome (Franco *et al.* 1982; Kozielska *et al.* 2008). In South-East England house fly populations, the *M* locus is mostly located on either autosome III or the X chromosome. In the majority of flies, both sexes had the genotype of XX and the Y chromosome was not present (Denholm *et al.* 1983). The *M* locus can also be located on autosome I, IV and V in some populations, but with a lower frequency (Hamm *et al.* 2015).

The geographical distribution of autosomal *M* loci and the dominant female determiner *Mdtra^D* follows a latitudinal gradient in house fly populations (Franco *et al.* 1982; Kozielska *et al.* 2008). “Standard” XX-XY populations with only the presence of *M^Y* and without the presence of *Mdtra^D* are mostly found in regions that are more distant from the equator. Autosomal *M* loci and the *Mdtra^D* allele are more abundant in populations at regions with lower latitude. In populations with *Mdtra^D*, often *M* loci exist on multiple chromosomes, of which at least one is homozygous (Franco *et al.* 1982; Feldmeyer *et al.* 2008; Kozielska *et al.* 2008). It was suggested that this gradient is of relatively recent origin but now seems a stable system in natural

populations (Franco *et al.* 1982; Kozielska *et al.* 2008; Meisel *et al.* 2016). Moreover, evidence has shown a selective advantage to carrying autosomal *M*, such as *M^{II}* and *M^{III}* (Hamm *et al.* 2005; Kozielska 2008). Yet, the factors that maintain this system with polymorphic sex determiners are not known. Several hypotheses have been proposed. Environmental factors such as temperature or humidity may play a role in maintaining polymorphic sex determining factors (Franco *et al.* 1982; Kozielska *et al.* 2008). Additionally, environmentally dependent sexual antagonistic selection could play a role (Meisel *et al.* 2016). Whether environmental factors and sexually antagonistic selection indeed shape the polymorphic house fly sex determination system or additional factors that need to be invoked require further investigation.

Recently, by investigating male-specifically expressed sequences, the first house fly *M* factor was identified as *Musca domestica* male determiner (*Mdmd*) (Sharma *et al.* 2017). Sequence analysis revealed that *Mdmd* is a paralog of *CWC22*, also known as *nucampholin* (*Mdnm* in house fly). The latter encodes a protein which is a required factor for the assembly of exon junction complex (EJC) (Steckelberg *et al.* 2012). It suggests that *Mdmd* originated from *Mdnm* and gained male determining function by neo-functionalization (Sharma *et al.* 2017). *Mdmd* sequence was found in houseflies that carry the *M* locus on autosome II, III, V or the Y chromosome but not on autosome I, suggesting that autosome I carries a male determining factor other than *Mdmd* and that intra-species variation exists for the male determining genes.

Previous studies revealed the complex nature of the different *M* loci (Sharma *et al.* 2017; Sharma 2018; Wu 2018). Characterization of the genomic sequences of the *M* locus via methods such as genome walking indicates that multiple copies of the *Mdmd* gene exist within the *M* locus on autosome III and autosome V (Wu 2018). The copy number of *Mdmd* seems to vary in different *M* loci (Sharma *et al.* 2017) and different copies are separated by intervening sequences, of which some are repetitive (Wu 2018). Comparison of *M^{III}* and *M^V* loci further revealed both common and locus-specific intervening sequences (Wu 2018). Yet, due to the complex nature of *M* loci and the limitation of the methods applied, much crucial information is still lacking. For instance, it is unclear how many functional *Mdmd* copies exist in the *M* locus. Moreover, besides *Mdmd*, not much is known about the sequence content of the intervening sequences, i.e. whether there are other genes in the *M* locus that contribute to male development. Such information is not only important regarding the functionality of the *M* loci, but may also shed light on the evolutionary history of *M* loci. To further address these topics, a necessary step would be to examine the

genomic sequence structure of *M* loci in detail.

The model of sex chromosome evolution requires that recombination arrest happens to chromosomes carrying sex-determining genes. However, recombination in house fly males is disputed. In some studies, recombination was rarely observed in males (Hiroyoshi 1961; Tsukamoto *et al.* 1961; Hiroyoshi 1964; Hiroyoshi 1977). Yet, evidence for recombination was also reported (Rubini *et al.* 1980; Feldmeyer *et al.* 2010) with frequencies as high as 30% for autosome II (Lester *et al.* 1979). Although the low recombination frequency of the *M*-carrying chromosome might be caused by the large genetic distance between the marker used and the *M* locus, these results indicate that recombination can still happen on the male-determining chromosome. Variation in recombination rate between *M* loci and autosomal markers could point towards different ages of these *M*-carrying chromosomes in houseflies.

Two hypotheses have been proposed regarding the evolutionary history of the polymorphic *M* loci. Sharma *et al.* (2017) proposed that the *M* locus on the current house fly Y chromosome started with *Mdmd* originating from a duplication of *Mdnm*. It subsequently underwent local amplification resulting in the formation of the large, composite complex region. The *M^Y* locus then translocated to other chromosomes as a large fragment. This hypothesis assumes an “ancient origin” of the Y and “recent origins” of *M*-carrying autosomes. In contrast, Meisel *et al.* (2017) proposed that the house fly Y chromosome is young and minimally differentiated from its ancient X chromosome partner. In fact, they referred to all the *M*-carrying chromosomes, including the current Y chromosome, as “neo-Y chromosomes” to reflect their recent acquisition of *M* and male determining function. They additionally proposed that the current Y chromosome is a “younger” neo-Y than the *M*-carrying autosome III. At his moment, the evolutionary trajectory of *M* loci in the house fly genome is still unresolved. Studying this can provide a better understanding of how a chromosome pair can gain sex determination function (e.g. by *M* locus translocation) and the following stages of proto-sex chromosome evolution.

Thesis overview

The goal of the current thesis is to further characterize various *M* loci in the house fly in terms of chromosomal location and genomic structure in order to get a better understanding of the evolution of sex determination loci and sex chromosomes. Referring to the variation in the chromosomal location of *M* loci, I aim to answer the

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following questions: 1) Does the frequency and genomic distribution of M loci vary at a more local population scale? 2) Do differences in recombination frequencies reflect inter-strain variation in recombination rates or different locations of M loci within autosomes? Referring to the genomic structure of M loci, I aim to answer the following questions: 1) What are the genomic structure and the sequence content of M loci? 2) What are the differences and similarities in DNA sequences between M loci on different chromosomes? Answering these questions will provide a better understanding of the dynamics of polymorphic sex determining systems and increase our insight into the evolutionary trajectories of “neo-Y” chromosomes.

In chapter 2, I investigate five neighboring Spanish populations for local variation in sex determiners. The chromosomal locations of M loci in males are determined by linkage mapping using backcrosses of males with females of the multi-marked *abys* strain. I measure the linkage of male function with one recessive marker on each of the five autosomes. As no marker is available for the sex chromosome pair, I additionally examine the sex chromosome composition with karyotyping. The *tra^D* allele frequency in females is also determined by diagnostic PCR. A strain from the Netherlands with a putative M^Y locus is used to systematically evaluate any deviations of Mendelian marker segregation that may impair the linkage mapping analysis. Small deviations from 50% are found for four of the five markers, indicating slight fitness disadvantages of the recessive markers, but larger deviations for one marker, indicating incomplete penetrance. I find that chromosomal localities of M loci differ strongly between populations, and so do the frequencies of various autosomal M . All females in populations with homozygous M males, carry the *tra^D* allele, whereas *tra^D* is absent in populations without homozygous M males. I further find evidence for recombination on the M -carrying autosomes and for occasional sex chromosome aneuploidy. These results indicate locally stable systems may either consist of hemizygous M and no *tra^D* (equivalent to a male heterogametic system), or homozygous M and *tra^D* (equivalent to a female heterogametic system). Local divergence in the composition of sex determiners also suggests limited gene flow between adjacent populations.

In chapter 3, I continue to investigate the chromosomal locations of M loci at a finer scale utilizing fluorescence *in situ* hybridization (FISH). I focus particularly on the localization of *Mdmd*-containing M loci and design an *Mdmd* specific probe for this purpose. I locate M loci on the Y chromosome, autosome II and autosome III near the centromeres, suggesting a tendency for M loci to locate in pericentromeric

regions. The *M* locus on the X chromosome is located in the middle of one chromosome arm. In addition, I compare the localization of *M* loci in populations of different geographical origins. No variation is found between populations from Spain and Italy for the chromosomal position of *M* on the X chromosome, autosome II and autosome III. This supports the hypothesis that each *M* translocation to an autosome was a single event and subsequently spread to different geographical regions. In addition to the *Mdmd* specific probe, I design a "mixed" probe, containing additional *M*-associated repetitive sequences for FISH. Using this "mixed" probe, I localize *M* but also discover *M* And Sex chromosome (MAS) located repeats, which differ in copy number between the X and Y chromosomes. MAS repeats not only localize on the *M*-carrying Y and X chromosome, but also on X chromosomes without *M*. Because MAS repeat regions likely overlap with the chromosomal locations of M^Y and M^X , I hypothesize that the translocation of *M* from the Y to the X is a non-allelic homologous recombination event mediated by MAS sequence.

In chapter 4, I further investigate the genomic structure of the *M* locus. A male house fly genome with an *M* locus on autosome III was sequenced with Pacbio SMRT sequencing and assembled (M3 genome). In the M3 genome, two *M* locus contigs are identified that together make up the M^{III} locus with the size of ~591 kb. In the *M* locus resides 88 *Mdmd* replicates, of which only one contains the complete ORF sequence and thus is likely the functional *Mdmd* gene, while others have degenerated to various degrees. Other than *Mdmd*, the M^{III} locus contains few sequences of known genes, although whether these non-*Mdmd* genes are functional is debatable. I find a high degree of sequence duplication throughout the *M* locus. The majority of the duplicated sequences are *Mdmd* replicates and their flanking sequences, however, repetitive sequences that did not contain *Mdmd* are also found. I propose that duplication of *Mdmd* and its flanking sequences may have a positive effect on sustaining this male determining region.

In chapter 5, I investigate variation in genomic structure between *M* loci on different chromosomes. I collect a series of Illumina sequence datasets including two datasets for the genome with M^{III} , one dataset for the genome with M^I and M^V each, three datasets for the genome with M^Y and two datasets for the female genome without *M* locus. I find that the copy number of *Mdmd* replicates varies between *M* loci. M^{III} appears to have the highest number of *Mdmd* replicates, whereas the M^V locus contains the fewest. I reveal that sequence variation also occurs between *M* loci on the Y chromosome, suggesting that M^Y in different populations evolved

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independently and at a different speed. Comparison of interspersed sequences among different M loci reveals high similarity between the M^{III} locus and the M^Y locus of one strain, whereas the M^I locus shows higher sequence similarity to the M^Y loci in two other strains suggesting that autosomal M loci may have originated from different haplotypes of M^Y . Additionally, the genomic region that contains the *Mdm* with the complete ORF sequences is universally present in all investigated M loci (M^I , M^{III} , M^V and M^Y), suggesting the importance of this region for the male determining function. Based on the results from this chapter and previous study, I propose that the M locus originated on the Y chromosome and sequentially translocated to autosomes.

In chapter 6, I summarize all my results on the geographical distribution and genomic structure of M loci of the house fly. I discuss how these findings contribute to our understanding of the evolutionary history of M loci. I also discuss how my discoveries fit the current theory of sex chromosome evolution. Based on my findings, I hypothesize two mechanisms, i.e. non-allelic homologous recombination and double strand breaks with homologous repair, as being responsible for M locus translocation and sex chromosome turnover in the house fly. I further identify remaining open questions and possible ways to approach them in future studies.

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