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Sex chromosome evolution in the house fly

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Chapter VI

General discussion

In this thesis, I have investigated the intriguing polymorphic sex determining system of the house fly (*Musca domestica*) (Dübendorfer *et al.* 2002; Hediger *et al.* 2010; Hamm *et al.* 2015; Sharma *et al.* 2017). In houseflies, a dominant male determining locus (*M*), that induces male development by interfering with the female determining factor *transformer* (*tra*), is not confined to the Y chromosome but can be found on both the Y or X sex chromosome (M^Y and M^X) and on any of the five autosomes (M^{I-V}). To add to this complexity, there exists a dominant allelic mutation of *tra*, termed tra^D, that is insensitive to the interference of *M* and by default directs female development when present in the genome (Dübendorfer *et al.* 2002; Hediger *et al.* 2010; Hamm *et al.* 2015; Sharma *et al.* 2017). The distribution of the different sex determining systems follows a latitudinal gradient, with increasing complexity towards the equator (Franco *et al.* 1982; Hamm *et al.* 2005; Feldmeyer *et al.* 2008; Kozielska *et al.* 2008). I investigated this polymorphic sex determining system at the genetic and genomic level to get a better understanding of the evolutionary dynamics of sex determination systems and the genomic processes underlying sex chromosome evolution in general. To obtain a more detailed understanding of the geographical distribution and the stability of polymorphic sex determining systems, I first analyzed the regional variation in genomic localization of sex determining factors at the population level using five neighboring Spanish populations. Second, I determined the physical location of *M* loci at the within chromosome level to gain more insight into *M* locus translocation history. Third, I sequenced and annotated the entire M^{III} locus, describing gene content and sequence duplications, to learn more about the genomic structure of this *M* locus and to formulate possible dynamics that have led to this structure. Fourth, I compared the genomic structure variation within and between *M* loci on autosome II, autosome III, autosome V and the Y chromosome to gain more insight into *M* locus translocation mechanisms at the genomic level and the evolutionary history of the polymorphic male determiners in the house fly.

Stability of the polymorphic sex determination systems

In chapter 2, polymorphism of sex determiners was investigated on a regional scale. In five neighboring Spanish house fly populations, frequencies of *M* loci in males and the tra^D allele in females were determined. I found that frequencies of male and female determiners vary strongly between these populations, despite the small

geographical distance between them. Two populations, SPA3 and SPA5 contained males with only the hemizygous M^{III} locus and females that did not possess the dominant tra^D allele. In SPA1 and SPA4 populations, all males were homozygous for the *M* locus on the X chromosome (M^X), whereas in SPA4 some males additionally possessed hemizygous M^I and/or M^{II} at low frequency. In both populations, all females possessed tra^D. In another population, SPA2, all females carried the tra^D allele, whereas both homozygous and hemizygous M^X males occurred, some of which were additionally hemizygous for M^{II}.

The results indicated a correlation between the presence of the tra^D allele in females and the number and homozygosity of *M* loci in males, which is consistent with predictions and the findings in Meisel *et al.* (2016). *M* loci can accumulate in presence of the dominant female-determining tra^D allele because they can also be inherited through females. We conclude that populations with (1) only one hemizygous *M* and no tra^D (male heterogametic system), and (2) with fixed homozygous *M* and tra^D in females (quasi female heterogametic system), may be stable states. The stability of the male heterogametic system is expected, because it safeguards a 0.5 sex ratio. Invasion of a population by males with a homozygous *M* would not change the male heterogametic system, as a homozygous *M* locus immediately becomes hemizygous in the next generation (all male) upon mating of a male with a non-tra^D female (that does not possess an *M* locus). It may result in various hemizygous *M* loci segregating independently in a population, but can never yield homozygous *M* males. Multiple hemizygous *M* loci in a population may however bias the sex ratio towards males. For instance, 75% of F1 offspring will be male when a male with two hemizygous *M* mates with a non-tra^D female, 2/3 of males will carry one *M* and 1/3 will carry two *M* loci. Sex ratio selection against individuals that produce male-biased broods will then take place to re-establish an equal sex ratio (Fisher 1958; Kozielska 2008). As for the quasi female heterogametic system, a 0.5 population sex ratio is expected to be stable as well. Fixation of tra^D, i.e. all females are heterozygous for the dominant tra^D allele, will lead to accumulation of *M*, both homozygous and multiple hemizygous ones upon invasion (or transposition if this would occur at high frequency), as the *M* locus can segregate to both males and females in such populations. This would however not alter the population sex ratio of 0.5.

Meisel *et al.* (2016) concluded that a population with a mix of sex determiners, i.e. males with hemizygous and homozygous *M* and females with and without tra^D, was stable based on a study of an American population in 1982 and 2014. However, stable

co-existence of males with multiple *M* factors and females with and without *tra*^{D} seems only possible if additional fitness factors are involved. A possibility of such selective factors can be linkage between an *M* locus and a recessive lethal gene that prevents homozygosity of certain *M* loci. An alternative possibility is linkage between an *M* locus/*tra* allele and a locus under positive selection, such as an allele for temperature tolerance (Delclos et al 2021). Verification of these verbal arguments about the dynamics of multiple sex determination factors in house fly populations requires theoretical modeling. They can be further tested by setting up experimental populations with various compositions of sex determiners and monitoring how frequencies of *M* loci and the *tra*^{D} allele change over generations. By applying different temperatures, this could potentially resolve why there exists a latitudinal cline for autosomal *M* loci and the *tra*^{D} allele.

Multiple factors have been proposed for maintaining the polymorphic sex determining system in natural house fly populations, including climate conditions such as temperature and humidity (Franco *et al.* 1982; Feldmeyer *et al.* 2008; Kozielska *et al.* 2008), and sexually antagonistic selection (Meisel *et al.* 2016). However, the strong differences between populations, with non-overlapping sex determining factors between neighboring Spanish populations suggest that additional factors are at play. Although the house fly is a highly abundant and widespread species, our results suggest limited gene flow between populations. Low genetic exchange between populations may enhance the effects of genetic drift, causing fixation and loss of alleles (Frankham 1996; Spielman *et al.* 2004; Meisel *et al.* 2016). Whether drift plays an important role in determining frequencies of sex determiners in natural populations requires further population genetic research into migration patterns and population sizes. In addition, environmental changes such as seasonal fluctuations in temperature, that affect population structure and size, may play a role. To further address the spatial and temporal changes in frequencies of sex determining factors requires monitoring the same populations over multiple generations and at various moments during the season.

Genomic mechanisms of *M* locus translocation

Possible genomic mechanisms responsible for *Mdmd* sequence duplication are double strand breakage and homologous repair that have been discussed in chapter 4. Here, I consider possible genomic processes responsible for the polymorphic male-determiners in the house fly. Based on my results, I propose two mechanisms of

genomic rearrangements that may be responsible for translocation of the *M* loci; non-allelic homologous recombination (NAHR) and double-strand breakage (DSB) and homologous repair.

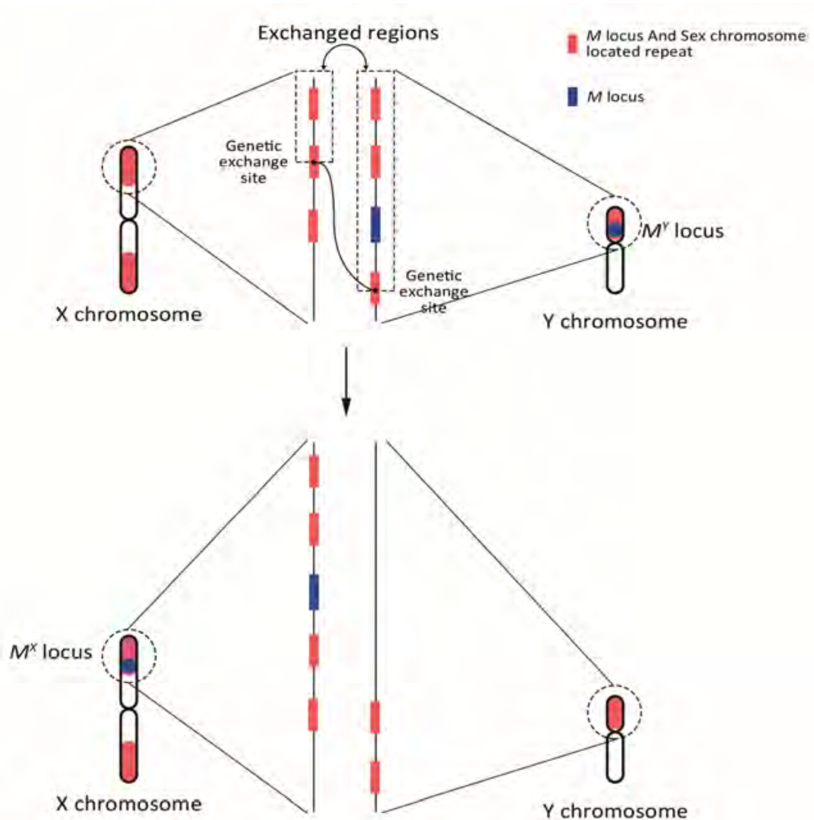

Figure 6.1 Schematic drawing of non-allelic homologous recombination (NAHR) that potentially mediated *M* locus translocation from the Y to the X chromosome. Both Y and X chromosomes contain large *M* locus And Sex chromosome (MAS) repeat regions. When unequal crossover happens between two MAS repeat sequences, it can result in translocation of the *M* locus from the Y to the X.

Non-allelic homologous recombination (Figure 6.1) refers to recombination when a

cross over event occurs between two non-allelic genomic sequences that have high sequence similarity (Hurles and Lupski 2006). Invoking NAHR is based on the discovery of the *M* locus And Sex chromosome (MAS) located repeats (Chapter 3). The MAS repeats exist as large block on the short arm of the Y chromosome and their location is adjacent or overlapping with the M^Y locus. MAS repeats are also found on both ends of the X chromosome with or without *M* locus. On the *M*-carrying X chromosome, the MAS repeats region is inseparable from the M^X locus based on my FISH results, suggesting their physical adjacency. Because of the sequence homology of the MAS repeat region on the X and Y, it may promote homologous pairing and recombination between the X and Y chromosomes. When non-allelic homologous recombination happens, it is possible for M^Y to relocate onto the X chromosome. This hypothesis can be tested by comparing the sequences of the M^Y and M^X loci, as it predicts high sequence identity between the two. Such sequence information of the M^Y and M^X locus needs to be obtained in future study.

The second potential mechanism for *M* locus translocation is the double-strand breakage (DSB) and homologous repairing mechanism (also known as duplication-dependent strand annealing, DDSA model) (Fiston-Lavier *et al.* 2007). My results suggest that the *M* loci with variable chromosomal locations are also a type of segmental duplication because several features of *M* loci fit this model of segmental duplication: 1) *M* loci translocated as multi-kb, large fragments (Chapter 5); 2) Different *M* loci show sequence similarity with each other (Chapter 5); 3) *M* loci locate mostly in pericentromeric regions (Chapter 3). Previous studies found that segmental duplication can be tandem or interspersed, and can be interchromosomal or intrachromosomal (Samonte and Eichler 2002; Stankiewicz and Lupski 2002). Although this mechanism was primarily proposed for explaining segmental duplications in *Drosophila* (Fiston-Lavier *et al.* 2007), it has also been reported for the silkworm *Bombyx mori*, and the mosquito *Anopheles gambiae* (Coulibaly *et al.* 2007; Fiston-Lavier *et al.* 2007; Zhao *et al.* 2013; Zhao *et al.* 2017). In addition, Kirsch *et al.* (2005) found interchromosomal segmental duplications of the pericentromeric region on the Y chromosome in human. In *Drosophila*, higher density of segmental duplication is also found in the pericentromeric regions of certain chromosomes (Fiston-Lavier *et al.* 2007), similar to our observations of *M* loci, further supporting the idea that the translocations of *M* are segmental duplication events.

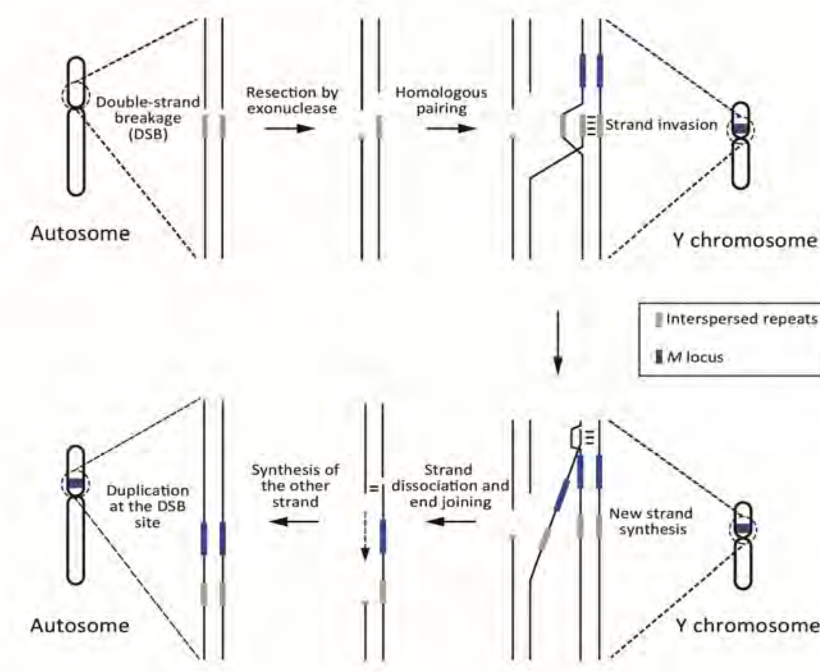

Figure 6.2 Schematic drawing of double-strand breakage (DSB) and homologous repair mechanism that potentially mediated inter-chromosomal *M* sequence duplication. The process starts with a DSB at the site of interspersed repeats on an autosome. The exonuclease digestion of two 5' ends leads to exposure of the protruding 3' ends at the DSB site. The exposed 3' end pairs with a homologous sequence located upstream of the *M* region on the Y chromosome. After pairing, the repair mechanism synthesizes a new strand at the exposed 3' end based on the template sequence. The newly synthesized strand then detaches from the template and reconnects with its original strand, resulting in the duplication of *M* sequence on the autosome.

Based on the DDSA model, I hypothesize that the *M* translocation process started with a DSB in a specific region, i.e. the pericentromeric region on autosome III, in or close to a copy of a repeated sequence, i.e. an interspersed repeats or transposable element (TE) (Figure 6.2). The DDSA model works as follows: at the DSB site, the exonuclease digestion of the two 5' ends leads to the exposure of the protruding 3' ends. The exposed 3' end then pairs with a homologous sequence on the template

strand (the *M* region in this case) from a different chromosome that is physically close to the exposed 3’ end due to the condensed arrangement of chromatids in the nucleus. After the homologous pairing, the repair mechanism initiates and synthesizes a new strand at the exposed 3’ end based on the *M* region sequence. The newly synthesized strand then detaches from the template and reconnects with its original strand, introducing the *M* region sequence to the DSB site. For the DSB/homologous repairing mechanism to work, the *M* locus or the upstream region of *M* should contain repeats that are also present in other genomic locations. This is indeed the case as I find many interspersed repeats such as TEs in the *M* locus (Chapter 4 and 5). What is more, Fiston-Lavier *et al.* (2007) described that the DSB/homologous repair mechanism can also cause tandem duplication and leave traces that allow for detection of such events in the sequence structure. I additionally examined the tandemly duplicated sequences in the *M* locus and indeed found such traces, supporting that the DSB/homologous repair mechanism is at least partially responsible for the complex *M* locus regions in the house fly genome. As M^{II} and M^{III} loci are found in the pericentromeric regions of autosomes II and III, we expect the existence of repeats in these pericentromeric regions that are also present in the *M* locus. This can be tested in future study.

DNA transposons may also contribute to translocation of the *M* locus, as they are known to be involved in gene duplication and translocation (Coates *et al.* 1997; Feschotte and Pritham 2007). In octoploid strawberry species, it was proposed that the sex determining region with variable chromosomal locations may be induced by active transposases (Tennessen *et al.* 2018). As we identified some DNA transposons within the *M* locus (Chapter 4), it is possible that one or more of them promoted *M* locus translocation. However, to provide evidence for such hypothesis requires clear definition of borders of different *M* loci and to find hallmarks for DNA transposons such as pairs of terminal inverted repeats (Wessler *et al.* 1995; Wessler 2006). Unfortunately, the exact borders of the house fly *M* loci remain to be determined before the role of transposons in *M* translocation can be substantiated.

Evolution of the polymorphic *M* loci of the house fly

The polymorphic sex determination system of the house fly is rather unique among insects. The Diptera suborder of Brachycera, which includes the house fly, has a recent common ancestor with a karyotype that consists of five autosomes (Muller elements A–E), a heterochromatic X chromosome (Muller element F), and a Y

chromosome with a male-determining locus (Vicoso and Bachtrog 2013; Meisel *et al.* 2020). Although the house fly has a pair of morphologically different XY chromosomes, its current Y chromosome has evolved from the ancestral X chromosome (Muller element F) of the common ancestor, as little genetic differentiation between the X and Y is found (Meisel *et al.* 2017). The ancestral X chromosome gained male determining function by acquiring the male determining gene *Musca domestica male determiner* (*Mdmd*) that originated from the duplication and translocation of a pre-existing gene named *nucampholin* (*Mdncm*) (Sharma *et al.* 2017). The ancestral Y, which is assumed to have been more diverged from the ancestral X and to contain an unknown male determiner, is considered to have become lost after having been substituted by the *Mdmd*-carrying ancestral X (Meisel *et al.* 2017). How *Mdmd* evolved from *Mdncm* and how polymorphic *M* loci originated remains unclear. Here, based upon my data from chapters 3, 4 and 5, I consider three hypothetical routes for the evolutionary history of *Mdmd*-containing *M* loci (Figure 6.3) and discuss which one is most plausible.

Route 1-M^I is the first *M* locus. This evolutionary trajectory states that *Mdncm* first duplicated locally and that the new copy became the paralog *Mdmd* by neofunctionalization. It assumes that *Mdncm* is located on chromosome I (Muller element B, which corresponds to 2L in *Drosophila*), which is likely the case based on synteny of the house fly Muller elements to *Drosophila*. The new *Mdmd* gene may have gone through minor on-site duplications and rearrangements of its neighboring sequences, possibly due to selection against disrupting *Mdncm* action, forming a primary M^I locus. Because of the high sequence similarity between *Mdmd* and *Mdncm*, unequal crossovers may still have happened between *Mdmd* sequences and *Mdncm* and disrupted the vital functions of both genes. This may have led to sequential translocation of *Mdmd* from the primay M^I locus to another chromosome, such as the ancestral X, causing subsequent loss of the ancestral Y chromosome. The remaining, *Mdmd-less* M^I, acquired another male determining mechanism, that is currently unknown, to form the current M^I. Interestingly, primary analysis of genomic data of M^I-possessing males discovered that some *Mdmd* remnant sequences are present in the M^I genome. If these sequences can be confirmed to locate on autosome I, this chromosome may indeed have harbored *Mdmd* in the past (Bopp, personal communication). On the ancestral X, the *M*-locus further expanded via local duplications and rearrangements, recombination arrest and sequence degeneration and evolved into the current neo-Y chromosome. In separate populations, the M^Y locus independently evolved at different speeds, which resulted in the observed M^Y

haplotypes with sequence variations. Different M^{Y} haplotypes further translocated to autosome II and autosome III as large fragments resulting in M^{II} and M^{III} loci that subsequently evolved further.

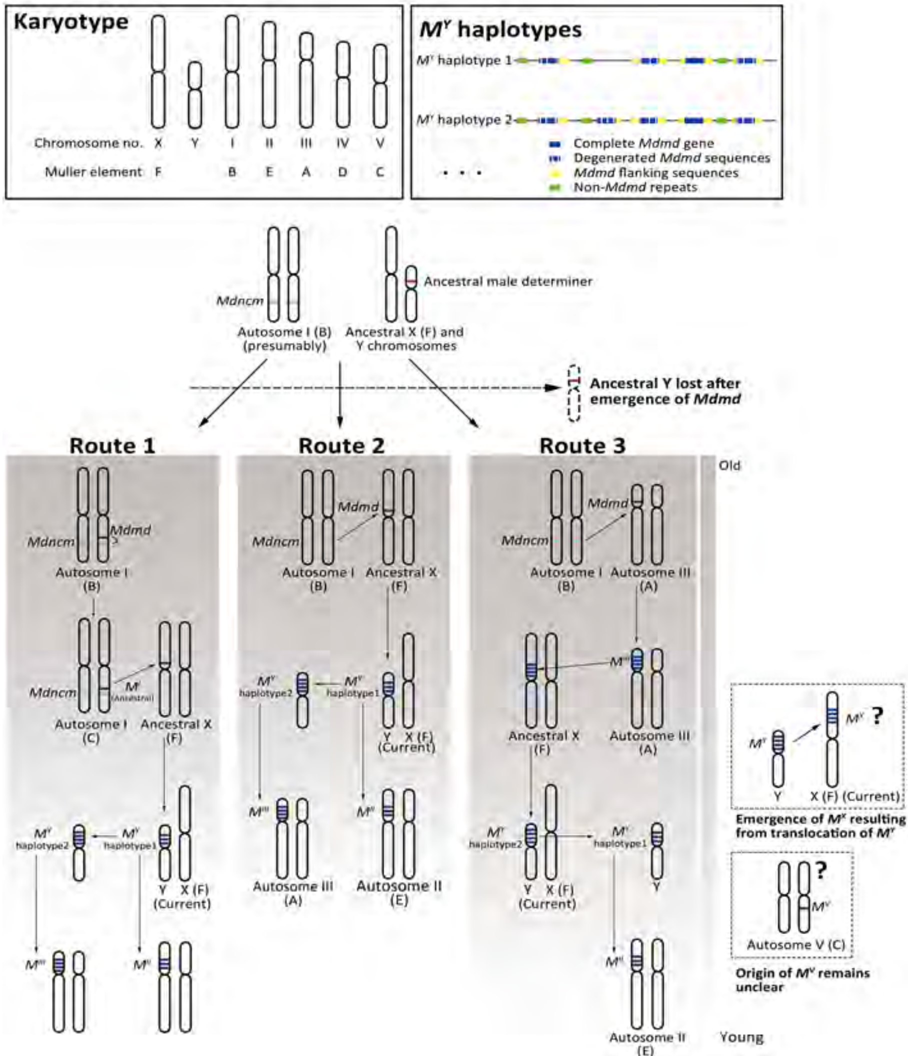

Figure 6.3 Hypothetical routes of polymorphic *M* loci evolution. The top boxes show schematic drawings of the karyotype of the house fly and different M^{Y} haplotypes. The common ancestor of Brachycera flies has a pair of ancestral XY chromosomes and an autosomal pair that carries the *Mdncm* gene (Muller element B, autosome I

in the house fly). Three potential step-by-step routes how polymorphic *M* loci evolved are shown. Route 1 assumes the *M* locus on autosome I originated first and subsequently translocated to the Y chromosome and autosomes II and III. Route 2 is similar to Route 1 except it assumes M^{Y} originated first. Route 3 assumes M^{III} originated first and then translocated to the ancestral X chromosome (that later became the Y chromosome) and autosome II. In all three routes, the current M^{X} locus is expected to have originated after M^{Y} (on the ancestral X) and the origin of the M^{V} locus remains unclear. The solid line boxes show schematic drawings of the karyotype of the house fly and zoomed-in sequence structure differences between M^{Y} haplotypes. The number of blue blocks on a chromosome indicates the complexity of various *M* loci.

Route 2-M^{Y} is the first *M* locus. If the *Mdncm* gene duplicated and simultaneously translocated, then the initially established *M* locus can be at another chromosome (Meisel *et al.* 2017; Meisel 2020). Route 2 is similar to most parts of Route 1, except at the beginning where the *M* locus originated on the ancestral X instead of on autosome V, turning the ancestral X into a proto-Y. Route 2 assumes that the composite structure of the male determining region was mainly formed through a series of on-site duplications that led to the size increase and the inclusion of repetitive sequences on the proto-Y. As similar sequence structures are found in *M* loci, the M^{Y} locus likely translocated as large fragments to other chromosomes. Under this scenario, the M^{V} locus is expected to be the result of translocation of another pre-existing *M* locus or the results of shrinkage after M^{Y} translocation. However, with the current information, we cannot pinpoint the translocation history of the M^{V} locus in this route.

Route 3-M^{III} is the first *M* locus. Interestingly, Meisel *et al.* (2017) suggested that the M^{III} locus might be “older” than the M^{Y} based on the finding of elevated heterozygosity between *M*-carrying autosome III and regular autosome III, but not for the X (Muller element F) and Y chromosomes. This leads to Route 3 of how the polymorphic *M* loci may have evolved. If M^{III} became the initial *M* locus, the original M^{III} locus translocated to the ancestral X and the subsequent trajectory is similar to Route 1 and 2. Other *M* loci are expected to have originated after the M^{III} locus. Considering the size of the current M^{III} locus, there are two possibilities why other the *M* loci are smaller and less complex. One is that M^{III} translocated several times in early stages of its existence, while it was still less complex. Another possibility is that M^{III} translocated as a large fragment after it had already undergone considerable

expansion. Other *M* loci may then have undergone subsequent sequence reduction, explaining why they are smaller and less complex than the current M^{III} locus, and why there exist multiple M^{Y} haplotypes. In all three routes, the origin of the M^{V} and the M^{X} loci remains unclear. However, if assume the current M^{X} is the result of NAHR between the XY chromosomes, the M^{X} locus is expected to have originated after M^{Y}. The M^{V} locus may be the youngest among different *M* loci and that is why it has not yet evolved into the more complicated structures like other "older" *M* loci, such as M^{Y}.

Among these three possible routes, I consider Route 2 to be the most plausible one for the following reasons. Route 2 assumes M^{Y} to be the first evolved *M* locus and that the current Y chromosome has existed for longer evolutionary time than other *M*-carrying chromosomes. This is consistent with the Y chromosome being the only degenerated *M*-carrying chromosome (being morphologically shorter than the X chromosome) (Chapter 3), while other chromosomes do not show visible, cytogenetic differences to their non-*M* carrying counterparts. One could, however, argue that autosome V and autosome III contain vital functional genes in proximity to the *M* locus and that mutation or degeneration of those genes negatively affected fitness, preventing degeneration. In contrast, the ancestral X chromosome may not contain vital genes which may have led to faster degeneration.

Another indication for *M*-carrying autosomes being "young" is that both our study and previous study observed recombination, albeit at low rates, between chromosome markers and the *M* loci on autosome II and autosome III (Chapter 2, Feldmeyer *et al.* 2010). According to the theory of sex chromosome evolution, recombination arrest is expected to be stronger for the more evolved sex chromosome (Charlesworth *et al.* 2005; Schenkel and Beukeboom 2016). The fact that *M*-carrying autosome II and autosome III are still recombining suggests that recombination arrest has not yet proceeded to the major part of these chromosomes and thus acquired their *M* locus more recently.

A difference between Route 3 and the other two routes is that it assumes the *M* locus translocated to different chromosomes and subsequently underwent sequence reduction rather than expansion, resulting in *M* loci that are missing some of the M^{III} sequences (the first established *M*). In Route 3, the M^{Y} locus is proposed to have originated from M^{III}. Different M^{Y} haplotypes must then have formed after the translocation, by different degrees of sequence reduction in geographically

separated populations. According to this hypothesis, the M^{III}-like M^{Y} haplotype went through less sequence reduction and the M^{II}-like M^{Y} haplotype with smaller size is more degenerated. Alternatively, the M^{II}-like M^{Y} originated earlier and from a smaller M^{III} locus than the M^{III}-like M^{Y}. As stated in Chapter 5, the M^{III} sequence was used as reference for conducting sequence comparison for different *M* loci. Thus, only differences between M^{III} and other *M* loci for sequences that are contained in M^{III} are considered and sequences that are not present in M^{III} but present in other *M* loci are undetected. To further investigate the evolutionary history of *M* loci, those possible undetected *M* sequences need to be looked at to see to what degree they are shared between different *M* loci. This requires good assembly of different *M* loci.

In conclusion, my study provides novel data on population frequencies and genomic structure of polymorphic sex determiners in the house fly. I demonstrated that sex determining systems vary strongly at a regional level in house fly populations. These results suggest that additional forces other than previously proposed environmental factors play a role in maintaining this polymorphic system. I described how a series of “young” male determiners is arranged at the genomic level. My results demonstrate that *M* loci consist of one complete and many partial *Mdmd* copies in addition to unique and repetitive sequences. I further proposed possible genomic mechanisms of *M* locus translocation based on comparing chromosomal locations and sequence structures of various *M* loci. I find evidence for degeneration by mutation accumulation in the *M* region, possibly as a result of recombination arrest, consistent with the model of sex chromosome evolution (Charlesworth *et al.* 2005; Bachtrog 2013; Schenkel and Beukeboom 2016). Based on my results, I propose several routes for the origin of the multiple *M* loci in the house fly genome. As the sizes of translocated *M* fragments differ between chromosomes, it is likely that several genomic mechanisms were simultaneously involved in *M* translocation and expansion, shaping the polymorphism that we observe today. There are many possible alternative routes for *M* locus evolution and more sequence information of more loci may help to resolve some of the uncertainties. My findings contribute to a better understanding of the evolutionary history of *M* loci in the house fly and sex chromosome evolution in general. My study also lays a foundation to further study polymorphic sex determining systems and test theories of sex chromosome evolution using house fly as a model.

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