The genomic basis of the response to female-limited X-chromosome evolution

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DOCTORAL DISSERTATION

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In this thesis, I attempted to achieve a better understanding of the nature of X-linked polymorphic loci using a female-limited X chromosome (FLX) experimental evolution in *Drosophila melanogaster*. I expected that expressing the evolved X chromosome will result in an increase in female fitness and a decrease in male fitness. I first investigated the effect of an experimentally evolved female-limited X chromosome on male reproductive traits (Paper I). Secondly, I examined how the genome-wide expression pattern responds to the presence of the evolved X chromosome (Paper II), and then I analysed the changes in allele frequencies across the genome (Paper III). Finally, I attempted to study the changes in genetic variances and covariances in sexually homologous traits in response to FLX evolution, as well as change in the cross-sex genetic correlations for these sexually homologous traits (Paper IV).
Contrary to the initial expectation, I found evidence of trade-offs between various components of male reproductive success rather than an overall decline (Paper I). However, I identified a more 'feminized' gene expression profile as the result of FLX evolution (Paper II) and found evidence of adaptation in the methodological control treatment which was a necessary part of the experimental design. The analysis of differences in allele frequencies between selection regimes showed no evidence of overrepresentation of SA loci on the X chromosome, but these results suggest an interesting avenue for future study of sexual conflict over sensory ability (Paper III). Finally, I found evidence of a breakdown in the intersexual genetic correlation for locomotory activity in FLX populations compared to control populations (Paper IV).
These results indicate that the X chromosome may not possess as many SA mutations as previously thought, and they are by nature difficult to study in a species with old, already highly degenerated sex chromosomes. However, the results presented here highlight the importance of sex-specific selection pressures in shaping the genetic architecture of many traits.

Key words: Sex chromosome, X chromosome, Sexual antagonism, experimental evolution, *D. melanogaster*

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Author contributions

1. JKA conceived the study. YM and GK designed experiments, collected data and analysed with JKA's help. YM wrote the first draft of this manuscript with input from JKA and KKLH.
2. JKA conceived the study. YM designed the experiment, collected the data, analyzed and wrote the first draft of this manuscript with input from JKA.
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4. JKA conceived the study. JKA, MT, and YM contributed to the design of the study. YM collected the data. All authors participated in the data analyses, interpreting the results and contributed to the writing and approved of the final manuscript.

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Abstract

Sexually antagonistic (SA) mutations that increase fitness in one sex and decrease it in the other sex (also known as intralocus sexual conflict) are central to the sexual antagonism hypothesis of sex chromosome evolution. It was suggested long ago (by Fisher, in 1931) that tight linkage to a sex-determining locus facilitates the accumulation of SA mutations even when their detrimental effect in one sex exceeds its benefit in the other. In male heterogametic species (XX/XY), the X chromosome is also thought to be a more favourable place for accumulation of SA mutations due to its inheritance mode, and this prediction has some empirical support. However other studies have found evidence of autosome linked SA mutations, and in some cases a much smaller number of X-linked SA variants were found compared to the random expectation. It is therefore still unclear whether SA mutations tend to accumulate disproportionately on the X chromosome, and whether the X chromosome tends to evolve toward the female optimum due to its female-biased expression (spending 2/3 of the time in females and 1/3 of the time in males on average). Furthermore, because of the difficulties in detecting SA alleles directly, we know very little about the nature of X-linked SA mutations and their evolutionary dynamics.

In this thesis, I attempted to achieve a better understanding of the nature of X-linked polymorphic loci using a female-limited X chromosome (FLX) experimental evolution in *Drosophila melanogaster*. I expected that expressing the evolved X chromosome will result in an increase in female fitness and a decrease in male fitness. I first investigated the effect of an experimentally evolved female-limited X chromosome on male reproductive traits (**Paper I**). Secondly, I examined how the genome-wide expression pattern responds to the presence of the evolved X chromosome (**Paper II**), and then I analysed the changes in allele frequencies across the genome (**Paper III**). Finally, I attempted to study the changes in genetic variances and covariances in sexually homologous traits in response to FLX evolution, as well as change in the cross-sex genetic correlations for these sexually homologous traits (**Paper IV**).

Contrary to the initial expectation, I found evidence of trade-offs between various components of male reproductive success rather than an overall decline (**Paper I**). However, I identified a more 'feminized' gene expression profile as the result of FLX evolution (**Paper II**) and found evidence of adaptation in the methodological control treatment which was a necessary part of the experimental design. The

analysis of differences in allele frequencies between selection regimes showed no evidence of overrepresentation of SA loci on the X chromosome, but these results suggest an interesting avenue for future study of sexual conflict over sensory ability (**Paper III**). Finally, I found evidence of a breakdown in the intersexual genetic correlation for locomotory activity in FLX populations compared to control populations (**Paper IV**).

These results indicate that the X chromosome may not possess as many SA mutations as previously thought, and they are by nature difficult to study in a species with old, already highly degenerated sex chromosomes. However, the results presented here highlight the importance of sex-specific selection pressures in shaping the genetic architecture of many traits.

Svensk sammanfattning

Sexuellt antagonistiska (SA) mutationer är genetiska varianter som ökar fit-ness (dvs överlevnad och reproduktionsframgång) hos ett kön men samtidigt minskar den hos det andra könet (detta kallas även inom-lokus sexuell konflikt, eller ”intralocus sexual conflict” på engelska). Sexuellt antagonistiska mutat-ioner är en central del i moderna hypoteser om könskromosomernas utveckling. Det föreslogs för länge sedan (av Fisher, 1931) att närliggande gener till en könsbestämmande gen har lättare för att ackumulera SA-mutationer även när den skadliga effekten hos det ena könet är större än den fördelaktiga effekten hos det andra. Hos heterogametiska arter (XX / XY), tros X-kromosomen också vara en mer gynnsam plats för ackumulering av SA-mutationer på grund av dess könsspecifika arvsmönster, vilket har bekräftats empiriskt flera gånger. Däremot finns det även andra studier som har visat den autosomala grunden för SA-mutationer, och i visa fall har många färre X-länkade varianter hittats jäm-fört med slumpmässiga förväntningar. Det är därför fortfarande oklart om SA-mutationer tenderar att ackumuleras på X-kromosomen mer än på autosomerna, samt om X-kromosom har en tendens att utvecklas mot den honliga optimum. Dessutom, på grund av svårigheterna med att direkt detektera SA-alleler, vet vi väldigt lite om X-länkade SA-mutationers allmänna egenskaper och deras evolutionära dynamik.

I denna avhandling försökte jag uppnå en bättre förståelse av X-länkade polymorfa loci med hjälp av experimentell evolution hos Drosophila melanogaster, där selektionstrycket på X-kromosomen begränsades till honor (experimentet kallas för FLX, efter ”Female-Limited X-chromsome”). Jag förväntade mig att uttryck av den utvecklade X-kromosomen skulle öka honlig fitness och minska hanlig fitness. Jag undersökte först effekten av experimen-tellt utvecklade X-kromosomer på hanliga reproduktionsegenskaper (Paper I). Därefter undersökte jag vilka genomgenomfattande skillnader i genuttrycksmönster orsakas av de utvecklade X-kromosomerna (Paper II), sedan analyserade jag förändringarna i allelfrekvens över genomet (Paper III). Slutligen studerade jag förändringarna i genetisk varians-kovarians som påverkar sexuellt homologa egenskapers respons till FLX-utveckling, samt genetiska korrelationer mellan dessa sexuellt homologa egenskaper (Paper IV).

I motsats till den ursprungliga förväntningen fann jag bevis på avvägningar mellan olika komponenter av hanlig reproduktionsframgång snarare än en övergripande nedgång (Paper I). Jag identifierade emellertid en mer 'feminiserad'

genuttrycksprofil som ett resultat av FLX-utvecklingen (Paper II) och fann bevis på anpassning till den metodologiska kontrollbehandlingen. Analysen av skillnaden i allelfrekvens mellan selektionsregimer visade inga bevis för överrepresentation av SA loci på X-kromosomen, men tydde på före-komsten av sexuell konflikt över sensorisk förmåga, vilket kan vara intressant att undersöka i framtida studier (Paper III). Jag hittade även att den genetiska korrelationen mellan könen för gångaktivitet bröts ner hos FLX-populationerna (Paper IV).

Dessa resultat indikerar att X-kromosomen kanske inte bär på så många SA-mutationer som vi har tidigare trott, och de är av sin natur svåra att studera hos en art med gamla, redan mycket degenererade könskromosomer. Däremot kunde jag visa att könsspecifika selektionstryck spelar en viktig roll i att forma den genetiska arkitekturen hos många egenskaper.

Абстракт

Бір жыныста фитнесті жоғарылататын және оны екінші жыныста төмендететін жыныстық антагонистік (ЖА) мутациялар (интралокустық жыныстық қақтығыс деп те аталады) жыныстық хромосома эволюциясының жыныстық антагонизм гипотезасының негізі болып табылады. Осыдан біраз бұрын (Фишер, 1931 жылы) бір жыныстағы зиянды әсері екіншісіндегі пайдасынан асып кетсе де, жынысты анықтайтын локуспен тығыз байланысы ЖА мутацияларының жинақталуын жеңілдететіндіегі айтылған. Аталығы гетерогаметикалық түрлерінде (XX / XY) X хромосомасы тұқым қуалаушылық режиміне байланысты ЖА мутацияларының жинақталуы үшін қолайлы жер болып саналады және бұл болжам эмпирикалық қолдауға ие. Алайда, басқа зерттеулерде аутосомалық байланысты ЖА мутациялардың дәлелдері табылды, ал кейбір жағдайларда кездейсоқ күтумен салыстырғанда X-байланысқан ЖА нұсқаларының саны аз екендігі байқалған. Сондықтан X хромосомасының аналықтағы басым экспрессясына (орта есеппен уақытының 2/3 бөлігін аналықта және 1/3 бөлігін аталықта жұмсауы) байланысты оның ЖА мутациялардың пропорционалды емес түрде жиналуы мен аналыққа бейім жиналуы әлі күнге анық емес. Сонымен қатар, ЖА аллельдерін тікелей анықтаудағы қиындықтарға байланысты, біз X-байланысқан ЖА мутацияларының табиғаты және олардың эволюциялық динамикасы туралы өте аз білеміз.

Осы тезисте мен дрозофила меланогастердің аналығына шектелген (АШ) X хромосома эксперименттік эволюциясын қолдана отырып, X байланысқан полиморфты локустардың қасиетін түсінуге тырыстым. Сол себепті, тәжірибелік эволюциядан өткен X хромосомасын экспрессялау аналық фитнесын жоғарылауына және аталық фитнесынің төмендеуіне әкеледі деп күттім. Мен алдымен (I мақала) тәжірибелік эволюциядан өткен X хромосоманың аталықтың репродуктивті белгілеріне әсерін эксперименталды түрде зерттедім. Екіншіден, мен бүкіл геномдық экспрессясының тәжірибелік эволюциядан өткен X хромосомасына жауабына анализ жасадым (II мақала), осыдан кейін бүкіл геном бойынша аллельдердің жиілігінің өзгеруін талдадым (III мақала). Соңында, мен АШ X хромосома эволюциясына жауап ретінде жыныстық гомологты белгілердегі генетикалық варианстар мен ковариантар өзгерісін, сондай-ақ осы жыныстық гомологиялық белгілердің жыныс араық генетикалық өзара әсерінің өзгеруін зерттеуге тырыстым (IV мақала).

Бастапқы тәжірибелік болжамымызға қарсы, аталық фитнесынің жалпы төмендеуын емес, қайта аталық репродуктивті белгілерінің компоненттері арасындағы өзара қарама-қайшылықты өзгерісті таптық (I мақала). Алайда, мен АШ Х хромосома эволюциясы нәтижесінде ‘аналыққа бейімделген’ гендік экспрессия профилін анықтадым (II мақала) және тәжірибелік жобалаудың қажетті бөлігі болған методикалық бақылау режимінде бейімделудің дәлелдерін таптым. Селекциялық режимдер арасындағы аллельдер жиілігінің айырмашылығын талдау нәтижесі Х хромосомасында ЖА локустарының артықша жинақтаыуын көрсеткен жоқ, бірақ бұл нәтижелер сенсорлық қабілеттіліктегі жыныстық қақтығысты болашақта зерттеудің қызықты бағытын ұсынады (III мақала). Соңында, мен бақылау популяцияларымен салыстырғанда АШ Х хромосома популяцияларындағы локомотивтік белсенділіктің жынысаралық генетикалық корреляциясының бұзылуының дәлелін таптым (IV мақала).

Бұл нәтижелер Х хромосомасында бұрын ойлағандай көп ЖА мутациясы болмауы мүмкін екенін және олардың табиғаты бойынша әлде қашан жоғарғы деңейде деградатцтияланған жыныстық хромосомалары бар түрлерде зерттеу қиын екенін көрсетті. Алайда, мұнда келтірілген нәтижелер көптеген белгілердің генетикалық архитектурасын қалыптастыруда жынысқа тән селекциялық қысымның маңыздылығын көрсетеді.

Introduction

Sex chromosomes are specialized chromosomes carrying the major sex-determining region that controls the sex of an individual (Bachtrog et al. 2011). Many eukaryotes have heteromorphic sex chromosomes that are usually differentially represented in males and females (e.g., 2/3 of the time in a homozygous or heterozygous state in females and 1/3 of the time in a hemizygous state in males, in XY systems), and are therefore expected to experience various sex-specific selection pressures that play an important role in the evolution of sexual dimorphism (Rice 1984). The unique characteristics of sex chromosomes have attracted considerable attention and the study of these chromosomes has become an active research field (Abbott et al. 2017, Bachtrog et al. 2014, Beukeboom and Perrin 2014, Ellegren 2011). The arrival of large-scale sequencing techniques and other genomic approaches offers new perspectives to study the genetic properties of sex chromosomes and the molecular evolution of sex-liked genetic variants (Bachtrog et al. 2014, Ellegren 2011). However, the role of polymorphic sex-liked genetic variants in sex chromosome evolution and their genome-wide effects are poorly understood.

In this thesis I have utilized experimental evolution to study the nature of X-linked polymorphic loci using the famous model organism *Drosophila melanogaster* as a study system.

Determination of sex

Sex is the mixing of genomes from two individuals of opposite sex via meiosis (meiotic sex), and is a common component of sexual reproduction in all eukaryotes (Mirzaghaderi and Horandl 2016). Sex is determined by a wide range of mechanisms, which can be classified into two main categories: environmental (in some amphibians and reptiles for example) and genetic sex determination mechanisms (Bull 1983). Genetics of sex determination is remarkably variable, even within a single type of system such as male heterogamety (Bachtrog et al. 2014, Beukeboom and Perrin 2014). For example, in humans, males are heterozygous for the sex determining region (XY) while females are homozygous carrying 2 X's (XX/XY system), and the Y is essential for developing into a male. However, in *D. melanogaster*, it is the ratio between the X and the autosomal chromosomes (A) that determines sex: females are 2X;2A and males are X;2A, and XO individuals lacking

a Y still develop into (sterile) males (Baker and Ridge 1980, Bridges 1921). Female heterogametic sex determination systems are found in some insects and birds (ZZ/ZW system) (Beukeboom and Perrin 2014).

In some algae and bryophytes sex is determined in the haploid phase (known as a UV sex determination system), where individuals with a U chromosome are female and those with a V chromosome are male (Bachtrog et al. 2011, Ellegren 2011). Haplodiploidy is another mode of sex determination occurring in 12% of animal species, including all ants, wasps and bees. In rare cases, some populations of the same species (e.g. houseflies) exhibit a mixed sex determination system (Beukeboom and Perrin 2014, Bull 1983), with elements from both XY and ZW systems. The genomic basis of sex determination is highly variable, and it is also the case that the main evolutionary driver(s) and exact mechanism(s) of turnover in sex determination systems still remain unclear (Bachtrog et al. 2014, Pennell et al. 2018).

Sex chromosome evolution

In most eukaryotic organisms, including animals, birds and insects, sex is determined by the presence of morphologically and genetically differentiated X and Y chromosomes (in male heterogamety), or Z and W chromosomes (in female heterogamety) (Charlesworth 1996, Vicoso and Bachtrog 2015). Such highly dimorphic sex chromosomes carry the genes that determine sex and have evolved independently multiple times in many different taxa (Charlesworth 1991, Vicoso and Charlesworth 2006). Morphologically distinct sex chromosomes (X and Y in mammals and some other model species such as *Drosophila*) are usually old, conserved, and are the most familiar and well-studied sex determination systems (Bachtrog et al. 2014).

One of the widely accepted models for the evolution of heteromorphic sex chromosomes (**Fig. 1**) proposes that sex chromosomes evolve from a pair of autosomes, which at some point gained a major sex-determination function through mutations in one or several genes (Bachtrog et al. 2011, Beukeboom and Perrin 2014, Charlesworth 1978). In transitions from hermaphroditism to separate sexes, the evolutionary sequence is thought to be that a recessive male-sterility mutation first spreads, generating a gynodioecious population (i.e., females and hermaphrodites), then at a different locus a dominant female-sterility mutation occurs, creating males and completing the transition to separate sexes. These two mutations are usually at different loci but may be tightly linked, forming proto-X and proto-Y chromosomes (Charlesworth 1996). In transitions from polygenic sex determination or environmental sex determination to genetic sex determination, fixation of a single mutation may be sufficient (van Doorn 2014).

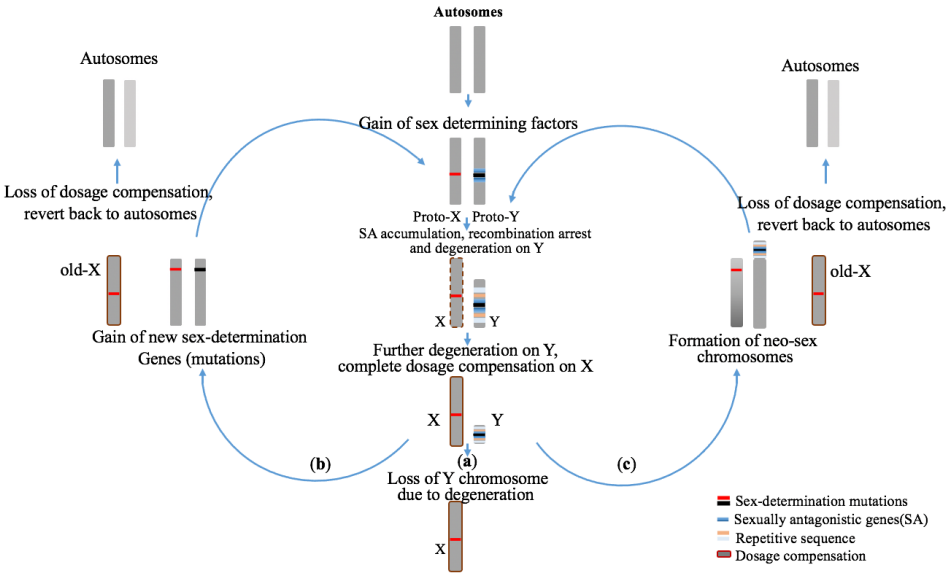

Figure 1. The dynamic cycle and multiple pathways of sex-chromosome evolution in a male heteromorphic system. Top centre: an autosome pair in a hermaphrodite (or a species with environmental sex determination) gains a sex-determining factor that evolves to become a highly heteromorphic pair of sex chromosomes, via cessation of recombination, degeneration and evo-lution of dosage compensation (a). Figure adapted from (Abbott et al. 2017, Wei and Barbash 2015). New sex chromosomes can evolve by either acquisition of new sex-determination genes or transposition of a sex-determining locus to an autosome (b), or fusion between autosomes and existing sex chromosomes (c).

After the establishment of the proto-sex chromosomes, selection favours a linkage between the sex-determining alleles and sexually antagonistic alleles (mutations that are advantageous in one sex but disadvantageous in the other sex). Accumulation of sexually antagonistic alleles close to the sex-determining loci leads to further expansion of the sex-determining region, increasing genetic differentiation between the two proto-sex chromosomes. Then suppression of recombination evolves in the heterozygous sex, because selection favours these genes to be inherited together (Abbott et al. 2017, Wei and Barbash 2015). Over time, and in the absence of recombination, the two chromosomes independently accumulate mutations and structural changes (inversions, repetitive sequences, or transposons) that make them progressively more different from each other (Ellegren 2011).

Next, the increase of the non-recombining segment causes the sex-limited Y chromosome to degenerate via mutation accumulation, selection, and genetic drift (Bachtrog et al. 2011). As the Y chromosome degenerates and loses gene function, the X chromosome becomes effectively haploid in males, while the rest of the genome is diploid. Therefore, the ratio between products of X-linked genes and autosomal genes is halved compared to that of XX females, creating a detrimental imbalance for dosage-sensitive genes (Wei and Barbash 2015). This unequal

expression can be solved by different dosage compensation strategies. For example, hyperexpression of X-linked genes in males in *Drosophila*, or random inactivation of one of the X chromosomes in female mammals (Graves 2015).

Sex chromosomes are labile, so frequent switches between autosomes and the chromosome pair that determines sex can occur rapidly over evolutionary time (Charlesworth 1991, Charlesworth and Charlesworth 2000). Transitions are particularly likely in species with relatively undifferentiated sex chromosomes and may be facilitated when the new sex-determining gene (or closely linked locus) has beneficial effects on fitness (Abbott et al. 2017, Vicoso and Bachtrog 2015). Transitions may also occur by chromosomal fusions between autosomes and existing sex chromosomes (to produce neo-sex chromosomes) (Wei and Barbash 2015).

X chromosome evolution and intralocus sexual conflict

Compared to all other chromosomes, the unique inheritance characteristics of the sex chromosomes were first noticed by German cytologist H. Henking. During the study of male meiosis of the *Pyrrhocoris apterus* in 1891, he noted an unpaired chromatin element segregating to only one half of the sperm and named it as element 'X' for its unknown nature (Beukeboom and Perrin 2014, Brown 2003). In the early 1900s, Nettie Stevens and Edmund Beecher Wilson independently discovered its important role in sex determination in insects (Abbott et al. 2017, Beukeboom and Perrin 2014, Brush 1978).

The unique characteristics of the X chromosome are mainly associated with its effect on sex determination and inheritance mode; males have only a single copy of the X chromosome while females possess two copies (Rice 1984, Schaffner 2004). This unequal inheritance of the X chromosome has an evolutionarily significant effect on mutation accumulation (Mallet et al. 2011) and recombination rate on the X chromosome (Schaffner 2004). Specifically, sexually antagonistic mutations which are beneficial for one sex but detrimental for the other sex may be more easily accumulated on the X chromosome, and eventually lead to the evolution of sexual dimorphism (Rice 1984).

Sexually dimorphic reproductive strategies and differences in variance in reproductive success between males and females can bring the sexes into evolutionary conflict and generate different selection pressures on many traits that maximize the fitness of one sex at the expense of the other sex (Andersson 1994, Ranz et al. 2003, Rice 1984). The result of divergent selection pressures in sexually differentiated species depends on there being two different sexes with females producing large macrogametes (eggs), and males making microgametes (sperm). This anisogamy, i.e., gametes of two different sizes, ultimately underlies the

evolution of sex differences in behavior and morphology, although ecological and demographic factors can play a more important proximate role (Andersson 1994, Kokko and Jennions 2008). Regardless of how sexual dimorphism evolves, it sets the stage for sexual conflict, within and between the sexes (Andersson 1994). It is an almost universal phenomenon in sexually reproducing organisms, occurring whenever traits shared by males and females have sex-specific optima that cannot be attained simultaneously, generating an evolutionary conflict of interest between the sexes (Burke and Bonduriansky 2017).

Sexual antagonism, specifically intralocus sexual antagonism (also known as intralocus sexual conflict, or occasionally ontogenetic sexual conflict), where alleles at a single locus have opposite fitness effects in the two sexes (Rice 1984), has important implications for the nature and magnitude of genetic diversity within populations (Connallon and Clark 2014, Gibson et al. 2002).

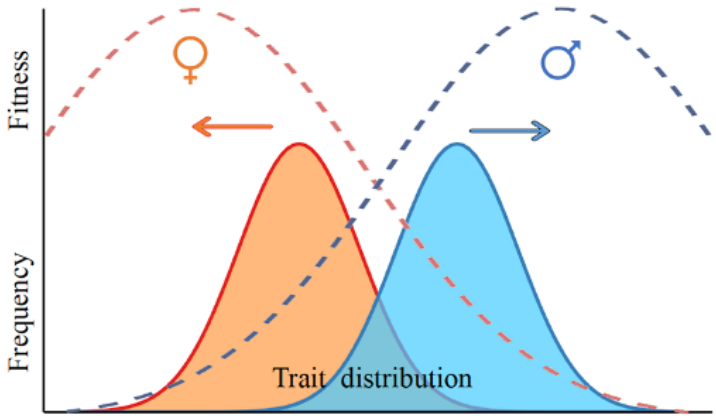

Figure 2. The relationship between sexual dimorphism and intralocus sexual conflict. Blue and orange areas indicate phenotypic distributions for a quantitative trait in males and females respectively, and dashed lines signify fitness surfaces for males (blue) and females (orange). Arrows indicate the discrepancy between the fitness optimum and the phenotypic mean in each sex.

Intralocus sexual conflict is common in a wide variety of traits in many taxa and has been found in both natural and laboratory populations (Abbott 2011, Stewart et al. 2010). These shared traits have a common genetic basis, which means there is often a strong, positive genetic correlation between the two sexes (Bonduriansky and Chenoweth 2009, Lande 1980), and the conflict may be mitigated or resolved by mechanisms leading to the evolution of sexual dimorphisms (Fig. 2), such as sex-specific gene expression, genomic imprinting, or reduced opposite-sex heritability (Pischedda and Chippindale 2006). Sexual antagonism is therefore often considered to be a relatively transient phenomenon (Stewart et al. 2010). In addition, sex-linkage may contribute to the resolution of intra-locus sexual conflict (Rice 1984), and the sex chromosomes can either increase or reduce this conflict, depending on

dominance and pleiotropy. For example, at the adult stage in a laboratory-adapted *D. melanogaster* the X chromosome was estimated to harbor 97% of the genome-wide sexually antagonistic variation (Gibson et al. 2002). When most of this type of conflict remains unresolved in the genome, it can contribute to the maintenance of additive genetic variance (Bonduriansky and Chenoweth 2009), but also result in reduced population mean fitness (**Fig. 2**) (Cox and Calsbeek 2009).

Thesis aims

In this thesis, I aimed to investigate the nature of polymorphic X-linked loci and their role in the sex-specific genetic architecture using experimentally evolved *Drosophila melanogaster* populations, in which selection pressures on the X chromosome had been manipulated for multiple generations.

In **Paper I**, after more than 100 generations of female limited X chromosome evolution (FLX), we analyzed the effect of the evolved X on male reproductive traits. The traits that we investigated, male attractiveness to females and performance in sperm competition, were selected because the FLX experimental evolution protocol should remove male-specific selection pressures on the X, and we therefore expected a negative effect of the evolved X on male reproductive traits.

In **Paper II**, after 95 generations of female limited expression of the X chromosome, we analyzed the genome-wide gene expression in fly heads in response to the presence of an evolved X chromosome. We also attempted to use this information to disentangle signatures of feminization of gene expression as the result of FLX evolution from the confounding effect of the balancer chromosome on gene expression, which was necessary to control the inheritance of the experimental X chromosome.

In **Paper III**, at generation 133 of FLX experimental evolution, we analyzed genome-wide allele frequency changes using next generation sequencing of pools of individuals. The main idea of using the FLX experiment protocol is to remove male selective constraints on X-linked polymorphic sites and fix them for female beneficial alleles, so we therefore expected to see increased frequency of alleles which were subject to sexually antagonistic selection in the ancestral population.

In **Paper IV**, after more than 147 generations of FLX evolution, we used quantitative genetic analyses to investigate the effect of experimentally evolved X chromosomes on the genetic architecture of sexually homologous traits and analyzed the divergence in within-sex (**G**) and between-sex (**B**) genetic variance-covariance matrices in experimentally evolved *D. melanogaster* populations.

General methodology

Study system, *Drosophila melanogaster*

The common vinegar fly *Drosohila melanogaster* is one of the most widely used and successful genetic model systems (Heberlein 2000). There are many advantages of using this organism for the study of human diseases as well as many other different fields, including regulation of gene expression, cell biology, neuro-biology, development, and behavior (Jennings 2011). First of all, the entire genome sequence and the annotations of the genome based on the finished sequence are publicly available, making it very simple to study and manipulate a particular gene or genomic region (Adams et al. 2000, FlyBase 2003). The *D. melanogaster* genome is around 60% homologous to the human genome; in addition about 75% of genes responsible for human diseases have homologs in these flies (Pandey and Nichols 2011).

Another advantage of *D. melanogaster* is their small size (2-3mm), short generation time (approximately 10 days), and ease of handling without any sophisticated tools, combined with inexpensive ways to culture them. All these advantages have established *Drosophila* as one of the leading animal models allowing researchers to explore the heritability of certain traits or behaviours over may generations in a short period of time (Pandey and Nichols 2011).

In addition, *D. melanogaster* is a sexually dimorphic species, where females are larger than males and males are completely melanised on their last three abdominal segments. Males also have sex combs on their front legs, which they use for courtship, and external genitals at the end of the abdomen (Demerec 1950). *D. melanogaster* is a male heterogametic species (XY), and the ratio between X and autosomal (A) chromosomes determines sex, where males are X:2A, and females are 2X:2A as discussed above (2X:2A individuals are female even if they also carry a Y chromosome) (Bridges 1925).

Another important factor is sex-linkage; for example, previous estimates suggest that the X chromosome of *D. melanogaster* accounts for 45% of the genome-wide fitness variation and 97% of the genome-wide sexually antagonistic variation (Gibson et al. 2002). This genomic structure has important implications for the evolution of sex chromosomes and evolution of complex traits, because X-linked sexually antagonistic variation contributes to negative intersexual heritability for

fitness (Gibson et al. 2002). However, note that more recent studies do not support enrichment of SA loci on the X (Frank and Patten 2020, Fry 2010). Nevertheless, the large size of the X (approximately 20% of the euchromatic genome) in relation to the rest of the genome means that X-linked loci are likely to make a large contribution to the variance in the many polygenic traits (Adams et al. 2000). In terms of the Y chromosome, the gene content on the Y is about 0.2% of the genome of *D. melanogaster,* with only a few functional genes on the Y chromosome (Adams et al. 2000). However, different types of polymorphisms on the Y chromosome differentially affect the expression of hundreds of X-linked and autosomal genes, probably via non-coding RNA (Lemos et al. 2008).

Experimental evolution

Experimental evolution is a highly useful research approach where it is possible to study different aspects of evolutionary changes occurring in experimental populations under a set of predefined selection conditions (Kawecki et al. 2012). The possibility to replicate the same gene pool and to maintain it under the same selection condition is one of the main attractive advantages of experimental evolution compared with other methods of evolutionary analysis (Schlötterer et al. 2015). Divergence between replicates will increase with time in response to selection that acts on pre-existing polymorphisms (Kawecki et al. 2012, Schlötterer et al. 2015). Therefore, the experimenter is able to disentangle these stochastic effects of the (genetically polymorphic) base population from the deterministic effects associated with the selection pressure (Kawecki et al. 2012, Schlötterer et al. 2015).

The arrival of next-generation sequencing technologies have now made it possible to study the underlying genomic response to selection (Barrick and Lenski 2013). Especially next generation sequencing of pooled samples (Pool-Seq) is a cost effective way to provide highly accurate estimates (Schlötterer et al. 2015). Thus, using Pool-Seq to study the genetic dynamics in experimentally evolved populations is a promising approach. Furthermore, due to its great flexibility of experimental designs, experimental evolution has been used as a powerful method to study sexual conflict and sex chromosome evolution (Abbott et al. 2020, Abbott et al. 2013, Hollis et al. 2014, Immonen et al. 2014, Innocenti et al. 2014, Lund-Hansen et al. 2020, Rice 1996, Rice 1998). These and many other advantages of experimental evolution, as well as several limitations (including relatively small sample size of study populations) are reviewed in (Kawecki et al. 2012, Schlötterer et al. 2015).

Experimental design

A seminal model formulated by Rice showed how an accumulation of sexually-antagonistic genes can be facilitated on the X chromosome compared to the autosomes (Rice 1984). In this work Rice predicted that SA alleles of sex-linked genes could spread even if the harm to benefit ratio is high. There is some empirical evidence to support this prediction and also showing that the X chromosome harbours substantial sexually antagonistic variance for fitness (Abbott et al. 2013, Gibson et al. 2002). Compelling empirical evidence for the existence of intralocus sexual conflict comes from the *D. melanogaster* genetic model system (Pischedda and Chippindale 2006). In prior experimental evolutionary studies with *D. melanogaster* researchers have been able to evaluate the evolutionary impact of intralocus sexual conflict (IASC), by using a sex-biased selection method that removes the opportunity for selection in one sex, which results in increased fitness in the other sex (Abbott et al. 2013, Prasad et al. 2007, Rice 1996, Rice 1998). So far, most of these sex-limited experiments have focused on the response to male-specific selection.

In this project, we studied the nature of X-linked polymorphic loci by carrying out female-limited X chromosome (FLX) experimental evolution in a laboratory-adapted population of *D. melanogaster*. We predicted that this protocol should select for feminization of the X, and that expressing the experimentally evolved X chromosome will result in expression of a more feminized phenotype in both sexes. To control the inheritance of the selected X chromosome we used an FM (First Multiple: FM, FM7a) balancer chromosome, which is an X chromosome with a series of inversions so that it cannot recombine with its homolog but should still act like a normal X chromosome. The FM balancer carries phenotypic markers, so we could distinguish the flies' genotypes by eye (**Fig. 3**).

There were four replicate populations of each of three experimental treatments: Female-limited X-chromosome (FLX), control wild type (Cwt), and a methodological control treatment to control for the confounding effect of the FM balancer chromosome in the FLX populations (CFM). The CFM treatment was handled in the same way as the FLX treatment, except that the X chromosome went through repeating cycles of two generations in females followed by one generation in males. This eliminated the sex-specific selection found in the FLX treatment since the 2:1 ratio of time spent in each sex was the same as the average wildtype X chromosome. The FLX and CFM treatments also had a "recombination box" to prevent clonal evolution of the selected X chromosomes. For more information about the recombination box see (Abbott et al. 2013, Prasad et al. 2007, Rice 1996). The Cwt treatment group was a group of wild-type flies, which were maintained under the same experimental conditions as the FLX and CFM treatments (virgin collection, smaller population size), but without sex-limited selection or the FM

balancer. I was thereby able to control for the experimental protocol itself and for any effects that may be caused by a reduction in effective population size.

The base population used to start the evolution experiment, LH_M, has been maintained as a large, outbred population generated from 400 inseminated females collected from central California in 1991 (Chippindale and Rice 2001).

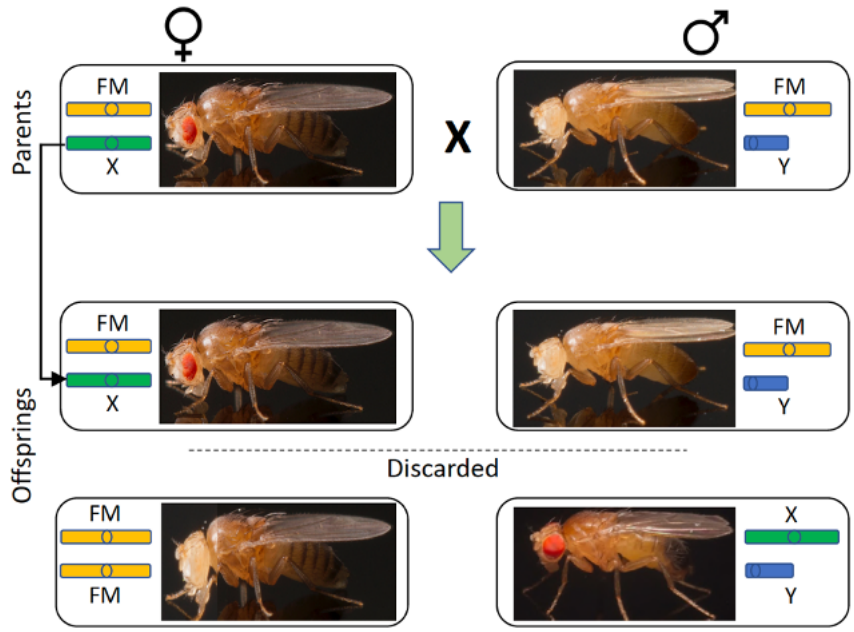

Figure 3. Protocol for the female-limited X-chromosome (FLX) evolution experiment and graphical interpretation of the selection regimes' effects on the fly performance. The evolving X-chromosome (green bar) is passed from mother to daughter with the help of an FM balancer chromosome (yellow bar). The parental cross produces four genotypes, of which the offspring above the dashed line are crossed to produce the next generation, and the offspring under the dashed line are discarded. The FM balancer carries several phenotypic markers, which can be used to phenotype offspring, as illustrated by the pictures next to the genotypes (fly pictures by Qinyang Li). Adapted from **Paper I** (Manat et al. 2021).

Predictions

If the X chromosome is highly polymorphic and enriched for SA loci, thus making it subject to large effects of IASC, then removing male selective constraints and generating long-term female-specific selection on the X chromosome should lead to more feminized X chromosomes (e.g., increase in expression of female-biased genes and decrease in expression of males biased genes, or fixation of alleles that result in a more feminized phenotype). To test this idea, and to provide a better

understanding of both the evolutionary dynamics and characteristics of X-linked polymorphic loci, I performed a female-limited X chromosome evolution experiment in *Drosophila melanogaster*. After limiting the expression of the X chromosome to females for multiple generations, we analysed the effect of the evolved X chromosome on some of the male reproductive traits (**Paper I**), genome-wide gene expression pattern (**Paper II**), genome-wide allele frequency changes (**Paper III**), and the quantitative-genetic structure of dimorphic traits by estimating the within- and between-sex additive genetic variance-covariance matrix (**Paper IV**).

Because there are three selection regimes in the experiment, a significant effect of selection regime can arise in a number of different ways, and there are three possible pairwise comparisons. These three pairwise comparisons can result in three general patterns (**Fig. 4** A, B, C), each of which has a unique interpretation. If the FLX regime is different from both CFM and CWT, this suggests an effect of FLX selection. If the CWT regime is different from both FLX and CFM, this suggests an effect of adaptation to the presence of the FM balancer. If the CFM regime is different from both FLX and CWT, this suggests an effect of adaptation to the presence of the balancer that is countered by FLX selection.

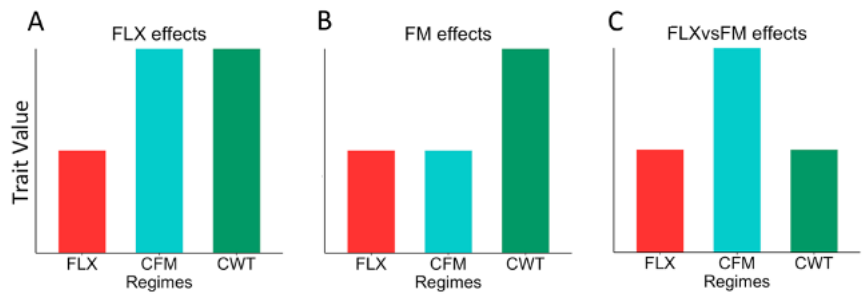

Figure 4. Potential outcomes of the experiments. (A) FLX effects. If the trait value of FLX flies different from CFM and Cwt flies', this should be a result of the FLX selection. (B) FM effects. If the trait value of FLX and CFM flies are different from Cwt flies', this suggests an effect of the FM balancer. (C) FLX versus FM effects. If flies from the FLX regime have different trait value than CFM flies' but similarly to Cwt flies', this suggests that the deleterious effects of a feminized X-chromosome seem to outweigh the FM effect. Adapted from **Paper I** (Manat et al. 2021).

Results and discussion

Female-limited X-chromosome evolution effects on male pre- and post-copulatory success **(Paper I)**

In **Paper I**, we analyzed the effects of the evolved X chromosome on male pre- and post-copulatory success, including measuring male attractiveness (as copulation latency and mating frequency), and sperm competitiveness (as both defense and offense ability). Different reproductive roles of the two sexes generates different selection pressures on many traits, and traits that are favored in one sex might be costly to the other sex (Andersson 1994, Arnqvist 2004, Parker 1979). Furthermore, due to stronger sexual selection in males in most species, male reproductive traits tend to evolve and diverge at higher rates, and male mating success is more variable than female reproductive success in fruit flies (Andersson 1994, Bateman 1948). Therefore, experimentally evolved X chromosome may reduce male reproductive traits through both IASC and interlocus sexual conflict. To test this idea, after more than 100 generations of FLX experimental evolution, we measured responses of male pre- and post-copulatory traits to the experimentally evolved female-limited X chromosome. Male attractiveness was measured as mating frequency and copulation latency, using a standard no-choice protocol (Katayama et al. 2014). Sperm defense and sperm offense were measured as production of target offspring when being the first (for defense) or second (for offense) male to mate (Clark et al. 1995, Parker 1970). Data was analyzed using linear mixed models (for copulation latency) or generalized linear mixed models (for mating frequency, sperm defense, and sperm offense) with replicate population nested within treatment (Arnqvist 2020).

When comparing the mating frequency and copulation latency between experimental evolution regimes, we found no evidence of decreased pre-copulatory success of the FLX males (p = 0.24 and p = 0.48 for mating frequency, and copulation latency respectively). For post-copulatory success, counter to our expectation, we found that FLX males' sperm defense ability significantly increased (p = 0,009, **Fig. 5a**). However, there was also evidence for a marginally non-significant decrease in sperm offense in the FLX males (p = 0,08, **Fig. 5b**).

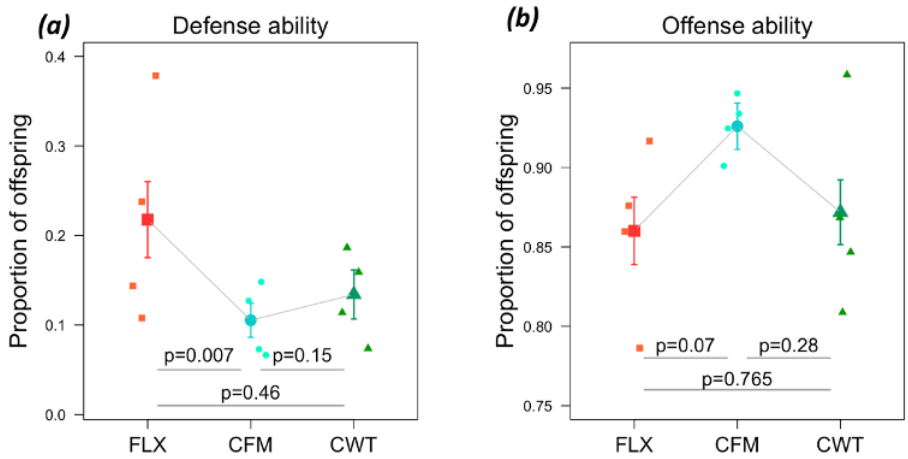

Figure 5. Male sperm competition. **(*a*)** Sperm defense ability measured as proportion of offspring sired by the target male. **(*b*)** Sperm offense ability measured as proportion of offspring sired by the target male. Points with error bars represent overall means and SEs, and individual points are the means for the four replicate populations. FLX: red, CFM: blue, CWT: green. *P*-values are from Tukey's HSD test. Adapted from **Paper I** (Manat et al. 2021).

Even if the increased sperm defense ability in FLX males was counter to our expectations we interpreted it as a result of the evolved X chromosome (see Paper I for more information). Moreover, it is consistent with the previously observed increase in body size in FLX flies (Lund-Hansen et al. 2020) assuming larger males transfer more sperm or are preferred under cryptic female choice (Moya-Laraño and Fox 2006, Parker 2006). We also hypothesized that the higher performance of CFM males in sperm offense is the result of autosomal and Y-linked adaptation to regain fitness in the presence of the balancer, which was outweighed by the negative effect of evolved X chromosome in FLX males. We therefore concluded that these changes in male reproductive traits indicate that the X chromosome in D. melanogaster contains polymorphic loci that are important for male fitness and potentially subject to IASC.

Differential gene expression in *Drosophila melanogaster* heads in response to female-limited X chromosome evolution **(Paper II)**

In **Paper II**, after 95 generations of the FLX experiment, we analyzed genome-wide gene expression differences between selection regimes in *Drosophila melanogaster* head tissue. Differential gene expression between the sexes mediates the development of highly dimorphic traits, despite males and females sharing most of

their genome (Bachtrog et al. 2014, Connallon and Knowles 2005, Ellegren and Parsch 2007). Such differences in gene regulation are therefore considered as a possible way to resolve IASC and achieve phenotypic dimorphism (Bonduriansky and Chenoweth 2009, Connallon and Knowles 2005, Ellegren and Parsch 2007, Lande 1980, Pennell and Morrow 2013). In this study, we therefore tried to determine how sex-limited selection affected on genome-wide patterns of gene expression between the different selection regimes (including FLX evolution).

Data was obtained by RNA sequencing of pooled samples of 25 fly heads from virgin individuals (three of each sex per combination of replicate population and selection regime), using the Illumina HiSeq2500 system. Quality control was carried out using *trimmomatic*, and selection regimes were compared using DEseq2 (Love et al. 2014).

To be able to detect sex-specific responses in gene expression to female-limited evolution of the X chromosome we carried out pairwise comparisons between selection regimes separately in males and females. The largest amount of significantly differentially expressed genes (q-value < 0.05) was found between selection regimes in females (1714 genes), while in males 778 genes were significantly differentially expressed (**Fig. 6**).

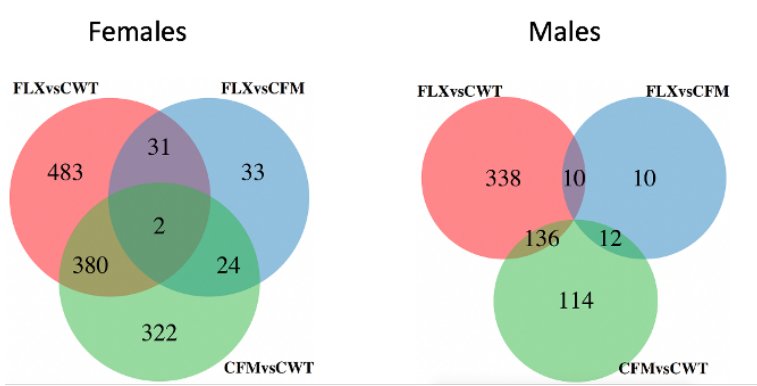

Figure 6. Number of significantly differentially expressed genes between selection regimes in females (left) and males (right). Adapted from **Paper II**.

Analysing the sexes separately also allowed us to disentangle the long-term effect of the FM balancer in FLX regime from the FLX selection effect which was detected as in our previous studies (Lund-Hansen et al. 2020, Manat et al. 2021). Therefore, we further categorized these differentially expressed genes into three classes: FLX effect, CFM effect and CFM vs. FLX effect (see Paper II for more details). Then we performed a number of analyses on each class, including the

chromosomal distribution, tests for over-representation of GO terms, tissue enrichment, tests for association with sex-specific fitness and sexual antagonism (Innocenti and Morrow 2010), and several other enrichment analyses (see Paper II for more details). Overall, in agreement with our expectation, we found some evidence of a feminized expression pattern in both sexes, that could explain the phenotypic trait responses observed in previous studies (Lund-Hansen et al. 2020, Manat et al. 2021). For example, the FLX effect class of genes was positively related to female fitness and negatively related to male fitness genes in both sexes. They also showed an overrepresentation of genes that previously have been identified as positively associated with sexual antagonism and sex-specific fitness by (Innocenti and Morrow 2010).

We also found potential confounding effects of using the balancer chromosome (FM7a), some of which were consistent with a reduction in the level of conflict over mating rate and fertilisation success (Manat et al. 2021). For example, overrepresentation of genes related to the intensity of sexual conflict in D. melanogaster (Innocenti et al. 2014) in the CFM effect class of genes in both males (up-regulated CFM effect genes) and this has previously been found in females (Innocenti et al. 2014). In summary, these results suggest a highly dynamic nature of intralocus sexual conflict on the X chromosome, and that this conflict can be partly resolved experimentally through rapid evolution of changes in gene expression under controlled experimental conditions.

Changes in allele frequency as the result of female-limited selection in *Drosophila melanogaster* **(Paper III)**

In **Paper III**, at generation 133 of the FLX experimental evolution, we examined genome-wide allele frequency changes and analyzed the divergence between selection regimes. If the X chromosome possesses a substantial number of polymorphic loci which are subject to sexually antagonistic selection, then the relatively long timescale of our experimental evolution study should gradually fix these loci for female beneficial alleles and result in changes in allele frequency. Therefore, we expected that under long-term positive selection on standing genetic variation will change the frequency of alleles with small effects (Hermisson and Pennings 2005).

DNA was extracted from two pools of 50 individuals of each sex for each replicate population and selection regime and sequenced using the Illumina NovaSeq system. Quality control was carried out using *trimmomatic*, and reads were then mapped to

the *D. melanogaster* reference genome (version 6.35) using *bwa mem* (Li and Durbin 2009). Consistent changes in allele frequencies were detected using quasibinomial generalized linear models, as recommended by Wiberg et al. (2017).

In total, we found 241 single nucleotide polymorphisms (SNPs), of which 225 in females and 18 in males, that significantly changed in frequency in response to our selection regimes (hereafter the "significant SNPs" with FDR < 0.1; **Fig. 7**).

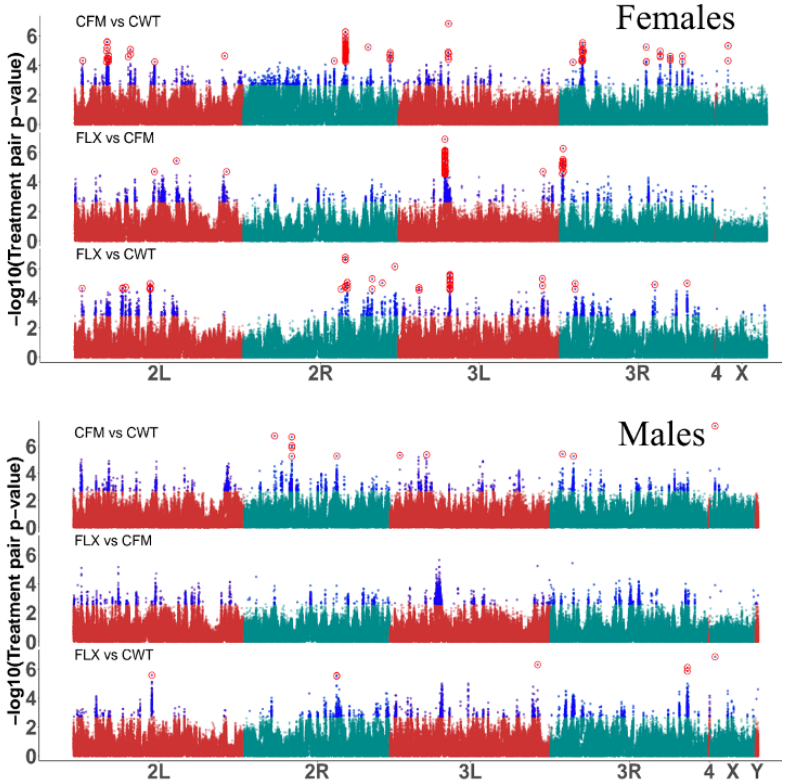

Figure 7. Manhattan plot of allele frequencies differences between regimes in females (upper panel) and males (lower panel). The x-axis shows genomic position (chromosomes 2L - Y). Each point represents the −log10(*p*-values) from the quasibinomial GLM. Blue points are the top 2,000 SNPs, red points (above the blue points) are SNPs that pass the genome-wide FDR<0.1 threshold. Adapted from **Paper III**.

A chromosomal distribution test of significant SNPs showed that most of the significant SNPs were located on other chromosomes than the X in both sexes. The results of the genetic variant annotation and functional effect prediction analysis showed that most of the significant SNPs (~75%) have various regulatory functions which are important for transcription and translation (Mattick 1994, Sonenberg 1994). We found some modest evidence of feminization due to the female-limited

selection in the females, where SA genes characterized by Ruzicka et al. (2019) were overrepresented among SNPs in the FLX vs CFM comparison. Other than this, we found no evidence of enrichment of genes previously identified as differentially expressed in these populations (Paper II), as well as no evidence for overrepresentation of genes characterized as SA genes in (Ruzicka et al. 2019). However, the overrepresented GO terms for sensory genes activity in females indicate possible changes in mate choice and mating behavior (Horth 2007). This is likely a result of adaptation in the females to the reduced intensity of sexual conflict, caused by the presence of the FM chromosome in the FLX regime (which makes the FM males blind). Most of the overrepresented GO terms in males related to translational efficiency which suggest some sort of compensation for low expression of FM-linked genes in males that might have been subject to loss of function mutations. In agreement with previous study (Ruzicka and Connallon 2020), our results suggest that the X chromosome may not possess as many SA mutations as predicted by theory (Rice 1984). However, they also provide additional evidence that adaptation to the FM balancer has resulted in a number of evolutionary changes which can plausibly be linked to altered sexual conflict dynamics.

Change in the B-matrix as a result of female-limited X chromosome evolution **(Paper IV)**

In **Paper IV**, we used quantitative genetic analyses to estimate the impact of experimentally evolved X chromosomes (for over 147 generations) on sexually dimorphic traits. This was done by analyzing the divergence in intersexual genetic variance-covariances matrices in six experimentally evolved populations of *D. melanogaster* (i.e., the FLX, CFM, and CWT populations from replicates 1 and 2). We first analyzed the phenotypic response in wing length, desiccation resistance and locomotory activity. We chose these traits because the wing length and desiccation resistance are highly dimorphic and rapidly respond to selection in males (Abbott et al. 2010, Gibson Vega et al. 2020), and locomotory activity is considered to be a sexually antagonistic trait (Abbott et al. 2020, Long and Rice 2007). We also wanted to select qualitatively different traits (morphology, physiology, and behavior), because there are predictions about the heritabilities of these classes of traits (highest in morphological traits and lowest in behavioural traits) (Roff and Mousseau 1987).

Wing length was estimated from photographs (Lack et al. 2016), and desiccation resistance was measured using a standard protocol (Kwan et al. 2008). Locomotory activity was measured in the same way as a previous study of these populations (Lund-Hansen et al. 2020). Phenotypic differences were then analyzed separately in males and females using linear mixed models (for wing length and desiccation

resistance) and generalized linear mixed models (for locomotory activity) (Arnqvist 2020). Quantitative genetic parameters (additive genetic variances, residual variances, and covariances) were estimated using *MCMCglmm* (Hadfield 2010), and the resulting genetic variance-covariance matrices were then compared using the tensor method developed by Hine (2009) and Aguirre et al. (2014).

Results of the analyses of phenotypic traits were generally qualitatively consistent with previous results (Lund-Hansen et al. 2020), where FLX females were less active (non-significantly so) and significantly larger. Only desiccation resistance showed a significant difference in this dataset, where desiccation resistance decreased in both FLX males and females ($p = 0.0002$ and $p = 0.035$ respectively) (**Fig. 8**).

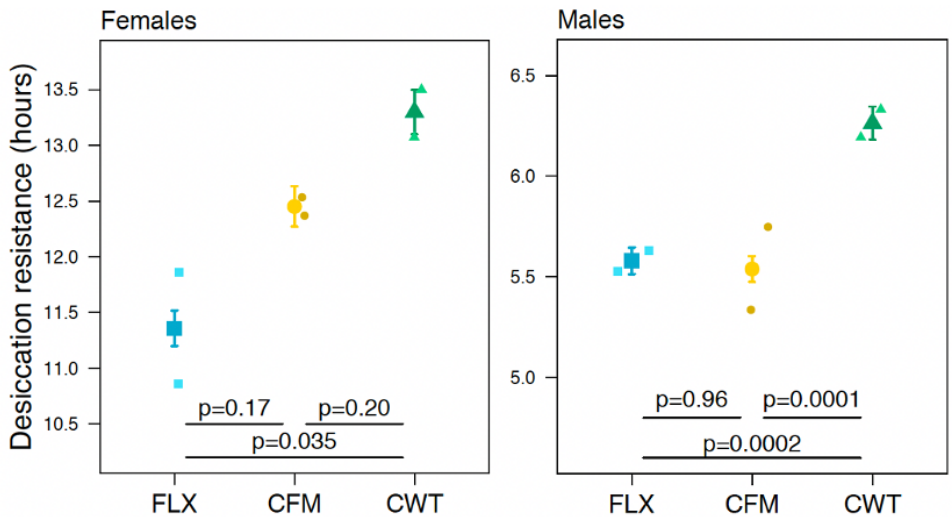

Figure 8. Desiccation resistance in hours at generations 147-156 in both sexes. Points with error bars represent means and standard errors (±SE), and individual points are the means for the two replicate populations. FLX = blue square, control FM (CFM) = yellow circle, and control wild type (CWT) = green triangle. p-values are from Tukey's HSD test (from Paper IV, Figure 4).

Heritability estimates were consistent with the general pattern of variation in the heritability of traits in *Drosophila* (Roff and Mousseau 1987). In males, the heritability of male wing length was ~0.57, desiccation resistance ~0.33, locomotory activity ~0.12, and in females, the heritability of wing length was ~0.56, desiccation resistance ~0.36, locomotory activity ~0.13 (averaged across all six populations). In males, the heritability of wing length was higher in FLX selection regimes than any other regimes, and we saw the same pattern in females (except rep2Cwt). For heritability of desiccation resistance, we found the opposite pattern; heritability was lower in FLX and higher in Cwt (in most cases). There were not any consistent differences in heritability of locomotory activity between the selection regimes.

However, by comparing intersexual genetic variance-covariance matrices between traits, we found an evidence of changes in the genetic architecture of locomotory activity as a result of the FLX selection regime (see Table 4, in Paper IV). Specifically, there was evidence of a breakdown in the intersexual genetic correlation for locomotory activity in FLX compared to the CFM and CWT selection regimes.

In summary, these results indicate that X-linked genetic variants likely have an important effect on the underlying genetic basis of these three traits, and that the FLX selection regime altered the genetic architecture of these traits despite the fact that there were no large changes in their overall heritabilities.

Conclusions

In this project, I tried to characterize X-linked polymorphic loci using an approach that was initially developed by (Rice 1996, 1998) for assessing the evolutionary impact of intralocus sexual conflict. I implemented this approach and restricted the expression of X chromosome to females for over 100 generations, and thus removed male-specific constraints. If the X chromosome is highly polymorphic and subject to sexually antagonistic selection, then this selection strategy is expected to allow the X chromosome to evolve towards the female optimum.

Our results show that the X chromosome of *D. melanogaster* is still polymorphic for many shared traits and subject to conflicting selection pressures between sexes. X-linked polymorphic loci have highly dynamic nature and play an important role in gnome-wide gene expression pattern and have regulatory effect. More specifically, we found the following major results:

I. Some evidence of resolution of sexual antagonism: Male sperm defence showed a pattern consistent with release from sexual antagonism, probably as a byproduct of changes in body size (which was previously has been found to be a sexually antagonistic trait; (Lund-Hansen et al. 2020)). Gene expression changes also showed some signatures of antagonism, where SA loci were overrepresented, and some modest evidence of allele frequency changes consistent with release from antagonism. We also detected evidence of antagonism in two new traits, desiccation resistance (Paper III and IV) and sensory ability (Paper III and Abbott et al. (2020)). Although it is currently unclear what sex-specific selective advantages underlie these patterns, one possible speculation with respect to sensory ability is that there is a conflict over investment in olfaction versus vision between the sexes, with olfaction more favoured in females (Paper III) and vision more favoured in males (Abbott et al. 2020). This hypothesis is worth further study. Contrary to our initial expectations, we did not find evidence of enrichment of SA loci on the X chromosome per se, but we found that X-linked genetic variation had important genome-wide effects.

II. Confounding effects of adaptation to the FM balancer: Data from Paper I, II and III all provide support for the hypothesis originally presented in Lund-Hansen et al. (2020), that the presence of the FM has resulted in altered sexual conflict dynamics, most likely a shift towards female control over mating rate (Lund-Hansen unpublished data). Overall, the presence of

the balancer seems to largely (but not exclusively) result in changes to traits related to interlocus sexual conflict. This was an unexpected by-product of the phenotypic markers on the FM chromosome and not taken into account in the original experimental predictions. However, now that several lines of evidence support the hypothesis that adaptation to the FM balancer occurs mainly in traits related to interlocus sexual conflict, this means that we can tentatively classify traits as mainly subject to sexual antagonism, interlocus sexual conflict, or both, depending on the changes seen as a result of the experimental evolution protocol. For example, body size (Lund-Hansen et al. 2020), wing size (Paper IV), and sperm defense (Manat et al. 2021) all show signatures of release from sexual antagonism (i.e., a pattern where the FLX individuals are different from CFM and CWT). Conversely, development time (Lund-Hansen et al. 2020), locomotory activity in females (Paper IV), and desiccation resistance in males (Paper IV) all show signatures of release from interlocus conflict (i.e., a pattern where the CWT individuals are different from FLX and CFM). Finally, sperm offense (Manat et al. 2021) and desiccation resistance in females (Paper IV) both show evidence of combined effects of sexual antagonism and interlocus conflict (sperm offense where release from antagonism counters the effects of interlocus conflict, and desiccation resistance where release from antagonism enhances effects of altered interlocus conflict). These findings will be useful in setting the stage for future work exploring interactive effects of sexual antagonism and interlocus sexual conflict.

III. Changes in genetic architecture: Papers II and III demonstrated that the experimental protocol has resulted in changes in expression and allele frequencies, clearly showing that sex-specific selection pressures can result in substantial evolutionary changes in a relatively short time. However, given previous results (Abbott et al. 2020, Long and Rice 2007), it was initially surprising that there was no obvious signature of release from sexual antagonism in locomotory activity. Results in (Manat et al. 2021) & Paper II, as well as data on mating behaviour (Lund-Hansen unpublished data), all suggest that interlocus sexual conflict dynamics have been altered as a result of the FM balancer, likely selecting for increased activity in one or both sexes in order to increase encounter rates between males and females. It is plausible that this selection occurred mainly on male activity, since females bearing the FM balancer as heterozygotes have relatively normal vision and can seek out males if desired whereas FM males are blind. In the FLX treatment any increase in male activity would be expected to occur mostly via changes in autosomal loci. However, based on previous results showing that low activity seems to be advantageous in females (Abbott et al. 2020, Long and Rice 2007), we would expect female-specific selection on X-linked loci in the FLX regime to reduce locomotory activity. Consistent with this, preliminary data where the evolved X and autosomes

were expressed separately in males and females show that individuals carrying evolved FLX autosomes have higher activity than individuals carrying evolved FLX X-chromosomes, but that there is no difference for CFM X chromosome versus CFM autosomes (Li, unpublished data). These conflicting sex-specific selection pressures on different parts of the genome seem to have resulted in a breakdown of the intersexual genetic correlation for locomotory activity, a completely novel result, to our knowledge. This finding should provide new insights into how intersexual genetic correlations evolve in nature.

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List of papers

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II. Manat, Y., and Abbott, J.K. Differential gene expression in *Drosophila melanogaster* heads in response to female-limited X chromosome evolution. Manuscript.

III. Manat, Y., Hansson, B., Ramnath, V., Abbott, J.K. Changes in allele frequency as the result of female-limited selection. Manuscript.

IV. Manat, Y., Tarka, M., Hansson, B., Abbott, J.K. Change in the B-matrix as a result of female-limited X-chromosome evolution. Manuscript.

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