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Evolutionary genetics and dynamics of transitions in sex determination

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CHAPTER I:

INTRODUCTION

Martijn A. Schenkel

Anisogamy and the evolution of sex determination cascades

Anisogamous species, which are prevalent among e.g. animals and plants, are characterised by a bimodal distribution of gamete sizes, with females producing large oocytes and males producing small spermatozoa. According to evolutionary theory, this dichotomy has become established by disruptive selection on gametes, which favours individuals that produce either small or large gametes but disfavors individuals producing gametes of intermediate sizes. Disruptive selection on gamete size is caused by two factors: (1) all individuals are selected to minimize the size of their gametes which is assumed to correspond to a lower investment in terms of resources, and (2) the fitness of the zygote created by fusion between two gametes depends on their combined resource contribution. The first factor promotes the evolution of 'male' sperm, whereas the second promotes the evolution of 'female' oocytes as a counteradaptation to offset the cost of mating with an individual that contributes less gametic resources (Parker et al., 1972). Following specialisation in gamete size, males and females may come to be exposed to different selective pressures (Parker, 2006; Roze & Otto, 2011). They undergo sex-specific adaptations towards their reproductive role, resulting in male-female divergence in among others physiology, morphology, and in case of animals behaviour (Cox & Calsbeek, 2009). As divergence between males and females develops over evolutionary time and comes to involve more traits, it becomes ever more apparent that those who fail to commit to develop into either of the canalised sexual phenotypes are bound to suffer fitness costs (Haldane, 1922). Such intersexual individuals show reduced or even no reproductive success. Mechanisms that canalise the process of sex determination (SD) become selectively favourable because they enforce proper embryonic sexual development, preventing individuals from developing as intersexes and thereby ensuring full reproductive capability.

Although SD mechanisms have a pivotal role in individual development and fitness, the manner in which sex is determined is highly evolvable (Bachtrog et al., 2014; Beukeboom & Perrin, 2014). Sex is set by an initial cue, which directs the state of an underlying genetic pathway that executes the process of sexual differentiation. Initial cues can be either environmental or genetic, and SD mechanisms are classified as environmental SD (ESD) or genetic SD (GSD) mechanisms. In ESD systems, different environmental factors can function as the

initial cue, such as temperature (Janzen, 1994), acidity (Parker et al., 2018), or social context (Moyer & Nakazono, 1978). Temperature is however the most common cue and will therefore be assumed to be the cue when discussing ESD systems in general. Variation in GSD systems occurs in even more ways than for ESD systems. First, different chromosomal systems have been described, including the various sex chromosome systems (XY male heterogamety, ZW female heterogamety, and haploid UV systems) as well as several non-sex chromosome systems (haplodiploidy, paternal genome elimination). Within and between different chromosomal systems, different genes evolved to generate the initial cue conferring sexual identity. The underlying genetic machinery that interprets this initial cue and actually carries out the process of SD appears to diverge too. Altogether, across the tree of life an astounding variety of SD mechanisms has evolved. Understanding the evolutionary processes that have driven this diversification is one of the major open questions in contemporary evolutionary biology (Bachtrog et al., 2014).

In this thesis, I study the evolution of novel GSD mechanisms, or SD transitions, focusing on the ultimate causes as well as the immediate consequences of these transitions in terms of early-stage sex chromosome evolution. Although SD transitions and sex chromosome evolution have mostly been considered as two separate processes, the two are clearly interconnected. First, SD is the primary function of sex chromosomes and GSD transitions can result in the formation of new sex chromosome systems. Additionally, similar evolutionary phenomena may be involved in SD transitions and sex chromosome evolution, such as sexually antagonistic selection and meiotic drive. In this introduction, I will first review the different evolutionary processes that are able to promote transitions in SD. Secondly, I will review the changes that sex chromosomes undergo throughout their evolution. Thirdly, I will review the genetics of SD in the housefly *Musca domestica*, whose multifactorial SD system has served as the main inspiration for this thesis and is therefore referred to in various chapters. Finally, I will provide an overview of the thesis and the topics addressed in each chapter.

Evolution of sex determination mechanisms

Differentiation between males and females is achieved by a genetic pathway whose state is set by an initial cue in both ESD and GSD systems. These pathways generally

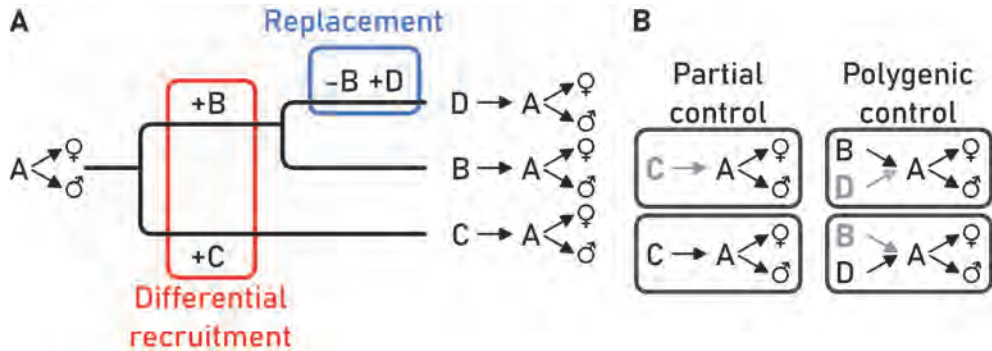


Figure 1: Types of evolutionary change in sex determination cascades. (A) A basic SD cascade with only a gene A can evolve to include other components. When different lineages recruit gene B respectively C, this results in two different SD cascades ($B \rightarrow A$ and $C \rightarrow A$). In both cases, a novel gene is added on top of the cascade. Alternatively, an existing SD gene can be replaced by another gene, e.g. B by D, resulting in a third SD system $D \rightarrow A$. (B) Novel SD genes may not spread throughout the entire species range, resulting in either partial control or polygenic control of the ancestral SD gene A. Under partial control, C may control A in one subpopulation, but not the other. Under polygenic control, A may be controlled by B in one subpopulation but D in another. Grey letters and arrows indicate the absence of an SD gene in that subpopulation.

consist of a regulatory cascade, where an initial cue regulates downstream target genes, who in turn regulate yet further downstream targets. SD systems have been theorized to evolve bottom-up, with the bottom-most components being most conserved and new components sequentially being added to the top of the SD cascade (Wilkins, 1995; Pomiankowski et al., 2004). Transitions in SD mechanisms can occur when a new SD gene replaces an existing SD gene at the top of the SD cascade, or when a new gene is added that regulates the formerly-topmost gene; variation in SD between species can be used to infer whether such transitions have occurred (Figure 1).

Environmental versus genetic sex determination

Evolution of SD cascades may also include transitions from ESD to GSD and vice versa, although the latter has received considerably less attention. Several hypotheses have been proposed for the evolution of ESD, though the differential fitness hypothesis (Charnov & Bull, 1977) is best supported (e.g Warner & Shine, 2008). Male and female fitness may be differently affected by environmental conditions, e.g. when males have higher fitness at low temperatures and females

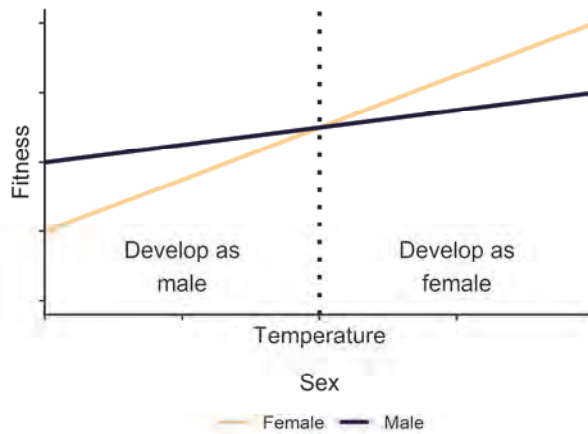


Figure 2: Sex differences in fitness under different environmental conditions can promote the evolutionary stability of environmental sex determination. Sex-by-environment (here assumed to be temperature) effects on fitness result in higher fitness for males when temperatures are low versus higher fitness for females when temperatures are high. This causes selection to promote temperature-sensitivity in SD whereby individuals develop as males under low temperatures but as females under high temperatures so that their fitness is maximized. The dotted line indicates the temperature value where sexes have equal fitness and indicates the transition point in environmental conditions between male versus female development.

have higher fitness at high temperatures (Figure 2). Selection then favours the evolution of an SD cascade with temperature sensitivity. GSD mechanisms may lack this flexibility which can result in a fitness reduction across the population so that it is disfavoured relative to ESD.

ESD comes with several drawbacks that may promote the evolution of GSD. First, environmental conditions are intrinsically variable, and can lead to substantial sex ratio biases under ESD. Biased sex ratios can lead to (local) extinctions due to one sex being rare or even absent (e.g. Santidrián Tomillo & Spotila, 2020). Sex ratio selection may then favour genetic variants that promote development into the rare sex, establishing a primitive GSD mechanism (Wilkins, 1995). Second, intralocus sexual conflict (IASC; see also Chapter 2) occurs when certain genetic variants are favoured in males but disfavoured in females and vice versa (Bonduriansky & Chenoweth, 2009). In GSD systems, this conflict can be resolved by the evolution of linkage between the SD gene and IASC loci, which has been implicated in sex chromosome evolution (discussed below). SD genes are transmitted exclusively through one sex, and linked regions are transmitted accordingly (Haig et al., 2014).

These regions are exposed to selection in only one sex, and may therefore become enriched for alleles that benefit this sex even if they would have severe fitness costs in the opposite sex (Jordan & Charlesworth, 2012). In ESD, such SD genes do not exist, no sex-limited region can be established, and the genetic load imposed by IASC is more likely to persist. This load can promote transitions to GSD as genes that promote development into one sex evolve near IASC loci (Muralidhar & Veller, 2018).

Evolutionary transitions in genetic sex determination

Within GSD systems, changes in the initial cue can occur due to replacement of the cue gene by a gene with an identical function or by recruitment of novel genes into the cascade (Figure 1); here, I will discuss which evolutionary processes can promote such changes to SD cascades. Once a new SD gene arises via mutation, multiple SD systems coexist, corresponding to the different SD genes. Transitions in SD occur when the newly-mutated SD gene spreads to fixation. During the transition, multiple SD systems can co-exist. In absence of fitness effects, a stable sex ratio can be achieved even when multiple SD genes coexist (Bull & Charnov, 1977). Shifts in the frequency of the one SD gene due to genetic drift cause a small sex ratio bias. Sex ratio selection then promotes the loss of this SD gene as well as the increase in the other SD gene. Allele frequencies at both SD loci will then shift and a new equilibrium will be reached. Reiterated bouts of this process can result in the complete invasion of an SD gene (Bull & Charnov, 1977; Veller et al., 2017), so that transitions in SD can occur via neutral evolution.

In many GSD systems, and particularly those with male or female heterogamety, the meiotic segregation of SD genes and sex chromosomes effectively enforces an equal sex ratio (Uller et al., 2007). Sex chromosome meiotic drive (Jaenike, 2001), i.e. elevated transmission rate of one of the sex chromosomes, can cause biased sex ratios. Hence, sex chromosome meiotic drive can promote the spread of other SD genes that restore the sex ratio. Alternatively, meiotic drive may also promote SD transitions despite a cost of sex ratio selection (Kozielska et al., 2010). In this scenario, the enhanced transmission rate of the driving SD gene is sufficient to outweigh the costs of sex ratio selection, allowing it to spread in the population.

Biased sex ratios may also be favoured (Hamilton, 1967; Uller et al., 2007), such as when a conflict occurs between parties whose fitness is maximized under different sex ratios (Werren & Beukeboom, 1998). This is most obvious for elements that exhibit sex-biased inheritance patterns versus those that do not, such as sex chromosomes versus autosomes (Haig et al., 2014). Many cytoplasmic elements like mitochondria and chloroplasts, but also a range of endosymbiotic bacteria, are solely transmitted through the female germline and hence transmission to males represents an evolutionary dead end for these elements. Consequently, selection on maternally-transmitted elements favours mechanisms that increase the production of female offspring. This can eventually lead to a transition from nuclear to cytoplasmic GSD. Other parties may also engage in conflict over the SD process, such as parents versus offspring (Werren et al., 2002; Pen, 2006; Kuijper & Pen, 2014). Both parents and offspring will be selected to maximize their own fitness and therefore seek to influence the SD process for their own benefit. Even in absence of full parental or zygotic control over sex, parent-offspring conflict can maintain variation in SD genes (Kozielska et al., 2006). Altogether, an individual's sexual identity is subject to conflict between various parties at different levels of selection, resulting in an amalgam of selective pressures to bias sex to either the male or female state.

Other explanations for SD transitions (which do not invoke sex ratio selection) have focused on the selective benefits conferred by certain SD genes or genes linked to new SD variants. First, SD genes may have a direct positive effect on fitness and may therefore be selectively favourable (Bull & Charnov, 1977). Direct selection on SD genes is however considered to be rare as it requires the SD gene not only to affect an individual's sex, but additionally to have (pleiotropic) effects on its fitness via its effect on some other phenotype (van Doorn, 2014). Other models for SD transitions invoke indirect selection on SD genes as a result of selection acting on nearby linked genes. Meiotic drive genes can be considered one such type of linked selection. Sexually antagonistic selection on nearby genes (i.e. IASC loci) can also promote the invasion of new SD genes (van Doorn & Kirkpatrick, 2007, 2010), similar to how it may establish transitions from ESD to GSD (Muralidhar & Veller, 2018). Interestingly, accumulation of IASC loci has been proposed as a possible driver of sex chromosome evolution (see "Sexually antagonistic selection and sex-specific adaptation"). IASC may therefore have an important role in both the evolution of SD

and sex chromosomes. Empirical support for this second role is however largely lacking and still often inconsistent (e.g. (Wright et al., 2017; Charlesworth, 2018)).

Finally, new SD genes may stabilize the existing SD cascade by improved sex-specific regulation of SD genes (Pomiankowski et al., 2004). In this scenario, SD genes do not confer a fitness benefit *per se*, but rather help prevent misexpression of certain SD genes to ensure fidelity of the SD program. Alternatively, downstream SD genes may confer a fitness benefit when more highly expressed, but are constrained in their expression as their sex-determining function prevents them from occurring in a homozygous state. The evolution of an upstream regulator may relieve this constraint; in this case, the new SD gene can spread due to selection on other, potentially unlinked loci.

Evolution of sex chromosomes

Transitions in SD systems can have profound influences at a genomic scale, as it can enable the evolution of a new sex chromosome system. Here, I will review the evolutionary changes new sex chromosomes undergo, focussing primarily on the initial processes. Depending on the means by which sex is determined, the chromosome carrying the topmost gene of the cascade (i.e., the primary cue gene) may become destined to evolve into a mature sex chromosome. If the primary signal without fail induces development into one specific sex, then the region linked to this gene is limited to one sex. This sex-specific inheritance is vital to sex chromosome evolution; hence, no sex chromosomes are found in haplodiploid systems, such as those as found in Hymenoptera, where the primary signal gene(s) is passed through both mothers and fathers. Sex chromosomes differ from their autosomal counterparts in various aspects (Table 1), and may be central to evolutionary processes such as speciation (e.g. Kitano & Peichel, 2012; Demuth *et al.*, 2014), sexual selection (Kirkpatrick & Hall, 2004), and genomic conflict (Werren & Beukeboom, 1998). Throughout their evolutionary history, sex chromosomes are thought to undergo several evolutionary steps to develop from near-homomorphic autosomes, which only differ with regards to the presence of the master sex-determining gene, to full-fledged heteromorphic sex chromosomes, whose gene content has diverged between the two sex chromosomes.

Table 1: Sex chromosomes differ from autosomes in a variety of ways. Adapted from Schenkel & Beukeboom (2016).

Trait	X	Y	Z	W	U	V	Autosome
Stage in which sex is determined	Diploid	Diploid	Diploid	Diploid	Haploid	Haploid	Either
Time in males	1/3	1	2/3	0	0	1	01/Feb
Time in females	2/3	0	1/3	1	1	0	01/Feb
Paternal inheritance	1 to daughters	1 to sons	1/2 to sons, 1/2 to daughters	0	0	1	1/2 to sons, 1/2 to daughters
Maternal inheritance	1/2 to sons, 1/2 to daughters	0	1 to daughters	1 to sons	1	0	1/2 to sons, 1/2 to daughters
Recombination suppression	Only in males	Yes	Only in females	Yes	Yes	Yes	No
Enriched for sexually antagonistic genes	Dominant female benefit, recessive male benefit	Male benefit (hemizygous)	Dominant male benefit, recessive female benefit	Female benefit (hemizygous)	Female-beneficial	Male-beneficial	Not enriched
Mutation accumulation	No	Yes ¹	No	Yes ¹	No ¹	No ¹	No
Effective population size	3/4	1/4	3/4	1/4	1/2	1/2	1

¹ Selection in the haploid phase of the life cycle can prevent the spreading of deleterious mutations, but is uncommon in diploid species other than plants.

Autosomes become sex chromosomes by acquiring a sex-determining function

A new sex chromosome arises when a chromosome pair acquires a sex-determining function (Ohno, 1967). This can occur by translocation of an existing SD gene or by the *de novo* evolution of an SD gene on a former autosome, resulting in a proto-sex chromosome. Alternatively, existing sex chromosomes may fuse with autosomes to form neo-sex chromosomes (Peichel et al., 2004; Pease & Hahn, 2012; Zhou & Bachtrog, 2012a). Multiple fusion events can result in complex neo-sex chromosome systems such as in the platypus *Ornithorhynchus anatinus* in which ten neo-sex chromosomes exist (Rens et al., 2004; see also Fabig et al., 2016). Both proto-sex chromosomes and neo-sex chromosomes undergo similar evolutionary trajectories, although the ancestral sex chromosome may have already undergone sex chromosome evolution to some extent. For the remainder of this introduction, I will assume that the sex chromosome arose by the evolution of a male-determining gene on a novel proto-Y-chromosome; however, W-chromosomes carrying a female-determining gene undergo similar evolutionary processes but in females instead of males. Likewise, X-chromosomes and Z-chromosomes evolve similar to each other as well.

Sexually antagonistic selection, sex-specific adaptation and recombination suppression

Similar to regular autosomes, young X- and Y-chromosomes are virtually identical to each other, differing only in a small region harbouring the SD gene. Old sex chromosomes may however be substantially differentiated from autosomes, and likewise the X- and Y-chromosomes be differentiated from each other. Sex chromosome differentiation is caused by the combined effect of recombination suppression on the Y-chromosome, and differences in exposure to selection in males and females for both the X- and the Y-chromosome. The presence of the SD gene establishes a male-linked region on the novel Y-chromosome which is transmitted only through males (Haig et al., 2014), resulting in it being exposed to selection in males only and sheltered from selection in females. For the novel X-chromosome, the opposite pattern holds but to a lesser extent; the region is more often

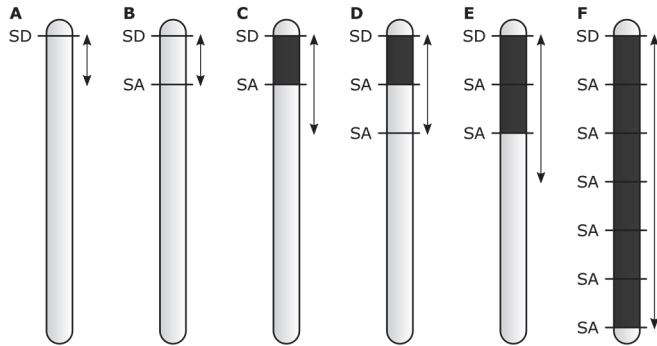


Figure 3: Sex determination, sexually antagonistic loci, and the suppression of recombination. (A) The presence of a sex-determining gene (SD) results in sex-linkage of the nearby region (denoted by the arrow). (B) This may allow genetic differentiation between males and females at a nearby locus under sexually antagonistic selection (denoted SA). (C) Linkage between the SD and SA loci is enhanced by suppression of recombination (dark grey). (D) Subsequently, the sex-linked region expands, allowing a second SA locus to differentiate between males and females. (E) Recombination becomes suppressed along a larger portion of the chromosome as it reaches this second SA locus. (F) Repetitions of these processes may culminate in a largely non-recombining chromosome, whose gene content has diverged significantly over time. Adapted from Rice (1996).

transmitted through females and accordingly is more often exposed to selection in females than in males. These different transmission and selection patterns promote the differentiation of this region between an Y- and X-chromosome. Sex-specific adaptation can then occur at IASC loci at which different alleles are favoured between males and females (Rice, 1984, 1987a). As the Y-chromosome is male-limited, it becomes enriched for male-beneficial (and potentially female-detrimental) alleles. For X-chromosomes, female-beneficial alleles spread more easily, especially when dominant. Recessive male-beneficial alleles on the X-chromosome may however persist, as these can be sheltered from counterselection in heterozygous females but are maintained by positive selection in males (assuming the Y-chromosome is fixed for the male-beneficial allele) (Rice, 1988; Graves, 2006).

On a new Y-chromosome, the sex-limited region is restricted to the immediate vicinity of the SD gene which becomes extended by the evolution of recombination suppression. Over time, this allows the entire Y-chromosome to become male-limited. Old Y-chromosomes generally show little to no recombination with the X-chromosome except for in a small region called the pseudoautosomal

region (Page et al., 1987). Several ultimate mechanisms can lead to recombination suppression, of which selection against intersexual development and the resolution of IASC are considered most common. If SD occurs not by a single dominant allele but a combination of different genes, recombination between these genes may result in intersexual development in the offspring. Such offspring are swiftly selected against, and thus a recombination-suppressing mutation may provide evolutionary stability to this complex (Haldane, 1922; Fisher, 1931). With regards to IASC, linkage between the master SD gene and an allele beneficial to the heterogametic sex results in a co-adapted gene complex that confers a fitness benefit. Reiterations of this process may allow the complex to extend outward as new loci become linked to the previously-fixed co-adapted gene complex (Rice, 1987a) (Figure 3; this model is commonly referred to as the "sexual antagonism" model of sex chromosome evolution). A novel sex chromosome harbours a limited number of IASC loci, but translocation may result in further enrichment of this chromosome (Connallon & Clark, 2011; Carvalho et al., 2015).

Chromosomal decay and suboptimal evolution in the absence of recombination

Although recombination suppression is initially favoured as it helps maintain coadapted gene complexes on the Y-chromosome, it will in the long term also lead to the Y-chromosome's decay. Recombination enables the formation of novel haplotypes and for selection to work independently on individual loci. In absence of recombination, chromosomes face deterioration or, at best, suboptimal evolution because deleterious mutations are more prone to accumulate as a result of genetic drift or via linked selection on beneficial mutations (Charlesworth & Charlesworth, 2000; Bachtrog, 2013). These effects have been formulated in the context of several related but distinct phenomena, being (1) Muller's ratchet; (2) Hill-Robertson interference; (3) background selection; and (4) genetic hitchhiking (See Box 1). Recently, an alternative but complementary process has been proposed, whereby deterioration of *cis*-regulatory elements of Y-linked genes via Muller's ratchet-like mutation accumulation promotes their selective silencing in conjunction with overexpression of X-linked counterparts (Lenormand et al., 2020).

The decay and reduced rate of adaptive evolution of the Y-chromosome following the evolution of suppressed recombination can cause the gene products

derived from this chromosome to function less efficiently than those of the X-chromosome. Intralocus competition over substrate, where the proteins generated by different alleles compete with each other over a substrate, then induces selection for an increase in gene expression for loci on the X-chromosome relative to the Y-chromosome (Rice, 1987b). Shifting this balance may occur through selective silencing on loci on the Y-chromosome, or by increased expression of those on the X-chromosome. Selective silencing may be achieved by transposons being incorporated into genes, effectively eliminating their expression (Steinemann & Steinemann, 1992, 1998), which under other circumstances might have a profoundly negative effect. Provided that the allele on the minor sex chromosome is not required to be functional through e.g. dosage sensitivity (Kaiser et al., 2011; Mank, 2013), the integration of transposons may actually provide a fitness benefit. Alternatively, the increased expression of loci on the X-chromosome may offer a way around such dosage sensitivity. This may actually represent a precursor state to full dosage compensation, and provides a fitness benefit to the upregulation of gene expression from the major sex chromosome (Charlesworth, 1978, but see Zhou & Bachtrog, 2012a).

Box 1: Recombination suppression results in mutation accumulation and suboptimal evolution on the Y-chromosome.

Recombination between two loci results in the formation of new haplotypes when both loci harbour two different alleles. Recombination breaks up the association between an allele and its genetic background, and allow selection to act independently on this allele. On the Y-chromosome, recombination becomes suppressed, and selection can act less efficiently. This inefficacy results in four population genetic processes that together underlie the accumulation of mutations on and suboptimal evolution of the Y-chromosome: (1) Muller's ratchet; (2) Hill-Robertson interference; (3) background selection; and (4) genetic hitchhiking.

Muller's ratchet (Muller, 1918, 1964) describes how (slightly) deleterious mutations may accumulate in the absence of recombination. Mutations may arise by chance and, provided they confer no or at worst a very minor fitness

disadvantage, may persist due to genetic drift (Rice, 1994). This may be the case for recessive mutations whose effect is sheltered by unmutated copies on the X-chromosome. As mutations keep on occurring, the number of chromosomes carrying a mutation is expected to increase, as in the absence of recombination these mutations cannot be purged (Rice, 1996a). In individuals carrying two different mutations, recombination results in one chromosome with two mutations and one without mutations. Without recombination, the constant mutation pressure leads to an increase in the number of chromosomes with a mutation, whereas the number of mutation-free chromosomes may only decrease in the absence of purifying selection (Rice, 1999). Genetic drift may result in changes in the frequency of mutation-free chromosomes, which can lead to the last mutation-free chromosome to eventually be lost. Alternatively, the last mutation-free chromosome may incur a mutation itself. After either of these events takes place, the least-loaded chromosome carries one mutation more compared to the ancestral state.

Hill-Robertson interference (Hill & Robertson, 1966) occurs when two beneficial mutations compete with each other. Here, an initial mutation is spreading but not fixed in the population when a second mutation occurs on a linked locus, but on a chromosome without the initial mutation. Without recombination, these mutations will not be brought together on a single chromosome and both mutations continue to compete with each other (Charlesworth & Charlesworth, 2000). Eventually, the mutation conferring a higher fitness benefit will become fixed through natural selection, resulting in a loss of the lesser beneficial mutation.

Background selection (Charlesworth et al., 1993; Peck, 1994) occurs when a beneficial mutation is unable to spread due to selection against the genetic background in which it arose. The presence of deleterious mutations, for example through Muller's ratchet, may prevent a beneficial mutation from sorting its effect. When a weakly beneficial mutation arises on a mutation-ridden chromosome, it may be lost when this chromosome acquires more mutations and subsequently transgresses the mutational load that causes it to be selected against (Rice, 1996a, 1999).

The spread of deleterious mutations may however also be facilitated by their linkage to loci under positive selection. Similar to the case of background selection, a novel beneficial mutation arises in a genotypic background with deleterious mutations. However, the selective benefit conferred by this mutation may surpass the selective deficit created by its background. This leads to genetic hitchhiking (Rice, 1987b), where deleterious mutations spread as a result of their association with a beneficial mutations. Recombination would allow the beneficial mutation to break free from its background resulting in a chromosome devoid of deleterious mutations while still in the possession of this single beneficial mutation.

Survivor genes on late-stage sex chromosomes

The differentiation of Y-chromosomes occurs by both adaptive evolution - resulting in enrichment for genes under IASC or with sex-specific functions - as well as by genetic decay due to the absence of recombination. Together these processes result in marked changes in the gene content of older Y-chromosomes. Primarily, SD genes are maintained, as mutations in these genes are likely to interfere with sexual differentiation and are thus not near-neutral, i.e. they do not have a small effect on fitness but instead are likely to be highly deleterious (Hawkins et al., 1992). This prevents such mutations from being maintained via genetic drift and therefore prevents mutations in these genes from accumulating via Muller's ratchet. IASC loci that came to be associated with the SD genes may also remain intact, provided that the selection pressure against their decay is sufficiently strong. These genes may not be essential, and therefore may accumulate mutations via Muller's ratchet, leading to their eventual demise. Formerly autosomal genes will also decay, either directly if dosage-insensitive or following evolutionary modification towards increased expression from alleles on the X-chromosome. As genes on the Y-chromosome become inactivated, large chromosomal lesions may be selectively neutral, leading to severe size reductions (Graves, 2006). Ultimately, the remaining gene content on the minor sex chromosome becomes increasingly sexualized during its evolution.

During early stages of the evolutionary process, gene numbers may have increased e.g. due to translocation of IASC loci, but gene numbers may also dwindle as other genes move away from the sex chromosomes (Emerson et al., 2004). This can be selectively advantageous for example for genes with functions in late gametogenesis. X-chromosomes often undergo meiotic silencing, which would prevent these genes from carrying out their function when X-linked (Wu & Xu, 2003). Translocation of X-chromosomal genes to the autosomes has been observed in, amongst others, *D. melanogaster* (Betrán et al., 2002; Vbranovski et al., 2009) and some mammals (Emerson et al., 2004), in which these genes indeed appear to be expressed predominantly in the testes. Effectively, these translocation events result in de-masculinization of the X-chromosome.

Turnover of sex chromosomes

Throughout their evolutionary development, sex chromosomes may face turnover and as a result a different chromosome pair may become the principal sex chromosomes via an SD transition. However, the presence of gametogenesis-related genes on the Y-chromosome observed in various species means it is required for male fertility (Lahn & Page, 1997). The Y-chromosome must thus be retained until these genes have taken up a new position in the genome. This can "lock in" a sex chromosome pair, preventing turnover to a new system even if selection otherwise favours this transition. However, sex chromosome turnover through the formation of neo-sex chromosomes is a common phenomenon in taxa such as fish (Kitano & Peichel, 2012; Kikuchi & Hamaguchi, 2013) and grasshoppers (Bugrov et al., 2016). Proto-sex chromosome formation may occur at elevated rates in species in which the SD gene shows transpositional activity such as the phorid fly *Megaselia scalaris* (Traut, 2010), strawberries (Wei et al., 2017; Tennessen et al., 2018), and the housefly *Musca domestica* (Green, 1980; Sharma, 2018). However, although mutational events may generate new SD genes and thereby create novel sex chromosomes, some selective benefit is generally needed to establish a full SD transition and concomitantly a new sex chromosome system.

The process of sex chromosome turnover effectively resets the degenerative cycle of sex chromosome evolution, but the evolution of sex chromosomes may also have a different outcome. The process of minor sex chromosome degeneration

caused Y-chromosomes to be considered as being born to die, leading to a species to go extinct as individuals of the heterogametic sex are no longer produced (Graves, 2006). This loss may also result in a change from an XY- or ZW-system to an XO- or ZO-system, though the Y-chromosome cannot be lost until its essential genes have translocated elsewhere.

Genetics of sex determination in *Musca domestica*

The housefly *M. domestica* has a multifactorial SD system in which different genes affect the SD process and can function as the SD gene. In this section, I first discuss the molecular mechanisms underlying SD in *M. domestica*; next, I discuss some previously-proposed hypotheses regarding the maintenance of multiple SD genes in this species and the support for these hypotheses.

Molecular genetics of sex determination in houseflies

The *M. domestica* SD cascade follows the generic structure that applies to most insect SD cascades, in which three functional components can be identified: (1) at the top, a primary signal conveys the sexual identity of its carrier; (2) a transducer then interprets and memorizes the signal and relays it downwards; and (3) a genetic switch that can direct development into males or females executes the order received from the transducer (Dübendorfer et al., 2002; Shearman, 2002). An overview of the SD cascade in *M. domestica* is provided in Figure 4. The key switch gene *dsx*, which directs sexual development, lies at the bottom of the SD cascade (Hediger et al., 2004). *Dsx* is expressed in individuals of both sexes, but its pre-mRNA is differently spliced in males (Dsx^M) and females (Dsx^F). Each splice variant codes for a different active form of DSX protein that bind to downstream targets to direct sexual development. In addition to *dsx*, an ortholog of *fruitless* (*fru*) regulates courtship behaviour (Meier et al., 2013). Like *dsx*, *fru* pre-mRNA is also sex-specifically spliced into several male-specific and female-specific isoforms, and its regulation is identical to that of *dsx*. Only *dsx* will be used for further discussion of the SD pathway of *M. domestica*.

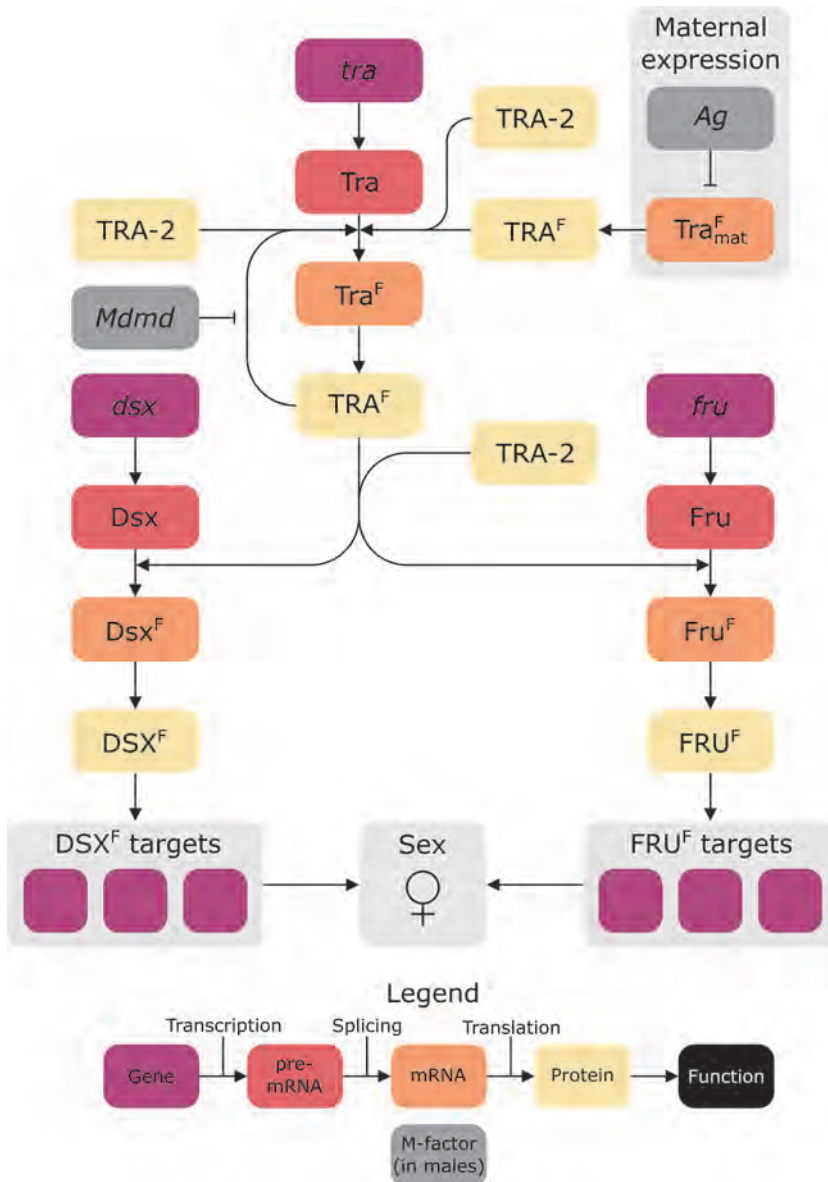


Figure 4: Known molecular components of the sex determination mechanisms in the housefly *Musca domestica*. The depicted mechanism outlines the process of female sex determination in a regular *tra/tra* female without M-factors. Boxes in dark grey indicate known M-factors that could inhibit (*Mdm*) or prevent (*Ag*) the *tra* autoregulatory loop to induce masculinization.

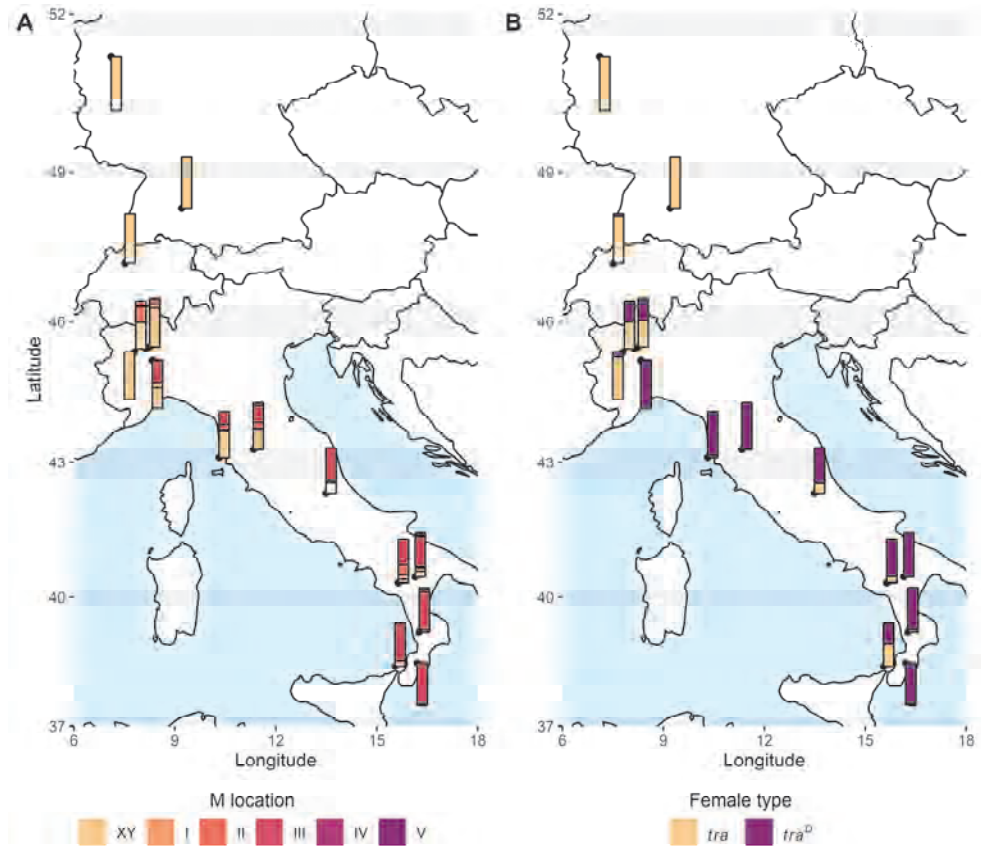


Figure 5: Variation in sex determination mechanisms in some European populations of *M. domestica*. (A) Frequencies of autosomal M-factors and (B) *tra^D*-bearing females increase with decreasing latitude in Europe. In (A), XY denotes an M-factor on the sex chromosomes rather than the autosomes. In (B), females are defined as *tra* if they have a *tra/tra* genotype, and *tra^D* if they have a *tra^D/tra* genotype. Black dots in the corner of each bar indicate the collection site. Modified from Kozielska et al. (2008).

Splicing of Dsx pre-mRNA is controlled by the *transformer (tra)* gene. *Tra* is active in females (Inoue & Hiroyoshi, 1986), and sets the splicing of *dsx* pre-mRNA to the female-specific mode in cooperation with *transformer-2 (tra-2)* (Burghardt et al., 2005; Hediger et al., 2010). *Tra* activity is maintained through an autoregulatory loop in which TRA^F/TRA-2 dimers bind to Tra pre-mRNA to splice it into the female-specific functional form, Tra^F. The loop is initiated by maternally-provided Tra^F mRNA in the zygote (Dübendorfer & Hediger, 1998), which is translated into TRA. Because *tra* is by default active in developing zygotes, additional factors are required to de-

activate it and achieve male development (so-called M-factors). Natural populations of *M. domestica* show distinctive variation in their M-factors (reviewed in Hamm *et al.*, 2015). In the standard scenario, which is commonly found in higher-latitude populations (northern hemisphere), an M-factor is located on a heteromorphic Y-chromosome (Hediger *et al.*, 1998). In this case, females carry two X-chromosomes, and males carry an X-chromosome and a Y-chromosome. In lower-latitude populations, M-factors have been mapped to autosomes I to V (Franco *et al.*, 1982), or may even be located on X-chromosomes (Denholm *et al.*, 1985). Moreover, individuals may be homozygous for certain M-factors or carry different M-factors. Autosomal M-factors increase in frequency with decreasing latitude, as found in populations of amongst others Japan (Inoue *et al.*, 1983; Tomita & Wada, 1989), Italy (Franco *et al.*, 1982), Africa (Feldmeyer *et al.*, 2008), North America (Hamm *et al.*, 2005), and Australia (Hamm & Scott, 2009). This is commonly associated with a loss of the Y-chromosome in these populations, and hence all individuals carry two X-chromosomes (Çakır & Kence, 1996).

M-factors found in different genomic locations may represent different ways to inhibit *tra* functionality, and thus be non-homologous genes that acquired a sex-determining function independently (Bopp, 2010), or may simply be duplicated copies of a single ancestral gene (Green, 1980; Çakır & Kence, 2000). The M-factor on autosome III has recently been identified as a neo-functionalized paralogue of the *M. domestica* orthologue of *nucampholin* (*ncm*), which is an essential co-factor for the spliceosome (Kelly *et al.*, 1997), and is termed *Musca domestica male-determiner* (*Mdmd*) (Sharma *et al.*, 2017). In addition, the M-factors on autosomes II to V as well as the X- and Y-chromosome are homologous to *Mdmd* (Li *et al.*, in prep). Although most M-factors are identical, it appears at least one different method to knock out the activity of the *tra* feedback loop exists in natural populations.

M-factor homozygosity in natural populations implies the presence of a feminizing factor which is epistatic to M-factors. Indeed, in populations with high incidences of autosomal M-factors, females carry a gain-of-function allele of *tra*, namely *tra^D* (Figure 5). *tra^D* appears to be insensitive to inactivation by M-factors, and thus its carrier always develops into a female. In developing zygotes, *Mdmd* induces male development by interfering with the *tra* feedback loop, presumably by preventing the action of the TRA/TRA2-complex (Hediger *et al.*, 2010). Following this, additional male-promoting factors may bind to the Tra pre-mRNA to directs its

splicing into the male-specific Tra^M isoform. The *tra^D* allele in turn may lack the binding sites for the male-promoting factors, and hence its pre-mRNA product is always spliced in the female-specific isoform. Male-specific splicing of Tra pre-mRNA would then not be a passive process, but rather it would require M-factor activity to be achieved. Interestingly, germline transplantations reveal *tra^D* do not need to be inherited maternally to induce female development. In turn, *Mdmd* acts maternally to induce male-specific splicing of Tra, resulting in all-male offspring even when the donor father was heterozygous for an M-factor. That is, the transplanted germline consists of a mixture of gametes that either do or do not carry *Mdmd*, yet all of them will lack the Tra^F mRNA required to kick-start the *tra* feedback loop in the developing zygote (Dübendorfer & Hediger, 1998). Furthermore, in *tra^D*-carrying mothers, the maternal investment of Tra^F mRNA is negated by *Mdmd* in those gametes that carry the regular *tra* allele, resulting in male development, whereas the gametes carrying the *tra^D*-allele will always develop into females. These findings illustrate that the interaction between *Mdmd* and *tra* is not necessarily restricted to the developing zygote, but rather it may already take place in the maternal germline. Accordingly, a maternal effect male-determining gene *Arrhenogenic* (Vanossi Este & Rovati, 1982) has been described in *M. domestica* that exploits this mechanism to induce maleness. Altogether, *M. domestica* features a large variation in SD mechanisms, wherein different genes involved in SD can confer the sexual identity. SD genes are found on different chromosomes, and M-factors in particular can be found on virtually every chromosome in this species.

Persistence of multifactorial sex determination across different populations

The presence of geographic variation in the frequencies of autosomal M-factors and the *tra^D* allele have drawn attention to their evolutionary history and the underlying selective pressures promoting their spread. Initially, the spread of *Mdmd* in *M. domestica* populations in Southern Europe was suggested to be due to linkage to an insecticide resistance gene (Franco et al., 1982). However, the frequency of M^{III} had not increased in Northern European populations by 2008, despite (prior) widespread use of insecticides in these regions (Kozielska *et al.*, 2008, but see Højland *et al.*, 2014). Because geographic variation in SD genes followed a latitudinal cline, it was hypothesized that the benefit of the different M-factors and *tra^D* may stem from their

selective benefit at increased temperatures as experienced by populations at lower latitudes (Franco et al., 1982; Çakır & Kence, 1996). Variation in the frequency of autosomal M-factors was best explained by seasonality in temperature, whereas variation in humidity and yearly mean temperature were shown to be best able to explain variations in the frequency of *tra^D* (Feldmeyer et al., 2008).

Temperature also affects the hypomorphic *man* mutant of *tra* (*tra^{man}*) (Schmidt, Hediger, Nöthiger, et al., 1997; Hediger et al., 2010). In heterozygous *tra^{man}/tra* females, maternal provisioning of Tra^F mRNA to the zygote is reduced, resulting in male-biased broods. Sex ratios produced by heterozygous females kept at 22 °C were less biased than those produced by females kept at 29 °C, suggesting *tra^{man}* may interfere more readily with *tra* function at elevated temperatures. Although temperature appears to play a role in maintaining autosomal M-factors and *tra^D*, it is still unresolved whether *tra^D* spread due to increased frequencies of autosomal M-factors, or whether these M-factors could spread only in the presence of *tra^D*. If M-factors of *M. domestica* indeed exhibit transpositional activity as previously suggested (Green, 1980), then this may cause male-biased sex ratios, and which would have favoured the spread of *tra^D* (Wilkins, 1995). Such a scenario would be in line with observations that *tra^D* alleles are identical in populations from almost all continents (Scott et al., 2014). However, as previously mentioned, autosomal M-factor homozygosity cannot occur in the absence of *tra^D* due to their restriction to males. An intriguing piece of evidence may come from the different viabilities associated with homozygosity for different M-factors. Homozygosity for M^I appears to cause lethality, as crosses between females carrying germlines transplanted from heterozygous M^I males and other such males failed to produce homozygous offspring (Schmidt, Hediger, Roth, et al., 1997). In other reports, homozygosity for autosomal M-factors appears to be similarly limited, except for M^{II} and M^{III} (reviewed in (Hamm et al., 2015)). This suggests most M-factors are associated with recessive lethal mutations, a characteristic commonly seen on diverged sex chromosomes. In the ancestral state, these M-factors thus may have been male-limited and their genomic neighbourhoods have evolved towards diverged sex chromosomes. Although their distribution appears to be linked to climatic factors, the evolutionary steps by which autosomal M-factors and *tra^D* arose remain undetermined.

Current state of the art

In this thesis, I explore the causes and consequences of transitions in SD mechanisms from a theoretical and experimental perspective. Previous (mostly theoretical) work on transitions in SD mechanisms has identified a variety of evolutionary phenomena that may promote or inhibit these processes described in my introduction. Despite providing a solid foundation for studying the evolution of SD, existing models of SD transitions are generally limited due to simplifying assumptions which can be overly restrictive and/or may not be fully validated. First, SD genes affect the evolution of nearby genomic regions and vice versa (van Doorn & Kirkpatrick, 2007, 2010), but this concept has not been fully explored. It is becoming increasingly clear that individual fitness and, by extension, adaptive evolution often has a complex genetic basis (e.g. (Hoban et al., 2016)). Second, many SD mechanisms involve a collection of interacting genes, yet these underlying mechanisms are often simplified to a system where sex is determined by presence/absence of a dominant allele at a single sex-determining locus that is replaced by a second locus with similar properties. Rather than being strictly dichotomous, sex is generally considered a threshold trait which commonly results in a male or female development; however, variation in SD genes and (environmental) perturbations of their activity may alter the outcome of this process so that these SD genes are in fact not fully dominant. It is not yet clear to what extent this affects the evolution of SD mechanisms. Third, multifactorial SD systems like that of *M. domestica* are poorly understood. Indeed, contemporary theories of SD evolution predict turnover in SD to occur in an identical manner throughout the entire population. Possibly, the evolution of multifactorial SD systems and simple SD systems (such as those described above) may be driven by the same evolutionary phenomena. For multifactorial SD the manner in which these phenomena affect SD transitions must however be more complex; this can be either that the selective pressures on SD genes must also work in a more complicated manner, or that these pressures are modulated by other phenomena in some yet unknown manner. Taken together, there is a rich body of work on transitions in SD mechanisms, but the complicated nature of SD and its consequences are generally inadequately addressed in existing models of SD transitions.

In many systems, transitions in SD mechanisms are associated with turnover in sex chromosomes as the new SD gene arises on an autosome. Various theories have been proposed regarding the early stages of sex chromosome evolution (i.e. shortly after the turnover in SD mechanism), but many commonly-used model organisms harbour sex chromosomes that have long been established and therefore have been subject to genetic degeneration. This has led to the development of other model systems (e.g. *Drosophila* spp. (Zhou & Bachtrog, 2012a)) in which turnover has taken place more recently and degeneration is less severe. In species with multifactorial SD, crosses between populations or strains harbouring different SD genes allow for the de novo establishment of a sex chromosome pair. In particular, *M. domestica* may be amenable to such approaches because, in this species, (potential) SD genes are found on every chromosome. Such approaches however also require a framework for assessing fitness, which (1) takes into account sex differences in individual fitness and (2) can be applied to study existing theories of early-stage sex chromosome evolution (e.g. the 'sexual antagonism' theory). Further studies on sex chromosome evolution would thus benefit from the development of new experimental model systems including a proper framework for assessing fitness.

Thesis overview

Chapter 2: Making sense of intralocus and interlocus sexual conflict

Sexual conflict arises in two main forms: intralocus and interlocus sexual conflict. Intralocus sexual conflict revolves around sex differences in the optimal phenotype and the associated genotype(s), and how this may result in conflicting selective pressures acting on loci that are shared between males and females. Interlocus sexual conflict occurs over the outcome of interactions between males and females with regard to reproduction. Both phenomena have a unique impact on the ecology and evolution of organisms, yet the distinction between them is often not sufficiently clear, resulting in errors in the design of experiments and interpretation of results. In this chapter, I outline the conceptual overlap between these two terms, as well as the various differences between them.

Chapter 3: Epistatic interactions between sex chromosomes and autosomes affect the stability of sex chromosomes systems

Sex chromosomes originate from autosomes by evolution of SD genes, which over time become associated with sexually antagonistic genetic alleles as recombination becomes suppressed. Sex chromosomes generally comprise but a small portion of the genome, and may interact with their autosomal counterparts to affect fitness. However, existing models of transitions in SD mechanisms generally ignore such interactions, and instead assume that sex chromosomes only affect fitness due to direct fitness benefits of the SD gene or by enrichment for sexually antagonistic genetic variants. Here, I extend recent models of SD transitions by incorporating epistatic interactions between the sex chromosome and an autosomal locus, and assess under what conditions this impedes or promotes transitions between SD mechanisms.

Chapter 4: Evolution of polymorphic sex determination system in heterogeneous environments

SD mechanisms are commonly classified into environmental (ESD) or genetic (GSD) SD mechanisms. However, genetic processes are commonly affected by environmental conditions, and, in many species, sex can be determined by an individual's genotype at a certain locus, but where the sex-determining capacity of this locus may be overruled or otherwise affected by the environmental conditions in which an individual's sex is determined. The impact of such genotype-by-environment effects on individual SD and the evolution of SD mechanisms has not yet been theoretically investigated. In this chapter, I develop a model in which sex is determined by the activity of a genetic factor, which is in turn modulated by environmental conditions. I determine under what conditions novel SD variants may evolve, and how this can lead to intraspecific variation in SD along environmental gradients. Finally, I apply this model to illustrate how the polymorphic SD system of the housefly may have evolved via adaptive evolution.

*Chapter 5: Establishment of baby-sex chromosomes using interpopulation crosses in the housefly *Musca domestica**

Sex chromosome evolution results in the degeneration of the Y-/W-chromosome following the evolution of recombination suppression. This erodes signs of past (adaptive) evolution on these chromosomes so that our current understanding of early sex chromosome evolution is mostly theoretical. Previous work has sought to exploit naturally-occurring young sex chromosome systems to study these early stages using e.g. interspecific comparisons. In this chapter, I used a novel approach to generate young sex chromosomes by exploiting the multifactorial SD system of *M. domestica*. By introgressing autosomal M-factors from Italian populations into a Dutch genetic background, I managed to establish a new XY chromosome pair (baby-sex chromosomes), where the M-carrying autosome from Italian strains is converted into a baby-Y chromosome. This approach can be used in a repeatable manner and allows for sex chromosome evolution to be studied in real time. I additionally discuss how these baby-sex chromosomes may be used to test the 'sexual antagonism' model of sex chromosome evolution.

*Chapter 6: Sex-specific fitness in the housefly *Musca domestica**

Fitness plays an essential conceptual role in evolutionary biology, but understanding how individual fitness is achieved and even maximized is generally difficult or even impossible as it is unclear which selective pressures affect individuals and to what extent they do so. Moreover, these selective pressures may not be constant, but rather different individuals may face different selective pressures. Fitness is optimized under different conditions for males and females. In this chapter, I discuss conceptual issues regarding estimating fitness in general and sex-specific fitness in particular. Based on the sex roles in the housefly, the development of sex-specific fitness proxies are discussed. Furthermore, artificial selection for increased female fitness (based on a fitness proxy) is used to illustrate the applicability of such proxies.

Chapter 7: Discussion

This chapter contains the final summarizing discussion of this thesis.

