Analysis of reproductive anomalies in hybridogenetic beetles (Chrysomelidae, *Altica lythri*)

Dissertation

zur Erlangung des Doktorgrades der

Naturwissenschaften (Dr. rer. nat.)

Fachbereich Biologie

der Fakultät für Mathematik, Informatik und Naturwissenschaften

an der Universität Hamburg

vorgelegt von

Alina Sanken

aus Hamburg

Hamburg, Mai 2023

Datum der Disputation: 07.07.2023

Prüfungsvorsitz: Prof. Dr. Jutta Schneider

Mitglieder des Prüfungskommitees:

Prof. Dr. Susanne Dobler

Prof. Dr. Bernhard Hausdorf

Dr. Kim Rohlfing

Dr. Frank Friedrich

Erstgutachterin: Prof. Dr. Susanne Dobler

Zweitgutachter: Prof. Dr. Bernhard Hausdorf

Table of content

1 Abstract

The flea beetle *Altica lythri* harbours three main mitochondrial (mtDNA) haplotypes (HT1, HT2, HT3), one of which shows a strong sex ratio distortion with exclusively female progeny (HT1). The females of this HT still need to mate to produce offspring, as unfertilised eggs to not develop into larvae. All three HTs may be infected with a *Wolbachia* strain that each belongs to the two supergroups found in this beetle species. These strains depict a strong correlation with the mtDNA HTs. One *Wolbachia* strain, *w*LytB, has started introgressing into other HTs (HT2), intermixing and forming double infections, whereas the infection of the all-female HT1 with *w*LytA1 remains separated.

In this thesis, the phenotype of HT1 has been addressed. The extreme sex ratio distortion in this HT led to the assumption that there are reproduction anomalies present that need to be uncovered. For this, I performed quaddRAD analyses, which is a method that makes it possible to screen the entire genome and call SNPs simultaneously. No reference genome is required, which makes quaddRAD the optimal method to use in non-model organisms like *A. lythri*. With the help of this method, I could show for the first time that the offspring of this all-female HT inherit the majority of their SNPs from the mother. This is not what would be expected for sexual reproduction but for individuals reproducing gynogenetically. Gynogenesis is a form of parthenogenesis, in which the females can be seen as sperm parasites as they require the trigger of insemination for the development of their unreduced eggs without using the paternal genome for next generation. The maternal inheritance of SNPs is on a par with the strong female bias in this HT. However, a small percentage of paternal genes leaked into the next generation, highlighting the faulty exclusion mechanism that takes place in gynogenetic eggs. It is expected for gynogenetic individuals to produce unreduced oocytes to ensure that the majority of the maternal genes are transmitted to the next generation. With the help of fluorescence microscopy of unfertilised eggs, I could show that *A. lythri* produces diploid oocytes instead of the regular haploid oocytes. The latter can be found in HT2, which would be needed for sexual reproduction.

Various possible discussion points will be given to address the cause of gynogenesis in this dissertation, one of which is the infection with reproduction-manipulating organisms like *Wolbachia*. The correlation between mtDNA HT and *Wolbachia* strain, especially for HT1, is striking and likely to be connected to the change in meiosis that led to the production of

female gametes with an unreduced chromosome set. The part that mitochondria and selfish elements might have in this phenotype is still unknown, as is the fate of the male pronucleus after the fusion with the gynogenetic egg. I will give discussion points and possible ways of why the male set of chromosomes might be detected and excluded, but more research and analyses need to be done to fully answer these questions. What has ultimately caused the transition from sexual to parthenogenetic reproduction in HT1 is not known yet, so this HT needs to undergo further research to help us understand why gynogenesis is the mode of reproduction in HT1.

2 Zusammenfassung

Der Flohkäfer *Altica lythri* besitzt drei mitochondriale (mtDNA) Haplotypen (HT1, HT2, HT3), von denen einer mit fast ausschließlich weiblichen Nachkommen eine starke Verschiebung des Geschlechterverhältnisses aufweist (HT1). Die Weibchen dieses HT müssen sich jedoch paaren, um Nachkommen zu produzieren, da unbefruchtete Eier sich nicht zu Larven entwickeln. Alle drei HTs sind mit je einem *Wolbachia*-Stamm infiziert, der jeweils zu einer der beiden Supergroups gehört, die bei dieser Käferart zu finden sind. Diese Stämme stehen in Korrelation mit den mtDNA HTs. Während der letzten Jahre ist einer der drei *Wolbachia*-Stämme, *w*LytB, zu HT2 übergegangen und hat zu Doppelinfektionen geführt. Der Stamm *w*LytA1 in HT1 ist hier nicht von betroffen und bildet keine Doppelinfektionen mit einem anderen Stamm.

In dieser Arbeit steht der Phänotyp von HT1 im Fokus. Die extreme Verschiebung des Geschlechtsverhältnisses in diesem HT führt zu der Hypothese, dass hier Anomalien in der Reproduktion/Meiose vorhanden sind, die diesen Phänotyp erklären könnten. Um diese zu untersuchen, habe ich quaddRAD-Analysen durchgeführt. Dies ist eine Methode, mit der SNPs, verteilt über das gesamte Genom, in allen zu untersuchenden Proben gleichzeitig detektiert werden können. Es wird kein Referenzgenom benötigt, welches quaddRAD zur optimalen Methode für Nicht-Modellorganismen, wie *A. lythri*, macht. Mit Hilfe dieser Methode konnte ich erstmals zeigen, dass die Nachkommen dieses rein weiblichen HT die Mehrzahl ihrer SNPs von der Mutter erben. Dieses Ergebnis entspricht nicht sexueller Reproduktion, sondern spricht für eine gynogenetische Fortpflanzung. Die Gynogenese ist eine Form der Parthenogenese, bei der die Weibchen als Spermienparasiten betrachtet

werden können, da sie den Auslöser der Befruchtung lediglich für die Entwicklung ihrer nicht reduzierten Eizellen benötigen. Hierbei gelangen die paternalen Chromosomen nicht in die nächste Generation. Die maternale Vererbung der SNPs stimmt mit dem starken Weibchenüberschuss in HT1 überein. Ein kleiner Prozentsatz der väterlichen Gene konnte dennoch in den Genomen der nächsten Generation gefunden werden, was auf einen fehlerhaften Ausschlussmechanismus in den gynogenetischen Eiern hindeutet. Fluoreszenzmikroskopische Präparate von unbefruchteten Eiern konnten zeigen, dass HT1 von *A. lythri* diploide Eizellen produziert, im Gegensatz zu den normalen haploiden Eizellen, die in HT2 für die sexuelle Fortpflanzung benötigt werden. Die Produktion nicht reduzierter Eizellen untermauert des Weiteren die mehrheitlich mütterliche Vererbung der SNPs an die nächste Generation.

In dieser Dissertation werden verschiedene mögliche Ursachen genannt, um dem Ursprung der Gynogenese auf den Grund zu gehen. Die Infektion mit fortpflanzungsmanipulierenden Organismen wie *Wolbachia* ist hier als einen möglichen Grund zu nennen. Die Korrelation zwischen mtDNA HT und *Wolbachia*-Stamm, insbesondere für HT1, ist auffällig und wahrscheinlich mit der Veränderung der Meiose verbunden, die in HT1 zur Bildung weiblicher Gameten mit einem nicht reduzierten Chromosomensatz führt. Welche Rolle Mitochondrien und *selfish elements* in diesem Phänotypen spielen können ist noch nicht bekannt, ebenso wie das Schicksal des männlichen Pronukleus nach der Verschmelzung mit dem gynogenetischen Ei. Abschließend werden mögliche Wege diskutiert, warum der männliche Chromosomensatz erkannt und ausgeschlossen werden könnte. Dennoch sind weitere Untersuchungen und Analysen erforderlich, um diese Fragen vollständig zu beantworten. Was letztendlich den Übergang von der sexuellen zur parthenogenetischen Reproduktion bei HT1 verursacht hat, ist noch nicht bekannt. Es muss weiter in die Erforschung dieses HT investiert werden, um zu verstehen, warum HT1 sich gynogenetisch fortpflanzt.

3 Aim of this thesis

The aim of the thesis focuses on uncovering the reason behind the extreme sex ratio distortion in HT1 of *A. lythri*. It is based on the findings of Jäckel (2011) and Jäckel, Mora and Dobler (2013), which show the absence of true parthenogenesis in the given HT and a strong correlation between mtDNA HTs of *A. lythri* and three strains belonging to two supergroups of *Wolbachia*.

Because of the observation that HT1 of *A. lythri* still needs to copulate in order to produce offspring, it is likely that this HT reproduces gynogenetically. Gynogenesis, a form of parthenogenesis, in which the parthenogenetic female requires the sperm of a closely related species as a trigger for the further development of the egg, is on a part with the extreme sex ratio distortion found in this HT, as exclusively female progeny hatches. This leads to the conclusion that the Y sex chromosome does not get transferred to the next generation. To see what happens with the male genetic material, I deducted a quaddRAD analysis. With the help of this method, I could call SNPs genome-wide, compare those found in the offspring with those found in the parents, and determine from whom the SNPs in the offspring have been inherited (chapters 6.4 and 6.5).

Another characteristic that can be found in gynogenetic females is an unreduced oocyte. To be able to inherit the entirety or majority of the maternal genome to the next generation, the number of chromosomes need to be unreduced and thus that of somatic cells. This would enable the gynogenetic females to exclude the male pronucleus and not use the paternal genetic material to restore ploidy in the zygote. This can be achieved via multiple mechanisms, which include pre- and postmeiotic doubling of chromosomes, apomixis, or automixis. To determine the chromosome count of the unfertilised eggs, I generated chromosome spreads (chapter 6.11).

Why this HT switched from sexual to asexual reproduction is not understood and could not be securely answered in the course of this doctoral thesis. However, it was possible to point out various possibilities of how this phenotype could have developed, which will hopefully help in shedding light onto the reproduction anomalies of HT1 of *A. lythri* (chapter 7) and further on how infection with selfish parasites, multiple mtDNA HTs, and hybridisation can affect the fate of a species. There are still a lot of open questions on hybridisation and its effects on species in general – how reproduction may be altered and what consequences that

might have on populations, meiosis, and sexes. So far, only model organisms have been given as examples for answering detailed research questions; for example, *Tribolium*, *Drosophila*, and *Poecilia*. However, not every model organism is a suitable comparison for the current research question. Being able to use non-model organisms without a reference genome opens many possibilities for research. For example, *A. lythri* can now be referred to when analysing hybridisation and its consequences on the fate of a species. Is it possible that hybridisation can alter meiosis to a degree that an organism switches from sexual to asexual reproduction? Is it possible that hybridisation is only the very first trigger that requires further incidences to induce a change in reproduction, like selfish elements or reproduction-manipulating parasites? Female biases can be found in various taxa, yet not all is known about its causes and consequences. To be able to use natural populations that show such a phenotype to answer still open questions about sex ratio distortion, altered meiosis, reproductive anomalies, and hydridisation presents an immense opportunity that should be used to help us understand the workings outside of Mendelian inheritance and what is seen as ‘normal’.

4 Introduction

4.1 Reproductive anomalies in the flea beetle *Altica lythri*

Mendelian reproduction describes the production of equal numbers of male and female offspring through the fusion of two haploid gametes. The parental chromosomes undergo multiple recombination events to form a new and diverse generation of individuals. In some organisms, however, it is common to find a sex ratio distortion with a strong female bias (Phillips, 1979; Kangas and Rutanen, 1993; Siede, 1998; Jäckel, Mora and Dobler, 2013). Various possible explanations exist for this phenomenon, including reproduction-manipulating microorganisms that can cause the death of genetic male offspring, their feminisation, or the induction of parthenogenesis (Werren, 1997; Stouthamer, Breeuwer and Hurst, 1999; Weeks, Velten and Stouthamer, 2003; Hagimori *et al.*, 2006; Duron *et al.*, 2008; Werren, Baldo and Clark, 2008; Kageyama, Narita and Watanabe, 2012; Zug and Hammerstein, 2012; Wei *et al.*, 2020). Parthenogenesis is a form of reproduction that has varying levels of dependency on paternal gametes, sometimes requiring the trigger of insemination and other times excluding one of the parental chromosome sets from the offspring altogether (see chapter Parthenogenesis).

The flea beetle *Altica lythri* shows a distinct sex ratio distortion with a strong female bias. One of the three main haplotypes (HTs) only produces female offspring, whereas the other HTs also produce male progeny. On the contrary to true parthenogenetic individuals, the females of the all-female HT need to copulate to produce offspring, as unfertilised eggs of this mtDNA HT do not hatch (Jäckel, Mora and Dobler, 2013).

This phenomenon raises intriguing questions about the underlying genetic mechanisms: what is happening inside this mtDNA HT and what happens with the male genetic material that gets introduced via copulation if the progeny is all-female. My aim was to address these questions using bioinformatical as well as histological methods to answer the underlying questions of why the all-female HT only produces female offspring and what happens with the male genetic material introduced by the sperm, and to speculate about why this mtDNA HT is as evolutionarily stable as it seems to be.

4.2 What is sex determination?

In all sexually reproducing species some mechanisms for sex determination is needed for the development of sexually reproducing individuals into female and male (Simpson and Douglas, 2013, pp. 388-390), and describes the development mechanisms an egg must undergo in order to develop into one of the two existing biological sexes. This mechanism involves a sex determination cascade that triggers the developmental determination of an embryo's sexual fate (Sanchez, 2008).

This cascade of gene activation and inactivation, which ends with biological, morphological and behavioural differences between the individuals, can be controlled by various factors. These factors include the composition of sex chromosomes, epigenetic stimuli such as temperature and pH level, or social stimuli like population structure. Sex chromosomes exhibit a high variation amongst the animal kingdom, with humans and the fruit fly *Drosophila melanogaster* exhibiting the best-known case. Females of these species are homogametic, meaning they have two sex chromosomes of the same type (XX), while males are heterogametic and have one X and one Y chromosome cells. In contrast, the orders of Lepidoptera and Trichoptera have heterogametic females with one Z and one W sex chromosome and homogametic males with two Z chromosomes. Other orders of the insect clade may have lost the Y chromosome completely, leaving the males gametically haploid (X0) and the females homogametic diploid (XX). Some insects have no sex chromosomes altogether and their sex determination cascade is entirely controlled by pathways that are conserved on their autosomes (Beukeboom and Perrin, 2014, pp. 37ff).

Apart from sex determination cascades, it is entirely possible for a female to undergo parasitic manipulation of the sex determination of the next generation (Beukeboom and Perrin, 2014, pp. 34 and 34). Selfish elements and inherited or acquired bacterial endosymbionts, such as the α-proteobacterium *Wolbachia pipientis* (Hertig and Wolbach, 1924; Cordaux, Bouchon and Grève, 2011), *Cardinium*, *Rickettsia*, γ-proteobacterium *Arsenophonus*, or *Spiroplasma* (Duron *et al.*, 2008; Moran, McCutcheon and Nakabachi, 2008; Werren, Baldo and Clark, 2008; Saridaki and Bourtzis, 2010) in arthropods and nematodes, or the fungus *Microsporidia* in crustaceans (Jahnke *et al.*, 2013) can increase their own chance of transmission through various mechanisms that increase the number of females in a population (see chapter Reproductive parasite *Wolbachia* in arthropods).

4.2.1 Sex determination in *Drosophila melanogaster*

To fully understand how the sexes can be determined in insects, it is important to start with the sex determination of species that has been analysed in detail. Sex determination, no matter in what organism, is controlled by a cascade of genes. In *Drosophila melanogaster*, MEIGEN 1830, this cascade (Figure 1, A) gets triggered by the balance or imbalance between one type of sex chromosomes (X) and the autosomes (Lucchesi, 1973). The amount of X-linked 'numerator' gene products gets compared to the amount of autosomally encoded 'denominator' proteins (Erickson and Quintero, 2007). This leads to an imbalance of X chromosomal genes to autosomal genes in the males because of their heterogamety (XY; X:A = 0.5) and a balance in the females because of their homogamety (XX; X:A = 1.0). The ratio between numerator and denominator gene products determines the activity state of the sex-determining gene *Sexlethal* (*Sxl*).

The correct splicing of *Sxl* mRNA in females leads to a female-specific splice variant of the corresponding protein, SXL^F. This causes the splicing of *Transformer* (*Tra*) pre-mRNA into a female-splice variant of the *Tra* gene product. The translated protein, TRA^F, is necessary for the regulation of the sex determination genes that follow downstream in the cascade, including the production of a female-specific splice variant of the *Doublesex* (*Dsx*) pre-mRNA, whose protein inhibits the expression of genes needed for male somatic sexual differentiation and causes the final development of the embryo into a female. Furthermore, TRA^F inhibits some of the dosage compensation components (DCC) present to prevent the formation of the active dosage compensation complex. This complex is needed to equilibrate the ratio between the X chromosomes between the sexes (Lucchesi, 1973; Conrad and Akhtar, 2012), which, in turn, additionally promotes the female sex differentiation (Shukla and Palli, 2012). In the males, the gene transcription of *Sxl* is prohibited in early embryogenesis due to the default splicing of the pre-mRNA. The resulting truncated and non-functioning protein does not mediate the *Tra* pre-mRNA splicing, which causes the male-specific, default splicing of the *Dsx* pre-mRNA due to the absence of a functioning TRA^F protein.

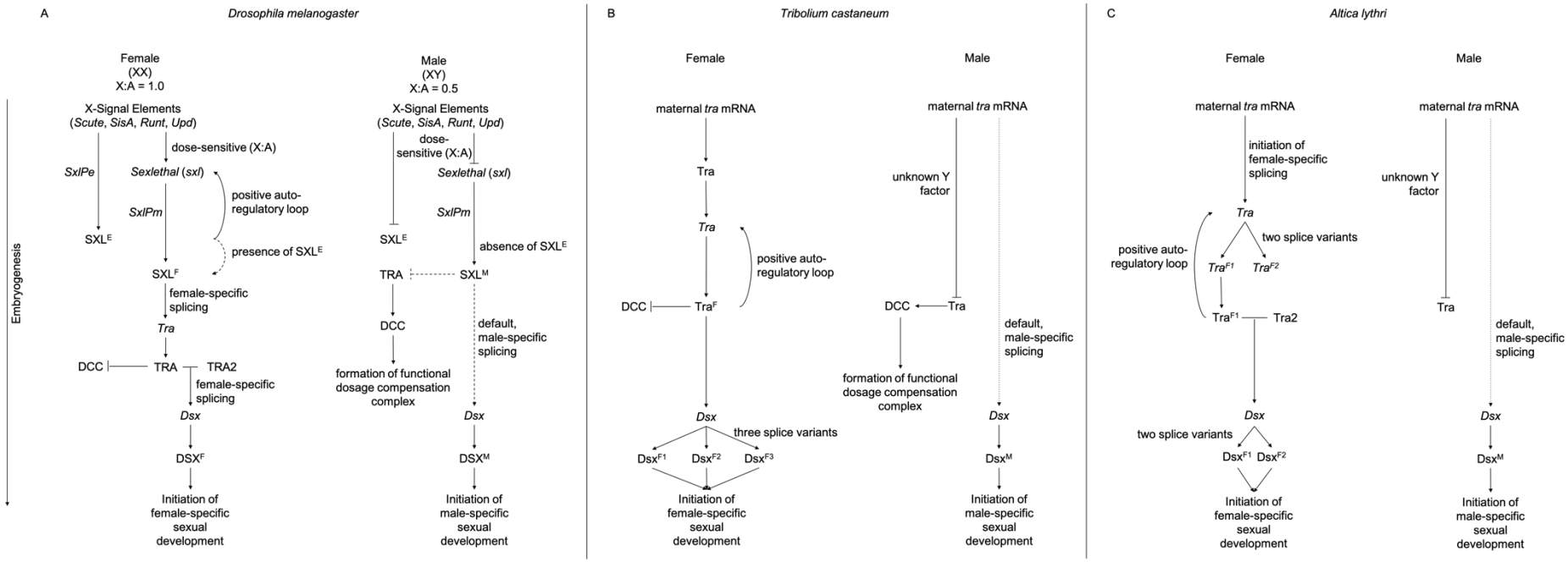


Figure 1 - Sex determination cascades of insect species. (A) *Drosophila melanogaster*, (B) *Tribolium castaneum*, (C) *Altica lythri*.

This variant of DSX (DSX^M) then represses any female-specific gene expression that would lead to female somatic sexual differentiation and causes the embryo to develop into a male (Erickson and Quintero, 2007). The lack of a functioning TRA^F protein, further allows for the formation of a functional dosage compensation complex, whose components get inhibited in the female differentiation pathway. Here, this complex upregulates the transcription of X-linked genes by twofold (Hamada *et al.*, 2005; Straub *et al.*, 2005; Park *et al.*, 2010; Prestel *et al.*, 2010) to compensate for the differences in the number and concentration of the X-linked genes (Lucchesi, 1973; Conrad and Akhtar, 2012).

4.2.2 Sex determination in *Tribolium castaneum*

A lot of research has gone into the *D. melanogaster* sex determination system, however, the red-brown flour beetle *Tribolium castaneum* is phylogenetically closer related to *A. lythri* than is *D. melanogaster*, which makes it more suitable for comparison. Just like in other insects, the sex determination pathway in *T. castaneum*, HERBST 1797, is highly conserved (Figure 1, B). It begins with the sex-specific splicing of the *tra* mRNA (Shukla and Palli, 2012), which is maternally transferred to the embryo and triggers the cascade. The resulting Tra protein acts as a splicing regulator for the zygotically transcribed *tra* pre-mRNA into the female-specific variant of *tra* mRNA, which then translates into the female-specific Tra (Tra^F) protein (Sanchez, 2008; Verhulst, van de Zande and Beukeboom, 2010; Bopp, Saccone and Beye, 2014). A positive feedback loop ensures the continuous production of Tra^F. This protein contains a conserved serine/arginine-rich (SR) domain and a putative auto regulatory domain that is conserved in all Diptera species, as well as Hymenoptera and Coleoptera (Rohlfing *et al.*, 2023). Tra^F then proceeds to splice the *dsx* pre-mRNA into three female-specific splice variants, *dsxf1*, *dsxf2*, and *dsxf3*. These three isoforms are crucial for development into a female organism. Tra^F inhibits parts of the DCC to keep the active dosage compensation complex from forming, which additionally promotes the female sex differentiation (Shukla and Palli, 2012).

In males, an unknown dominant Y-factor inhibits the translation of the maternally transmitted *tra* mRNA and either degenerates it or inhibits its autoregulation (Shukla and Palli, 2014). This step is not yet entirely understood and still requires more research to answer its functionality. However, the absence of the initial Tra protein in females that originates from the maternally supplied *tra* transcript, leads to the default splicing of the *tra* pre-mRNA and forms a

truncated, non-functioning protein (Beukeboom, Kamping and van de Zande, 2007). As a result, the *dsx* pre-mRNA undergoes the default, male-specific splicing and produces the male isoform *dsxm* (Cline and Meyer, 1996). The missing functional Tra protein in males allows for the formation of a functional dosage compensation complex, whose components get inhibited in the female differentiation pathway. In males the functional dosage compensation complex upregulates the transcription of X-linked genes by twofold (Hamada *et al.*, 2005; Straub *et al.*, 2005; Park *et al.*, 2010; Prestel *et al.*, 2010) to compensate for the differences in the number and concentration of the X-linked genes (Lucchesi, 1973; Conrad and Akhtar, 2012).

4.2.3 Sex determination in *Altica lythri*

The sex determination cascade of *A. lythri* has recently been investigated, but is not yet fully understood. However, similarities between main cascade components found in *A. lythri*, AUBÉ 1843 and *T. castaneum* have been identified. The primary sex determination signal is not fully understood yet and still requires more research. The main differences that have been detected so far between the two organisms are the amount of splice variant each generates (Figure 1, C). In *A. lythri*, the *dsx* pre-mRNA gets spliced into three isoforms, the male-specific *dsxm*, as well as the two female-specific *dsxf1* and *dsxf2* (Rohlfing *et al.*, 2023). Further isoforms are formed during the splicing of the *tra* pre-mRNA, which generates three variants, the male-specific *tram*, and the two female-specific *traf1* and *traf2*. Tra^{F1}, the protein translated from the *traf1* mRNA, is the longest of the three variants and shows a 20% identical match across the entire sequence and a 38% match of the highly conserved SR domain when compared to the homologue in *T. castaneum* (Rohlfing *et al.*, 2023). The existence of the conserved auto-regulatory domain in Tra^{F} of *A. lythri* conjectures a positive feedback. This is further supported by findings that during ontogeny, *traf* mRNA is constantly expressed, whereas the concentration of tram mRNA decreases significantly during the development.

The only difference in the functioning of the cascade in *A. lythri* lies in the interaction between Tra^{F} and Tra2, which likely form a splicing complex that is necessary for the consecutive splicing of the *dsx* pre-mRNA into the female variant, which then leads to the initiation of the female sex differentiation of the embryo. In contrast to Tra, Tra2 does not show sex-specific expression in Diptera, Hymenoptera, Lepidoptera, and Coleoptera (Belote and Baker, 1982; Burghardt *et al.*, 2005; Niu *et al.*, 2005; Salvemini *et al.*, 2009; Nissen, Müller and Beye, 2012;

Suzuki *et al.*, 2012; Shukla and Palli, 2013), including *A. lythri*. It is constitutively expressed in both sexes at high concentrations, however only the female individuals have a functioning Tra protein that can then form a complex with Tra2. In males, the male-specific isoform of *tra* (*tram*), which gets produced by default when the trigger of the positive feedback loop is missing, forms a truncated, non-functioning protein, which then further causes the default splicing of the *dsx* pre-mRNA and sets off the male differentiation (Rohlfing *et al.*, 2023).

4.3 Reproductive parasite *Wolbachia* in arthropods

Because the highly conserved nature of sex determination cascades in insects, reproduction-manipulating parasites can alter the outcome of these pathways. Infections are widespread among insects, arthropods and nematodes (Duron *et al.*, 2008; Hilgenboecker *et al.*, 2008; Cordaux, Bouchon and Grève, 2011) and with an infection level of about 65% of all insects (Hilgenboecker *et al.*, 2008), *Wolbachia* are one of the most prevalent reproduction-manipulating bacteria in insects and other arthropods in general (Werren, 1997; Stouthamer, Breeuwer and Hurst, 1999; Werren and Windsor, 2000; Duron *et al.*, 2008; Werren, Baldo and Clark, 2008; Zug and Hammerstein, 2012; Wei *et al.*, 2020).

Based on phylogenetic analyses, these bacteria might have spread through horizontal transfer among hosts (Werren, Baldo and Clark, 2008; Stahlhut *et al.*, 2010; Kageyama, Narita and Watanabe, 2012). They are intracellular, maternally inherited microorganisms that act as selfish elements by altering their host's mtDNA population structure through induction of a linkage disequilibrium between the involved bacterial strain and the mtDNA HT (Hurst and Jiggins, 2005) as both are maternally inherited. Male hosts equal evolutionary dead ends for these parasites, so it is common that an infection with *Wolbachia* causes a feminises the sex ratio of the host, which promotes their own transmission (Turelli and Hoffmann, 1991).

Feminisation of the hosts may happen through multiple mechanisms (Kageyama, Narita and Watanabe, 2012). The induction of parthenogenesis causes the production of only female offspring, thus securing the transmission of these sexual parasites (Lehtonen *et al.*, 2013) to the next generation. Another way of altering the sex ratio of their host's population is by the feminisation of genetic males. This occurs in the isopod species *Armadillidium vulgare* (Kageyama, Narita and Watanabe, 2012) and the shrimp species *Gammarus duebeni* (Kageyama, Narita and Watanabe, 2012), wherein *Wolbachia* disrupts the secretion of androgenic hormones by inactivation of the corresponding gland, thus effectively feminising

its host. In the butterfly *Eurema mandarina*, individuals bearing the *Wolbachia w*Fem strain produce all-female offspring, no matter whether these individuals are genetically male or female (Kageyama, Narita and Watanabe, 2012; Kern *et al.*, 2015; Kageyama *et al.*, 2017; Miyata *et al.*, 2017). The mechanism behind this phenotype is not yet fully understood. The phenomenon of male killing (Werren, Baldo and Clark, 2008) can be separated into two types, the early and the late male killing (Hurst, 1991; Nakanishi *et al.*, 2008). Early male killing is usually bacteria-induced and kills the male offspring during their embryonic or early larval developmental stages. Late male killing is caused by *Microsporidia* or RNA viruses and kills the males during later larval developmental stages. Apart from those mechanisms of sex ratio distortion, it is also possible for *Wolbachia* to selectively indirectly improve the fitness of infected females by causing cytoplasmic incompatibility (CI) between infected sperm and uninfected eggs. This results in embryonic lethality due to the different cytoplasmic contents in the sperm and egg of diploid hosts and reduces the fitness of uninfected females in the population (Werren, 1997; Poinsot, Charlat and Merçot, 2003). In haplodiploid organisms, CI can cause haploid male progeny. As an example, the order Hympenoptera can be named. Here, it is likely that *Wolbachia* modify male chromosomes that lead to improper condensation and ultimate loss of the male genetic material upon fertilisation of an uninfected egg (Breeuwer and Werren, 1993; Vavre *et al.*, 2001). Fusion with an infected egg leads to the paternal chromosomes being rescued and the formation of haploid male offspring (Vavre *et al.*, 2001, 2002).

Overall, it can be said that these reproduction-manipulating organisms have an effect on the sex ratio within their host population (Jiggins, 2003; Becking *et al.*, 2017), alter sex determination pathways through manipulation at various stages of the larval development (Kageyama, Narita and Watanabe, 2012) and can influence speciation of their host species (Bordenstein, O'Hara and Werren, 2001; Engelstädter and Hurst, 2009; Miller, Ehrman and Schneider, 2010).

4.3.1 *Rickettsia*, *Spiroplasma*, and other reproductive parasites

Other reproductive parasites, beyond *Wolbachia*, are known to plague arthropods (Weeks, Velten and Stouthamer, 2003; Hagimori *et al.*, 2006; Duron *et al.*, 2008; Kageyama, Narita and Watanabe, 2012 and citations therein) through similar reproduction anomalies in their hosts. *Rickettsia* is known to mediate male-killing as well as parthenogenesis, where no sperm is

needed from potential fathers and the resulting offspring are genetic clones of their mothers. *Spiroplasma*, as well as *Flavobacteria*, can cause male-killing only, which is a phenomenon that can be observed in several genera (Werren, Baldo and Clark, 2008). *Cardinium*, on the other hand, causes parthenogenesis and feminisation of the genetically male offspring to produce phenotypic females. It is possible for multiple male-killing bacteria to coexist in a single population (G. D. D. Hurst *et al.*, 1999; Gregory D. D. Hurst *et al.*, 1999), so it is likely that an individual is infected with multiple species of reproduction-manipulating microorganisms.

These sex ratio distorters occur in our model organism *A. lythri* at various frequencies, as has been documented by Jäckel *et al.* (unpublished data). Next to *Wolbachia*, *Rickettsia* and *Spiroplasma* are very common and can be detected in up to 100% of all individuals within a given population. *Cardinium* can be found sporadically in the flea beetle, whereas *Flavobacterium* infections have not been detected. However, neither do these parasites differentiate between the HTs that prevail in this species (more on that in chapter 4.4.1), nor has it been possible to determine an association between *Rickettsia*, *Spiroplasma* and *Wolbachia*, or *Rickettsia*, *Spiroplasma* and the HTs (Jäckel *et al.*, unpublished data). They all act and occur independently from each other but could potentially also interact (Goto, Anbutsu and Fukatsu, 2006; Semiatizki *et al.*, 2020).

4.4 The model organism *Altica lythri*

Altica lythri belongs to the flea beetle genus Altica (Coleoptera, Alticinae) that has 26 known species in Europe (Jäckel, Mora and Dobler, 2013). All these species can easily be distinguished by their mitochondrial cytochrome oxidase I genes (COI). *A. lythri* shows three distinct mitochondrial haplotypes (HTs), which are non-monophyletic (Jäckel, Mora and Dobler, 2013). The mtDNA HTs are well separated, with a pronounced sequence divergence of 2.1 to 4.6% *p*-distance, which means that this mitochondrial polyphyly must originate from interspecific hybridisation with introgression of mtDNA from two other species (Jäckel, Mora and Dobler, 2013). One of the three mtDNA HTs (HT1), together with one variant (HT1*) of 0.6% sequence divergence (Jäckel, Mora and Dobler, 2013), shows a pronounced sex ratio distortion with a female bias (Mohr, 1966; Kangas and Rutanen, 1993; Siede, 1998) (Figure 2).

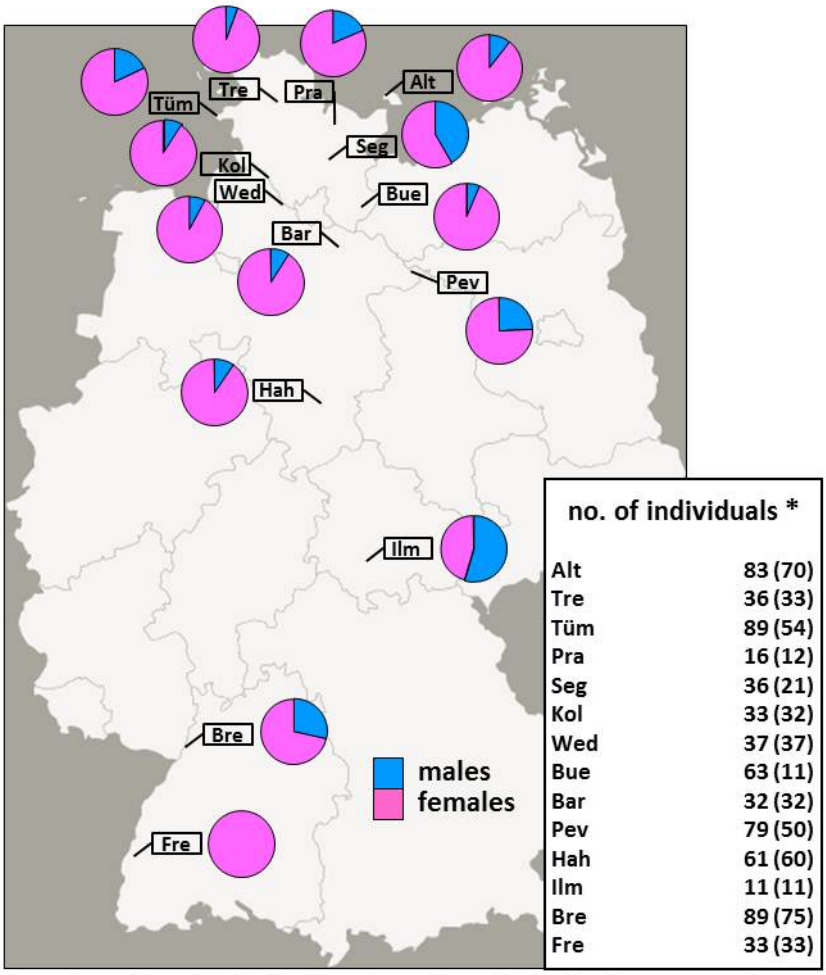


Figure 2 - Distribution of *A. lythri* populations in 2011 and their sex ratios across Germany. Pink depicts female individuals; blue depicts male individuals. n = 698. Sample collection sides, Alt: Altenteil, Schleswig-Holstein; Tre: Treia, Schleswig-Holstein; Tüm: Tümlauer Koog, Schleswig-Holstein; Pra: Pratjau, Schleswig-Holstein; Seg: Bad Segeberg, Schleswig-Holstein; Kol: Kollmar, Schleswig-Holstein; Wed: Wedel, Schleswig-Holstein; Bue: Büchen, Schleswig-Holstein; Bar: Barendorf, Niedersachsen; Pev: Pevestorf, Niedersachsen; Hah: Hahndorf, Niedersachsen; Ilm: Ilmenau, Thüringen; Bre: Bretten, Baden-Württemberg; Fre: Freiburg, Baden-Württemberg. Figure generated by Regina Jäckel.

The females of these haplotypes do not produce male progeny and thus deviate from the 1:1 sex ratio of sexual species predicted according to Fisher's model of sex ratio equality (Fisher, 1930; Hamilton, 1967), while the other two HTs with the corresponding variant (HT2, HT2* and HT3) show a relatively even sex ratio with about 50% male offspring (Jäckel, Mora and Dobler, 2013). *A. lythri* is not the only species in this genus that shows such a pronounced distorted sex ratio (Mohr, 1966; Kangas and Rutanen, 1993; Siede, 1998; Jäckel, Mora and Dobler, 2013). *A. ericeti* (Phillips, 1979), *A. oleracea* with only 9.0% males (Phillips, 1979; Jäckel, Mora and Dobler, 2013), as well as *A. palustris* with 4.1% males can be named as other examples found in this genus (Jäckel, Mora and Dobler, 2013).

The sex ratios of these species are visibly female-biased. The reason for these alterations, however, is not yet fully understood. One possible explanation for the phenomenon of the extreme female bias in these *Altica* species may be *Wolbachia* or other reproductive manipulating bacteria.

4.4.1 *Wolbachia* in *A. lythri*

All of the above-mentioned reproduction-manipulating organisms have various ways to distort the sex ratio of their host's population. They achieve this by killing any male offspring (Werren, Baldo and Clark, 2008), feminising male progeny (Kageyama, Narita and Watanabe, 2012), causing the induction of parthenogenesis (Kageyama, Narita and Watanabe, 2012) or CI (Poinsot, Charlat and Merçot, 2003; Werren, Baldo and Clark, 2008). By doing so *Wolbachia* promote their own transmission through major selection on mtDNA level (Nunes, Nolte and Schlötterer, 2008).

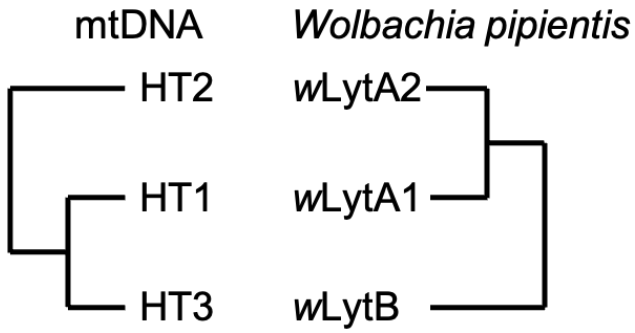


Figure 3 - Correlation between *A. lythri* mtDNA HTs and *Wolbachia pipientis* strains. Based on Jäckel, Mora and Dobler, 2013.

Jäckel, Mora and Dobler (2013) found that each mtDNA HT is associated with a specific *Wolbachia* strain. By sequencing the gene encoding the bacterial cell division protein, *ftsZ*, and the gene transcribing for a surface protein, *wsp*, it was shown that three *Wolbachia* strains are present in the populations, each belonging to the supergroups A or B (Jäckel, Mora and Dobler, 2013). Depending on the analysed population, up to 100% of the individuals were infected with one of the three strains. Each strain shows a consistent association with one HT (Jäckel, Mora and Dobler, 2013) (Figure 3). *w*LytA1 can only be found in HT1, *w*LytA2 has only been detected in HT2 individuals, and *w*LytB has originally only been found in HT3 (Jäckel, Mora and Dobler, 2013). In the meantime, however, *w*LytB has been spreading into HT2, causing a double infection for the latter HT (own observation). This correlation between the

mtDNA HTs and the different *Wolbachia* strains depicts a linkage disequilibrium caused by both being maternally transmitted (Jäckel, Mora and Dobler, 2013).

Because HT1 of *A. lythri* is the mtDNA HT that shows the strong sex ratio distortion with exclusively females, it is possible that the infection with the *w*LytA1 can be part of the reason behind it. This is not fully tested yet, and two things in particular speak against this hypothesis. The variant of HT1, HT1*, is mostly uninfected but still shows the same sex ratio as HT1. Furthermore, a similar *Wolbachia* strain (*w*AolA1) seems to prevail in *A. oleracea*, a sister species of *A. lythri*, at an infection rate of 41.7% (Jäckel, 2011; Jäckel, Mora and Dobler, 2013). This species produces 9.0% males (Phillips, 1979; Jäckel, Mora and Dobler, 2013), whereas *A. lythri* HT1 produces 100% female offspring.

So, whether *Wolbachia* can be named as the sole cause of these sex ratio distortions is yet to be determined. Multiple possible explanations for this phenomenon have arisen during the analyses conducted, but the cause of this distinct sex ratio distortion is yet to be pinpointed. With the help of the data generated, we have come closer to its resolution.

4.5 Meiosis and its alterations

The strongly biased sex ratio towards the females might originate from alterations in meiosis causing the males to vanish. Meiosis is an essential mechanism for organisms that reproduce sexually (Figure 4, A). In general, it can be understood as a special combination of cell divisions that produces gametes in sexually reproducing organisms. After two rounds of chromosome segregation, one major crossing-over event, and one round of DNA replication, the formation of four gametes with a unique haploid genome each is complete (Kleckner, 1996; Nasmyth, 2001; Kitajima, Kawashima and Watanabe, 2004; Gerton and Hawley, 2005; Ables, 2015). The fusion of two haploid gametes, one from each parent organism, then forms the new diploid zygote to start embryogenesis of the recombined progeny.

There are organisms in the animal kingdom that show meiosis alterations that lead to an unreduced chromosome set in the gametes and can be summarised in three types: apomixis, also called restitutional meiosis (Mirzaghaderi and Hörandl, 2016) (Figure 4, B), doubling of chromosomes, and automixis (Lampert, 2008).

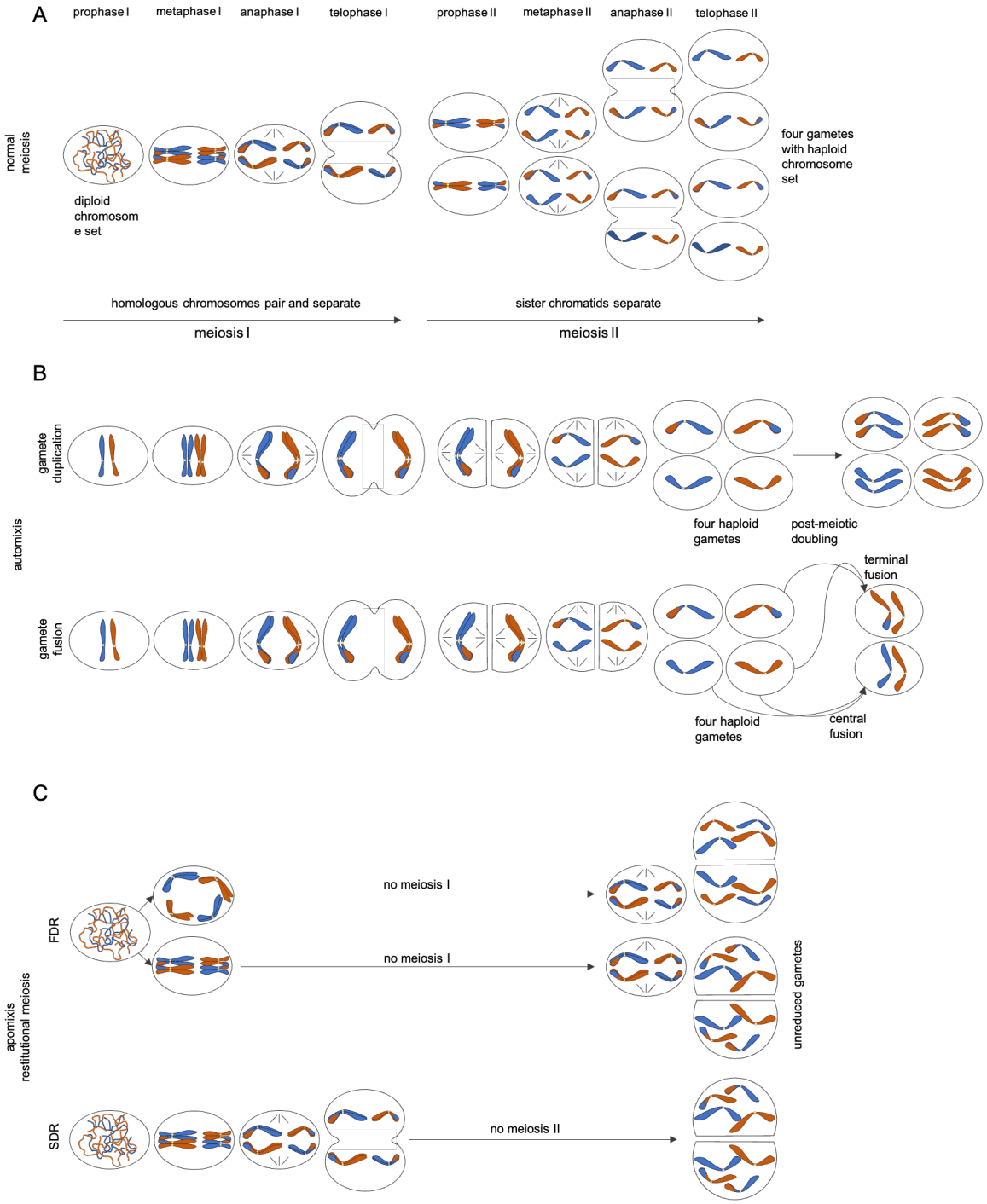


Figure 4 - Possible alterations of meiosis in animals. (A) Normal meiosis with first and second division, (B) Automixis with the two variations of gamete duplication followed by a post-meiotic doubling of chromosomes and gamete fusion followed by either terminal or central fusion of gametes. (C) Apomixis or restitutional meiosis with first division restitution (FDR) and no first meiotic division and second division restitution (SDR), where the second meiotic division is omitted. (B) and (C) show the production of unreduced gametes. Based on Mirzaghaderi and Hörlandl, 2016.

Organisms whose meiosis is repressed are called apomictic. In these cases, the oocyte is produced by mitosis, which means there is no segregation or recombination taking place and the meiosis does not occur (Rasch, Monaco and Balsano, 1982), which leaves the offspring as genetical clones of the mother. This form of altered meiosis can be found in the amazon molly *Poecilia formosa* (Turner *et al.*, 1980) and other gynogenetic fishes like *Carassius auratus gibelio* (Uzzell, 1970), in which the first meiotic division is suppressed. This phenomenon is called first division restitution (FDR), whereas the suppression of the second meiotic division is known as second division restitution (SDR) (Bretagnolle and Thompson, 1995) (Figure 4, C). Another way to accomplish a diploid chromosome count in gametes is a pre- (for examples, see Macgregor and Uzzell, 1964; Uzzell, 1970; Cuellar, 1976) or post-meiotic doubling (Mirzaghaderi and Hörandl, 2016 and citations therein) of chromosomes. Here, an additional doubling step occurs before or after meiosis takes place, respectively. This enables the chromosomes to segregate and recombine normally, but because this happens between identical homologous chromosomes and no recombination between the parental chromosomes takes place, the resulting progeny are genetical clones of the mother.

The last possible altered meiosis type is called automixis, in which meiosis is maintained and the diploid stage is restored after meiosis is complete, usually by fusion of two meiotic products (Mogie, 1986; Lampert, 2008). Automixis can be divided into two forms of meiotic fusion: central and terminal fusion. In the case of central fusion, the first polar body combines with the oocyte. This enables the cell to maintain most of the maternal heterozygosity, and the resulting offspring inherit a high number, albeit not all, of maternal alleles.

Terminal fusion, on the other hand, describes the fusion of the egg nucleus with the second polar body, which leads to mostly homozygous offspring that inherit approximately half of the maternal alleles (for examples, see Olsen and Marsden, 1954; Uzzell, 1970; Watts *et al.*, 2006).

Despite the advantages of sexual recombination, the costs of meiosis are high. The recombination of genes through the formation of haploid gametes and the following fusion of parental chromosome sets can break up favourable gene combinations that might have led to an evolutionary advantage (Mirzaghaderi and Hörandl, 2016). Meiosis not only includes a potential for errors (Lehtonen, Jennions and Kokko, 2012), but also the risk that comes with mate searching, mate finding and mating (Maynard Smith, 1978). However, not every organism is dependent on recombination to distribute their chromosomes (Gerton and

Hawley, 2005; Page and Hawley, 2005) and thus is not facing the same challenges recombination and distribution of chromosomes during sexual reproduction present.

4.6 Parthenogenesis

The production of exclusively female offspring in HT1 does not seem to follow sexual reproduction, as that would result in 50% male and 50% female progeny. Apart from an altered meiosis, parthenogenesis is a probable explanation for the observed phenotype of *A. lythri*. Reported in about 600 beetle species from at least twenty families (Boucher, Dutrillaux and Dutrillaux, 2015; Blackmon, Ross and Bachtrog, 2017), parthenogenesis is an abundant phenomenon in the insect world. In Coleoptera, especially in Polyphaga, the most common parthenogenetic development is apomictic or ameiotic (Furth, 1994).

Parthenogenesis is generally described as the development of the parthenogenetic progeny that does not need the egg to be fertilised with sperm and thus develops without the contribution of a father. In this case, the egg is usually unreduced (diploid or polyploid, depending on the organism) and has a complete set of chromosomes (Grebelnyi, 2009; Galis and Alphen, 2020). This form of reproduction is always associated with the disintegration of meiosis, which ultimately causes the genetic recombination to cease to exist. The progeny only receives maternal genetic information without the recombinational changes that would have been introduced by the father (Grebelnyi, 2009). Due to the missing production of male offspring, females spend the entirety of eggs on female progeny, which doubles the advantage of this type of reproduction over sexual reproduction (Maynard Smith, 1978). However, it has been discussed that the advantages of parthenogenetic populations are not caused by the increased breeding rate because of the lack of males, but rather their higher heterozygosity and genotypic consistency of individuals (Suomalainen, 1969; Suomalainen and Saura, 1973). Despite the advantages over sexual reproduction, parthenogenesis is much less common in metazoans than its recombining counterpart (Simon *et al.*, 2003; Neaves and Baumann, 2011) and has evolved repeatedly in a wide range of taxa (van der Kooi, Matthey-Doret and Schwander, 2017).

4.6.1 Obligate and sperm-dependent parthenogenesis

Various types of parthenogenesis exist in nature, all of which can be summarised under obligate, sperm-dependent, facultative, or sporadic parthenogenesis (Galis and Alphen,

2020). They all differ from sexual reproduction in a way that excludes one set of parental genetic information and allows the other set to fully control the further development of the embryo. Most of the types lead to a sex ratio distortion that results in a female bias and the exclusion of the paternal chromosomes.

True parthenogenesis, which also goes under the names obligate parthenogenesis or thelytoky, is a rare form of parthenogenesis and is estimated to occur in only about 100 vertebrate and 1000 invertebrate species worldwide (Hodgsen, 2009). It describes the development of an embryo from an unfertilised egg (Vershinina and Kuznetsova, 2016).

Sperm-dependent parthenogenesis includes gynogenesis, hybridogenesis, androgenesis, as well as pseudo-arrhenotoky, and describes a type of parthenogenesis, in which the sperm of a related species is necessary to trigger the development of the unfertilised egg and can thus be called sexual or sperm parasitism (Kokko, Heubel and Rankin, 2008; Lehtonen *et al.*, 2013). It is widespread and has evolved independently in at least 24 genera belonging to seven phyla. Gynogenesis describes the development of an unfertilised egg after the trigger of insemination by a close relative (Hubbs and Hubbs, 1932; Beukeboom and Vrijenhoek, 1998). The spermatozoon penetrates the egg, and its pronucleus gets excluded (Grebelnyi, 2009). The following development of the embryo is then fully controlled by the maternal genome, leading to all-female offspring. Examples for gynogenesis are vast and can be found in many species, like the diploid and triploid races of tropical fishes of the genera *Poecilia* and *Poeciliopsis* (Poeciliidae) (Hubbs and Hubbs, 1932; Miller and Schultz, 1959; Schultz, 1961, 1966, 1969), as well as some insects like the triploid beetle *Ptinus mobilis* (Ptinidae) that require the sperm of the males of the diploid species *Ptinus clavipes* (Moore, Woodroffe and Sanderson, 1956; Woodroffe, 1958; Sanderson, 1960) to reproduce.

Hybridogenesis describes a form of parthenogenesis in which the offspring develop from fertilised eggs. The paternal genes supplied by the fusion of the egg with the spermatozoon reveal themselves in the progeny's phenotype (Grebelnyi, 2009). This mode of reproduction leads to all-female offspring, which have chromosomes from both parents. While the paternal genes are apparent in the phenotype, they get eliminated during the initial stage of oogenesis, which means that only the maternal set is kept in the mature egg. This has the ultimate consequence that each following generation can only emerge after one haploid set of chromosomes is borrowed from a male of a closely related species (Grebelnyi, 2009).

Androgenesis is similar to gynogenesis in the way that it is a natural type of reproduction to clone or multiply identical genotypes, however, although androgenetic progeny arises from fertilised eggs, they inherit only paternal genes that have been introduced by the spermatozoon (Grebelnyi, 2009). Genes contained in the egg nucleus are lost.

Pseudo-arrhenotoky (Borsa and Kjellberg, 1996) requires fertilised eggs for the production of male offspring, but the paternal set of chromosomes is later eliminated during embryogenesis (Hodson *et al.*, 2017) or not transmitted to the progeny. This ensures the female control of the sex ratio of the population.

4.6.2 Facultative and sporadic parthenogenesis

Facultative parthenogenesis describes the mode of reproduction when females can produce both parthenogenetically as well as sexually. The most common type is arrhenotoky. Females of this reproduction type sexually produce diploid females and parthenogenetically produce haploid males, which is also called haplodiploidy (Hamilton, 1967; Borsa and Kjellberg, 1996). It has evolved independently at least twenty times and is estimated to prevail in about 20% of all animal species. It is possible for the arrhenotokous sexually reproducing generation with the offspring hatching from fertilised eggs to switch to asexual reproduction with unfertilised eggs, and back again (Riparbelli, Gottardo and Callaini, 2017). This alternating between sexual and asexual reproduction is called cycling parthenogenesis. These groups generally reproduce parthenogenetically and exploit resources during favourable conditions, but can switch to sexual reproduction when the environmental conditions are turning critical (Borsa and Kjellberg, 1996; Simon *et al.*, 2003). Sporadic parthenogenesis, also called tychoparthenogenesis, is a rare form of parthenogenesis and occurs in otherwise sexually reproducing organisms when the presence of males is sparse (Stalker, 1954; Carson, 1967; Kramer and Templeton, 2007).

Jäckel, Mora and Dobler (2013) undertook a series of experiments trying to discern what kind of parthenogenesis *A. lythri* exhibits. None of the unfertilised eggs laid by HT1 females developed, leading to the conclusion that *A. lythri* needs to mate in order to produce offspring, so thelytoky, the type of parthenogenesis in which the eggs do not require the stimulus of insemination to develop into fully functioning organisms (Furth, 1994; Jäckel, Mora and Dobler, 2013) is out of the question, as are all facultative and sporadic

parthenogenesis types. Further research is still underway to analyse what the reason for the distorted sex ratio in *A. lythri* really is.

4.7 Paternity analyses

To fully understand what is happening on a genomic level of HT1, I conducted paternity analyses to determine the parentage in the offspring of the HTs 1 and 2. Jäckel *et al.* (unpublished data) used microsatellites to answer this question. The results showed a maternal inheritance of the loci in question, but for some all-female offspring, the paternal locus has been detected. The variability with one to three loci was not high enough, so a more advanced method and a higher sample size had to be chosen to show more polymorphisms. Using SNPs, it was possible to answer whether the fathers contribute genetic material to the exclusively female HT1 offspring and determine the type of reproduction present in this species. SNPs have been used to answer various questions in research so far, varying from population demographic studies to conversation genomics and paternity investigations (Stetz *et al.*, 2016; Carvalho *et al.*, 2017; Kleinman-Ruiz *et al.*, 2017; Çilingir *et al.*, 2017; Hernández-Rangel *et al.*, 2018). SNPs are advantageous for addressing studies of parentage and relatedness data, as they are abundant in the genome and show a lower mutation rate than microsatellites (Brumfield *et al.*, 2003; Peterson *et al.*, 2012; Puritz *et al.*, 2014; Thrasher *et al.*, 2018). To generate SNP data, it was important to screen whole genomes within families consisting of known parents and their offspring. For this, I used double digest restriction-site DNA sequencing (ddRADseq), or more specifically the modified ddRAD protocol, quadruple barcode ddRAD (quaddRAD; Franchini *et al.*, 2017). quaddRAD is a method to simultaneously discover and screen a large number of anonymous SNPs that are distributed throughout the entire genome to create detailed genetic information on species that have not been established as model organisms (Amores *et al.*, 2011; Catchen *et al.*, 2011) without the need to have a corresponding reference genome (Peterson *et al.*, 2012; Schweyen, Rozenberg and Leese, 2014; Thrasher *et al.*, 2018).

quaddRADseq (Figure 5) is based on restriction-site DNA sequencing (RADseq) (Baird *et al.*, 2008), which was the first method developed to ensure that the analysis of the same homologous regions in the samples would be constant between individuals (Peterson *et al.*, 2012), which led to the development of a reduced representation sequencing library solely meant for polymorphism discovery (Altshuler *et al.*, 2000) with taking advantage of the

sequence specificity of restriction enzymes. This method is fitting for systems without an available reference genome. However, due to the lack of analysis efficiency, up to over half of the sequence data had to be discarded due to various errors in sequence reads, number of variable sites in each sequenced region (Emerson *et al.*, 2010; Hohenlohe *et al.*, 2011; Pfender *et al.*, 2011; Peterson *et al.*, 2012), as well as the introduction of false biases in the data (Felsenstein, 2006; Pollard *et al.*, 2006; Carling and Brumfield, 2007; White *et al.*, 2009).

ddRADseq (Peterson *et al.*, 2012) was thus developed to eliminate the random shearing and end repair of genomic DNA fragments that is a major part of the sample preparation for RADseq. It uses size selection to recover a number of regions distributed randomly throughout the entire genome, and maximises the number of samples per sequencing lane, a process called multiplexing, by introducing a two-index combination tagging approach.

Compared to RADseq, ddRADseq uses a double restriction enzyme digest and allows for the precise selection of defined genomic fragment sizes and thus permits an overall higher control of what type of fractions will be represented in the final library. It allows for a higher sample throughput by increasing the multiplexing potential (Peterson *et al.*, 2012), as well as incorporating an in-line barcode system (Craig *et al.*, 2008) and a standard Illumina multiplexing read index to improve sequencing and the downstream analysis. ddRADseq increases the possibilities in handling and analysing a rising amount of data. However, there are two disadvantages that have not been taken into account in the development of ddRADseq, namely PCR duplicates, which can lead to false genotype calls (Pompanon *et al.*, 2005), as well as a potentially unequal representation of different loci in different subpools.

quaddRAD (Franchini *et al.*, 2017) (Figure 5) is an advanced ddRAD protocol, which addresses these two disadvantages and further increases the level of possible multiplexing. Just like ddRADseq, quaddRADseq utilises a digestion with two restriction enzymes, one frequent and one rare cutter, to divide the genome into fragments of various sizes. This step is combined with the ligation of adapters to the fragmented DNA. By the introduction of a short, four-base sequence at the distal region of each Illumina adapter, it is possible to determine PCR duplicates (Casbon *et al.*, 2011; Tin *et al.*, 2015), which can later be discarded and thus excluded from the final bioinformatical analysis.

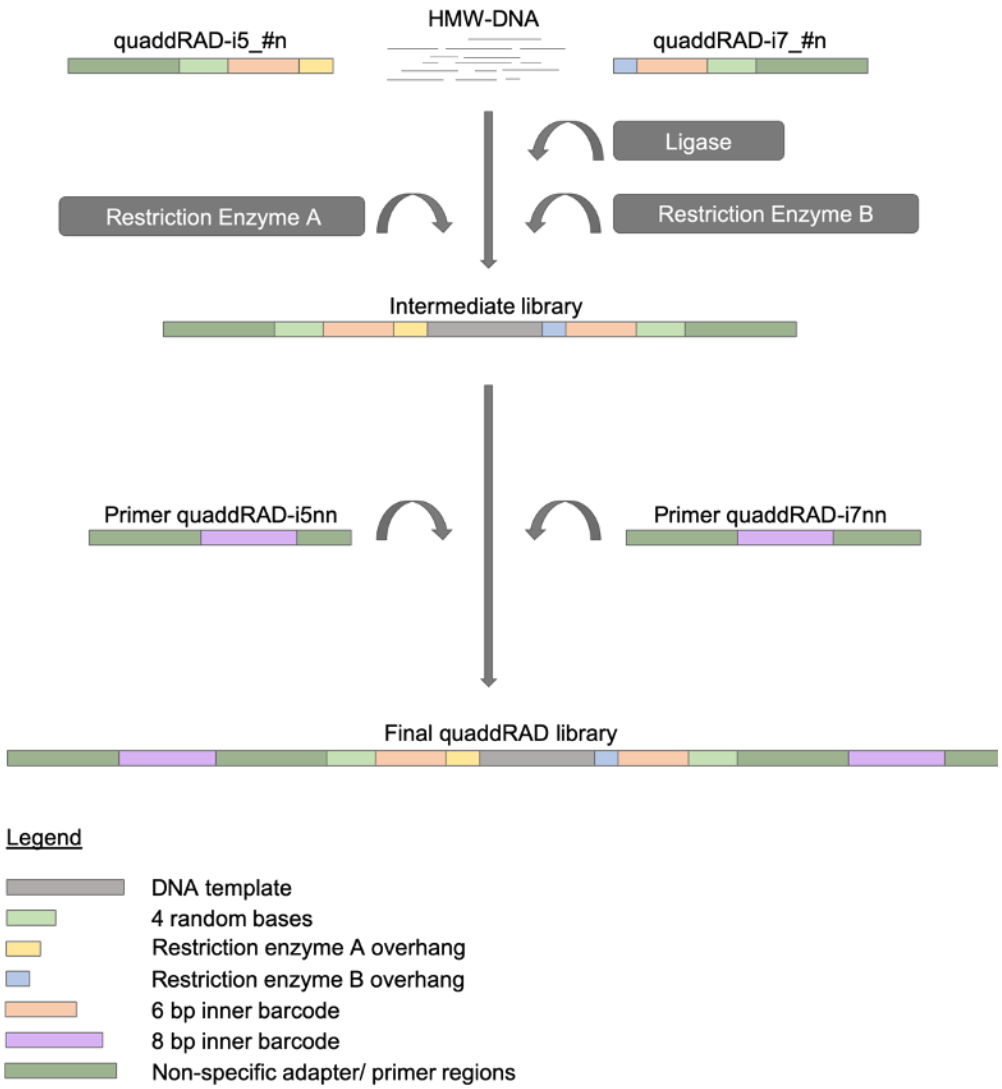


Figure 5 - Model of quaddRADseq. Based on Franchini *et al*., 2017.

Following the digestion and ligation step, primer sequences are added via PCR. The samples, which will receive the same primer combination are combined equimolarly, forming subpools. Here, it is important that each sample in a subpool has different adapter sequences ligated to it. This step further introduces unique sequences that will help distinguish between the samples in the end. The subpools are then combined into one final pool consisting of all samples in the analysis and are then size-selected using a device like the Blue Pippin (Sage Science), which leads to an even distribution of fragment sizes within the final pool. In the ddRAD protocol by Peterson *et al.* (2012), the size selection gets performed on the subpools, causing a possibility for a size selection error and can thus lead to the samples not being comparable in the final analysis steps. Performing the size selection on one library minimises the interlibrary fragment size variability and thus maximises the number of orthologous loci among all subpools (Franchini *et al.*, 2017). The adapter and primer combinations, and thus

their sequences, allow for an increase in uniquely identifiable samples per subpool and library without the need of additional oligonucleotide synthesis and thus higher sequencing costs due to longer fragments (Peterson *et al.*, 2012). With the changes that come with the quaddRAD protocol, it is possible to identify and eliminate PCR duplicates and thus remove the possibility of false genotype calling (Pompanon *et al.*, 2005) as well as to increase the sample multiplexing potential within one pool (Franchini *et al.*, 2017) while working with a non-model organism.

4.8 Histological analyses

While paternity analyses using SNPs can clarify what happens to the paternal contribution during mating with a HT1 female of *A. lythri*, they cannot explain everything that is happening in this HT, as they only show the final result of what happened inside the reproductive system of the female beetles, both before and after insemination. To get additional insights into the mode of reproduction, I looked at undeveloped and unfertilised but ovipositioned eggs to count the chromosomes of HT1 and HT2 gametes. By checking the undeveloped eggs, I wanted to determine whether differences could be seen between these two HTs. It was important to understand how the reproductive system of the females looked like and when the fertilisation with stored sperm happens.

In insects, the pair of ovaries is joined with a pair of lateral oviducts which connect into a genital chamber (Suzuki, 1988). This genital chamber often forms a pouch, known as the bursa copulatrix, which is meant to receive the penis or spermatophore and is connected to the spermatheca, where the sperm is stored until fertilisation (Figure 6; based on Suzuki, 1988; Simões, 2012). Once the eggs leave the ovaries, travel through the lateral oviduct, they reach the genital chamber, which the spermatheca is connected to, and usually will be fertilised when passing the spermatheca duct.

In insects, as well as most animals, the female gamete arrests in metaphase II until the continuation of the second meiosis division is triggered by the fusion with a sperm (Masui and Markert, 1971; Choi *et al.*, 1996; Simpson and Douglas, 2013, p.322; Gruss, 2018). If the female beetle is unfertilised, meaning the spermathecae are empty, the egg remains in metaphase II, even after oviposition. This made it possible to use fully developed eggs, which are sturdier than those that do not have a fully hardened chorion (Simpson and Douglas, 2013, p.349), for the microscopy analyses. Understanding the structure of the reproductive

system, it was possible to produce chromosome spread of unfertilised eggs and compare the chromosome counts amongst the HTs 1 and 2.

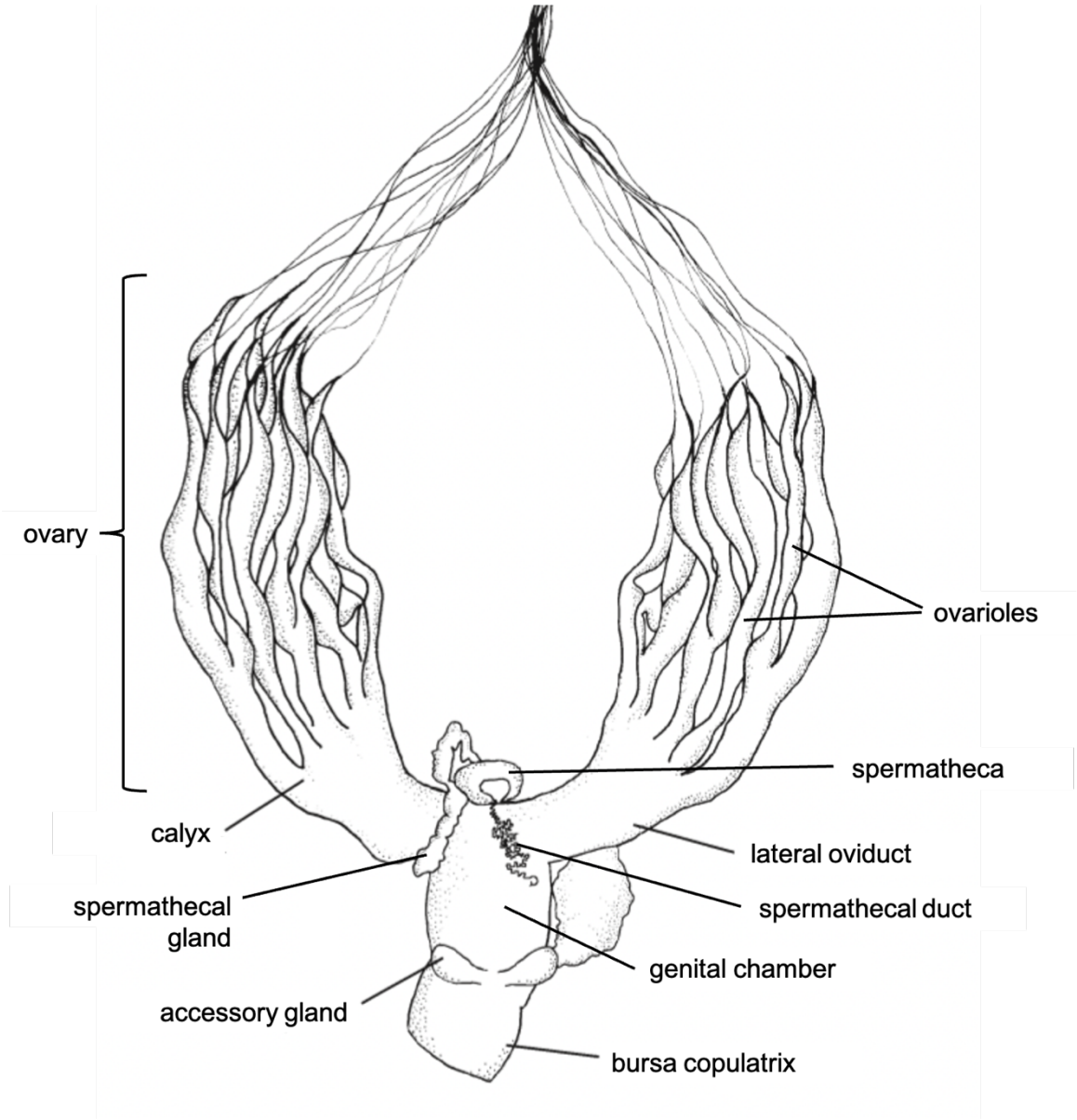


Figure 6 - Model of the reproductive system of *Stolas conspera* (Germar, 1824) as a representative for Coleoptera, Chrysomelidae. Based on Suzuki (1988) and Simões (2012).

5 Material and Methods

5.1 Paternity analysis

5.1.1 Beetle collection, captive breeding, and sample preparation

The flea beetle *Altica lythri* is monophagous on plants of the genus *Epilobium* (Jäckel, Mora and Dobler, 2013), which belongs to the family of the willowherb Onagraceae. They can be found near ponds or calm rivers. Its life cycle lasts for one year, starting with the imagines feeding for some time before hibernating in the leaves on the ground or in the upper soil layers. In early spring, the males emerge before the females, distribute amongst the host plants and, once the females emerge, mate with them (own observation). The females lay eggs at the base of leaves, so that the larvae that hatch about three weeks after oviposition can feed on the leaves of the host plant to grow, pupate, and hatch as the next generation of imagines in late summer of the same year. The beetles used for this study were collected during spring 2019, summer 2020, spring 2021, and spring 2022 from populations in Büchen (53°28'42.4"N 10°37'56.2"E), Güster (53°32'24.1"N 10°41'10.0"E), and Pevestorf (53°03'57.8"N 11°27'23.2"E), the first two are locations in Schleswig-Holstein, Northern Germany, the latter is a location in Niedersachen, Northern Germany.

The sex of the collected beetles was determined morphologically by analysing the differences in the shape of their last abdominal sternites (Jäckel, 2011). The mtDNA haplotype (HT) was determined by PCR-RFLP, which is described below. The beetles were separated by population, sex and mtDNA HT, and placed into *Drosophila* rearing tubes with moistened florist foam, a piece of *E. hirsutum* leaf and a foam stopple. They were kept in a climate chamber at 15 °C and constant conditions of a light and dark cycle of 14:10h.

For the paternity analysis, the female beetles needed to be virgin to ensure that the male beetle was added to the female's tube would be the father of the offspring. To determine the fertilisation status of the females, up to ten ten-days-old eggs were squashed between two microscope slides, covered with a cover slip, and checked for signs of cell division. If no cell division or larva forming could be detected, these females were used for paternity analyses. To obtain the offspring, mtDNA HT1, 2 and 3 female beetles were put together with one HT2 or HT3 male each and kept in a rearing tube. The eggs were transferred to Petri dishes with a piece of moist florist foam to keep them from drying out and labelled according to the mother. I waited for a minimum of seven larvae to hatch and grow to a length of about

6 - 8 mm before killing the offspring by freezing at -80 °C, putting them in separate tubes and labelling accordingly. The parent animals were frozen at -80 °C as soon as enough larvae had survived and grown big enough for further analysis. For a list of samples, see Table 5.

5.1.2 Identification of mitochondrial haplotypes

To identify which mitochondrial HT the beetles had, the DNA from live beetles was extracted using their fresh faeces. For this, the beetles were placed in a 1.5 mL reaction tube for 3 h. Once enough material was in the tubes, the beetles were placed back into their rearing tubes and the DNA was extracted using the innuPREP DNA Forensic Kit (neoLab, Heidelberg, Germany) and the manufacturer's animal tissue protocol.

The mitochondrial haplotypes were determined using a previously established protocol (Jäckel, 2011). The amplification of the cytochrome oxidase subunit I (COI) gene was conducted using Taq polymerase (5 U/µL, Thermo Fisher Scientific) in 25 µL reactions (1x Taq buffer, 2 mM $MgCl_2$, 100 µM of each dNTP, 200 µM of each oligonucleotide (S1634 (ATTGGAGATGAYCAAATTTATAAYGT) and A2969_Alyt (AGTTCAGAGTATGAGTGTTCAG)), 2 µg bovine serum albumin, 1 µL DNA in 35 cycles [95 °C 2 min, 35x (95 °C 30 s, 47 °C 30 s, 72 °C 60 s), 72 °C 10 min]. With the help of a 1% agarose gel in 1x TAE (10x: 48.5 g Tris, 11.42 mL acetic acid, 200 µL 0.5M EDTA), the thickness of each PCR product band and thus the approximately amount of PCR product for the enzyme digestion was determined. Figure 7 (A) shows an exemplary agarose gel of a COI-PCR, which depicts the varying band thicknesses. Exemplary samples 1 to 3, 5 to 7, and 9 show a strong band. For those seven samples, 8 µL PCR product would have been used in a 20 µL reaction. Exemplary samples 4 and 8 show a weaker band for which 10 and 12 µL, respectively, would have been given into the reaction, and exemplary sample 11, showing a very weak band, would have been repeated. The fragments with a length of approximately 400 bp are unspecific.

Figure 7 (B) shows the result of the next step; the enzyme digestion. For this, the previously determined amount of PCR product was given to 2 µL of enzyme buffer R and 0.2 µL of the endonuclease *Hinf*I (10 U/µL, Thermo Fisher Scientific). This reaction was then incubated overnight at 37 °C. The restriction fragments were then separated on a 1.5 % agarose gel (TBE; 108 g Tris, 55 g boric acid, 40 mL 0.5M EDTA, pH 8.3) for 3 h at 100 V, stained with ethidium bromide, and visualised under UV light. The resulting fragment patterns were assigned to the

mtDNA haplotypes based on prior knowledge of their sequences and cut sites (Jäckel, 2011; Figure 7, C).

5.1.3 RNA extraction and sex determination of the offspring

To document the sex ratio of the offspring, the total RNA was extracted using a combination of RNAmagic (Bio-Budget, Krefeld, Germany) and the RNeasy Plus Mini Kit (Qiagen, Venlo, Netherlands). The heads of the larvae were cut off, put into a sterile 1.5 mL reaction tube respectively, and frozen in liquid nitrogen before being ground using a Teflon pestle (Corning, Kaiserslautern, Germany). The resulting powder was then mixed with 500 µL RNAmagic, vortexed thoroughly, and incubated for 15 min at room temperature (RT). Afterwards, the samples were centrifuged at max. g for 10 min at RT and the interphase was transferred to a fresh tube. After the addition of 100 µL chloroform, the samples were vortexed, incubated at RT for another 10 min and centrifuged at max. g for 10 min at 4 °C. The aqueous phase was transferred onto gDNA eliminator columns provided by the RNeasy Plus Mini Kit (Qiagen) and further purified using the manufacturer's protocol for the RNeasy Plus Mini Kit (Qiagen).
Following the extraction, the RNA was reverse-transcribed into cDNA using the SuperScript™ III Reserve Transcriptase kit (Invitrogen, Massachusetts, United States of America). For this, 0.35 µg RNA was reverse-transcribed with an oligo-$(dT)_{17}$ primer in a total volume of 20 µL. Afterwards, 2 µL of the cDNA was used to amplify the target gene *dsx*, a gene that has three splice variants (two female-specific (1179 bp and 1351 bp) and one male-specific (759 bp) (Rohlfing *et al.*, 2023)). The amplification was done in 12.5 µL reactions (1x HF buffer, 2 mM MgCl2, 200 µM of each dNTP, 200 µM of each oligonucleotide (Aly_dsx_for (ACTTCAGAACGACAACGAG) and Aly_dsx_rev (GCGTTACTCTTGATTCAGC)), 2 µL cDNA) in 35 cycles [98 °C 3 min, 35x (98 °C 10 s, 58 °C 30 s, 72 °C 1 min), 72 °C 7 min]. The amplified fragments were then separated on a 1.5% agarose gel, stained with ethidium bromide and visualised under UV light.

5.1.4 Illumina library preparation

For the preparation of the Illumina library, the DNA of the offspring and parents was extracted. For this, I used the DNeasy Blood & Tissue Kit (Qiagen) and the provided Animal Tissue (Spin-Column) protocol. The only significant change I made to the procedure was to

change the elution volume from 200 µL elution buffer to 100 µL water. This makes it possible to continue the analysis with higher concentrated and salt-free DNA.

The novel paired-end ddRAD protocol quaddRAD (quadruple barcode design; Franchini *et al.*, 2017) is based on the ddRAD protocol by Peterson *et al.* (2012). It eliminates disadvantages like the accumulation of PCR artefacts and duplicates (Schweyen, Rozenberg and Leese, 2014), the false genotype calls due to skewed allele frequencies (Pompanon *et al.*, 2005) and includes the creation of a single library containing all individuals in the analysis. This promises even size selection and distribution of fragments.

Following the genomic DNA extraction, I prepared the quaddRAD library according to the quaddRAD protocol (Franchini *et al.*, 2017). For the double digestion and adapter ligation, I used one frequent cutter restriction enzyme (*Pst*I) and one rare cutter restriction enzyme (*Msp*I). The adapters used are listed in Franchini *et al.* (2017) (Table 6). To check whether the first step of the library preparation worked out, I conducted a test PCR with the primers given in Franchini *et al.* (2017) (Table 7), which I ran on a 1% TAE (48.5 g Tris, 11.42 mL 100% acetic acid, 200 µL 0.5M EDTA) agarose gel. If the samples showed no smear but a strong, yet short fragment, I repeated the digestion and adapter ligation step. The expected result was a smear. The restricted and ligated DNA fragments were washed between each step using magnetic beads (AMPure XP from Beckman Coulter) to get rid of the very small fragments that might otherwise dominate in the PCR and disturb the reaction. After the indexing PCR was performed according to the protocol, the libraries were pooled equimolarly and fragments of a specific size selected using the BluePippin (Sage Science). The fragment target size was usually set to around 550 bp, depending on the fragment sizes detected in the sample. Afterwards, as the last step before sequencing, the final sample library was checked on the TapeStation (4150 system, Agilent) to check if the correct fragment size has been eluted. The entire procedure is based on the protocol by Xu and Hausdorf (2021).

5.1.5 Sequencing and paired-end data treatment

The quaddRAD protocol uses modified Illumina adapters that include short four redundant bases in the sequencing distal region to enable the identification and removal of PCR duplicates after sequencing. Furthermore, by the addition of four barcodes that incorporate two inner and two outer stretches of six bases, it is possible to increase the sample multiplexing compared to the original ddRAD protocol (Peterson *et al.*, 2012). Each primer

contains a specific Illumina index barcode and an Illumina flowcell adapter sequence (Kess *et al.*, 2015) to allow the binding and sequencing process on the flowcell.

The sequencing data was evaluated using the programme pipeline Stacks (Catchen *et al.*, 2011, 2013). All commands are listed in chapter 12.3. For ustacks, I selected a minimum number of raw reads required to form a stack (putative allele), -m, of ten, and a number of mismatches allowed between stacks (putative alleles) to merge them into a putative locus, -M, of three. For cstacks, I used an -n (number of mismatches allowed between putative loci during the construction of the catalogue) of one. The last step of this pipeline that required specifying parameters was populations, with a set minimum number of two samples a locus must be present in to process the locus (-p 2), and a minimum of 100% of individuals in a population required to process a locus (-r 1). The concluding summarising of the results was done via two Python3 scripts written by C. Zeng (chapter 12.4), followed by the final visualisation using the R package GGPLOT2 (Wickham, 2016). The functions used were 'ggplot' for the overview (Figure 10) and 'facet_wrap' for the detailed, zoomed-in view (Figure 11) in RStudio.

5.1.6 Statistics

All analyses were performed using R and Microsoft Excel. To determine the influence of haplotypes on the inheritance modes, I built binomial Bayesian mixed effects model using the RSTANARM package in R (Goodrich *et al.*, 2022). The binary response variable was two haplotypes (dummy coded as 0 and 1), and the predictor variables were "maternal gametes", "paternal gametes" and "sexual reproduction". Families were included as a random effect and the model was run for 6000 iterations, such that the effective sample sizes of all variables were at least >5000. The quaddRAD data for predictor variables (Table 3) is compositional, meaning that every value of the predictor variables sums up to 1, therefore the predictor variables were highly correlated. To avoid problems with multicollinearity in the mixed effects model, the predictor variables were subjected to isometric log-ratio transformation using the package COMPOSITIONS (van den Boogaart, Tolosana-Delgado and Bren, 2008). Model diagnosis was performed by checking for chain convergence, autocorrelation, divergent transitions and plots of observed versus simulated data.

5.2 Histological analysis of meiosis products

5.2.1 Determining the optimal digestion time for the removal of follicle cells

The goal of this part of the study was to determine the number of chromosomes in each HT. The eggs of insects are encased in a layer of follicle cells (Simpson and Douglas, 2013, p. 349), which are involved in the production of the chorion while the egg is in the ovary. Although they should be in apoptosis after oviposition, it is possible that singular nuclei of said cells survive until the moment of fixation. In that case, it is possible to find them under the microscope as the antibody and 4',6-diamidino-2-phenylindole (DAPI) are able to bind to the chromosomes. To remove the potential of staining their nuclei, it is possible in some cases to remove them from the eggs using potassium hydrogen (KOH). For this, eggs of various developmental stages were treated with 10%, 5% and 2% KOH. To check the correct incubation time, only laid eggs were further used in this procedure due to their size. They were placed on a clean microscopy slide, one per slide, and put underneath a light microscope. Once in focus, one drop of 2% KOH was added to the egg and pictures were taken after 15 s, 30 s, 45 s, 60 s, 90 s, 2 min, 2min 30 s, 3 min, 3 min 30 s, 4 min, 4 min 30 s, 5 min, and 11 min to determine the optimal incubation time needed to remove the follicle cells from the chorion.

5.2.2 Hypotonic treatment of laid eggs

To separate the egg nucleus from the follicle cell nuclei, I dissected beetle ovaries and tried to separate the egg nucleus from the follicle cells surrounding the chorion with hypotonic treatment. Eggs of early developmental stages were used as the chorion hardens and thickens upon oviposition (Simpson and Douglas, 2013, p. 349). The ovaries were prepared in 0.1M phosphate-buffered saline (PBS) and the eggs were taken out carefully separated from the ovarian tissue using fine forceps before they were transferred to 0.044M potassium chloride (KCl) solution (Sasai *et al.*, 1996) for 30 min to increase the osmotic pressure inside the egg. After the incubation, the egg was poked using a very fine glass needle (own production by heating up thin glass rods and pulling them apart to create a fine, sharp tip) to trigger the yolk flow out of the egg, which should carry the nucleus with it and away from the follicle cells, which are still alive and functioning at early developmental stages of the egg.

5.2.3 Fixation, preparation and storage of the chromosomes

This protocol is based on the fixation protocol that can be found in Jäckel (2011). I used eggs of HT1 and 2. They were one to three days old to ensure that there would not be any error introduced by the eggs drying out before fixation, which can alter the stability of the chorion and make preparation and staining more difficult (own observation).

For the fixation, eggs of each HT were placed separately into 1.5 mL Eppendorf tubes each, 20 µL of Clarke's fluid (ethanol and acetic acid, 3:1) were added and they were incubated for one hour at RT. This step prefixed the egg and helped with the final fixation of the chromosomes. Afterwards, the eggs were macerated on an adhesion microscopy slide (SuperFrost® Plus, Thermo Scientific) using one pointed and one flat dissection needle. This way, I could pull apart the egg and allow the nucleus to flow out of the egg and get in contact with the fixation buffer consisting of acetic acid and methanol (3:1). Methanol was chosen instead of ethanol to increase the spreading of the chromosomes while the methanol dries (Deng *et al.*, 2003). After the maceration and final fixation of the samples, the slides were air-dried and stored in a dark container at 4°C until they were labelled with antibodies and stained.

5.2.4 Antibody labelling, DNA staining and storage

To determine the chromosome number of HT1 and HT2, I labelled the centromeres of the chromosomes with a specific antibody, one that binds to the centromere identifier (CID) protein (Henikoff *et al.*, 2000; Blower and Karpen, 2001; Khetani and Bickel, 2007). The eggs of unfertilised beetles are arrested in the metaphase (Masui and Markert, 1971; Choi *et al.*, 1996; Simpson and Douglas, 2013, p. 322; Gruss, 2018), meaning that the chromosomes are in their condensed state, easy to label, stain and count.

For the antibody labelling, the macerated and fixed samples were blocked for one hour at RT using a liquid blocker pen to contain the buffer on and around the sample. The labelling process was done as can be read in chapter 6.10, and all liquids were cautiously taken off each sample using tissue paper. Before the DAPI-staining and sealing, the samples were air-dried and the liquid blocker pen was removed using 96% ethanol and a Q-tip. They were stored in the dark at 4°C until use.

5.2.5 *Sf*9 cell culture and cell preparation

The antibody staining did not give clear result, so I checked the specificity of the anti-CID antibody using *Spodoptera frugiperda* (*Sf*9) insect cells (Fisher Scientific, Invitrogen™ *Sf*9 cells). These cells were cultivated in 10 to 25 mL Insect-Xpress medium (Lonza, BioWhittaker®), depending on the culture flask size. The cells were split regularly by replacing the old with fresh medium and transferring a fraction of this cell suspension to a sterile culture flask and topped off with fresh medium to reach a total volume of 10 to 25 mL, depending on the flask size. The cells were incubated at 27 °C.

5.2.6 Testing of the anti-CID antibody specificity using *Sf*9 cells

For the antibody testing, cover slips were placed into each well of a 12-well plate and 1 mL containing 1.5 x 10^5 cells was added from a flask that had been incubating for three days. After 3 h, the cells had sufficiently attached to the slides to continue with the further treatment. They were treated with 0.005% colchicine solution (Merck) to inhibit the formation of microtubules in dividing cells for 20 min (Jäckel, 2011) before fixing them for 5 min using 4% paraformaldehyde (PFA). After the fixation incubation, the PFA was removed, the cells were wrapped in aluminium foil and stored at 4 °C until further use. To perforate the cell membrane, the cells were treated with Tris-buffered saline (TBS; 200mM Tris, 1500mM NaCl) containing 0.2% Triton-X for 5 min, before it was removed and a commercial blocking buffer for immunofluorescence (Immunofluorescence Blocking Buffer, Cell Signaling Technology, Massachusetts, United States of America) was added for 1 h. Multiple concentration and incubation times were tested for the primary antibody (anti-CID IgG, abcam, Cambridge, United Kingdom): 1:500 antibody dilution for 2 h, 1:100 antibody dilution for 1 h, and 1:100 antibody dilution overnight. All of these dilutions were achieved using blocking buffer that has been diluted 1:10 with sterile, deionised water. The samples were then washed three times using freshly diluted 1x PBS (10x PBS: 80 g NaCl, 11 g Na_2HPO_4, 2 g KH_2PO_4, 2 g KCl, pH 7.4) for 5 min before the secondary antibody was added. I used a goat anti-rabbit antibody (Alexa Fluor™ 488 goat anti-rabbit IgG (H+L), Invitrogen), diluted 1:1000 in 1:10 diluted blocking buffer. The samples were incubated for 1 h at RT in the dark. After the incubation, the solution was taken off and the samples were washed three times for 5 min in freshly diluted 1x PBS in the dark. Once the samples were air-dried, they were stained with

DAPI mounting medium (dianova, BIOZOL GmbH, Hamburg, Germany) and sealed with a cover slip and nail varnish.

5.2.7 Fluorescence and light microscopy

Fluorescence and light microscopy were performed using the Olympus BX51 fluorescence microscope with the 20x objective to get an overview and 40x and 100x objectives (UPlanFL N) for detailed pictures. The pictures were taken with the mounted Olympus DP71 camera and the software cellSens 1.16. The channels used were FITC for the antibody test using the goat anti-CID antibody and the DAPI channel for detection of the stained DNA material. The fluorescence lamp connected to the microscope was the X-Cite Series 120 (EXPO).

The exposures varied depending on the amount of signal the camera was detecting. For the antibody test, 20 ms were enough to get a sufficient DAPI signal, as were 160 ms for the FITC channel. For the chromosomes, however, 700 ms in the DAPI channel were used as the chromosomes are very small and the signal thus not as strong for a single nucleus as it was staining 1.5 x 10^5 *Sf*9 cells.

6 Results

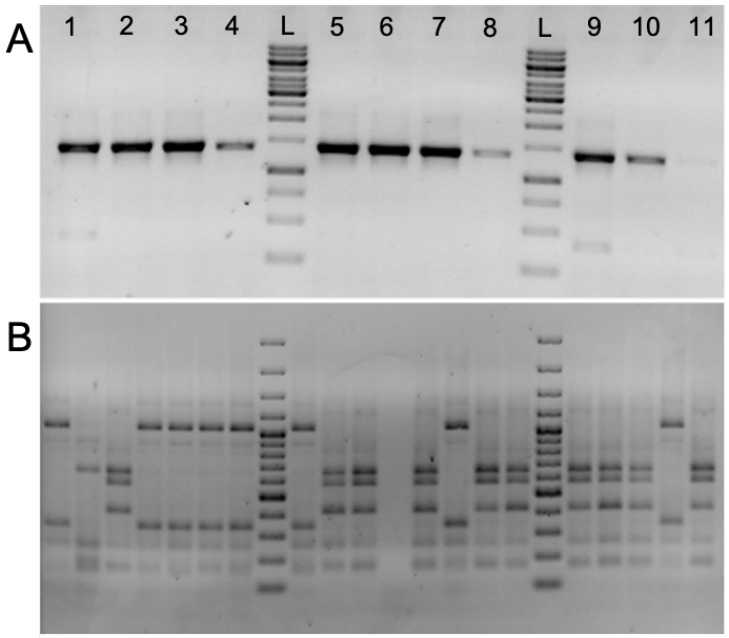
6.1 Rearing and crossing of beetles

Before performing the selected crosses of beetles for paternity analyses, the fertilisation status of the females had to be determined. For this, females were kept in isolation until the start of egg-laying, then three-day-old eggs were squashed and checked for signs of cell division. The mated females were excluded and the analysis only continued with the unfertilised female beetles. After this step, the HT was determined (see chapter 6.2) to ensure the correct females were placed and left together with a male. The males had either HT 2 or 3, depending on which HT was available. CI has not been shown in *A. lythri* (Jäckel, 2011), so the crosses of all females with HT2 or HT3 males produce viable larvae. The eggs, which were usually oviposited in the fold of the tissue paper covering the plug, were removed regularly and placed in a small petri dish with a piece of moist florist foam. After about three weeks, the larvae hatched. The offspring were fed until they reached about 7 mm in length and frozen at -80 °C until DNA and RNA extraction. The RNA extraction was needed for the sex determination of the progeny and was done using RNA extracted from the heads of the larvae (see chapter 5.1.3), while the DNA extraction was necessary to prepare the samples for sequencing (see chapter 5.1.4).

Because only unfertilised females were used for the analysis, the biological parents of the progeny are known, making the following paternity analysis possible as it was known that the SNPs found in the offspring could only originate from either the mother or the designated father. In total, 41 crosses with HT1 females were generated, 34 crosses with HT2 females, and seven crosses with HT3 females, but only six HT1 crosses, seven HT2 crosses, and one HT3 cross could be used in the final analysis. This was due to rearing problems like the lack of suitable feeding material, not enough offspring, or the failing of the sequencing.

6.2 Haplotyping

For the determination of the HT of each male and female, the DNA needed to be extracted. This step required the beetles to stay alive as they were still needed to produce offspring. To determine the HT of both parents without killing the beetles a sample of DNA was extracted from fresh faeces.



C

mtDNA haplotype	Banding pattern *Hinf*I digest
HT1	164 – 648 – 573
HT1*	164 – 648 – 237 – 186 – 150
HT2	1049 – 336
HT2*	164 – 885 – 336
HT3	164 – 1070 – 151

Figure 7 - Determination of the mtDNA haplotype. (A) Exemplary 1% agarose (TAE) gel of COI-PCR products. The fragment is 1385 bp long, as indicated to the right. (B) Exemplary 1.5% agarose (TBE) gel result of restriction fragment length polymorphism (RFLP) analysis of COI fragments digested with *Hinf*I. The banding pattern of the sample is specific for each mtDNA HT. (C) Table of banding patterns resulting from the COI-PCR product digestion using the restriction enzyme *Hinf*I overnight at 37 °C.

Afterwards, a COI-PCR was performed, as described in chapter 5.1.2. The PCR product of the cytochrome oxidase subunit I (COI) gene (as can be seen in Figure 7, A) has a fragment size of 1385 bp. The beetles haplotyped and used in the analysis are listed in the supplementary (chapter 12.1).

6.3 Sex determination of the progeny

The sex ratio of sexual reproduction is predicted to result in 50% of all offspring being female, which follows the 1:1 sex ratio of sexual species as described in Fisher's model of sex ratio equality (Fisher, 1930; Hamilton, 1967). While the HTs 2 and 3 roughly show this sex distribution, HT1 of *A. lythri* and other species in this genus do not (Phillips, 1979; Jäckel, Mora and Dobler, 2013).

To determine the sex ratio of the offspring that was used in the paternity analysis, RNA was extracted from the larvae's heads, followed by cDNA synthesis and a PCR amplification of *dsx*. This gene plays a major role in *A. lythri*'s sex determination cascade, as it is the last step of activation and inactivation of genes to determine the sexual differentiation of the progeny. The *dsx* pre-mRNA undergoes sex-specific splicing due to the formation of a splicing complex consisting of Tra^F and Tra2. This complex causes the consecutive splicing of the *dsx* pre-mRNA into the female variants, called *dsxf1* and *dsxf2*, whereas a missing splicing complex in males leads to the default splicing, which results in the male-specific isoform, *dsxm* (chapter 4.2.3). The resulting PCR products have length of 1179 bp and 1351 bp for *dsxf1* and *dsxf2*, respectively, and 759 bp for *dsxm* (Rohlfing *et al.*, 2023). Whereas it is possible to find both female- and male-specific splicing isoforms in females, only default splicing takes place in male individuals. This is because Tra^F is not present and thus cannot form the splicing complex with Tra2, ultimately leading to only the default splicing to take place. In Figure 8 (A), an exemplary *dsx* PCR 1.5% TAE agarose gel is shown, depicting the varying fragment sizes of the *dsx* PCR fragments that occur in *A. lythri*, depending on the sex of the tested individual.

In the gel, samples 1, 3, and 5 show the female splice variants with the fragment lengths of 1179 bp and 1351 bp. Sample 1 also shows a weak band of the male variant at the length of 759 bp. In female individuals, it is possible for the default splicing to occur, however, as soon as Tra^F exists and is able to form the splicing complex needed for the female-specific splicing of the *dsx* pre-mRNA, the individual develops into a female. Samples 2 and 4 do not show the two longer fragments of 1179 bp and 1351 bp and only have a male-specific band at 759 bp. The other two bands at around 500 bp and 300 bp are unspecific.

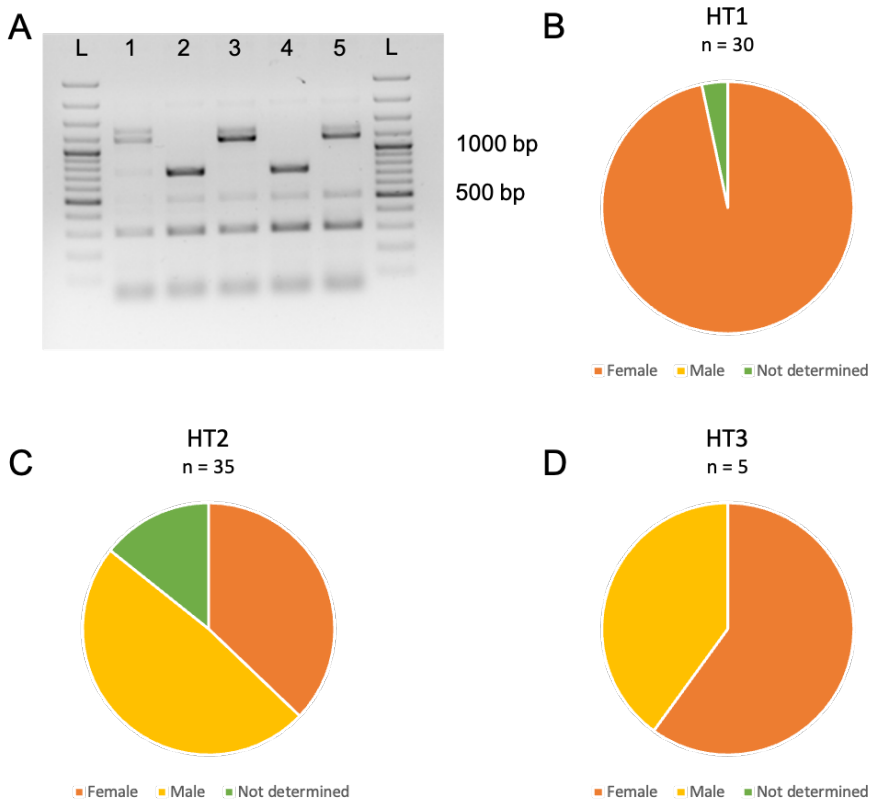


Figure 8 - Sex ratio of progeny used in quaddRAD paternity analysis. (A) Exemplary 1.5% agarose gel depicting the PCR products generated by a *dsx*-amplifying PCR. Fragment sizes are indicated to the right. Samples 1, 3, and 5 show female splice variants, samples 2 and 4 only show male splice variants. (B) Sex ratio of HT1 offspring, n = 30. (C) Sex ratio of HT2 offspring, n = 35. (D) Sex ratio of HT3 offspring, n = 5.

Upon amplifying the splice variants of *dsx* for each offspring used in the paternity analysis, it was possible to generate the sex ratio of the HTs that went into this analysis (Figure 8, B-D). For HT1 (Figure 8, B), six families could be analysed by sequencing and bioinformatics, meaning a total of 30 offspring were checked. The sex of one offspring could not be determined because the RNA extraction from the head had failed. However, all of those successfully extracted showed the female splice variants of *dsx* and no male splice variants could be found without the female equivalent also present, making the offspring exclusively female. HT2 showed a different pattern (Figure 8, C). Here, a total of seven families were used for the final analysis, meaning 35 offspring were extracted and their cDNA *dsx* amplified. The sex of five larvae could not be determined due to a mistake in handling the samples for RNA extraction, leaving a total of 30 larvae to test. Thirteen of them showed the female splice variants, 17 showed only the male-specific band. For HT3 (Figure 8, D), only one family was used for the final analysis due to sequencing failures and high levels of missing data in the other extracted families, resulting in five offspring that were sexed. Three of them showed the female-specific banding patterns, and the other two showed the male-specific ones.

Overall, for HTs 2 and 3, both males and females were detected via the amplification of the *dsx* splice variants. For HT1, exclusively female offspring was detected, which agrees with the findings of Jäckel, Mora and Dobler (2013).

6.4 quaddRAD data

To be able to determine paternity and inheritance patterns within families, large amounts of SNPs were generated by the quaddRAD method. The first step towards the determination of how the progeny SNPs have been inherited is the preparation of the samples. After the DNA extraction, the DNA was cut into shorter fragments using two restriction enzymes, *Msp*I and *Pst*I. This step causes specific cut ends which enabled the adapters to be ligated to the ends of the now cut fragments. After a washing step using AmPure beads (chapter 5.1.4), the samples were checked to see whether the fragment digestion and adapter ligation had worked. For this, a test PCR was performed. The primers for the indexing PCR were used for this step. They include sequences that bind to those found in the adapters and thus allow the polymerase to produce PCR products. If the adapter sequences were not ligated properly to the DNA fragments, the PCR reaction does not work. The expectancy of a successful digestion and ligation is a smear for each sample on a control agarose gel. Since the enzymes cut at different intervals and locations, a variety of fragment sizes was generated that can be seen as a smear on an agarose gel (Figure 9). If a sample showed a strong band at around 150 bp, the digestion and ligation step was repeated because in this case not enough long fragments were present to continue with the analysis. In case of too short fragments, the chance of determining shared SNPs amongst the family (mother, father, five offspring) is very low and the analysis would not have been successful.

Once every sample showed the expected smear and thus included the uniquely assigned adapter sequences, the indexing PCR was performed using the Phusion polymerase. After another washing step, the library was size-selected using the BluePippin and the fragment size was measured using the Tapestation (chapter 5.1.4) before the library was sent to sequencing. Fourteen families consisting of mother, father, and five offspring were used to create the following data. Six of them had the mtDNA HT1, seven HT2, and one HT3.

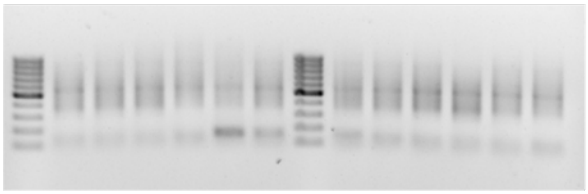


Figure 9 - Exemplary 1% TAE agarose gel picture of a test PCR after adapter annealing for quaddRAD. The ladder used was the 100 bp ladder. All samples show the expected smear and the analysis could be continued according to protocol.

I used the Stacks pipeline (Catchen *et al.*, 2011) to analyse the reads obtained from sequencing. The first step for this analysis is to generate stacks. These stacks consist of identical Illumina reads with the minimum of ten raw reads (-m 10) required to form a stack. Every stack below that number will be excluded from the first part of this analysis and saved as secondary stacks. Once a minimum of ten reads has been assembled into a stack, these stacks are then merged into putative loci. The number of allowed mismatches is determined by the -M parameter. Here, the previously discarded secondary stacks are used to further improve the stack depth or coverage. Generally speaking, the more reads could be added to a given stack, the higher the stack depth or coverage of the putative loci. Because this analysis is suitable for organisms without a reference genome, the stacks are required to build a *de novo* genome. The programme ustacks then checks each locus at each nucleotide position for present polymorphisms and generates a consensus sequence to record SNPs. Once the SNPs have been called, a catalogue is created out of the stacks from the parents to conclude a set of all possible loci. These are needed for the comparison with the loci and SNPs found in the progeny. This comparison serves to determine what percentage of loci are shared amongst the parents and their offspring. It is possible that slight changes in the sequences of the DNA fragments exist due to the filtering steps in the sample preparation (beads wash and size selection). To be able to fully compare the offspring with their parents, it is important to only look at those loci that are present and shared in all samples within one family.

After the Stacks pipeline (Catchen *et al.*, 2011) had identified the loci and formed stacks out of raw reads, the HT1 samples had a mean of 973,138 raw reads per individual, the HT2 samples had a mean of 668,885 raw reads per individual, and the HT3 samples had 747,989 raw reads per individual on average. The coverages of the stacks formed after ustacks were relatively even, although they spanned a range of over 100. For HTs 1, 2, and 3, the minimum coverages were 28.98, 27.70, and 47.88, respectively, and the maximum coverages were 159.53 for HT1, 156.65 for HT2, and 89.43 for HT3. The number of loci found per family after

cstacks and gstacks changed mildly, but on average all families of HT1 had 99.2% of all detected loci aligned to the *de novo* genome and shared 11.5% amongst each family. The HT2 families showed a similar picture with 99.6% of all loci found being aligned and 10.1% being shared amongst each family, whereas due to the low sequencing success for HT3, the family included in the analysis had 98.9% of all found loci aligned yet only shared 5.3% amongst them. In total, a mean of 1466 shared loci were a part of the final analysis within HT1, an average 1310 loci were shared within HT2, and 498 loci could be found in HT3 that all individuals shared within one family. It is not possible to determine how many reads got excluded throughout the Stacks analysis as some of those excluded in secondary stacks are used to increase stack depth, while others do not meet the required parameter setting (-M +2 as the default setting) and are ultimately excluded from the analysis. The detailed number of raw reads, minimum and maximum coverage of the stacks, number of primary and secondary stacks formed, number of loci found and aligned, as well as number of paired-end reads, genotyped and shared loci, and the number of individuals analysed in one HT (n) can be found in Table 1.

Table 1 - Sequence information for all quaddRAD runs and HTs, generated by the Stacks (Catchen *et al.*, 2011) pipeline.

Family	Raw reads	Min coverage	Max coverage	Primary stacks	Secondary stacks	Loci	Paired-end reads	Alligned paired-end reads	Genotyped loci	Shared loci	n
HT1.1	4341502	32.05	88.41	68697	591704	9794	4936764	4920015	9713	1774	7
HT1.2	7807019	31.53	146.34	82971	900260	14386	8881080	8855499	14272	1529	7
HT1.3	4839551	32.98	59.80	80216	519408	10319	4646718	4635813	10242	1099	7
HT1.4	2695017	30.86	47.27	59721	384597	7953	3035364	3024562	7902	1599	7
HT1.5	14208146	28.98	159.53	111785	1422228	21739	14936868	14902165	21579	819	7
HT1.6	6980551	50.93	73.65	100426	701803	12941	7673718	7659122	12836	1976	7
HT1 Total	40871786	28.98	159.53	503816	4520000	77132	44110512	43997176	76544	8796	42
HT2.1	6957224	31.67	159.65	78103	992415	17527	8281922	8281922	17462	1240	7
HT2.2	2997562	27.70	60.88	57229	453885	15953	3690406	3687783	15902	1096	7
HT2.3	4385758	36.59	81.51	70099	647117	13884	5439712	5436476	13810	941	7
HT2.4	4075399	35.69	87.47	65454	620359	11426	5024340	5022010	11376	1879	7
HT2.5	5295658	49.87	111.55	70831	748844	13400	6389326	6385994	13338	1577	7
HT2.6	5945392	32.37	111.44	65705	721557	12764	5970580	5958701	12696	2031	7
HT2.7	3118378	36.17	59.79	33683	1543639	5942	1682514	1682309	5909	408	7
HT2 Total	32775371	27.70	156.65	407421	5727816	90896	36478800	36455195	90493	9172	49
HT3.1	5235923	47.88	89.43	49418	2384525	9558	3418208	3417941	9450	498	7
HT3 Total	5235923	47.88	89.43	49418	2384525	9558	3418208	3417941	9450	498	7
Total	157766160	27.70	159.65	994338	12632341	355172	168015040	167740624	352974	36932	196

6.5 Allele inheritance in the HTs

With the help of a Python3 script written by Cen Zeng (see chapter 12.4), it was possible to sort through the loci and SNPs extracted after the Stacks pipeline and determine which SNP in the offspring was inherited from which parent. This way, the mode of inheritance of each shared SNP could be determined. Stacks filtered out the PCR duplicates to avoid false genotype calling, assigned the sequences to the individual samples with the help of the unique primer and adapter sequences, and determined the SNPs that were then compared using the Python3 script. The script selected the most likely mode of inheritance for each SNP found at the location of a specific SNP that has been detected in both parents and at least four out of five offspring.

'Maternal gametes' and 'paternal gametes' describe the strict inheritance of one SNP from the mother or the father, respectively. If the mother has the SNP 'AA' at a given locus, the father 'TT', and the five offspring 'AA', the script counted this SNP as inherited from the mother alone ('maternal gametes'). If the offspring showed 'TT' at this location, the script would detect this SNP as 'paternal gametes'. However, if the offspring showed 'AT' instead, this SNP would have been inherited from both parents, as the 'A' originates from the mother and the 'T' from the father, and the script would have marked this SNP location as inherited through 'sexual reproduction'. Sometimes, because of the composition of some SNPs, it was not always possible to determine the definite way this SNP had been inherited. Therefore, the script relates the most likely mode of inheritance. 'Maternal or sexual reproduction', 'maternal, paternal or sexual reproduction' and 'paternal or sexual reproduction' all fall under this category of not clear inheritance due to the SNP composition. For example, for 'maternal or sexual reproduction', the mother might have 'AA' for a SNP, the father 'AT', and the offspring 'AA'. In this case, it cannot be decided for sure whether the SNP found in the offspring has been inherited from the mother only ('AA') or through sexual reproduction ('AA' or 'AT'), as both are possible. The inheritance mode 'maternal, paternal or sexual reproduction' can be explained similarly. If the mother shows a 'GT' at one location, the father a 'GT' at the same location, it is impossible to determine which 'G' and which 'T' comes from which parent, so that the script chooses the 'maternal, paternal or sexual reproduction' inheritance type. 'Missing data' describes those locations in which for one specific SNP in a locus, one or two offspring did not have a SNP detected in the sequences.

HTs 2 and 3 show a very similar pattern (Table 2, Figure 10). A mean of 2.45% of the SNPs in the HT2 offspring (Table 2, HT2) were detected as being inherited from the mother only, 38.09% were inherited either from the mother or through sexual reproduction, meaning from both mother and father, and 17.57% of the HT2 SNPs in the progeny were either coming from the mother, from the father, or through sexual reproduction.

Table 2 - Mean values of percentages of which mode the offspring SNPs have been inherited by per HT.

Inheritance	HT2	HT3	HT1
Maternal gametes	2.45	3.29	0.92
Maternal or sexual reproduction	38.09	37.27	96.86
Maternal, paternal or sexual reproduction	17.57	18.23	0.89
Paternal or sexual reproduction	18.66	17.35	0.36
Paternal gametes	2.73	3.21	0.11
Sexual reproduction	15.42	15.1	0.42
Missing data	5.08	5.54	0.45

Same goes for the 'paternal or sexual reproduction' type, which had a mean of 18.66% of all HT2 offspring SNPs assigned to it. In all HT2 families, 2.73% of the offspring SNPs were inherited from the father, while 15.42% were inherited through sexual reproduction. The missing data rate lies at 5.08%. Overall, the inheritance types are relatively equal in their distribution, as none of the modes are predominant. For HT3 the sample size is small with one analysed family, so more individuals need to be added to this HT specifically. However, a pattern similar to that of HT2 is visible when looking at the data (Table 2, HT3). 3.29% of the SNPs found in the offspring were inherited only from the mother, 37.27% were inherited either from the mother or through sexual reproduction, and 18.23% could have been inherited through maternal gametes, paternal gametes, or sexual reproduction. Only paternal gametes as a base for inheritance are concluded for 3.21% of the SNPs in the offspring, whereas 17.35% were inherited from either the father or through sexual reproduction. A definite sexual reproduction inheritance could be detected for 15.1% of all SNPs found, and 5.54% of the data in the offspring was missing. Just like HT2, HT3 shows a relatively even distribution of inheritance types with no predominant mode.

HT1 (Table 2, HT1) shows a different picture, which can also be seen in Figure 10. The majority of the SNPs detected in the offspring (96.86%) were inherited from the mother or through sexual reproduction, whereas 0.92% were inherited solely from the mother and the origin of 0.89% of the SNPs could not be securely determined. 0.36% were inherited from either the father or through sexual reproduction, 0.11% of the SNPs originated from the father, and 0.42% came from sexual reproduction. 0.45% of the data was missing in the offspring. HT1,

contrary to HTs 2 and 3, does not show an equal distribution of inheritance types. Most of the different modes are under the 1% mark, whereas the maternal or sexual inheritance is dominant with 96.86% of all SNPs detected in the offspring being inherited from the mother, which is 2.5-times more than could be seen in HTs 2 and 3.

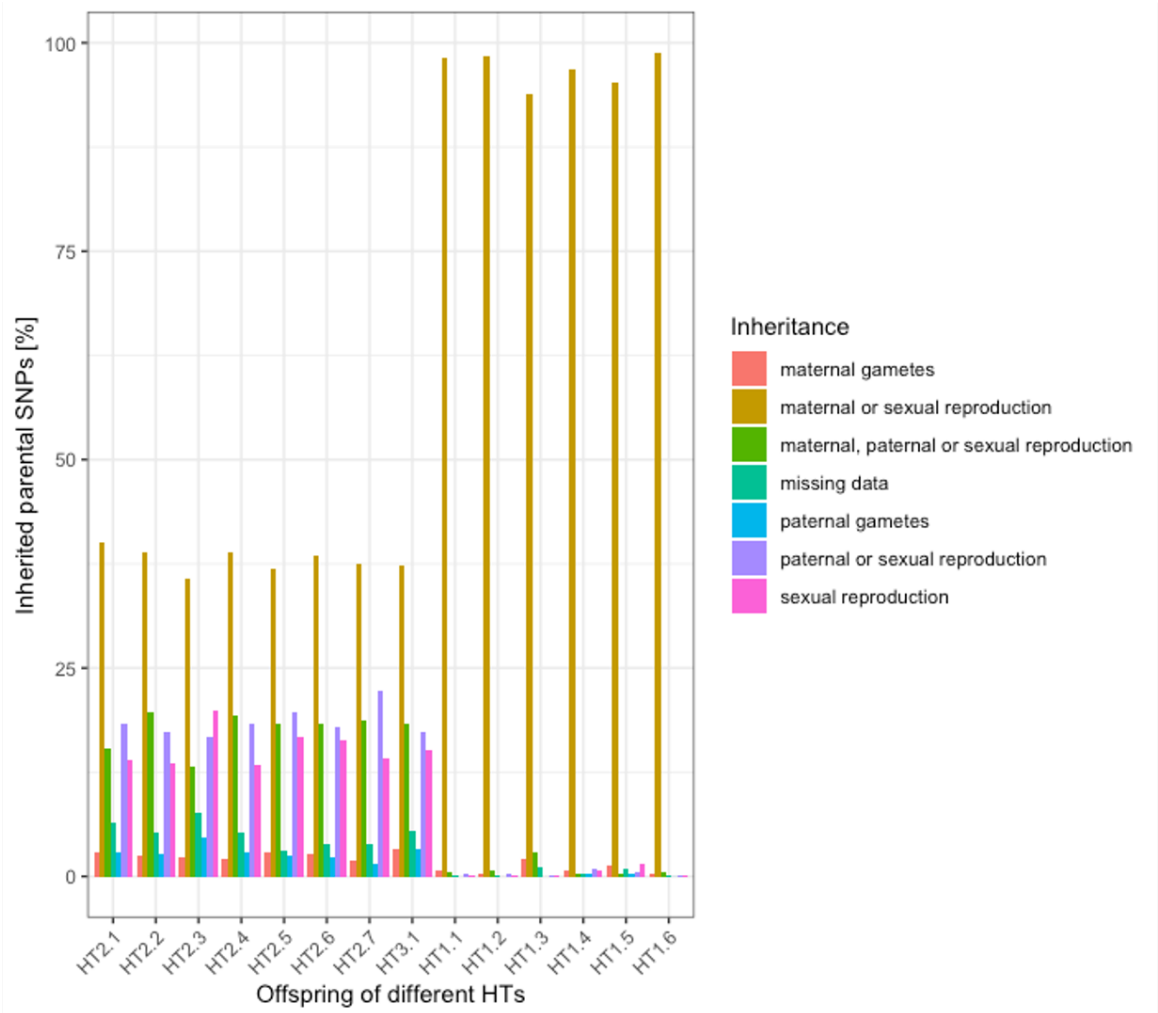


Figure 10 - Result of the inheritance analysis. The different colours depict the various types on inheritance determined by the Python3 script (C. Zeng). The script compares one specific SNP in a locus detected in a minimum of six out of seven individuals within a family and checks whether each offspring SNP was either inherited from the mother, the father, or a combination of both. Sometimes it is not possible for the script to determine the exact origin, depending on the composition of the SNP, so it then lists all possible inheritance modes, as can be seen in the legend on the right side. Shown in this figure are types of inheritance an offspring SNP was gained by. The Y axis shows the percentage of inherited parental SNPs in the offspring of all three HTs. Seven HT2 families were analysed (HT2.1 through HT2.7), one HT3 family (HT3.1), and six HT1 families (HT1.1 through HT1.6), depicted on the X axis. P-values via student's t-test – 'maternal gametes': 6.00939^{-9}, 'maternal or sexual': 3.46198^{-65}, 'maternal, paternal or sexual reproduction': 1.0749^{-39}, 'paternal or sexual reproduction': 4.05887^{-19}, 'sexual reproduction': 1.53001^{-32}.

6.6 Statistics

The difference between the inheritance modes of HT1 and HT2 is stark. Therefore, I performed additional statistical tests to further underline the results. I used two packages in R (version 2022.12.0+353; van den Boogaart, Tolosana-Delgado and Bren, 2008), COMPOSITIONS (version 2.0-4) for compositional data analysis and RSTANARM (version 2.19.2; Goodrich *et al.*, 2022) for Bayesian applied regression. I built a mixed model to rule-out the influence of families on the detected differences between the HTs in inheritance types. The quaddRAD data (Figure 10) is compositional, meaning that every value per sample sums up to 1, so it first had to be transformed via isometric log-ratio transformation, which is suitable for compositional predictor variables containing 0 values. The transformed variables (V1, V2, V3) were then used for the Bayesian mixed effects regression model. The results can be seen in Table 3. For better understanding, I combined 'maternal gametes' and 'maternal or sexual reproduction' into 'maternal gametes', 'paternal gametes' and 'paternal or sexual reproduction' into 'paternal gametes', and 'maternal, paternal or sexual reproduction' and 'missing data' into 'unknown'. This enabled me to perform the statistical analyses. The values of these inheritance modes then get transformed and the model generates three variables that describe whether the original data (and inheritance modes) are dependent on each other.

Table 3 - Statistical analysis using the packages COMPOSITIONS (V2.0-4) and RSTANARM (V2.19.2) in R (V2022.12.0+353). Significance values of the transformed variables (V1, V2, V3). The effective sample size has to be >5000 for the simulation to be robust.

Transformed variables	Effective sample size	Mean (log odd)	SD	2.5%	25%	50%	75%	97.5%
Intercept	9,689	-2.3	1.1	-4.6	-2.9	-2.2	-1.5	-0.4
V1	10,257	-4.0	1.5	-7.1	-5.0	-3.9	-3.0	-1.4
V2	10,301	-4.5	1.9	-8.4	-5.7	-4.4	-3.2	-1.2
V3	9,776	-1.5	1.3	-4.4	-2.3	-5.7	-0.5	0.9

The transformed variable V1 (Table 3) describes the comparison of the percentage of maternally inherited SNPs versus those inherited from the father amongst the two HTs. Maternal gametes were significantly different between the two HTs (estimate ± SD = -4.0 ± 1.5, 95% credible interval = -7.1 - -1.4, Table 3). Since the variables V1 and V2 (which are the transformed variables generated from the 'maternal' and 'paternal' data) were significant in the mixed effects model, it is likely that the variation in the two predictor variables were greater between the two HTs than the variation between the families. V2, which is the comparison of the percentage of paternally inherited SNPs versus those inherited through sexual reproduction amongst the two HTs, is also significant. Whereas V3, which is the

comparison of the percentage of sexually inherited SNPs versus the rest, is not significant. However, isometric log-ratio transformations of the three predictor variables (V1, V2 and V3) are not easy to interpret. As a much simpler test, yet with limitations concerning the test's assumptions, student's t-test can be used to compare the two HTs (Table 4). When compared between HT1 and HT2, the differences for all inheritance types (Figure 10) are highly significant. All of the tested inheritance modes show p-values of p<0.0001.

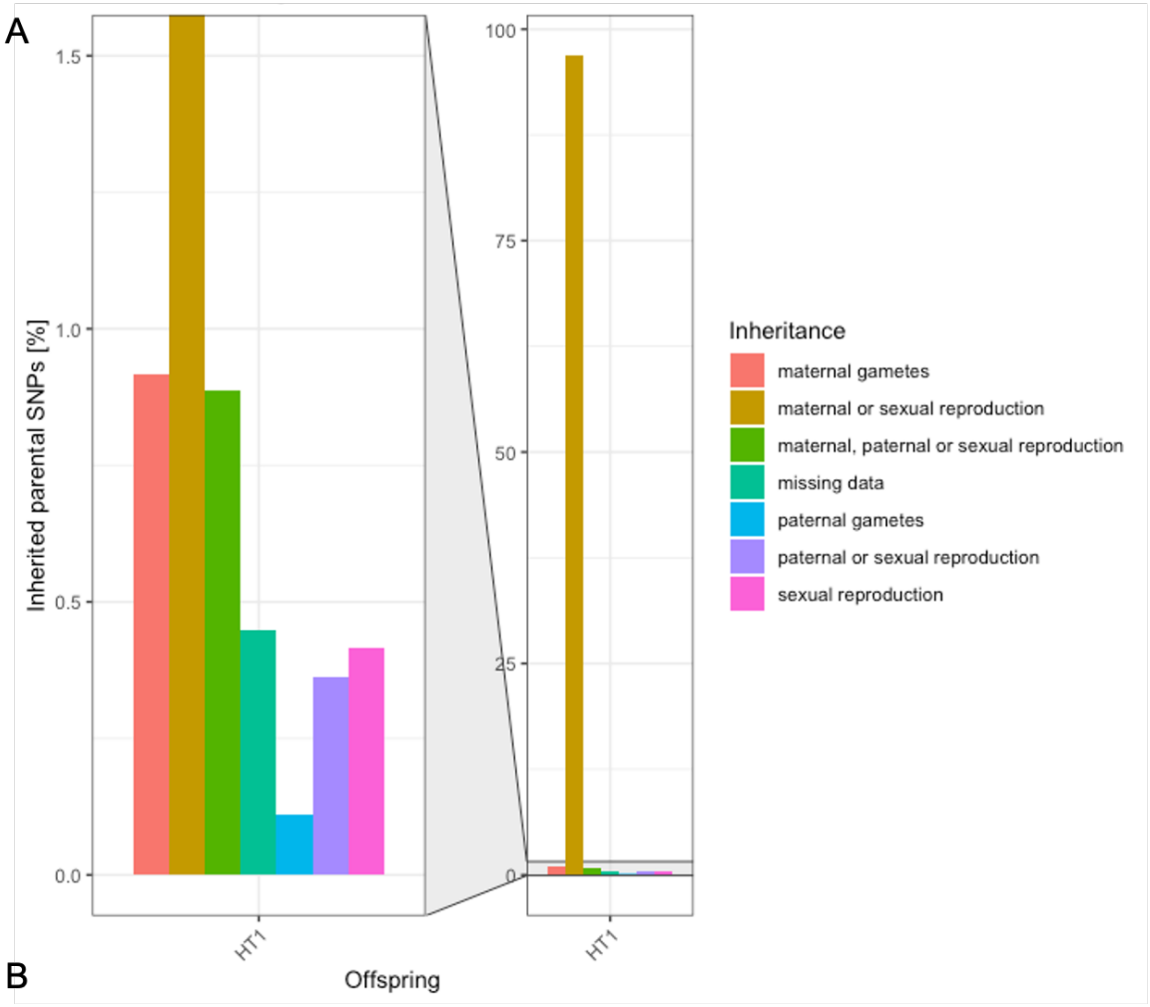
Table 4 - Student's t-test results. HTs 1 and 2 were compared based on the inheritance mode. All values are highly significant.

		maternal gametes	maternal or sexual reproduction	maternal, paternal or sexual reproduction	paternal or sexual reproduction	paternal gametes	sexual reproduction
HT1	Mean	0.92	96.86	0.89	0.36	0.11	0.41
	SD	0.95	3.09	1.80	0.34	0.21	0.53
HT2	Mean	2.45	37.85	17.39	18.83	2.70	15.47
	SD	0.96	3.18	3.06	2.85	1.07	2.96
	t-test	0.01E-09	3.46E-65	1.07E-39	4.06E-36	1.49E-19	1.53E-32
	p-values	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001

6.7 Paternal genes found in all-female HT1 offspring

HT1 females only produce female offspring, leading to the assumption that the fathers are merely sperm donors and their genetic material does not get transferred to the next generation. The quaddRAD analysis, however, shows that some paternal alleles were found in the all-female HT1 offspring (Figure 11, A) despite no male individuals in this HT. This analysis revealed negative values throughout the intervals (2.5% to 97.5%; Table 3) and is thus highly significant. This is further supported by the student's t-test I performed (Table 4).

I chose a ustacks parameter -m (minimum number of raw reads required to form a stack (putative allele)) of 10 to minimize the likelihood of missing reads representing a parental allele and to ensure on the other hand that the sequences used would cover the maximum of loci possible without risking the loss of data. Nevertheless, paternal genes have been detected in the offspring of HT1 with a total of 0.89% being passed on by either the father alone or through sexual reproduction (Figure 11, A). When looking at the corresponding SNPs (Figure 11, B), one particular locus ID is especially interesting. Figure 11 (B) shows a section of the SNPs inherited from the father to some degree. It shows the SNPs at loci 61 and 556 being inherited from either the father or through sexual reproduction and the SNPs at locus 2393, being inherited entirely from the father. Here, the SNP composition is clear enough for the script to determine its definite origin and is identical for all five offspring.



Locus ID	SNP location	Mother	Father	Offspring 1	Offspring 2	Offspring 3	Offspring 4	Offspring 5	SNP origin
61	55	TT	TG	TG	TG	TG	TG	TG	Sexual or paternal
61	200	TT	TC	TC	TC	TC	TC	TC	Sexual or paternal
61	243	TT	TG	TG	TG	TG	TG	TG	Sexual or paternal
556	176	AA	AG	AG	AG	AG	AG	AG	Sexual or paternal
556	187	GG	GA	GA	GA	GA	GA	GA	Sexual or paternal
556	249	CC	CA	CA	CA	CA	CA	CA	Sexual or paternal
2393	29	AA	CC	CC	CC	CC	CC	CC	Paternal only
2393	136	AA	TT	TT	TT	TT	TT	TT	Paternal only
2393	198	CC	TT	TT	TT	TT	TT	TT	Paternal only
2393	213	TT	AA	AA	AA	AA	AA	AA	Paternal only
2393	220	TT	CC	CC	CC	CC	CC	CC	Paternal only

Figure 11 - Mean inheritance types of all HT1 families in the analysis. (A) This figure focusses on the under 1.0% inheritances of SNPs detected in the all-female HT1 offspring. The Y axis shows the inherited parental SNPs in percent, which have been found in the HT1 progeny. (B) Loci found in HT1 offspring with corresponding SNPs. The highlighted locus shows exclusive paternal inheritance, while the non-highlighted one shows sexual or paternal inheritance.

Taken together, these results point towards a leakage of paternal genes in the all-female HT1. The combination of the majority of all SNPs found in the offspring having been inherited from the mother (Table 2, Figure 10) and under 1% of all progeny SNPs coming from the father leaves one to speculate that something is going on during the meiosis and/or the fertilisation of the egg that differs from the general process of sexual reproduction.

6.8 Preparation of ovaries and eggs

To address the assumption that there is a distinctive meiotic process in the eggs and to determine whether the production of the female gametes and/or the fertilisation of such are altered in HT1, I determined the chromosome counts of unfertilised eggs of this HT and compared them to those found in HT2. I extracted the ovaries from HT1 females and used eggs of various developmental stages to check the chromosome count.

In insects, the pair of ovaries is joined with a pair of lateral oviducts which connect into a genital chamber (Suzuki, 1988). Once the eggs leave the ovaries, they travel through the lateral oviduct until they reach the genital chamber, to which the spermathecae are connected (Figure 6). As the egg passes the spermathecal duct, it gets fertilised before oviposition. To prevent the sperm stored in the spermatheca to fertilise the egg, the undeveloped eggs were directly taken out of the ovaries.

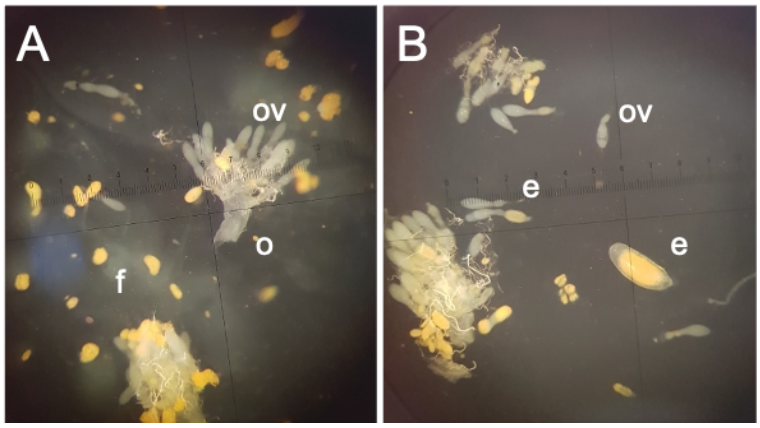


Figure 12 - Preparation of ovaries and eggs. The beetles were sedated by placing them in a 1.5mL tube and putting it on ice for 5 to 10 min. Afterwards, the head was cut off using a pair of small scissors, the beetle was placed into the preparation dish under a binocular with the magnification of 3.2x, and the elytra and wings were removed before 1x PBS buffer was added to ensure the osmotic stability of the cells, and the beetle was opened on the dorsal side. (A) Picture of an extracted ovary (o), consisting of eight ovarioles (ov) and surrounded by fat cell accumulations (f). (B) Picture of eggs (e) of various sizes, still encased in ovary tissue, as well as empty ovarioles (ov) that were severed from the ovary.

However, the preparation and further treatment of these eggs caused several problems. The separation of the eggs from the ovarian tissue (Figure 12) proved to be a difficult undertaking and often resulted in either the egg bursting or the tissue being unremovable. After a successful separation of the eggs from the surrounding tissue, the undeveloped eggs, which still lacked the strong protection of the chorion that hardens after oviposition (Simpson and Douglas, 2013, pp. 328-331), were nearly impossible to transfer to the fixation liquid. If that step was successful, the procedure failed during the fixation step, as the size of the eggs was too small and would cause them to get lost. Due to these difficulties, I decided to use eggs of

unfertilised females. This way, I could ensure the absence of sperm and use eggs that have been laid. After oviposition, the chorion is sturdy, the follicle cells surrounding the egg dry out, and – if no fertilisation has happened – the meiosis should still be arrested in metaphase (Masui and Markert, 1971; Simpson and Douglas, 2013, p. 322; Gruss, 2018), which makes the following counting of the chromosomes possible because of their condensation stage. Meiotic divisions are not completed in the ovary and only resume once fertilisation by a sperm cell occurred. Because of this phenomenon, no colchicine treatment was necessary that is otherwise used to arrest the chromosomes in metaphase by hindering the microtubules from forming.

6.9 Removal of follicle cells that surround the egg

During oocyte development and final egg production, follicle cells play a major role. They surround the egg and are responsible for the production of the vitelline envelope proteins, the ultimate determination of the anterior and posterior ends of the embryo as they produce the ligands that control the axes, as well as the production of the chorion, the egg wall (Simpson and Douglas, 2013, pp. 328-331). The latter hardens after oviposition and forms a sturdy barrier between the egg's contents and the surrounding environment, and the follicle cells dry out.

However, dried-out and dying cells also have nuclei that can be labelled with antibodies and stained with DAPI, so two procedures were used to get rid of said outer-most layer of cells. The first was hypotonic treatment. The eggs were taken out and carefully separated from as much ovarian tissue as possible before being transferred to 0.044M potassium chloride (KCl) solution (Sasai *et al.*, 1996) for 30 min. This would cause the ion content within the egg to be higher than that of the surrounding medium, leading to influx of water in the egg and increasing the interior pressure. In theory, by poking the egg using a very fine glass needle, the egg would burst at the point of contact and the contents would flow out, including the nucleus, leaving behind the follicle cells. Undeveloped eggs, however, were too delicate for this procedure. They had to be held in place using forceps to enable a controlled damaging of the developing egg envelope. The handling damaged them before a targeted wounding could take place, thus eliminating the effects of the hypotonic treatment. Since this proved unsuccessful, eggs after oviposition were used. This was not as successful as hoped either as

the chorion was too strong and withstood the osmotic pressure, so that even after poking into the chorion, the egg rarely burst open.

I then conducted a procedure to remove the follicle cell layer using potassium hydroxide (KOH), which is a common way to remove any unwanted cell layers in histology. For this, the eggs after oviposition were incubated in 10%, 5% and 2% KOH. After 30 s of incubation with 10% and 5% KOH, the eggs were so delicate that handling them led to the chorion to burst and the egg contents to flow out into the KOH solution.

10% and 5% KOH seemed to be too highly concentrated, so I conducted a time series under a light microscope using 2% KOH to determine the optimal incubation time that would cause the digestion of the follicle cells but keep the chorion still intact and strong enough for the following handling of the sample, which is depicted in Figure 13. What could be noticed was that the egg swelled and slightly changed its shape. The egg used was older than 3 d, meaning that it showed signs of mild dehydration under the microscope after KOH was added, as can be seen in Figure 13 (B-G). These signs disappeared after 2 min of KOH incubation. Also, whereas at the beginning of the time series (Figure 13, A) the light did not penetrate the egg, as soon as KOH was added that changed. Usually in microscopy, the addition of a liquid to the specimen helps to show a clearer picture (own observation), so that some light was able to shine through the egg's most outer layers right after KOH was added (Figure 13, B) was to be expected. After 11 min, the egg was so fragile that the addition of a cover slip immediately caused the chorion to burst. Because of this, I did not continue with the procedure of removing the follicle cells from the egg. KOH was too damaging to the protective structure to ensure that no exterior material would be able to enter the egg and thus potentially alter any further procedures.

Due to the arrest in metaphase II, the chromosomes of the oocyte's pronucleus are highly condensed, while the chromosomes of the follicle cells should be in a relaxed stage as transcription and translation is taking place in their nuclei. This made the differentiation between follicle nuclei and egg nucleus possible, even without removing the follicle cells prior to the staining of the chromosomes.

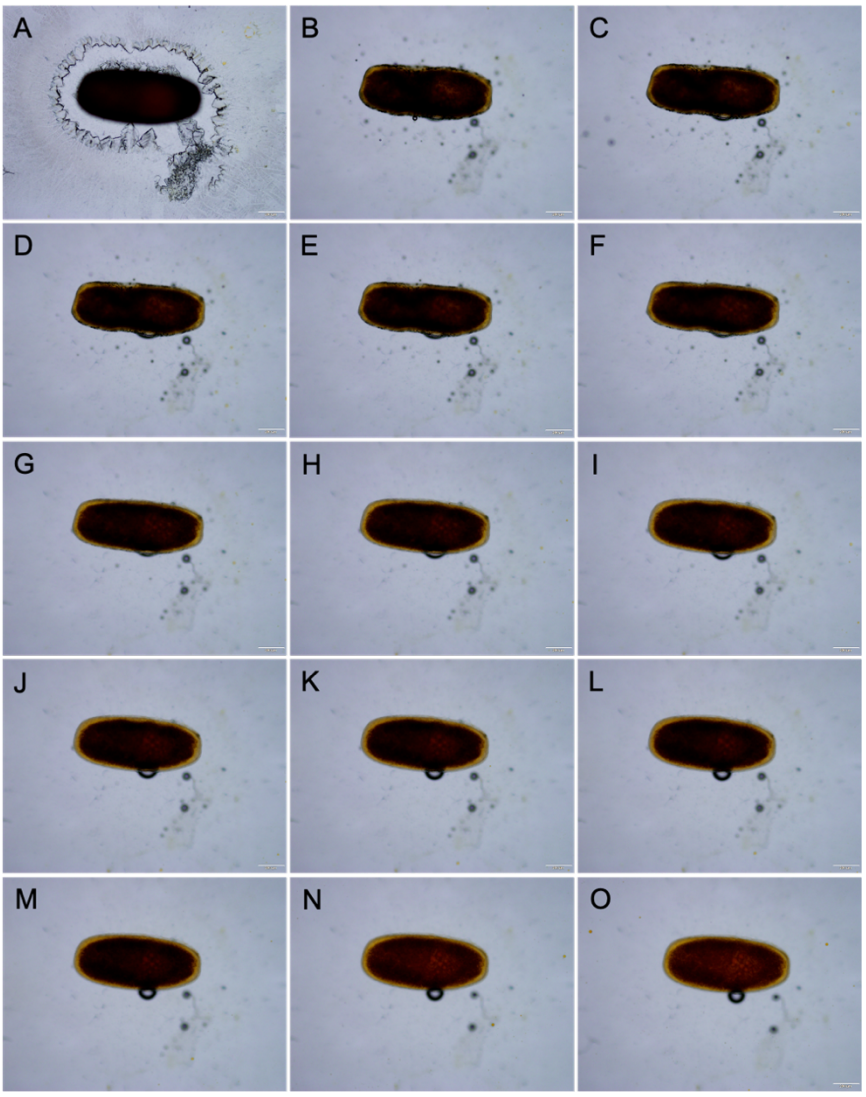


Figure 13 - Time line of 2% KOH incubation. (A) Egg under light microscope without KOH added. The crystalline structures surrounding the egg are most likely due to the 1x PBS buffer they were stored in to prevent major dehydration. (B) Egg immediately after KOH was added, t = 0 s incubation time. (C) t = 15 s. (D) t = 30 s. (E) t = 45 s. (F) t = 60 s. (G) t = 90 s. (H) t = 120 s. (I) t = 2 min 30 s. (J) t = 3 min. (K) t = 3 min 30 s. (L) t = 4 min. (M) t = 4 min 30 s. (N) t = 5 min. (O) t = 11 min. After 11 min, the procedure was stopped as no further changes could be detected. The size bar indicates 200 µm.

6.10 Testing the anti-CID antibody using *Sf*9 cells

The chromosomes of *A. lythri* are very small (Segarra and Petitpierre, 1982; Jäckel, 2011). They are visible under 400x magnification, but structures like the chromatids can only be seen under 1000x magnification. To help with the counting, and thus finding, of the metaphase chromosomes of the unfertilised eggs, the use of an anti-CID antibody was tested. The centromere identifier (CID) protein (Henikoff *et al.*, 2000; Blower and Karpen, 2001; Khetani and Bickel, 2007) is a protein located at the centromere of metaphase chromosomes. By working with a specific antibody, it should be possible to label this structure and therefore

help with the counting of the chromosomes, as each chromosome would show one fluorescent signal per centromere.

The epitope of the anti-CID antibody is not known. The antibody has been developed for *D. melanogaster*, and due to a fragmented and unannotated genome I could not compare the *D. melanogaster* CID sequence to that of *A. lythri*. Because of that, I decided to check the specificity of this primary antibody using *Sf*9 cells. These insect cells can be cultivated in cell culture, transferred to cover slips in a defined cell count, and then fixed, labelled, stained, and checked under a fluorescence microscope. For this, three different parameters were tested. For all of the antibody dilutions 1:10 diluted blocking buffer was used. The first dilution tested was 1:500 for the primary antibody with a 1 h incubation at room temperature (RT). Abcam recommends the 1:100 dilution of this antibody, so the first dilution was followed by a 1:100 dilution for 2 h at RT as well as a 1:100 dilution with an overnight incubation at 4 °C. The results of these tests can be seen in Figure 14.

Pictures A to C (Figure 14, A-C) show the negative controls for the 1:500 primary antibody solution after a 2 h-incubation at RT. No antibody signal could be detected using the FITC channel. Pictures G to H (Figure 14, G-H) show the negative control for the 1:100 primary antibody solution after a 1 h-incubation where no antibody signal could be detected. Pictures M to O (Figure 14, M-O) show the last negative control for the 1:100 primary antibody solution after an incubation at 4 °C overnight. Here, no fluorescence signal could be detected, meaning that all tested dilutions and incubation times do not cause unspecific signals of the secondary antibody.

Figure 14 (D-F) shows the cells after a 2 h-incubation at RT with the primary antibody, Figure 14 (J-L) shows a 1 h-incubation at RT with a 1:100 antibody dilution, and Figure 14 (P-R) shows the result after incubating overnight at 4 °C with a 1:100 primary antibody dilution. A significant difference between the signal strength of the three tested incubation methods could not be detected.

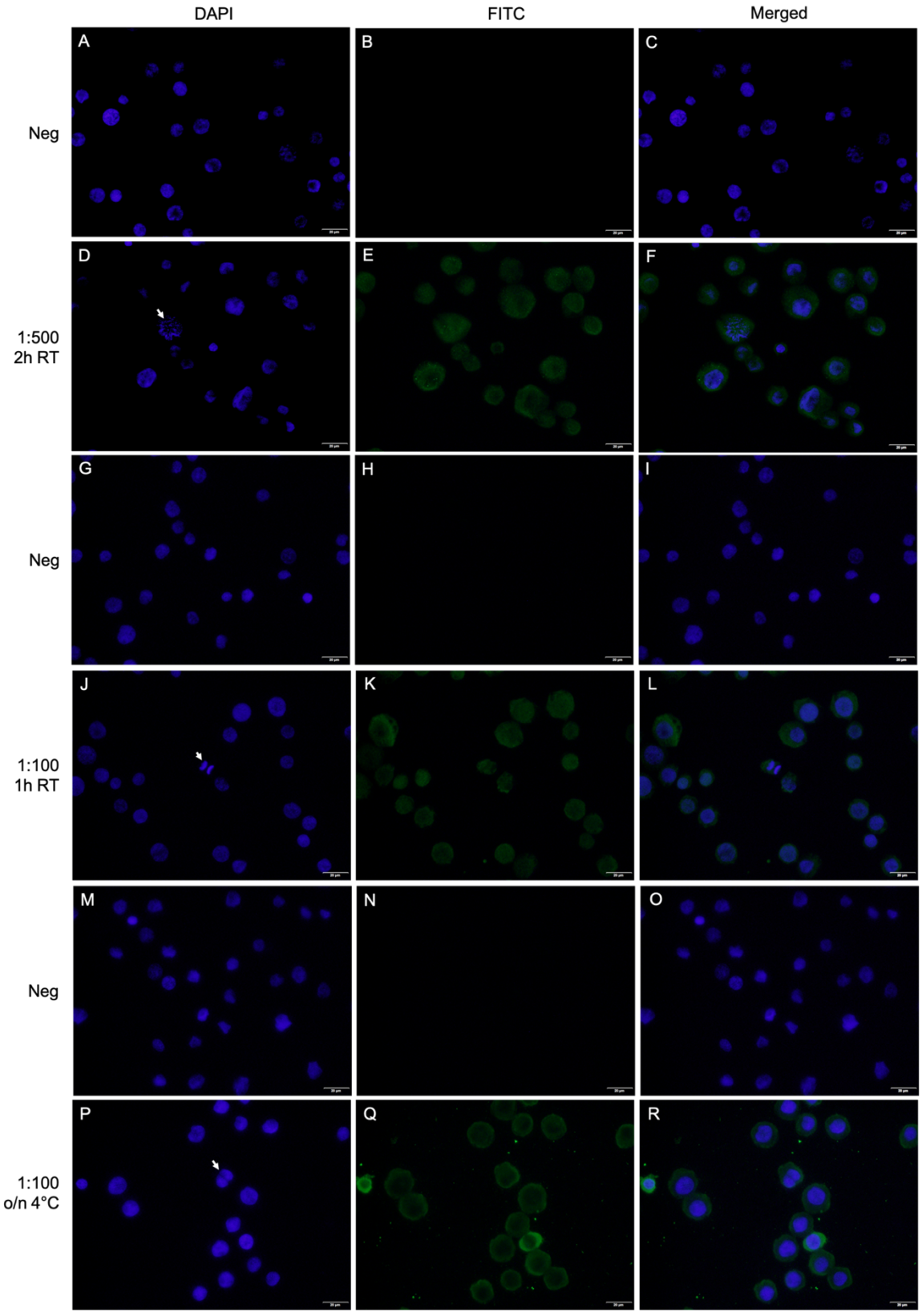

DAPI
FITC
Merged
A
B
C
Neg
D
E
F
1:500
2h RT
G
H
I
Neg
J
K
L
1:100
1h RT
M
N
O
Neg
P
Q
R
1:100
o/n 4°C

Figure 14 - Antibody labelling of *Sf*9 cells. The incubation times and conditions of the first antibody (anti-CID) are written on the left-hand side, the fluorescence filters at the top, merged indicates merged images of DAPI (depicted in blue) and FITC (depicted in green) signals. (A-C) Negative control, no primary antibody for the 2 h at room temperature (RT) incubation. (D-F) Primary antibody incubation for 2 h at RT in a dilution of 1:500 in 1:10 diluted blocking buffer. (G-I) Negative control, no primary antibody for the 1 h at RT incubation. (J-L) Primary antibody incubation for 1 h at RT in a dilution of 1:100 in 1:10 diluted blocking buffer. (M-O) Negative control, no primary antibody for the overnight at 4°C incubation. (P-R) Primary antibody incubation for overnight at 4°C in a dilution of 1:100 in 1:10 diluted blocking buffer. The arrows highlight different mitosis stages: late prophase (D), anaphase (J), telophase (P). Exposure time for the DAPI channel was 20 ms and for the FITC channel 160 ms. The size bar indicates 20 µm.

However, what could be seen was the signal distribution in the cells. I expected the signals to be specific as the primary antibody has been developed and tested to bind at CID in *D. melanogaster*. As can be seen in Figure 14, the green fluorescence signal (FITC) shows the entirety of the cell. The chromosomes are stained blue and varying stages of cell division can be seen. Figure 14 (D) shows a nucleus in late prophase with chromosomes that have started to condense (white arrow). Figure 14 (J) includes a nucleus in anaphase of mitosis, the stage in which homologous chromosomes separate (white arrow). Figure 14 (P) depicts a nucleus in telophase, where the homologous chromosomes are separated and the new nuclear envelope starts to form (white arrow).

The cells were treated with PBS with Triton, which permeabilises cell membranes. I thus expect the antibody to be able to penetrate the cell and the nucleus and bind at the target protein CID. Metaphase cells, like Figure 14 (D) might show (white arrow), do not have a nuclear envelope anymore as it dissolves at the end of prophase, or more specifically during the prophase stage diakinesis, so the antibody would have had no problem binding at the target protein.

The merged images (Figure 14, last column) show a clear distinction between the two fluorescence signals. The DAPI signal, which labels the chromosomes and the antibody signal which should label the CID proteins at the centromeres are well separated from each other. They do not overlap. Furthermore, the antibody signal is not concentrated as it would be if it had bound to CID. In conclusion, it can be said that the CID antibody binds nonspecifically in unknown structures of the membrane. The downstream analysis was thus performed without the antibody, and the chromosome spreads were stained using only DAPI.

6.11 Determining the chromosome count of unfertilised oocytes

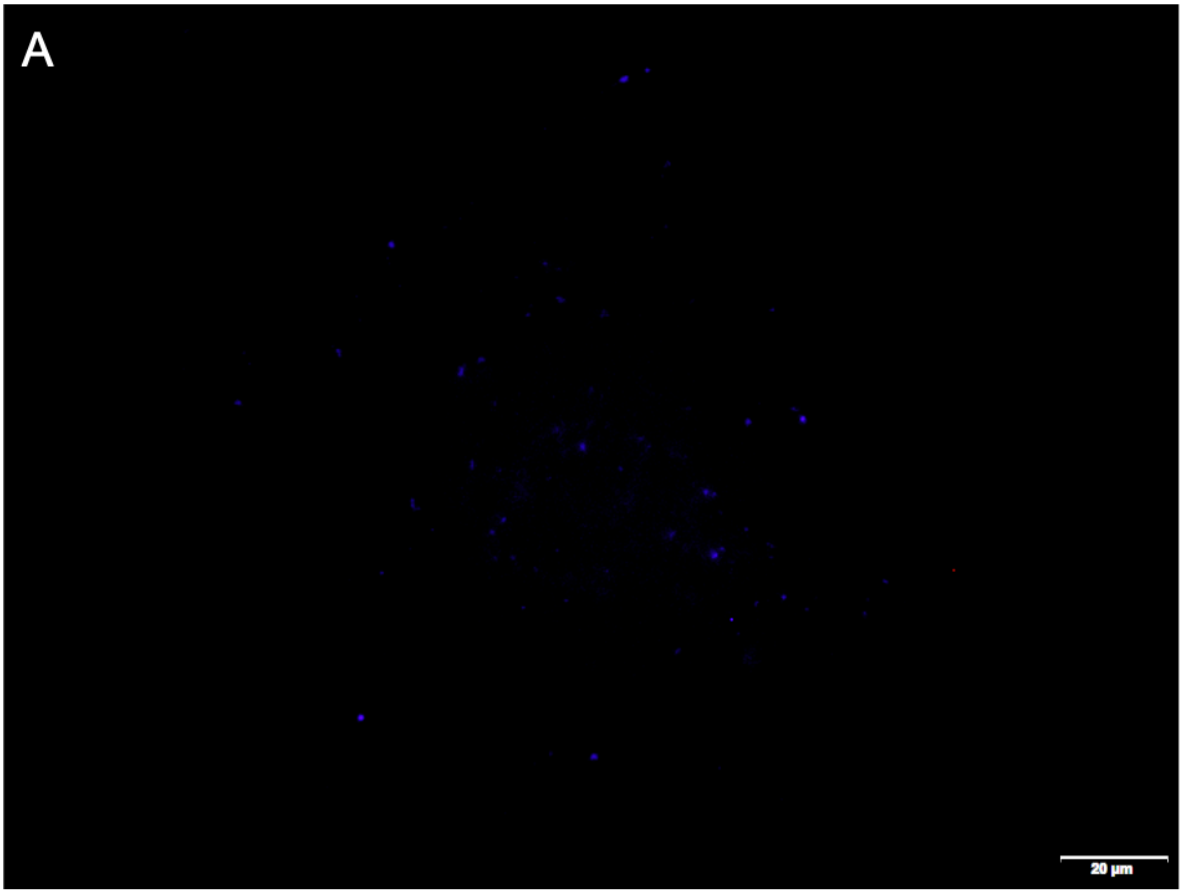
The non-mendelian reproduction indicated by the quaddRAD data (Figure 10 and Figure 11) indicates that the meiosis of HT1 is altered to some degree. For this, prepared chromosome spreads from unfertilised eggs from HTs 1 and 2, which were then stained using DAPI, were analysed to determine the metaphase II chromosome count of each HT.

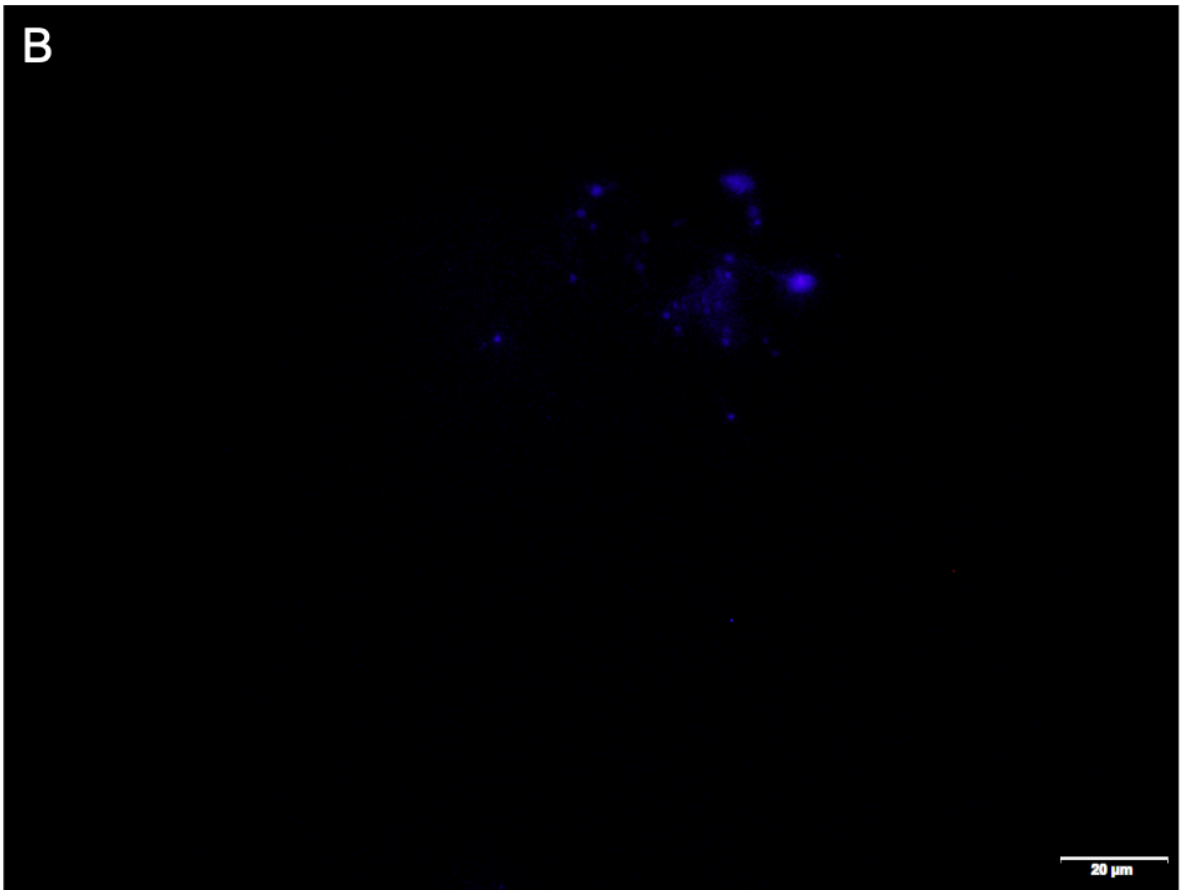
The results can be seen in Figure 15. In total, I counted eight nucleus samples from unfertilised eggs for each HT. If the eggs were arrested in metaphase II, a haploid chromosome set would be expected indicating that normal sexual reproduction takes place. In this case, meiosis should not lead to a differing chromosome count. This was expected to be the case for HT2.

The quaddRAD data showed an even distribution of inheritance types of the SNPs found in the offspring (Figure 10), which points to no alterations of meiosis in this HT. HT1, on the other hand, showed a divergence from the expected sexual reproduction with the majority of the SNPs found in the offspring originating from the mother (Figure 10 and Figure 11). Here, it is likely that meiosis has been altered to cause this phenotype.

The chromosomes found in HT2 can be seen in Figure 15 (B and D). For this HT, I counted 12 chromosomes. In two cases, I counted 11 or 13 chromosomes. This discrepancy can be caused by poor picture quality or looking at a less optimal focal plane. Because fragments of the chorion were still present on the microscopy slide, the sample was thicker than the chromosomes and finding the right focal plane was an important step in documenting these data.

With a diploid chromosome set of 2n = 24 (Segarra and Petitpierre, 1982; Jäckel, 2011), *A. lythri* follows the estimated primitive karyotype of the Alticinae (11^{II} + XY) (Virkki, 1970; Phillips, 1979; Segarra and Petitpierre, 1982). Thus, a chromosome count of 12 in an unfertilised egg equals a haploid chromosome set, which was expected based on the offspring sex ratio as well as the quaddRAD data.


A
20 µm


B
20 µm

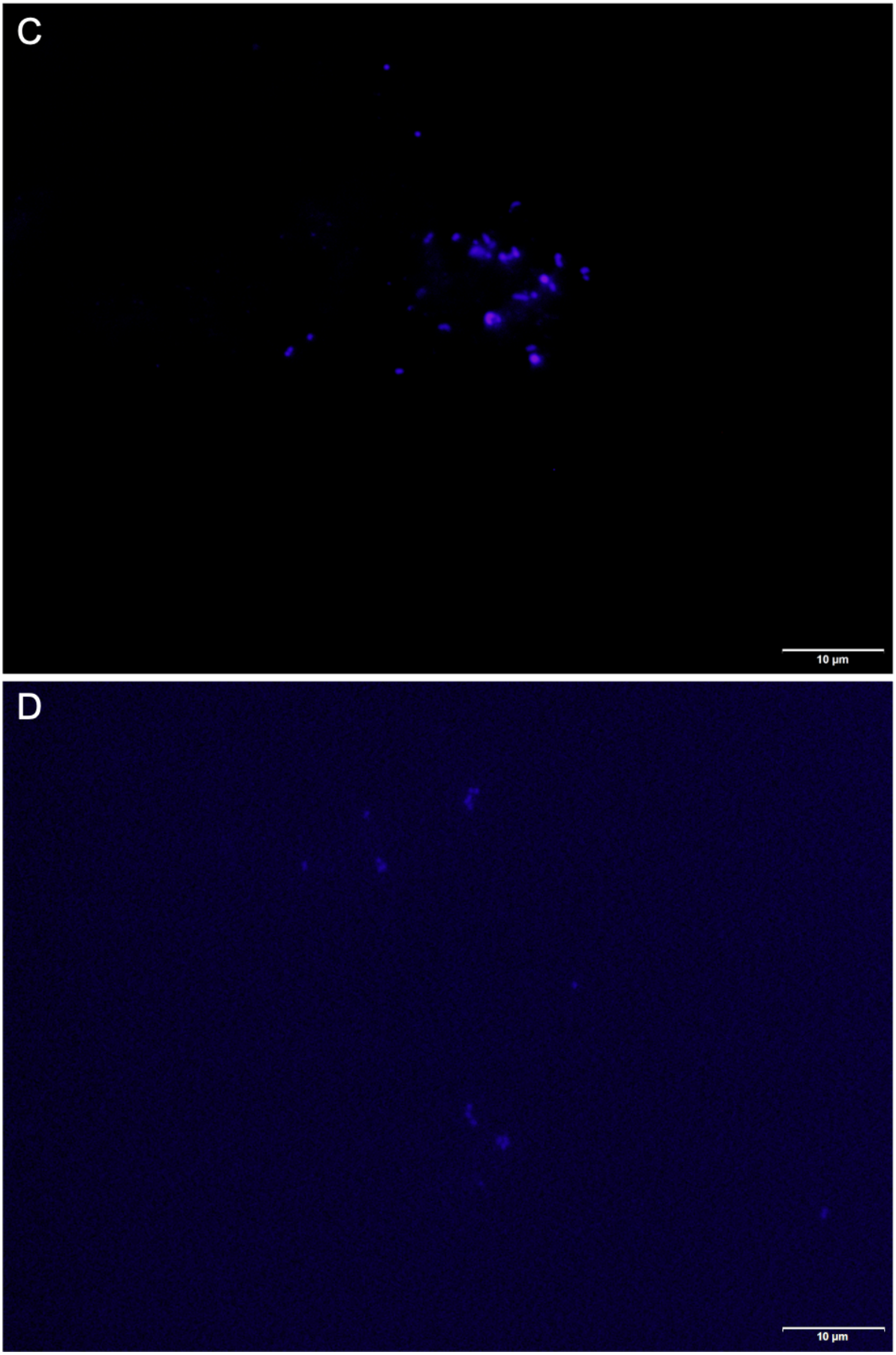


Figure 15 - Chromosomes of unfertilised eggs, stained with DAPI. (A) HT1 nucleus. (B) HT2 nucleus. The size bar indicates 20 µm. (C) Part of a HT1 nucleus. (D) HT2 nucleus. The size bar indicates 10 µm. n = 8 for each HT.

Figure 15 (A and C) shows chromosomes found in an unfertilised HT1 egg. Here, Figure 15 (C) only depicts part of the chromosome set as they were spread apart rather far. For this HT, I counted 24 chromosomes, apart from one case where I counted 23 chromosomes. This discrepancy could also be due to the focal plane because of the thickness of the samples. Considering the diploid chromosome set of 24, unfertilised HT1 eggs do not show a reduced chromosome set but an unreduced set of 24. This further supports the quaddRAD data pointing to altered meiosis in this HT. Comparing the images Figure 15 (C) and Figure 15 (D), which show the chromosomes in a high magnification (size bar indicates 10 µm), no difference in size or thickness can be detected in their composition between the HTs.

7 Discussion

The observation that inspired this thesis was the extreme female-biased sex ratio displayed by *A. lythri* (Mohr, 1966; Kangas and Rutanen, 1993; Jäckel, Mora and Dobler, 2013). This sex ratio distortion is not exclusive to *A. lythri*, as it occurs in various forms in other species of the genus (Phillips, 1979; Jäckel, Mora and Dobler, 2013). The characteristic that distinguishes *A. lythri* from the other species, however, is the differentiation of three main mitochondrial (mtDNA) haplotypes (HTs) within populations. These HTs show noticeable differences in their sex ratios. While HT2 and HT3 females produce both female and male offspring in approximately equal quantities, HT1 exclusively produces female progeny (Jäckel, Mora and Dobler, 2013).

This phenomenon can be explained several ways. Reproduction-manipulating bacteria can be found in many insect taxa. Whereas *Rickettsia*, *Cardinium*, and *Spiroplasma* have been detected to various degrees of infection in *A. lythri* (Jäckel *et al.*, unpublished data), two out of eight supergroups of *Wolbachia* have been found infecting the mtDNA HTs of *A. lythri*. These supergroups show a strong correlation between mtDNA HTs and their strains, each HT having its specific *Wolbachia* stain – *w*LytA1 can be found in HT1, *w*LytA2 in HT2, and *w*LytB in HT3. This may be part of the explanation for the strong sex ratio distortion that prevails in HT1 (Jäckel, Mora and Dobler, 2013). Another explanation is parthenogenesis. Females that reproduce parthenogenetically harbour only the genetical material that comes from the mother, exclude either the maternal or paternal chromosome set after insemination, or can switch from parthenogenetic to sexual reproduction (see chapter 4.6). The phenomenon of

parthenogenesis can take many forms and might be seen as part of the explanation as to why only females exist in HT1.

In the following subchapters, I will address the strong female bias in HT1. I will discuss what is happening on a genomic level with the help of quaddRAD data, present results from chromosome counts that I generated using histological methods, and speculate about other possible explanations that might help shed light on what is happening at the cellular and genomic level in the exclusively female HT1.

7.1 Hybridisation as cause for the diversity in HTs

Hybridisation can influence both evolution and speciation (Abbott *et al.*, 2013). Reproduction between individuals of genetically defined populations (Barton and Hewitt, 1985) can produce progeny that might have an advantage over their parent species (Abbott *et al.*, 2013). The effects of hybridisation on speciation are vast (Abbott *et al.*, 2013). The generation of new hybrid taxa, the differentiation through gene flow and increased speciation through introgression are only a few that can be named (Abbott *et al.*, 2013). Hybridisation and introgression of genes or mtDNA have been reported throughout the entire tree of life. Approximately 25% of all plant species and 10% of all animal species are estimated to show signs of introgression (Mallet, 2005), covering a wide range of taxa, both within insects like Lepidoptera (Schmidt and Sperling, 2008), Hymenoptera (Linnen and Farrell, 2007), Coleoptera (Jäckel, Mora and Dobler, 2013), Orthoptera (Kawakami *et al.*, 2007) and others, like fishes (Mims *et al.*, 2010) and mammals (Berthier, Excoffier and Ruedi, 2006; Good *et al.*, 2008).

The hybrid swarm theory (Seehausen, 2004) states that hybridisation can be given as an explanation for non-overlapping mitochondrial and morphology-based pedigrees. This is on a par with the hypothesis that hybridisation plays an important role in mitonuclear co-adaptation (Hill, 2016). Thus, hybridisation affects not only speciation, but sexual dimorphism, sex ratio distortion, sexual conflict, as well as local adaptation of a given species (Runemark *et al.*, 2018). An example for mitochondrial introgression can be found in the snail *Brotia pagodula* (Köhler and Deein, 2010), which shows *p*-distances in the mitochondrial marker genes COI and 16S of 10 - 13% (Köhler and Glaubrecht, 2006). For this species, interspecific hybridisation is given as an explanation for the high differences between mitochondrial and nuclear genomes (Köhler and Deein, 2010). *A. lythri* also shows such

incongruence between the mtDNA and the nuclear genomes. While monophyletic on a nuclear level (Jäckel, Mora and Dobler, 2013), the flea beetles show a non-monophyletic mtDNA gene tree based on the mtDNA marker COI (Jäckel, Mora and Dobler, 2013). The three HTs are highly divergent with *p*-distances of 2.1 - 4.6%, higher than when compared to other species groups, which showed lower genetic divergence within a species (Jäckel, Mora and Dobler, 2013). Furthermore, the three mtDNA HTs of *A. lythri* clustered in a way that separated them from each other by many other *Altica* species (Jäckel, Mora and Dobler, 2013), further highlighting the non-monophyletic origin of the mtDNA genomes. Two out of these three mtDNA HTs thus most likely originated from other closely related species, having introgressed through hybridisation (Jäckel, Mora and Dobler, 2013). One of the possible outcomes of mitonuclear co-adaptation following hybridisation is sex ratio distortion (Runemark *et al.*, 2018).

7.2 Paternity analysis in *A. lythri*

To answer the question of why HT1 of *A. lythri* produces exclusively female offspring, I conducted paternity analyses using quaddRAD. This method allowed me to call SNPs genome-wide, which made it possible to use full genomes of the mother, father, and five offspring, and compare those with each other on a SNP level. I could thus determine which SNP has been inherited from the mother, from the father, or through sexual reproduction.

The distorted sex ratio in HT1 already hinted at a distortion of SNP inheritance that is visible in the quaddRAD data. HT2 and HT3 functioned as control groups in this analysis, as those two HTs show a relatively equal sex ratio in their offspring. Thus, I hypothesised that these two HTs reproduce sexually, as the sex ratio follows Fisher's model of sex ratio equality with a 1:1 ratio in sexual species (Fisher, 1930; Hamilton, 1967), which was further supported by the quaddRAD data. HT2 and HT3 showed SNP inheritance that was relatively equal over all assigned inheritance types, which led to the conclusion that these two HTs acquired their SNPs to equal parts from the mother and the father. HT1, on the other hand, showed a strongly distorted SNP inheritance compared to HT2 and HT3. In HT1, the majority of the SNPs have been inherited only from the mother, which is on a par with the observation of exclusively female offspring in this HT. This depiction of data, taken together with the fact that HT1 needs to copulate in order to produce exclusively female offspring, points towards gynogenesis. This is a special form of parthenogenesis, in which gynogenetic female require

the trigger of insemination to trigger the development of the egg (Schlupp, 2005). According to Grebelnyi (2009), the sperm fuses with the gynogenetic egg, only to be immediately excluded from the oocyte. It has been discussed in the literature that this exclusion mechanism might be faulty and cause the leakage of paternal genetic material into the gynogenetic offspring (Schartl, Nanda, *et al.*, 1995; Lamatsch *et al.*, 2000; Lamatsch and Stöck, 2009; Lamatsch *et al.*, 2011; Suzuki *et al.*, 2020). This supports the findings of my quaddRAD data, which show that a very small percentage of paternal genetic material has indeed been found in the exclusively female HT1 offspring. Why this might happen will be discussed in the following subchapters.

7.3 Gynogenesis as a possible cause of the sex ratio distortion in *A. lythri*

A pronounced sex ratio distortion as seen in *A. lythri* can have various explanations. Male-killing, feminization of genetic males, and parthenogenesis are some of them. Male-killing can be taken out of the equation as this type of sex ratio distortion implies the death of half of the progeny. Both HT1 and HT2, however, have the same fertility as well as hatching rates (Jäckel, 2011). Furthermore, with the help of RT-PCR, Rohlfing *et al.* (2023) developed a method to determine the phenotypic sex of indistinguishable eggs by amplifying the *dsx* variants present. They were able to show that male offspring could not be detected in eggs that are three-hours old (Rohlfing *et al.*, 2023), which means that the males do not arise before the eggs are laid. That, in turn, leads to the conclusion that males do not exist in HT1. Feminisation is also unlikely for HT1, however, it is not possible to fully exclude this pathway to sex ratio distortion yet.

Because unfertilised eggs do not hatch, HT1 still requires the trigger of insemination, even though the majority of the maternal genome is transmitted to the next generation. This points towards a specific form of parthenogenetic reproduction, gynogenesis, which is characterised by females that need to mate with males of a closely related species to produce all-female offspring (Hubbs and Hubbs, 1932; Beukeboom and Vrijenhoek, 1998). This form of reproduction is often assumed to be caused by hybridisation (Leung and Angers, 2018). It can also be found in natural populations of *Poecilia* and *Poeciliopsis* (Poeciliidae) (Hubbs and Hubbs, 1932; Miller and Schultz, 1959; Schultz, 1961, 1966, 1969; Nanda *et al.*, 1995; Schartl, Nanda, *et al.*, 1995), as well as in some insects like the triploid beetle *Ptinus mobilis* (Ptinidae)

that is dependent on the sperm of the diploid species *P. clavipes* (Moore, Woodroffe and Sanderson, 1956; Woodroffe, 1958; Sanderson, 1960) to reproduce.

To determine the reproduction type of each HT, I conducted a quaddRAD analysis (see chapter 6.4). The Python3 script I used assigned suitable inheritance modes for each SNP detected in all members of the family. The inheritance mode 'maternal gametes' describes the inheritance of a given SNP only by the mother. In this case, all offspring would show the same SNPs as those found in the mother. The mode 'paternal gametes' describes the same phenomenon but with the SNP originating from the father. The mode 'maternal or sexual reproduction' is one of three inheritance modes that describe the inheritance of a SNP which could have multiple origins. Here, the SNP composition did not allow the script to securely determine the inheritance mode as the SNPs of the parents were too similar. Same goes for the modes 'maternal, paternal or sexual reproduction' and 'paternal or sexual reproduction'. The inheritance type 'sexual reproduction' describes the case in which a SNP has been inherited through sexual reproduction. Here, one allele would clearly originate from the mother and the other from the father. 'Missing data' describes all of the data that got excluded from any of the other inheritance types due to the way I set the parameters. For example, whenever a certain SNP could not be detected in two offspring, this SNP would be included in 'missing data'. The way I analysed the data, however, compared the SNPs found in both mother and father with those found in the offspring. Because females do not harbour Y chromosomes, the quaddRAD analysis cannot cover the male sex chromosome, as SNPs found on it would have been excluded under 'missing data' because no such SNPs would have been detected in the mother or in genotypic female offspring. Therefore, further analysis has to go into this topic to fully determine whether feminisation of genetic males is possible in *A. lythri*.

With the help of the quaddRAD data, I could show that HT2 and HT3 reproduce sexually. These two HTs show an equal distribution of the inheritance types of the SNPs found in their offspring with none of the inheritance modes being predominant. This is what would be expected for sexual reproduction, as their progeny inherited SNPs from both parents in relatively equal ratios. HT1, on the other hand, depicted a predominantly maternal inheritance of the SNPs detected in the offspring with a maternal inheritance of over 97%, which leads to the conclusion that this HT in particular reproduces gynogenetically. Despite

the small leakage of paternal alleles, it can be said that the majority of the paternal genome does not appear in the next generation.

The pronounced sex ratio distortion with a strong female bias in HT1 might thus be caused by a form of parthenogenesis. True parthenogenesis is characterised by the development of unfertilised eggs into fully functional individuals, which can, for example, be observed in bdelloid rotifers (Rotifera) (reviewed in Bengtsson, 2009; Vershinina and Kuznetsova, 2016). Jäckel (2011) reported that unfertilised eggs from HT1 females do not hatch, so they need the trigger of insemination, if not the entire male pronucleus, for the larvae to develop. The phenomenon that only female offspring is produced in this HT leads to the assumption that the male set of chromosomes is excluded. For sexual reproduction, as used by HT2 and HT3 of *A. lythri*, two haploid chromosome sets fuse with each other to form a new diploid nucleus. However, HT1 does not use the male haploid pronucleus in the further development of the larvae. This can be seen in the quaddRAD data, which shows that over 97% of the SNPs found in the offspring originate exclusively from the mother. This result, taken together with the observation of exclusively female offspring in HT1, leads to the assumption that the female pronucleus has to be diploid in this HT. I could verify this hypothesis in the histological analyses (see chapter 6.11).

The combination of chromosomes that takes place in Mendelian or sexual reproduction is important to counteract disadvantageous mutations and supports the new generation's ability to adapt to environmental changes, among others (Mirzaghaderi and Hörandl, 2016). If HT1 eggs were to use the male genetic material introduced into the zygote through fusion of the sperm with the egg, the nuclear envelope of the male pronucleus would have to break down, the male chromosomes could then mix with the female counterparts, and the first mitotic division would occur (Serbus *et al.*, 2008). In this case, according to normal Mendelian inheritance, half of the male chromosomes would be inherited by next generation, which would lead to approximately half of the offspring bearing XY sex chromosomes and developing into genetic male individuals, while the second half would be homogametic with two X chromosomes and developing into genetic females. This is not the case for HT1 of *A. lythri*, which produces exclusively female progeny.

7.4 Paternal leakage in HT1 of *A. lythri*

In gynogenetic females, the maternal genome fully controls the subsequent development of the all-female offspring (Grebelnyi, 2009). In *A. lythri*, the quaddRAD data showed that over 97% of all SNPs detected in the all-female offspring were, in fact, inherited from the mother. However, under 1% of the SNPs found in the progeny originated from the father. According to Grebelnyi (2009), the sperm fuses with the gynogenetic egg, which introduces the male pronucleus into the oocyte of gynogenetic females. A recombination with the maternal set of chromosomes is not described, as the pronucleus is separated immediately after fusion of the sperm with the egg and eliminated (Grebelnyi, 2009). However, the mechanism that controls the exclusion of the male pronucleus from the oocyte might fail (Lamatsch and Stöck, 2009). A phenomenon called 'leaky gynogenesis' or 'paternal leakage' (Schartl, Nanda, *et al.*, 1995; Lamatsch *et al.*, 2000; Lamatsch and Stöck, 2009; Leung and Angers, 2018; Suzuki *et al.*, 2020) describes occasional paternal introgression and resulting genetic recombination in gynogenetic offspring. It has been reported for paternal chromosomes to recombine with maternal chromosomes with no effect on ploidy level (Hedges, Bogart and Maxson, 1992; Spolsky, Phillips and Uzzell, 1992), but also an altered ploidy such as the emergence of triploidy can be caused by the introgression (Goddard and Dawley, 1990; Tomiuk and Loeschcke, 1992; Goddard and Schultz, 1993; Saura, Lokki and Suomalainen, 1993; Lamatsch *et al.*, 2000).

An example for paternal leakage is the Crucian carp *Carassius auratus gibelio* (Jia *et al.*, 2008). This species has an all-female triploid gynogenetic form, which greatly influences the sex ratio of its populations with 0 - 37% female individuals on average (Fan and Shen, 1990; Jia *et al.*, 2008). In the before-mentioned study, Jia *et al.* (2008) used 15 microsatellite loci to analyse the F1 generation of the gynogenetic females. They detected three possible outcomes apart from clonal inheritance. Either, the gynogenetic offspring inherited paternal alleles for some microsatellite loci, the offspring was missing maternal alleles, or the offspring showed novel alleles that were most likely a result of sexual recombination of the gynogenetic and male pronuclei (Brohede, 2002; reviewed in Schlupp, 2005; Jia *et al.*, 2008). This was also detected in the flatworm *Schmidtea polychroa* (D'Souza *et al.*, 2004). Similar to *A. lythri*, Jia *et al.* (2008) could detect occasional paternal introgression with genetic recombination in the all-female offspring of gynogenetic females of *C. auratus gibelio* at an average rate of 0.63%.

In the example of gynogenetic fishes like the Amazon molly *Poecilia formosa*, it has been described that the exclusion mechanism that aims at destroying the paternal chromosomes to keep them from recombining with the maternal set can stop before the entire pronucleus is eliminated (Schlupp, 2005), causing microchromosomes to enter the oocyte (Schartl, Nanda, *et al.*, 1995; Schlupp, 2005; Lamatsch *et al.*, 2011). *P. formosa*, the hybrid of *P. mexicana limantouri* and *P. latipinna* (Turner *et al.*, 1983; Vrijenhoek, 1989; Schartl, Wilde, *et al.*, 1995), is dependent on the sperm of the sexual species *P. mexicana* or *P. latipinna* (Schartl, Wilde, *et al.*, 1995), and the gynogenetic offspring frequently inherits microchromosomes from the father (Schartl, Nanda, *et al.*, 1995; Schlupp, 2005; Lamatsch *et al.*, 2011). The paternal genetic contribution to the next generation is expected to be small (Schartl, Nanda, *et al.*, 1995), considering that *P. formosa* produces unreduced eggs (Rasch, Monaco and Balsano, 1982), which would not allow the male to contribute a lot of genetic material. Furthermore, the microchromosomes are defined as very small fragments of paternal chromosomes, leaving a highly limited amount of paternal genetic material that can be introduced into the progeny (Schlupp, 2005).

7.5 Possible causes for the paternal leakage in HT1

As described previously, under 1% of all inherited SNPs in the HT1 offspring originated from the father. Because the paternal part of alleles in the offspring is under 1%, it is possible that this percentage was caused by misassignments in the method, the handling, or sequencing errors. To ensure that I can exclude these possibilities, I chose the parameters in the Stacks pipeline accordingly (see chapter 5.1.5). By increasing the -m parameter (minimum number of raw reads required to form a stack (putative locus)) to 10, I could optimise the probability of recovering all alleles without losing too much valuable data. This eliminates false positive results due to possible handling issues. I can thus say that the under 1% of paternal alleles found in the offspring truly originate from the father.

One locus in particular was of special interest; the locus with the locus ID 2393 in family 19_Bü_114 x 19_Bü_170 of HT1 has been inherited solely from the father. This locus could only be detected in this family. For all other families, this locus has not been detected. A possible reason for this, which would also relativise the result of this locus in particular, would be an occurring error in the indexing PCR step. The DNA undergoes various steps in preparation for sequencing (see chapter 5.1.4), which might all be potential causes for this

result, however, during the PCR the DNA gets replicated over and over again. Although the Phusion polymerase harbours a proof-reading ability, it is still possible for mistakes to occur during a PCR. This might have been the case for the mother sample. If, by accident, the polymerase changed the DNA sequence at this locus, it could cause such a result with this locus being entirely inherited from the father. It is unlikely for this possible error to have occurred during the bioinformatical analysis after sequencing, as the reads are checked by forward and reverse reads to ensure the accuracy of the sequence. Should the reverse read not match the forward read, or should the reverse read not exist, it is impossible to continue the Stacks pipeline for this sample and it gets excluded and thus the entire family will be taken out of the analysis. This, however, did not happen for any of the families, including family 19_Bü_114 x 19_Bü_170 of HT1. The fact that this locus could not be found in any of the other families might be a hint towards what is potentially the answer. The maternal allele might be missing from the other families, which could be the reason why this locus does not appear in any of the other analysed families. In this case, a PCR error seems more likely.

However, with the help of the quaddRAD data, I show the presence of loci that include paternal genetic material in the offspring, which has been introduced by mostly sexual reproduction. This is indicated by a combination of maternal and paternal alleles at a given locus. Despite the possibility of an error in the PCR, it is important to discuss further possible reasons for an exclusively paternal locus in gynogenetic offspring. We do not know on which chromosome this locus occurs, as the genome of *A. lythri* is still rather fragmented and the chromosomes have thus not been annotated yet. It is possible that this locus occurs on a telomeric region of a chromosome and could have been separated from the paternal chromosome during a faulty elimination of the paternal pronucleus from the oocyte. However, because the chromosomes of *A. lythri* are incredibly small (Segarra and Petitpierre, 1982; Jäckel, 2011), it might be difficult to detect possible broken-off pieces of excluded chromosomes under the microscope using only DAPI staining. By blasting this locus, it might be possible to gain further insight into which chromosome this locus originates from. Microchromosomes, a phenomenon described in *P. formosa* (Schartl, Nanda, *et al.*, 1995; Schlupp, 2005; Lamatsch *et al.*, 2011), have not been described for *A. lythri* as of yet (Segarra and Petitpierre, 1982; Jäckel, 2011). Furthermore, such microchromosomes would also not have been detected in the quaddRAD analysis, as each locus would need a counterpart in the maternal genome to show up in the results.

7.6 Paternal leakage could counteract the consequences of Muller's ratchet

In well investigated gynogenetic species like *P. formosa* (Schlupp, 2005) and *C. auratus gibelio* (Jia *et al.*, 2008), such leakage of paternal genetic material has been described in detail. It is not yet fully understood how or why that happens, but one explanation has been discussed in the literature as to why gynogenetic offspring sometimes inherit parts of the paternal genome.

Muller's ratchet (Muller, 1964) is a phenomenon that describes the genetic decay caused by a lack of sexual recombination in parthenogenetic organisms. Due to the accumulation of deleterious mutations that cannot be rectified because of the lack of recombination and re-organisation that occurs in sexually reproducing species both during gamete production and the intermixing of haploid chromosome sets in sexual reproduction (Muller, 1964; Dimijian, 2005), it is believed that parthenogenetic species or forms thereof would go extinct within 10^4 to 10^5 generations (Lynch and Gabriel, 1990; Gabriel, Lynch and Bürger, 1993). It is not known how old the hybrid species *A. lythri* is, so it is not possible to say whether it has already reached 10^4 to 10^5 generations of being gynogenetic, but contrary to this estimation, many asexual species are evolutionary older than 10^4 to 10^5 generations (Hedges, Bogart and Maxson, 1992; Quattro, Avise and Vrijenhoek, 1992; Spolsky, Phillips and Uzzell, 1992). It is possible that *A. lythri* is older than 10^5 generations but is genetically stable enough because of the detected paternal leakage found in the quaddRAD data. The inclusion of microchromosomes in *P. formosa* can be given as an example for how gynogenesis might be a type of reproduction that combines the advantages of sexual with those of asexual reproduction (Schartl, Nanda, *et al.*, 1995), and thus evading extinction due to an accumulation of deleterious mutations. Whether occasional paternal leakage in HT1 of *A. lythri* is enough introduction of new genetic material to counteract the effect of Muller's ratchet and part of this mtDNA HT's survival is uncertain but unlikely due to the very low percentage of leakage into the gynogenetic offspring.

7.7 Production of unreduced oocytes in HT1

To be able for the oocyte to exclude the paternal haploid set of chromosomes that would be needed in sexual reproduction, the female gamete would have to be unreduced and would have to harbour the full set of chromosomes that can also be found in somatic cells of this

organism. To determine whether that hypothesis was, in fact, true for *A. lythri*, I generated a set of chromosome spreads from unfertilised HT1 and HT2 eggs. Here, the HT2 eggs functioned as a comparison for the HT1 eggs, as I could show with the quaddRAD data that HT2 reproduces sexually, so the expected chromosome count in an unfertilised oocyte would be reduced and thus haploid. This is also what I detected upon counting the chromosomes. HT1, on the other hand, would require an unreduced chromosome set to be capable of transferring over 97% of the maternal genetic material to the gynogenetic offspring. Indeed, the unfertilised eggs of HT1 showed double the chromosome count than HT2 did. This means that HT1 eggs are not reduced and depict a diploid chromosome set of 24 chromosomes, which is the number that can be found in somatic cells (Segarra and Petitpierre, 1982; Jäckel, 2011).

Gynogenesis, like any other type of parthenogenesis, needs a trigger to appear in a species. What this trigger is and whether gynogenesis can be named mainly responsible for the observed sex ratio distortion in HT1 is not clear. In the following subchapters, I will address this question and highlight possible causes for such a phenotype.

7.8 The cause for the production of unreduced oocytes during meiosis in HT1

The goal of meiosis is the formation of four gametes that each harbour a unique reduced (here: haploid) genome (Kleckner, 1996; Nasmyth, 2001; Kitajima, Kawashima and Watanabe, 2004; Gerton and Hawley, 2005; Ables, 2015). In this case, the fusion of two haploid gametes, one from each parent organism, produces a unique diploid individual. *A. lythri* is a diploid organism with a 2n of 24 chromosomes, including X and Y sex chromosomes (Segarra and Petitpierre, 1982). As described above, the quaddRAD data shows that HT1 of *A. lythri* produces all-female progeny that inherit the majority of their alleles from their mother. This leads to the assumption that the meiosis of this HT is altered in a way that makes it possible for the majority of the maternal genome to be transferred to the next generation, essentially forming clones of the mother. This can, among other possibilities, be achieved by producing oocytes with an unreduced genome, so in case of *A. lythri* producing oocytes with 24 chromosomes.

To determine the chromosome count of unfertilised oocytes of *A. lythri*, I compared freshly laid HT1 and HT2 eggs. Before fertilisation, the chromosomes should normally be arrested in the metaphase II stage (Masui and Markert, 1971; Choi *et al.*, 1996; Simpson and Douglas,

2013, p. 322; Gruss, 2018), which made it possible for me to count the highly condensed chromosomes at this stage. HT2 showed a haploid chromosome count of 12 chromosomes. This was to be expected as this HT produces both male and female offspring in a relatively equal sex ratio. This was furthermore supported by the quaddRAD data, which showed an equal distribution of the inheritance modes of the SNPs found in the offspring. This confirms that HT2 reproduces sexually. HT1, on the other hand, produces exclusively female progeny. That taken together with the results obtained by quaddRAD show that the majority of the genetic material in the all-female offspring originates from the mother. Upon checking the chromosome count of unfertilised HT1 eggs, I found that it showed a diploid chromosome set with 24 chromosomes in the unfertilised eggs. This result is a characteristic found in gynogenetic females (Rasch, Monaco and Balsano, 1982; Presgraves, 2000) and further supports the result obtained by quaddRAD that this HT reproduces gynogenetically.

How *A. lythri* achieves the diploid chromosome set in the HT1 oocytes cannot yet be decided. Generally, to restore the ploidy of the organism in the gametes, several mechanisms can be found in the animal kingdom. The ploidy restoration can be achieved by apomixis, wherein meiosis is repressed and the oocyte essentially gets produced by mitosis (Rasch, Monaco and Balsano, 1982; Lampert, 2008), resulting in genetical clones of the mother. Pre- or post-meiotic doubling (Macgregor and Uzzell, 1964; Uzzell, 1970; Cuellar, 1976; Mirzaghaderi and Hörandl, 2016 and citations therein) of the chromosomes are other mechanisms, wherein meiosis is fully functional but due to doubling of the chromosomes, recombination between the chromosomes of the haploid nuclei does not take place. Automixis describes the maintaining of meiosis and the restoration of the diploid chromosome set by fusion of two meiotic products (Mogie, 1986; Lampert, 2008). Depending on which polar body fuses with the oocyte, the level of heterozygosity in the offspring is determined. A fusion of two meiosis products can be found in the wasp *Muscidifurax uniraptor*, in which the ploidy of the oocytes is restored by the fusion of two daughter cells, both of which are haploid after a normal first meiotic division, after meiosis I was completed (Gottlieb *et al.*, 2002).

Like *A. lythri*, the triploid spider beetle *P. clavipes* f. *mobilis* produces gametes with an unreduced chromosome count (Sanderson, 1960). The chromosomes of these females are bigger than those of their sexual form. In *A. lythri*, no visible difference in size and thickness could be detected between the chromosomes of HT1 and HT2 eggs, which suggests that the state and composition of the chromosomes is not changed but only their numbers. For

P. clavipes f. *mobilis*, Sanderson (1960) describes that no chromosome reduction takes place during meiosis. This might be due to the triploidy of the species. In the Amazon molly *P. formosa* (Turner *et al.*, 1980), the Crucian carp *C. auratus gibelio* (Uzzell, 1970) and *Trichogramma* wasps (Huigens and Stouthamer, 2003), apomixis is given as an explanation for their unreduced gametes. *C. auratus gibelio* shows a suppression of the first meiotic division, also known as first division restitution (FDR; Bretagnolle and Thompson, 1995).

The suppression of the first meiotic division or part thereof is the most likely explanation for the unreduced chromosome set in HT1 of *A. lythri*. As is normal for insects (Masui and Markert, 1971; Choi *et al.*, 1996; Simpson and Douglas, 2013, p. 322; Gruss, 2018), the oocytes arrest in metaphase II in *A. lythri*, awaiting fertilisation. Meiosis is not completed before fusion with the sperm. For the chromosome counts, HT2 served as a control to compare with HT1 because both the sex ratio of the offspring and the quaddRAD data showed that HT2 reproduces sexually. Sexual reproduction includes two meiotic divisions, at the end of which the oocyte is reduced and the chromosomes separated into sister chromatids. This is what could be seen for the unfertilised HT2 egg. Therefore, the HT2 egg was arrested in metaphase II. The HT1 egg, on the other hand, showed double the number of chromatids in the nucleus, which leads to the assumption that the production of the unreduced nucleus happens before the egg is arrested in metaphase II.

Automixis requires a reduction of chromosomes before metaphase II is completed to be able to fuse a polar body and the oocyte to restore ploidy. Once this has happened, mitosis continues and no arrest takes place as no sperm is required to restore ploidy. In case of *A. lythri*, HT1 requires insemination to produce offspring. Without this trigger, the oocyte does not start mitosis and thus the development of the larva does not begin. Therefore, an arrest of the chromosome division is present in unfertilised HT1 oocytes. To my knowledge, there is no report about an arrest taking place at a later stage than metaphase II, which makes a fusion of meiotic products as it happens in automixis very unlikely for HT1.

To completely exclude this possibility, and that of pre- and post-meiotic doubling of the chromosomes, more research would be needed that addresses the chromosome counts at different states of meiosis. This proved to be difficult as the fixation and extraction of the undeveloped oocytes was close to impossible with the fixation and preparation protocol I used to determine the chromosome count. However, according to what we know about the meiosis and meiosis arrest of HT1, post-meiotic doubling of the chromosomes can be omitted

from the discussion because meiosis is arrested in metaphase II when the oocyte awaits insemination, as explained above. A further support for this can be seen when comparing the chromosome spreads from HT2 and HT1 (see chapter 6.11). HT2 reproduces sexually and therefore is expected to show a regular meiosis. Compared to HT2, HT1's chromosomes looked the exact same, apart from the number, which was doubled. When chromosomes are arrested in metaphase, they are highly condensed and located on the equatorial plane. Because there were no visible differences in the thickness and size of the chromosomes in HT1 and HT2, it is likely that the chromosomes in HT1 are present as chromatids in the metaphase II arrest. Antibodies targeting telomere-associated proteins would make it possible to visually determine the number of telomeres present in one chromosome. One signal on each side of the chromosome would point towards single chromatids (an example for this can be seen in Cesare, Heaphy and O'Sullivan, 2015), whereas two signals located next to each other would depict a chromosome consisting of two sister chromatids. Using telomere-binding antibodies would determine whether the HT1 chromosomes really are present as single sister chromatids, as should be the case for HT2. By labelling the telomeres, the fluorescence signals could be compared between the two HTs.

One explanation that could explain the quaddRAD as well as the histological data would be apomixis, the complete suppression of the first meiotic division. This way, the chromosome pairs would not undergo the separation of homologue chromosomes, thus the diploid set would remain and enter the second meiotic division, in which the sister chromatids would be separated. The resulting gametes would harbour double the chromosome number they would have if the first meiotic division had happened, which is on a par with the visual comparison between the high magnification images of HT1 and HT2 chromosomes.

Another explanation, which might also be interesting in consideration that HT1 reproduces gynogenetically, are missing or skipped anaphase and telophase I. The anaphase stage in meiosis I is necessary to separate the homologous chromosomes from each other, whereas the telophase stage is necessary to split off half of the chromosomes to form the polar bodies (Figure 16, A). This happens at the end of the first meiotic division, forming the first polar body, as well as in the second meiotic division, resulting in the second and third polar body. This separation is needed to produce the reduced chromosome count in gametes. If, instead of the entire meiosis I, anaphase I and telophase I are skipped, one half of the homologous

chromosomes would not be separated from the other and the developing gamete would retain its full chromosome set.

7.9 Possible fates of the paternal pronucleus after fertilisation of the HT1 oocyte

Once the sperm fuses with the egg, the second meiotic division takes place while the male chromosomes decondense and the male nuclear envelope breaks down (Serbus *et al.*, 2008). This allows the male chromosomes to recognise the female pronucleus, drift towards it, and intermix with the maternal chromosomes at a later stage. Two polar bodies containing maternal chromosomes form in the process (Figure 16, A). In gynogenesis, the sperm fuses with the egg (Grebelnyi, 2009) and thus triggers the development of the unreduced oocyte (Rasch, Monaco and Balsano, 1982; Presgraves, 2000) into a larva. By fusing with the oocyte, the male pronucleus enters the female gamete and is immediately excluded from the egg (Schlupp, 2005; Grebelnyi, 2009). However, this procedure can be prone to mistakes (Schartl, Nanda, *et al.*, 1995; Lamatsch *et al.*, 2000, 2011; Schlupp, 2005; Lamatsch and Stöck, 2009; Suzuki *et al.*, 2020). One example, which causes microchromosomes in *P. formosa*, is the premature abortion of this exclusion mechanism (Schlupp, 2005). How the exclusion of the male pronucleus works is not yet fully understood.

In *A. lythri*, just under 1% of paternal alleles get transferred to the next generation, yet the fate of the rest is not known. It is also possible that the paternal chromosomes get methylated in a way that makes them unable to reorganise with the maternal set. Langley *et al.* (2014) reviewed various roles of DNA methylation, one of which highlighted the methylation state of maternal DNA, which can differ from that of paternal DNA. The maternal DNA methylation has to be discarded and resolved to resemble that of the paternal DNA to be able to continue with the zygotic transcription. According to Langley *et al.* (2014), it is therefore likely that a mechanism exists that is able to distinguish between maternal and paternal alleles, which is needed to reach the same methylation levels for the continuation of transcription and thus larval development. This has been shown in early zebrafish embryos (Jiang *et al.*, 2013; Potok *et al.*, 2013; reviewed in Langley *et al.*, 2014).

Genome methylation can be found in eukaryotes as a genome modification that affects gene expression (Curradi *et al.*, 2002) and stabilises a condensed chromatin conformation by recruiting methyl-binding proteins (Bird and Wolffe, 1999). The methylation rate of insect genomes ranges from 0 - 3% (Field *et al.*, 2004). Whereas possible functions in

D. melanogaster (transposon regulation), the peach-potato aphid *Myzus persicae* (gene regulation), the mealybug *Planococcus citri* (imprinting), and the cabbage moth *Mamestra brassicae* (genome stability) have been investigated in detail (Field *et al.*, 2004), no DNA methylation has been detected in Coleoptera (Field *et al.*, 2004; Glastad *et al.*, 2011). It would thus be prudent to use whole-genome bisulphite sequencing to analyse the methylcytosine content (as was done for zebrafish, see Jiang *et al.*, 2013; Potok *et al.*, 2013) in HT1 eggs and HT2 or HT3 sperm of *A. lythri*. Possible differences in methylation degree might help to answer the question of how the paternal pronucleus gets recognised and ultimately excluded after fusion with a gynogenetic HT1 egg. Assuming the nucleus enters the gynogenetic egg as stated by Grebelnyi (2009), it would need to be excluded as quaddRAD is also able to detect inactivated chromosomes that were transmitted to the offspring.

The elimination of the paternal genome would be needed if it were not excluded from the zygote. However, as *A. lythri* is a diploid species with a somatic chromosome count of 2n = 24 (Segarra and Petitpierre, 1982; Jäckel, 2011) and paternal genome elimination (PGE) can mainly be found in haplodiploids (Ross, Shuker and Pen, 2011), PGE is highly unlikely to occur in HT1 fertilised eggs. It is defined as the transfer of only those chromosomes inherited from one particular parent (Burt and Trivers, 2006).

Another possible explanation of how the gynogenetic egg recognises the paternal pronucleus is an altered chromosome condensation. During a normal fertilisation of an oocyte, the plasma membrane and the nuclear envelope of the male pronucleus instantly dissolve. With the help of maternally supplied histones, the male chromatin gets remodelled before it is surrounded by a maternally supplied nuclear envelope, as could be shown for *Drosophila* (Loppin *et al.*, 2005). This is followed by a replication and further condensation of the paternal chromatin to prepare for the first mitotic division before both pronuclei migrate towards each other with the help of microtubules and motor proteins. Once they have reached close proximity, the nuclear envelopes break down, the spindle apparatus forms, and the chromosomes get pulled apart at anaphase, resulting in two diploid daughter cells (Serbus *et al.*, 2008). An alteration of this chromosome condensation can, for example, be seen in cytoplasmic incompatibility (CI) of diploids (Serbus *et al.*, 2008). CI embryos are characterised by a severe disruption of the first mitotic division, which is due to highly tangled (Ryan and Saul, 1968) and wrongly condensed (Breeuwer and Werren, 1990) paternal chromatin. This leads to fragmentation of the paternal chromosomes during the first mitotic division. It is

possible for some of these fragments to come into contact with and be incorporated into the nuclei of the daughter cells (Reed and Werren, 1995; Tram and Sullivan, 2002).

In *A. lythri*, *Wolbachia*-induced CI cannot be given as an explanation for a possibly faulty paternal chromosome condensation as CI does not exist in this species (Jäckel, Mora and Dobler, 2013). However, the condensation of chromatin, whether it be maternal or paternal, may still be a factor that might influence the exclusion of the male pronucleus in gynogenetic eggs. The paternal chromatin cannot be condensed differently as it has to be condensed in a way that makes it compatible for fertilisation of HT2 and HT3 eggs, as those two crosses produce viable offspring (own observation). This leaves the chromatin of the gynogenetic mother. Is it possible that the condensation of gynogenetic chromatin differs to a degree that makes it impossible for the male genetic material to intermix? It would be interesting to see whether the gynogenetic maternal pronucleus is faster or slower in its progress through meiosis. If the condensation states of both pronuclei do not match, it would not be possible for them to mix and form a recombined nucleus that enters the first mitotic stage. A different developmental stage resulting from an altered chromosome condensation in the mother might be what causes the detection of the paternal pronucleus and ultimately results in its exclusion from the zygote. To check this hypothesis, male and female markers would be needed to label maternal and paternal chromosomes within a zygote using fluorescence *in situ* hybridisation (FISH) to determine potential differences in the stages of the first mitotic division.

More research needs to be invested into this hypothesis to get an idea of whether the composition and/or the condensation is involved in the exclusion of the paternal pronucleus. Using devices, such as micromanipulators that are used in *in vitro* fertilisation (Palermo, 1992; Bogolyubov, 2007), might make it possible to secure the oocyte in one place during the fixation step would make further analyses easier to execute.

Another possibility lies in the exclusion of the pronucleus, which happens as soon as the male pronucleus enters the unreduced female gamete (Schlupp, 2005; Grebelnyi, 2009). One explanation as to how this might happen can be seen in Figure 16. During telophase II of a normal meiosis, the last two polar bodies are formed.

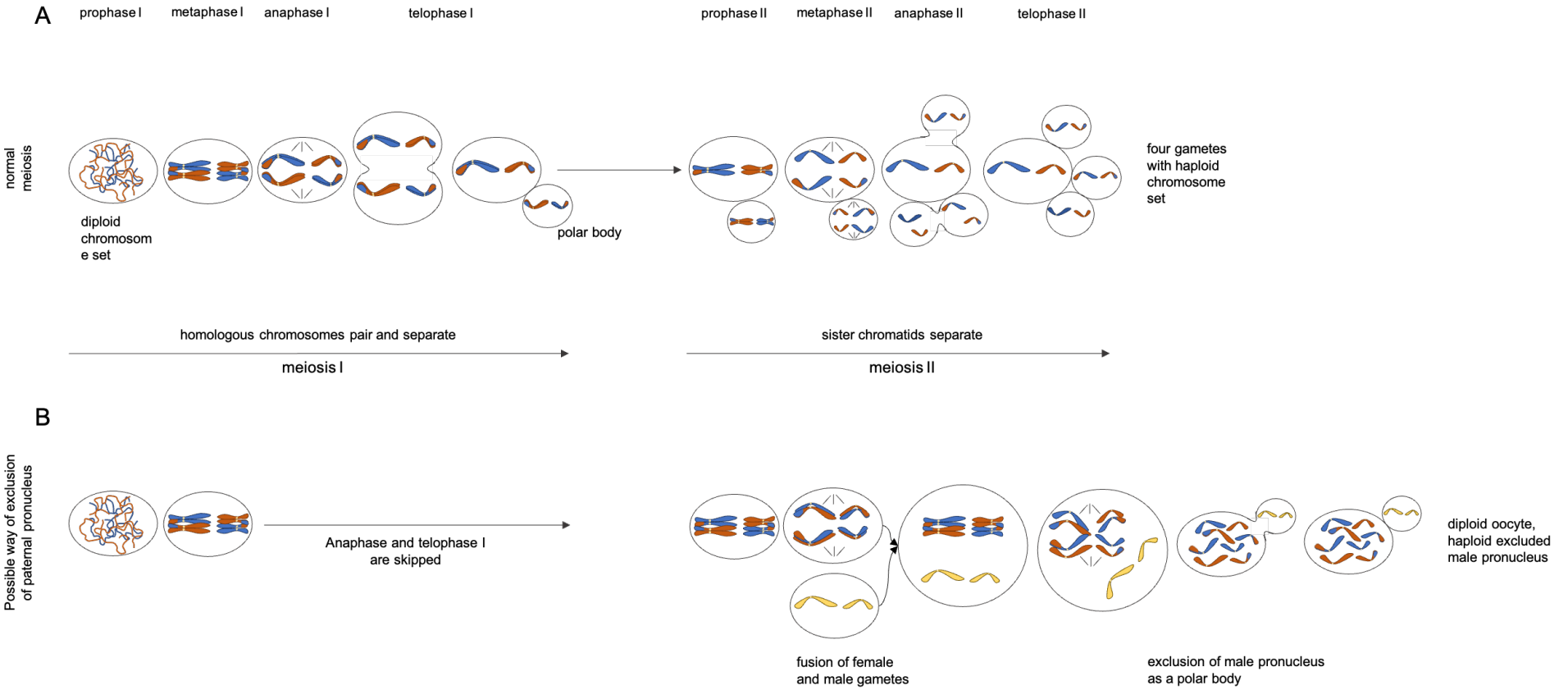


Figure 16 - Possible fate of paternal pronucleus after fertilisation of gynogenetic egg. (A) Regular meiosis of female, production of four haploid gametes, (B) Suggestion for meiosis in a gynogenetic egg producing a diploid gamete (blue and red chromosomes), following fertilisation by a haploid sperm and exclusion of male pronucleus (yellow chromosomes) from diploid gynogenetic oocyte.

In gynogenetic eggs, the male pronucleus is not needed to restore ploidy, so it might be possible that the oocyte excludes the male haploid chromosome set (coloured in yellow in Figure 16) as if it were forming a polar body in telophase II (Figure 16, B), effectively using the male chromosomes to form the polar body. Producing only one polar body containing the male set of chromosomes would allow the oocyte to keep its already, in case of *A. lythri*, diploid chromosome set and dispose of the male haploid chromosome set. This hypothesis needs more research to establish its accuracy.

7.10 Are *Wolbachia* involved in the sex ratio distortion of *A. lythri*?

I have established that gynogenesis, a form of parthenogenesis, can be one cause of strong female bias in *A. lythri*. However, gynogenesis may not be the only cause of female bias, as *A. lythri* is infected with the reproduction-manipulating microorganism *Wolbachia. Wolbachia* is known to induce CI, male-killing, feminisation and parthenogenesis as a means to promote its own transmission (see chapter 4.3) and can lead to a distorted sex ratio.

A. lythri is infected with three *Wolbachia* strains, each of which is closely associated with one mtDNA HT. In recent years, the strain *w*LytB has slowly introgressed into HT2, forming double infections with *w*LytA2 in HT2 (own observation). Such double infections have not been detected for HT1 and *w*LytA1. Because *w*LytA1 and *w*LytA2 are evolutionarily closer than *w*LytA2 and *w*LytB (Jäckel, Mora and Dobler, 2013), it is likely that *w*LytA1 is the most dominant and does not allow another strain to infect its host due to incompatibilities.

The ways in which *Wolbachia* can manipulate the sex determination cascade to induce a strong female bias and promote their own transmission are vast. Generally, it is expected that most insects produce a relatively balanced sex ratio in their offspring (Compton and Tu, 2022). However, it is always possible for selection to push the even sex ratio towards one sex and lead to meiotic drive (the bias in the distribution of gametes of a specific chromosome or genotype during meiosis, Compton and Tu, 2022). This has been documented in *D. simulans* (Tao, Araripe, *et al.*, 2007; Tao, Masly, *et al.*, 2007; Lin *et al.*, 2018) and the mosquito *Aedes aegypti* (Craig, Hickey and VandeHey, 1960; Newton, Wood and Southern, 1976).

7.10.1 Sex determination genes as possible targets for *Wolbachia* manipulation

One mechanism that can be manipulated by the bacterial endosymbionts is the dosage compensation that initiates most of the insects' sex determination cascade (Fukui *et al.*,

2015). Examples for this can be found in *Drosophila*, in which *Wolbachia* and *Spiroplasma* target the dosage-compensated X chromosome in males to cause the death of male offspring (Veneti *et al.*, 2005; Harumoto, Fukatsu and Lemaitre, 2018). The moth *Ostrinia furnacalis* is another example for the manipulation of the dosage compensation through *Wolbachia*, which is lethal for male embryos (Kiuchi *et al.*, 2014). One protein in particular is targeted, namely Masc. This protein is required for both masculinisation and dosage compensation (Kiuchi *et al.*, 2014) and the downregulation of its mRNA leads to unnaturally increased expression of Z-linked genes in male embryos, which causes male-specific embryonic death (Fukui *et al.*, 2015).

Tra, a gene that is integrated in the sex determination cascade of insects further downstream from the dosage compensation complex, is considered to be another prime candidate for manipulation (Beukeboom, 2012). Due to it being both maternally and zygotically expressed, it would be an optimal target for manipulation (Pane *et al.*, 2002; Lagos *et al.*, 2007; Hasselmann *et al.*, 2008; Concha and Scott, 2009; Hediger *et al.*, 2010; Verhulst, Beukeboom and van de Zande, 2010). This can be seen in dipterans and hymenopterans, whose *tra* activity is regulated through an autoregulatory loop (Beukeboom, 2012). A disruption of this autoregulation leads to male development of the embryo. Microbial intervention would be expected to prevent or counteract a disruption of the loop to ensure female development of the progeny (Beukeboom, 2012). Furthermore, so-called *M*-factors located on the Y chromosome would also be a logical target for reproduction-manipulating organisms, as the blocking of these masculinisers would cause the development of female embryos (Beukeboom, 2012). However, an altered amount of *tra* splice products has not been detected in 3-hour-old eggs (Rohlfing *et al.*, 2023), leaving this as an unlikely cause which ultimately led to gynogenesis. Because *dsx* is directly downstream of *tra*, a manipulation thereof is unlikely to be part of the cause for the emergence of gynogenesis.

Overall, there are various factors and stages in which the sex determination of *A. lythri* could have been affected through *Wolbachia* infection. The failure of dosage compensation (Kiuchi *et al.*, 2014; Fukui *et al.*, 2015), a step that has yet to be found in *A. lythri*'s sex determination cascade, as well as the interference with upstream genes (Sugimoto *et al.*, 2010; Sugimoto and Ishikawa, 2012) can all be listed, amongst gynogenesis, as possible causes for the strong sex ratio distortion in *A. lythri*. Hormonal manipulation as can be found in *Daphnia magna* (Negri *et al.*, 2010) and *A. vulgare* (Rigaud, Juchault and Mocquard, 1997; Bouchon, Cordaux

and Grève, 2008) are unlikely candidates for *A. lythri*. To fully exclude *Wolbachia* as a possible reason for the sex ratio distortion, the beetles would need to be fed with tetracycline. If *Wolbachia* was involved in this phenotype, a change in such should be visible after an elimination of the majority of the infection in the beetles. However, because HT1 shows a diploid chromosome set in unfertilised eggs, it is unlikely that an antibiotic treatment will lead to an even sex ratio within a few generations.

7.11 Are mitochondria involved in the sex ratio distortion found in *A. lythri*?

Through hybridisation, *A. lythri* has gained two additional mitochondrial haplotypes, adding up to a total of three in this species. Mitochondria are haploid organelles (Jelić *et al.*, 2015) that reproduce asexually and are uniparentally transmitted, usually via the mother (Birky, 1995, 2001). This uniparental inheritance prevents different cytoplasmic lineages from mixing, which is most likely to reduce any competitive interactions between different mitochondria (Perlman *et al.*, 2015), meaning that one individual can only harbour one mitochondrial variant. However, the uniparental transmission leads to one sex being an evolutionary dead end for these mitochondria. There are only a limited number of genes in a mtDNA genome, each is required for paramount biological processes (Boore, 1999), thus any change in their function needs to be rectified by natural selection (Takahata, 1984) to counteract any negative consequences for the individuals carrying these mitochondria.

Due to the absence of selection on mtDNA genomes that takes place in females (Jelić *et al.*, 2015), the risk of accumulating deleterious mutations is high in males, generating a male-specific genetic load, a phenomenon called Mother's curse (Frank and Hurst, 1996; Nachman *et al.*, 1996; Gemmell, Metcalf and Allendorf, 2004; Runemark *et al.*, 2018). Parthenogenesis might lead to an accelerated accumulation of deleterious mutations (Perlman *et al.*, 2015), which affect the reproductive fitness of males (Gemmell and Sin, 2002; Innocenti, Morrow and Dowling, 2011), as has been described for *D. melanogaster* (Innocenti, Morrow and Dowling, 2011). This process would further cause potential male individuals to suffer from the consequences, which might be neutral or even advantageous in females (Frank and Hurst, 1996; Camus, Clancy and Dowling, 2012).

Correlations between HT frequencies and environmental factors like infection with endosymbionts or the interaction with the nuclear genome of the cell has been a topic for discussion (Schmidt *et al.*, 2001; Mishmar *et al.*, 2003; Grossman *et al.*, 2004; Ruiz-Pesini *et*

al., 2004; Bazin, Glémin and Galtier, 2006) and has hinted at the presence of positive selection on mtDNA genomes (Jelić *et al.*, 2015). Mitonuclear interactions might be the reason for the upkeeping of mtDNA polymorphism (Rand, Clark and Kann, 2001) as a strong linkage disequilibrium between mtDNA HTs and nuclear genes has been described (Jelić *et al.*, 2015). These interactions have been found both occurring interspecifically and intraspecifically (Wolff *et al.*, 2014), even causing sex-specific selection of various mitonuclear types in both males and females (Jelić *et al.*, 2015) to maintain mitochondrial polymorphism (Babcock and Asmussen, 1996, 1998; Rand, Clark and Kann, 2001). This has been reported for a variety of taxa (Wolff *et al.*, 2014; Jelić *et al.*, 2015), with *D. melanogaster* (Rand, Clark and Kann, 2001; Dowling *et al.*, 2007) as one example for a species with notable sex-specific mitonuclear interaction.

The infection with reproduction-manipulating parasites, like *Wolbachia* and *Rickettsia*, can decrease the mtDNA variation by promoting the parasites' own transmission through further infection of previously uninfected individuals, and thus increasing the frequency of their associated mitochondrial genome (Turelli, Hoffmann and McKechnie, 1992; Ballad *et al.*, 1996; Jiggins, 2003; Shoemaker *et al.*, 2004). This can also be seen in *A. lythri*, wherein a strong correlation between the mtDNA HTs and three *Wolbachia* strains has been detected (Jäckel, Mora and Dobler, 2013). This also means that the effective population size of mitochondria in the still uninfected individuals gets reduced and causes a further decline in mtDNA variation. Uninfected individuals, while still detectable at the start of this project, are less likely to be found (own observation) as the infection rate and double infection with the strains *w*LytA2 and *w*LytB are increasing (own observation).

It is unlikely that the entire mitochondrial genome is responsible for triggering the gynogenesis that prevails in HT1, as no such case has been previously reported (Perlman *et al.*, 2015). The structure of the mitochondrial genome, on the other hand, provides a compelling explanation for the sex ratio distortion.

An example for this phenomenon can be seen in the booklouse *Liposcelis bostrychophila*, which shows an extreme sex ratio distortion. This species reproduces sexually (Perlman *et al.*, 2015) and is commonly infected with *Rickettsia* (Yusuf and Turner, 2004; Perlman, Hunter and Zchori-Fein, 2006; Behar, McCormick and Perlman, 2010). Two types of females have been detected in laboratory cultures – so-called nondistorter females that produce a relatively equal sex ratio in the offspring and the distorter females that produce exclusively female

offspring (Perlman *et al.*, 2015). The inheritance of this severe sex ratio distortion is strictly maternal, however. No gynogenesis is involved because the daughters of the distorter females show paternal alleles (Perlman *et al.*, 2015), unlike HT1 of *A. lythri* (see chapter 6.4). What has been found in the distorter females is a change in the mitochondrial genes when compared to the regular type that produces an equal amount of male and female offspring. The genes show an approximately 53 - 77% similarity at protein-coding level between the two types of mitochondria (Perlman *et al.*, 2015). Furthermore, the gene order as well as the genome structure of the mitochondria is highly divergent, and the morphology differs significantly between the distorter and normal mitochondria with the distorter females' mitochondria looking aged and very damaged, which (Perlman *et al.*, 2015) explained as a result of CI in mitochondria that are located in tissues with high metabolic activity.

Whether the genes on the mitochondria, their order on the genome, the presence or absence of promotors, or a possible size difference between the mtDNA genomes of HT1, HT2 and HT3 are in any way connected to *A. lythri*'s phenotype still requires further research. It would be interesting to generate mitochondrial maps of each HT, compare them with one another, and determine whether the different mitochondria can be given as part of the explanation as to why HT1 of *A. lythri* switched to gynogenetic reproduction.

7.12 Are selfish elements involved in the female bias found in *A. lythri*?

Apart from cytoplasmically inherited endosymbionts like *Wolbachia*, *Rickettsia*, *Spiroplasma*, and *Cardinium*, selfish elements on sex chromosomes (Hodson and Perlman, 2019) have to be considered when addressing the strong sex ratio distortion in *A. lythri*'s HT1. Selfish elements are genes that corrupt the regular inheritance rules to increase their own transmission. Together with genomic conflict, they have a massive impact on species evolution (Burt and Trivers, 2006; Rice, 2013), the evolution of sex determination (Hurst and Werren, 2001; Bachtrog *et al.*, 2014), and meiosis (Fishman and Willis, 2005; Malik and Bayes, 2006). When such selfish genetic elements are located on sex chromosomes, they commonly lead to the elimination of gametes that harbour the alternate sex chromosome in the heterogametic sex, pushing the sex ratio of their host into a strong female bias (Jaenike, 2001).

The HT1 of *A. lythri* shows a strong sex ratio distortion with an extreme female bias. The underlying mechanism that ultimately causes this female bias is gynogenesis as discussed

above, however, the ultimate cause that triggers gynogenesis is not yet understood. Selfish genetic elements located on the X chromosome can be discussed as a possible cause for it, together with infection with reproduction-manipulating endosymbionts and mitonuclear interactions.

The booklouse species *Liposcelis* sp. is known to harbour a selfish genetic element (Perlman *et al.*, 2015; Hamilton *et al.*, 2018). This species is highly female-biased and includes two types of females; nondistorter and distorter females that carry a maternally transmitted selfish genetic element that leads to the production of only female progeny. The all-female offspring inherit this element (Hodson and Perlman, 2019), effectively genetically isolating the distorter from the nondistorter females (Hamilton *et al.*, 2018). Although infected with *Rickettsia* (Yusuf and Turner, 2004; Perlman, Hunter and Zchori-Fein, 2006; Behar, McCormick and Perlman, 2010), the distorter females reproduce sexually and the microbial infection has been described to not be the cause of the strong sex ratio distortion in this species (Perlman *et al.*, 2015).

Furthermore, next to the selfish genetic elements located on the X chromosome of distorter females, these individuals also harbour several additional genes that could not be detected in nondistorter females (Hamilton *et al.*, 2018). It has been discussed that part of these genes has been horizontally transferred from the *Wolbachia* genome to the distorter female genome (Hamilton *et al.*, 2018; Hodson and Perlman, 2019), a characteristic that would be interesting to determine for *A. lythri*. The HT1* is a variant of HT1 of *A. lythri* with only minor sequence changes in the mitochondrial genome (Jäckel, Mora and Dobler, 2013). The reproductive phenotype corresponds to that found in HT1; the females of HT1* exclusively produce female offspring. This HT, however, is very rarely infected with *Wolbachia*. It is therefore possible that HT1* might have once been infected with reproduction-manipulating bacteria that have then horizontally transferred genes responsible for reproduction manipulation to the HT1* genome before this HT lost the parasites. This would explain the extreme sex ratio distortion in this HT, which may be in part connected to their *Wolbachia* infection with the strain *w*LytA1. This hypothesis is being tested by Cen Zeng in her PhD thesis, in which she is searching for integrated *Wolbachia* genes in the HT1* genome.

8 Conclusion

The reproduction of the flea beetle *A. lythri* shows anomalies in one of its mtDNA HTs. Whereas HT2 and HT3 reproduce sexually and produce both male and female offspring in equal ratio, HT1 shows a strong sex ratio distortion with an extreme female bias. The quaddRAD results of this thesis have revealed that HT1 reproduces gynogenetically. With the help of chromosome spreads of unfertilised eggs, I could show that HT1 produces unreduced oocytes with a diploid chromosome set. Gynogenetic females require the sperm merely as a trigger for the start of mitosis and thus the development of the larvae. The male pronucleus, if it enters the gynogenetic egg, gets excluded after its fusion with the egg to retain its already diploid chromosome set that it transfers almost unchanged to the next generation. The paternity analysis using quaddRAD showed a leakage of paternal genetic material into the gynogenetic offspring. This leads to the assumption that the underlying exclusion mechanisms, which are required to exclude the paternal pronucleus from the egg, seem to be faulty. A selective force stabilising this paternal leakage might be the phenomenon of Muller's ratchet, which describes the accumulation of deleterious mutations that ultimately might lead to the extinction of the species if not rectified by sexual recombination.

But what is the underlying mechanism for the gynogenetic reproduction that can be observed in HT1 of *A. lythri*? The unreduced chromosome set of the unfertilised eggs shows that there has to be an alteration in meiosis, but what caused this alteration? Although it is likely, it remains unknown whether the *Wolbachia* infection prevalent in all mtDNA HTs of *A. lythri* is in any way connected to this reproduction anomaly. The strong correlation between the HTs and the strains suggests such a connection. The strong linkage disequilibrium between the *Wolbachia* strain *w*LytA1 and mtDNA HT1 is a sign that, apart from possible different defence mechanisms that have not been uncovered yet, something might be happening on a molecular level that keeps this correlation so prominent. HT2 and HT3 also show *Wolbachia* infections, however, their corresponding strains have slowly intermixed and begun to form double infections that can only be detected in HT2 and HT3. Selfish elements, genes on mitochondria, or hybridisation are further possible explanations that lead to mitonuclear conflicts and can possibly help answer the phenomenon of the origin of gynogenesis in *A. lythri*'s HT1 in the future.

9 Outlook

There are still open questions which, once answered, will help interpret the current results and ultimately understand the reproductive anomalies in *A. lythri*. One of the main open questions that has high potential to help us understand the mechanisms of gynogenesis would be determining the origin of the locus ID 2393. This locus, found in one HT1 family via quaddRAD sequencing, has been detected in the exclusively female offspring and has been entirely inherited from the father in all 19_Bü_114 x 19_Bü_170 offspring. It would be interesting to test the hypothesis whether this locus is part of a telomeric region of a male chromosome, which could have broken off in the sometimes-faulty mechanism which excludes the paternal chromosomes from the unreduced female gamete, as has been described in literature. With a well annotated genome, it would be possible to locate the chromosome this locus is based on. Blasting this sequence against similar genomes already available might be problematic, as quaddRAD sequences are very short and could thus map unspecifically in the blast search.

Furthermore, to fully understand what is happening during meiosis in HT1 female gametes, determining the chromosome counts at various stages of meiosis would be vital. However, this is a difficult task to fulfil and might only be possible with a suitable protocol and maybe even devices that help to fix the oocyte in one place to ensure that it would stay secure during the fixation procedure. If that were to be successful, and the undeveloped eggs would be taken out of an unfertilised female, it might be possible to remove any additional tissue surrounding the undeveloped eggs and fix them without further damaging the gametes. If possible, micromanipulators could be used to hold the oocyte in one place while fixing it (Palermo, 1992). Bogolyubov (2007) fixed oocytes of the flour beetle *Tenebrio molitor* to prepare them for microinjections with fluorescence-inducting anti-RNA.

While no major differences have been detected when the HT1 chromosomes of the unfertilised egg have been compared to those of an HT2 egg, it would be helpful to use telomere-binding antibodies to fully determine the structure of the chromosomes. Because HT2 reproduces sexually, which is both supported by this HT producing an equal sex ratio in their offspring as well as the even distribution of inheritance types in the quaddRAD data presented here, I concluded that the chromosomes in this HT's unfertilised egg have to be present as single chromatids. The eggs are arrested in metaphase II and only resume the second meiosis, and later the first mitotic division, once a male pronucleus has fused with the

egg. Thus, finding no visible differences between the HT1 and HT2 chromosomes at a 1000-times magnification, I drew the conclusion that the HT1 chromosomes are also present as single chromatids, albeit twice as many due to the missing reduction during meiosis. Telomeric antibodies would further underline this assumption and we would be able to determine the definite constitution of the HT1 chromosomes at this stage of the egg.

To check the chromosomes for possible differences in their methylation, whole-genome bisulphite sequencing can be performed to determine the methylcytosine content in HT1 eggs and HT2 or HT3 sperm. If differences in such would be found, it might help to come closer to the answer of how egg recognises the male chromosomes as foreign. To check the hypothesis of the possible fate of the male pronucleus as a polar body, female- and males-specific markers combined with FISH would help to get an idea of what actually happens with the male pronucleus.

Furthermore, it would be interesting to see whether the phenotype of exclusively female offspring can be altered by treating multiple generations of the beetles with the antibiotic tetracycline. This would test the hypothesis that *Wolbachia* is directly involved in this changed reproduction. To either exclude or support mitochondria as a possible cause for any reproduction anomalies, as discussed earlier, mitochondrial maps of each mtDNA HT can be generated and compared with one another. That way, differences in the number, types, and order of genes, promotor regions, as well as the size of the mitochondrial genome between the HTs can be determined.

Another possible way of addressing the strong female bias and its ultimate cause might be to look at the sister species of *A. lythri*, *A. oleracea*. Beetles of this species harbour only one mtDNA HT, but also show a strong sex ratio distortion with a high female bias. However, in this species, 9% of the produced offspring is male. It would be interesting to see what distinguishes the females producing both males and females from those that only produce females. Is it something similar as has been found in the booklouse *L. bostrychophila* that has both distorter and nondistorter females in their populations or do the females that exclusively produce female progeny harbour *Wolbachia*-derived genes that they have acquired via horizontal gene transfer?

A lot of questions remain open thus far, but this PhD project has helped to get closer to understanding what is happening in the mtDNA HT1 of *A. lythri*. In this thesis, I have shown that HT1 of *A. lythri* reproduces asexually via gynogenesis, while HT2 and HT3 reproduce

sexually. This is on a par with the observed phenotype of the exclusively female offspring of HT1 and the equal sex ratio of that of HT2 and HT3. I have detected paternal leakage in the gynogenetic HT1. I have shown that the unfertilised oocytes of HT1 are unreduced with a diploid chromosome count of 24, while HT2 showed a reduced chromosome set of 12 chromosomes. However, to fully answer the open questions listed above, more research needs to be conducted.

10 Bibliography

Abbott, R. *et al.* (2013) 'Hybridization and speciation', *Journal of Evolutionary Biology*, 26(2), pp. 229–246. Available at: https://doi.org/10.1111/j.1420-9101.2012.02599.x.

Ables, E.T. (2015) '*Drosophila* oocytes as a model for understanding meiosis: An educational primer to accompany "Corolla Is a novel protein that contributes to the architecture of the synaptonemal complex of *Drosophila*"', *Genetics Society of America*, 199(1), pp. 17–23. Available at: https://doi.org/10.1534/genetics.114.167940.

Altshuler, D. *et al.* (2000) 'An SNP map of the human genome generated by reduced representation shotgun sequencing', *Nature*, 407(6803), pp. 513–516. Available at: https://doi.org/10.1038/35035083.

Amores, A. *et al.* (2011) 'Genome evolution and meiotic maps by massively parallel DNA sequencing: Spotted gar, an outgroup for the teleost genome duplication', *Genetics*, 188(4), pp. 799–808. Available at: https://doi.org/10.1534/genetics.111.127324.

Babcock, C.S. and Asmussen, M.A. (1996) 'Effects of differential selection in the sexes on cytonuclear polymorphism and disequilibria', *Genetics*, 144(2), pp. 839–853. Available at: https://doi.org/10.1093/genetics/144.2.839.

Babcock, C.S. and Asmussen, M.A. (1998) 'Effects of differential selection in the sexes on cytonuclear dynamics: Life stages with sex differences', *Genetics*, 149(4), pp. 2063–2077. Available at: https://doi.org/10.1093/genetics/149.4.2063.

Bachtrog, D. *et al.* (2014) 'Sex determination: Why so many ways of doing It?', *PLoS Biology*, 12(7), p. e1001899. Available at: https://doi.org/10.1371/journal.pbio.1001899.

Baird, N.A. *et al.* (2008) 'Rapid SNP discovery and genetic mapping using sequenced RAD markers', *PLoS ONE*. Edited by J.C. Fay, 3(10), p. e3376. Available at: https://doi.org/10.1371/journal.pone.0003376.

Ballad, J.W.O. *et al.* (1996) 'Reduced variation in *Drosophila simulans* mitochondrial DNA', *Genetics*, 144(4), pp. 1519–1528. Available at: https://doi.org/10.1093/genetics/144.4.1519.

Barton, N.H. and Hewitt, G.M. (1985) 'Analysis of hybrid zones', *Annual Review of Ecology and Systematics*, 16(1), pp. 113–148. Available at: https://doi.org/10.1146/annurev.es.16.110185.000553.

Bazin, E., Glémin, S. and Galtier, N. (2006) 'Population size does not influence mitochondrial genetic diversity in animals', *Science*, 312(5773), pp. 570–572. Available at: https://doi.org/10.1126/science.1122033.

Becking, T. *et al.* (2017) 'Diversity and evolution of sex determination systems in terrestrial isopods', *Scientific Reports*, 7(1), p. 1084. Available at: https://doi.org/10.1038/s41598-017-01195-4.

Behar, A., McCormick, L.J. and Perlman, S.J. (2010) '*Rickettsia felis* infection in a common household insect pest, *Liposcelis bostrychophila* (Psocoptera: Liposcelidae)', *Applied and Environmental Microbiology*, 76(7), pp. 2280–2285. Available at: https://doi.org/10.1128/AEM.00026-10.

Belote, J.M. and Baker, B.S. (1982) 'Sex determination in *Drosophila melanogaster*: analysis of transformer-2, a sex-transforming locus.', *Proceedings of the National Academy of Sciences*, 79(5), pp. 1568–1572. Available at: https://doi.org/10.1073/pnas.79.5.1568.

Bengtsson, B.O. (2009) 'Asex and evolution: A very large-scale overview', in I. Schön, K. Martens, and P. Dijk (eds) *Lost Sex*. Dordrecht: Springer Netherlands, pp. 1–19. Available at: https://doi.org/10.1007/978-90-481-2770-2_1.

Berthier, P., Excoffier, L. and Ruedi, M. (2006) 'Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*', *Proceedings of the Royal Society B: Biological Sciences*, 273(1605), pp. 3101–3123. Available at: https://doi.org/10.1098/rspb.2006.3680.

Beukeboom, L.W. (2012) 'Microbial manipulation of host sex determination: Endosymbiotic bacteria can directly manipulate their host's sex determination towards the production of female offspring', *BioEssays*, 34(6), pp. 484–488. Available at: https://doi.org/10.1002/bies.201100192.

Beukeboom, L.W., Kamping, A. and van de Zande, L. (2007) 'Sex determination in the

haplodiploid wasp *Nasonia vitripennis* (Hymenoptera: Chalcidoidea): A critical consideration of models and evidence', *Seminars in Cell & Developmental Biology*, 18(3), pp. 371–378. Available at: https://doi.org/10.1016/j.semcdb.2006.12.015.

Beukeboom, L.W. and Perrin, N. (2014) *The Evolution of Sex Determination*. 1st edn. Oxford University Press.

Beukeboom, L.W. and Vrijenhoek, R.C. (1998) 'Evolutionary genetics and ecology of sperm-dependent parthenogenesis', *Journal of Evolutionary Biology*, 11(6), pp. 755–782. Available at: https://doi.org/10.1046/j.1420-9101.1998.11060755.x.

Bird, A.P. and Wolffe, A.P. (1999) 'Methylation-induced repression— Belts, braces, and chromatin', *Cell*, 99(5), pp. 451–454. Available at: https://doi.org/10.1016/S0092-8674(00)81532-9.

Birky, C.W. (1995) 'Uniparental inheritance of mitochondrial and chloroplast genes: Mechanisms and evolution', *Proceedings of the National Academy of Sciences*, 92(25), pp. 11331–11338. Available at: https://doi.org/10.1073/pnas.92.25.11331.

Birky, C.W. (2001) 'The inheritance of genes in mitochondria and chloroplasts: Laws, mechanisms, and models', *Annual Review of Genetics*, 35(1), pp. 125–148. Available at: https://doi.org/10.1146/annurev.genet.35.102401.090231.

Blackmon, H., Ross, L. and Bachtrog, D. (2017) 'Sex determination, sex chromosomes, and karyotype evolution in insects', *Journal of Heredity*, 108(1), pp. 78–93. Available at: https://doi.org/10.1093/jhered/esw047.

Blower, M.D. and Karpen, G.H. (2001) 'The role of *Drosophila* CID in kinetochore formation, cell-cycle progression and heterochromatin interactions', *Nature Cell Biology*, 3, pp. 730–739.

Bogolyubov, D. (2007) 'Localization of RNA transcription sites in insect oocytes using microinjections of 5-bromouridine 5'-triphosphate', *Folia Histochemica Et Cytobiologica*, 45(2), pp. 129–134.

van den Boogaart, K.G., Tolosana-Delgado, R. and Bren, M. (2008) 'compositions: Compositional data analysis', *CRAN* [Preprint].

Boore, J.L. (1999) 'Animal mitochondrial genomes', *Nucleic Acids Research*, 27(8), pp. 1767–1780. Available at: https://doi.org/10.1093/nar/27.8.1767.

Bopp, D., Saccone, G. and Beye, M. (2014) 'Sex determination in insects: Variations on a common theme', *Sexual Development*, 8(1–3), pp. 20–28. Available at: https://doi.org/10.1159/000356458.

Bordenstein, S.R., O'Hara, F.P. and Werren, J.H. (2001) '*Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*', *Nature*, 409(6821), pp. 707–710. Available at: https://doi.org/10.1038/35055543.

Borsa, P. and Kjellberg, F. (1996) 'Experimental evidence for pseudo-arrhenotoky in *Hypothenemus hampei* (Coleoptera: Scolytidae)', *Heredity*, 76(2), pp. 130–135. Available at: https://doi.org/10.1038/hdy.1996.20.

Boucher, S., Dutrillaux, A.-M. and Dutrillaux, B. (2015) 'Parthenogenetic reproduction demonstrated in the diploid Spasalus puncticollis (Le Peletier & Serville 1825), n. stat., from the Antilles (Coleoptera, Scarabaeoidea, Passalidae)', *Comptes Rendus Biologies*, 338(11), pp. 738–744. Available at: https://doi.org/10.1016/j.crvi.2015.09.001.

Bouchon, D., Cordaux, R. and Grève, P. (2008) 'Feminizing *Wolbachia* and the evolution of sex determination in isopods', in K. Bourtzis and T. Miller (eds) *Insect Symbiosis, Volume 3*. CRC Press (Contemporary Topics in Entomology), pp. 273–294. Available at: https://doi.org/10.1201/9781420064117.ch12.

Breeuwer, J.A.J. and Werren, J.H. (1990) 'Microorganisms associated with chromosome destruction and reproductive isolation between two insect species', *Nature*, 346(6284), pp. 558–560. Available at: https://doi.org/10.1038/346558a0.

Breeuwer, J.A.J. and Werren, J.H. (1993) 'Effect of genotype on cytoplasmic incompatibility between two species of *Nasonia*', *Heredity*, 70(4), pp. 428–436. Available at: https://doi.org/10.1038/hdy.1993.60.

Bretagnolle, F. and Thompson, J.D. (1995) 'Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants', *New Phytologist*, 129(1), pp. 1–22. Available at: https://doi.org/10.1111/j.1469-

8137.1995.tb03005.x.

Brohede, J. (2002) 'Heterogeneity in the rate and pattern of germline mutation at individual microsatellite loci', *Nucleic Acids Research*, 30(9), pp. 1997–2003. Available at: https://doi.org/10.1093/nar/30.9.1997.

Brumfield, R.T. *et al.* (2003) 'The utility of single nucleotide polymorphisms in inferences of population history', *Trends in Ecology & Evolution*, 18(5), pp. 249–256. Available at: https://doi.org/10.1016/S0169-5347(03)00018-1.

Burghardt, G. *et al.* (2005) 'The transformer2 gene in *Musca domestica* is required for selecting and maintaining the female pathway of development', *Development Genes and Evolution*, 215(4), pp. 165–176. Available at: https://doi.org/10.1007/s00427-004-0464-7.

Burt, A. and Trivers, R. (2006) *Genes in Conflict: The Biology of Selfish Genetic Elements*. Harvard University Press. Available at: https://doi.org/10.4159/9780674029118.

Camus, M.F., Clancy, D.J. and Dowling, D.K. (2012) 'Mitochondria, maternal inheritance, and male aging', *Current Biology*, 22(18), pp. 1717–1721. Available at: https://doi.org/10.1016/j.cub.2012.07.018.

Carling, M.D. and Brumfield, R.T. (2007) 'Gene sampling strategies for multi-locus population estimates of genetic diversity (θ)', *PLoS ONE*. Edited by K. Scheffler, 2(1), p. e160. Available at: https://doi.org/10.1371/journal.pone.0000160.

Carson, H.L. (1967) 'Selection for parthenogenesis in *Drosophila mercatorum*', *Genetics*, 55(1), pp. 157–171. Available at: https://doi.org/10.1093/genetics/55.1.157.

Carvalho, M. *et al.* (2017) 'Genetic diversity and structure of Iberian Peninsula cowpeas compared to world-wide cowpea accessions using high density SNP markers', *BMC Genomics*, 18(1), p. 891. Available at: https://doi.org/10.1186/s12864-017-4295-0.

Casbon, J.A. *et al.* (2011) 'A method for counting PCR template molecules with application to next-generation sequencing', *Nucleic Acids Research*, 39(12), pp. e81–e81. Available at: https://doi.org/10.1093/nar/gkr217.

Catchen, J. *et al.* (2013) 'The population structure and recent colonization history of Oregon

threespine stickleback determined using restriction-site associated DNA-sequencing', *Molecular Ecology*, 22(11), pp. 2864–2883. Available at: https://doi.org/10.1111/mec.12330.

Catchen, J.M. *et al.* (2011) '*Stacks* : Building and genotyping loci *de novo* from short-read sequences', *G3 Genes*|*Genomes*|*Genetics*, 1(3), pp. 171–182. Available at: https://doi.org/10.1534/g3.111.000240.

Cesare, A.J., Heaphy, C.M. and O'Sullivan, R.J. (2015) 'Visualization of telomere integrity and function *in vitro* and *in vivo* using immunofluorescence techniques', *Current Protocols in Cytometry*, 73(1). Available at: https://doi.org/10.1002/0471142956.cy1240s73.

Choi, T. *et al.* (1996) 'The Mos/mitogen-activated protein kinase (MAPK) pathway regulates the size and degradation of the first polar body in maturing mouse oocytes', *Proceedings of the National Academy of Sciences*, 93(14), pp. 7032–7035. Available at: https://doi.org/10.1073/pnas.93.14.7032.

Çilingir, F.G. *et al.* (2017) 'Conservation genomics of the endangered Burmese roofed turtle: Conservation Genomics of *Batagur trivittata*', *Conservation Biology*, 31(6), pp. 1469–1476. Available at: https://doi.org/10.1111/cobi.12921.

Cline, T.W. and Meyer, B.J. (1996) 'VIVE LA DIFFÉRENCE: Males vs females in flies vs worms', *Annual Review of Genetics*, 30(1), pp. 637–702. Available at: https://doi.org/10.1146/annurev.genet.30.1.637.

Compton, A. and Tu, Z. (2022) 'Natural and engineered sex ratio distortion in insects', *Frontiers in Ecology and Evolution*, 10, p. 884159. Available at: https://doi.org/10.3389/fevo.2022.884159.

Concha, C. and Scott, M.J. (2009) 'Sexual development in *Lucilia cuprina* (Diptera, Calliphoridae) is controlled by the Transformer gene', *Genetics*, 182(3), pp. 785–798. Available at: https://doi.org/10.1534/genetics.109.100982.

Conrad, T. and Akhtar, A. (2012) 'Dosage compensation in *Drosophila melanogaster*: epigenetic fine-tuning of chromosome-wide transcription', *Nature Reviews Genetics*, 13(2), pp. 123–134. Available at: https://doi.org/10.1038/nrg3124.

Cordaux, R., Bouchon, D. and Grève, P. (2011) 'The impact of endosymbionts on the evolution of host sex-determination mechanisms', *Trends in Genetics*, 27(8), pp. 332–341. Available at: https://doi.org/10.1016/j.tig.2011.05.002.

Craig, D.W. *et al.* (2008) 'Identification of genetic variants using bar-coded multiplexed sequencing', *Nature Methods*, 5(10), pp. 887–893. Available at: https://doi.org/10.1038/nmeth.1251.

Craig, G.B., Hickey, W.A. and VandeHey, R.C. (1960) 'An inherited male-producing factor in *Aedes aegypti*', *Science*, 132(3443), pp. 1887–1889. Available at: https://doi.org/10.1126/science.132.3443.1887.

Cuellar, O. (1976) 'Cytology of meiosis in the triploid gynogenetic salamander *Ambystoma tremblayi*', *Chromosoma*, 58(4), pp. 355–364. Available at: https://doi.org/10.1007/BF00292843.

Curradi, M. *et al.* (2002) 'Molecular mechanisms of gene silencing mediated by DNA methylation', *Molecular and Cellular Biology*, 22(9), pp. 3157–3173. Available at: https://doi.org/10.1128/MCB.22.9.3157-3173.2002.

Deng, W. *et al.* (2003) 'A new method for improving metaphase chromosome spreading', *Cytometry*, 51A(1), pp. 46–51. Available at: https://doi.org/10.1002/cyto.a.10004.

Dimijian, G.G. (2005) 'Evolution of sexuality: Biology and behavior', *Baylor University Medical Center Proceedings*, 18(3), pp. 244–258. Available at: https://doi.org/10.1080/08998280.2005.11928075.

Dowling, D.K. *et al.* (2007) 'Intergenomic epistasis for fitness: Within-population interactions between cytoplasmic and nuclear genes in *Drosophila melanogaster*', *Genetics*, 175(1), pp. 235–244. Available at: https://doi.org/10.1534/genetics.105.052050.

D'Souza, T.G. *et al.* (2004) 'Occasional sex in an "asexual" polyploid hermaphrodite', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1543), pp. 1001–1007. Available at: https://doi.org/10.1098/rspb.2004.2675.

Duron, O. *et al.* (2008) 'The diversity of reproductive parasites among arthropods: *Wolbachia*

do not walk alone', *BMC Biology*, 6(1), p. 27. Available at: https://doi.org/10.1186/1741-7007-6-27.

Emerson, K.J. *et al.* (2010) 'Resolving postglacial phylogeography using high-throughput sequencing', *Proceedings of the National Academy of Sciences*, 107(37), pp. 16196–16200. Available at: https://doi.org/10.1073/pnas.1006538107.

Engelstädter, J. and Hurst, G.D.D. (2009) 'The ecology and evolution of microbes that manipulate host reproduction', *Annual Review of Ecology, Evolution, and Systematics*, 40(1), pp. 127–149. Available at: https://doi.org/10.1146/annurev.ecolsys.110308.120206.

Erickson, J.W. and Quintero, J.J. (2007) 'Indirect effects of ploidy suggest X chromosome dose, not the X:A ratio, signals sex in *Drosophila*', *PLoS Biology*. Edited by P. Becker, 5(12), p. e332. Available at: https://doi.org/10.1371/journal.pbio.0050332.

Fan, Z. and Shen, J. (1990) 'Studies on the evolution of bisexual reproduction in crucian carp (*Carassius auratus gibelio* Bloch)', *Aquaculture*, 84(3–4), pp. 235–244. Available at: https://doi.org/10.1016/0044-8486(90)90089-6.

Felsenstein, J. (2006) 'Accuracy of coalescent likelihood estimates: Do we need more sites, more sequences, or more loci?', *Molecular Biology and Evolution*, 23(3), pp. 691–700. Available at: https://doi.org/10.1093/molbev/msj079.

Field, L.M. *et al.* (2004) 'DNA methylation in insects', *Insect Molecular Biology*, 13(2), pp. 109–115. Available at: https://doi.org/10.1111/j.0962-1075.2004.00470.x.

Fisher, R.A. (1930) *The genetical theory of natural selection*. Oxford: Claredon Press.

Fishman, L. and Willis, J.H. (2005) 'A novel meiotic drive locus almost completely distorts segregation in *Mimulus* (monkeyflower) hybrids', *Genetics*, 169(1), pp. 347–353. Available at: https://doi.org/10.1534/genetics.104.032789.

Franchini, P. *et al.* (2017) 'quaddRAD: a new high-multiplexing and PCR duplicate removal ddRAD protocol produces novel evolutionary insights in a nonradiating cichlid lineage', *Molecular Ecology*, 26(10), pp. 2783–2795. Available at: https://doi.org/10.1111/mec.14077.

Frank, S.A. and Hurst, L.D. (1996) 'Mitochondria and male disease', *Nature*, 383(6597), pp.

224–224. Available at: https://doi.org/10.1038/383224a0.

Fukui, T. *et al.* (2015) 'The endosymbiotic bacterium *Wolbachia* selectively kills male hosts by targeting the masculinizing gene', *PLOS Pathogens*. Edited by E.A. McGraw, 11(7), p. e1005048. Available at: https://doi.org/10.1371/journal.ppat.1005048.

Furth, D.G. (1994) 'A new case of parthenogenesis in beetles: *Longitarsus melanurus* (Melsheimer) (Coleoptera: Chrysomelidae)', *Journal of the New York Entomological society*, 102(3), pp. 310–317.

Gabriel, W., Lynch, M. and Bürger, R. (1993) 'Muller's ratchet and mutational meltdowns', *Evolution*, 47(6), pp. 1744–1757. Available at: https://doi.org/10.1111/j.1558-5646.1993.tb01266.x.

Galis, F. and Alphen, J.J.M. (2020) 'Parthenogenesis and developmental constraints', *Evolution & Development*, 22(1–2), pp. 205–217. Available at: https://doi.org/10.1111/ede.12324.

Gemmell, N.J., Metcalf, V.J. and Allendorf, F.W. (2004) 'Mother's curse: the effect of mtDNA on individual fitness and population viability', *Trends in Ecology & Evolution*, 19(5), pp. 238–244. Available at: https://doi.org/10.1016/j.tree.2004.02.002.

Gemmell, N.J. and Sin, F.Y.T. (2002) 'Mitochondrial mutations may drive Y chromosome evolution', *BioEssays*, 24(3), pp. 275–279. Available at: https://doi.org/10.1002/bies.10062.

Gerton, J.L. and Hawley, R.S. (2005) 'Homologous chromosome interactions in meiosis: diversity amidst conservation', *Nature Reviews Genetics*, 6(6), pp. 477–487. Available at: https://doi.org/10.1038/nrg1614.

Glastad, K.M. *et al.* (2011) 'DNA methylation in insects: on the brink of the epigenomic era: DNA methylation in insects', *Insect Molecular Biology*, 20(5), pp. 553–565. Available at: https://doi.org/10.1111/j.1365-2583.2011.01092.x.

Goddard, K.A. and Dawley, R.M. (1990) 'Clonal inheritance of a diploid nuclear genome by a hybrid freshwater minnow (*Phoxinus eos-neogaeus*, Pisces: Cyprinidae)', *Evolution*, 44(4), pp. 1052–1065. Available at: https://doi.org/10.1111/j.1558-5646.1990.tb03825.x.

Goddard, K.A. and Schultz, R.J. (1993) 'Aclonal reproduction by polyploid members of the clonal hybrid species *Phoxinus eos-neogaeus* (Cyprinidae)', *Copeia*, 1993(3), p. 650. Available at: https://doi.org/10.2307/1447226.

Good, J.M. *et al.* (2008) 'Ancient hybridization and mitochondrial capture between two species of chipmunks', *Molecular Ecology*, 17(5), pp. 1313–1327. Available at: https://doi.org/10.1111/j.1365-294X.2007.03640.x.

Goodrich, B. *et al.* (2022) 'rstanarm: Bayesian applied regression modeling via Stan. R package version 2.21.3', *CRAN* [Preprint].

Goto, S., Anbutsu, H. and Fukatsu, T. (2006) 'Asymmetrical interactions between *Wolbachia* and *Spiroplasma* endosymbionts coexisting in the same insect host', *Applied and Environmental Microbiology*, 72(7), pp. 4805–4810. Available at: https://doi.org/10.1128/AEM.00416-06.

Gottlieb, Y. *et al.* (2002) 'Diploidy restoration in *Wolbachia*-infected *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae)', *Journal of Invertebrate Pathology*, 81(3), pp. 166–174. Available at: https://doi.org/10.1016/S0022-2011(02)00149-0.

Grebelnyi, S.D. (2009) 'Unisexual reproduction: Either maternal or paternal inheritance', *Russian Journal of Developmental Biology*, 40(3), pp. 145–149. Available at: https://doi.org/10.1134/S1062360409030023.

Grossman, L.I. *et al.* (2004) 'Accelerated evolution of the electron transport chain in anthropoid primates', *Trends in Genetics*, 20(11), pp. 578–585. Available at: https://doi.org/10.1016/j.tig.2004.09.002.

Gruss, O. (2018) 'Animal female meiosis: The challenges of eliminating centrosomes', *Cells*, 7(7), p. 73. Available at: https://doi.org/10.3390/cells7070073.

Hagimori, T. *et al.* (2006) 'The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects', *Current Microbiology*, 52(2), pp. 97–101. Available at: https://doi.org/10.1007/s00284-005-0092-0.

Hamada, F.N. *et al.* (2005) 'Global regulation of X chromosomal genes by the MSL complex in

Drosophila melanogaster', *Genes & Development*, 19(19), pp. 2289–2294. Available at: https://doi.org/10.1101/gad.1343705.

Hamilton, P.T. *et al.* (2018) 'Genetics and genomics of an unusual selfish sex ratio distortion in an insect', *Current Biology*, 28(23), pp. 3864–3870. Available at: https://doi.org/10.1016/j.cub.2018.10.035.

Hamilton, W.D. (1967) 'Extraordinary sex ratios: A sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology.', *Science*, 156(3774), pp. 477–488. Available at: https://doi.org/10.1126/science.156.3774.477.

Harumoto, T., Fukatsu, T. and Lemaitre, B. (2018) 'Common and unique strategies of male killing evolved in two distinct *Drosophila* symbionts', *Proceedings of the Royal Society B: Biological Sciences*, 285(1875), p. 20172167. Available at: https://doi.org/10.1098/rspb.2017.2167.

Hasselmann, M. *et al.* (2008) 'Evidence for the evolutionary nascence of a novel sex determination pathway in honeybees', *Nature*, 454(7203), pp. 519–522. Available at: https://doi.org/10.1038/nature07052.

Hedges, S.B., Bogart, J.P. and Maxson, L.R. (1992) 'Ancestry of unisexual salamanders', *Nature*, 356(6371), pp. 708–710. Available at: https://doi.org/10.1038/356708a0.

Hediger, M. *et al.* (2010) 'Molecular characterization of the key switch *F* provides a basis for understanding the rapid divergence of the sex-determining pathway in the housefly', *Genetics*, 184(1), pp. 155–170. Available at: https://doi.org/10.1534/genetics.109.109249.

Henikoff, S. *et al.* (2000) 'Heterochromatic deposition of centromeric histone H3-like proteins', *Proceedings of the National Academy of Sciences*, 97(2), pp. 716–721. Available at: https://doi.org/10.1073/pnas.97.2.716.

Hernández-Rangel, S., M. *et al.* (2018) 'A genomic toolbox for population assignment and monitoring of a conservation dependent Amazonian crocodylian (*Melanosuchus niger*: Alligatoridae: Crocodylia)', *Pan-American Journal of Aquatic Sciences*, 13(2), pp. 179–187.

Hertig, M. and Wolbach, S.B. (1924) 'Studies on *Rickettsia*-like microorganisms in insects', *The*

Journal of Medical Research, 44(3), pp. 329-374.7.

Hilgenboecker, K. *et al.* (2008) 'How many species are infected with *Wolbachia*? – a statistical analysis of current data: *Wolbachia* infection rates', *FEMS Microbiology Letters*, 281(2), pp. 215–220. Available at: https://doi.org/10.1111/j.1574-6968.2008.01110.x.

Hill, G.E. (2016) 'Mitonuclear coevolution as the genesis of speciation and the mitochondrial DNA barcode gap', *Ecology and Evolution*, 6(16), pp. 5831–5842. Available at: https://doi.org/10.1002/ece3.2338.

Hodgsen, A.N. (2009) 'Reproduction and sex in invertebrates', in. EoLSS, pp. 1–27.

Hodson, C.N. *et al.* (2017) 'Paternal genome elimination in *Liposcelis* booklice (Insecta: Psocodea)', *Genetics*, 206(2), pp. 1091–1100. Available at: https://doi.org/10.1534/genetics.117.199786.

Hodson, C.N. and Perlman, S.J. (2019) 'Population biology of a selfish sex ratio distorting element in a booklouse (Psocodea: *Liposcelis*)', *Journal of Evolutionary Biology*, 32(8), pp. 825–832. Available at: https://doi.org/10.1111/jeb.13484.

Hohenlohe, P.A. *et al.* (2011) 'Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout', *Molecular Ecology Resources*, 11, pp. 117–122. Available at: https://doi.org/10.1111/j.1755-0998.2010.02967.x.

Hubbs, C.L. and Hubbs, L.C. (1932) 'Apparent parthenogenesis in nature, in a form of fish of hybrid origin', *Science*, 76(1983), pp. 628–630.

Huigens, M.E. and Stouthamer, R. (2003) 'Parthenogenesis Associated with *Wolbachia*', in K. Bourtzis and T.A. Miller (eds) *Insect Symbiosis*. 0 edn. CRC Press, pp. 265–284. Available at: https://doi.org/10.1201/9780203009918-20.

Hurst, G. D. D. *et al.* (1999) 'Invasion of one insect species, *Adalia bipunctata*, by two different male-killing bacteria', *Insect Molecular Biology*, 8(1), pp. 133–139. Available at: https://doi.org/10.1046/j.1365-2583.1999.810133.x.

Hurst, Gregory D. D. *et al.* (1999) 'Male–killing *Wolbachia* in two species of insect',

Proceedings of the Royal Society of London. Series B: Biological Sciences, 266(1420), pp. 735–740. Available at: https://doi.org/10.1098/rspb.1999.0698.

Hurst, G.D.D. and Jiggins, F.M. (2005) 'Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts', *Proceedings of the Royal Society B: Biological Sciences*, 272(1572), pp. 1525–1534. Available at: https://doi.org/10.1098/rspb.2005.3056.

Hurst, G.D.D. and Werren, J.H. (2001) 'The role of selfish genetic elements in eukaryotic evolution', *Nature Reviews Genetics*, 2(8), pp. 597–606. Available at: https://doi.org/10.1038/35084545.

Hurst, L. (1991) 'The incidences and evolution of cytoplasmic male killers', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 244(1310), pp. 91–99. Available at: https://doi.org/10.1098/rspb.1991.0056.

Innocenti, P., Morrow, E.H. and Dowling, D.K. (2011) 'Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution', *Science*, 332(6031), pp. 845–848. Available at: https://doi.org/10.1126/science.1201157.

Jäckel, R. (2011) *Einfluss von* Wolbachia*-Endosymbionten auf Populationsstruktur, prägame Isolation und Speziationsmechanismen bei* Altica lythri *Aubé, 1843 (Coleoptera, Chrysomelidae)*. Universität Hamburg.

Jäckel, R., Mora, D. and Dobler, S. (2013) 'Evidence for selective sweeps by *Wolbachia* infections: phylogeny of *Altica* leaf beetles and their reproductive parasites', *Molecular Ecology*, 22(16), pp. 4241–4255. Available at: https://doi.org/10.1111/mec.12389.

Jaenike, J. (2001) 'Sex chromosome meiotic drive', *Annual Review of Ecology and Systematics*, 32(1), pp. 25–49. Available at: https://doi.org/10.1146/annurev.ecolsys.32.081501.113958.

Jahnke, M. *et al.* (2013) 'Effects of feminizing microsporidia on the masculinizing function of the androgenic gland in *Gammarus duebeni*', *Journal of Invertebrate Pathology*, 112(2), pp. 146–151. Available at: https://doi.org/10.1016/j.jip.2012.11.008.

Jelić, M. *et al.* (2015) 'Sex-specific effects of sympatric mitonuclear variation on fitness in

Drosophila subobscura', *BMC Evolutionary Biology*, 15(1), p. 135. Available at: https://doi.org/10.1186/s12862-015-0421-2.

Jia, Z.Y. *et al.* (2008) 'Inheritance of microsatellite DNA in bisexual gynogenesis complex of Fangzheng silver crucian carp, *Carassius auratus gibelio* (Bloch)', *Journal of Fish Biology*, 73(5), pp. 1161–1169. Available at: https://doi.org/10.1111/j.1095-8649.2008.01946.x.

Jiang, L. *et al.* (2013) 'Sperm, but not oocyte, DNA methylome Is inherited by zebrafish early embryos', *Cell*, 153(4), pp. 773–784. Available at: https://doi.org/10.1016/j.cell.2013.04.041.

Jiggins, F.M. (2003) 'Male-killing *Wolbachia* and mitochondrial DNA: Selective sweeps, hybrid introgression and parasite population dynamics', *Genetics*, 164(1), pp. 5–12. Available at: https://doi.org/10.1093/genetics/164.1.5.

Kageyama, D. *et al.* (2017) 'Feminizing *Wolbachia* endosymbiont disrupts maternal sex chromosome inheritance in a butterfly species: *Wolbachia* disrupts sex chromosome inheritance', *Evolution Letters*, 1(5), pp. 232–244. Available at: https://doi.org/10.1002/evl3.28.

Kageyama, D., Narita, S. and Watanabe, M. (2012) 'Insect sex determination manipulated by their endosymbionts: Incidences, mechanisms and implications', *Insects*, 3(1), pp. 161–199. Available at: https://doi.org/10.3390/insects3010161.

Kangas, E. and Rutanen, I. (1993) 'Identification of females of the Finnish species of *Altica Müller* (Coleoptera, Chrysomelidae)', *Entomologica Fennica*, 4(2), pp. 115–129. Available at: https://doi.org/10.33338/ef.83755.

Kawakami, T. *et al.* (2007) 'Differential gene flow of mitochondrial and nuclear DNA markers among chromosomal races of Australian morabine grasshoppers (*Vandiemenella, viatica* species group)', *Molecular Ecology*, 16(23), pp. 5044–5056. Available at: https://doi.org/10.1111/j.1365-294X.2007.03572.x.

Kern, P. *et al.* (2015) 'Double trouble: combined action of meiotic drive and *Wolbachia* feminization in *Eurema* butterflies', *Biology Letters*, 11(5), p. 20150095. Available at: https://doi.org/10.1098/rsbl.2015.0095.

Kess, T. *et al.* (2015) 'Low-cost ddRAD method of SNP discovery and genotyping applied to the periwinkle *Littorina saxatilis*', *Journal of Molluscan Studies*, 82, pp. 104–109. Available at: https://doi.org/10.1093/mollus/eyv042.

Khetani, R.S. and Bickel, S.E. (2007) 'Regulation of meiotic cohesion and chromosome core morphogenesis during pachytene in *Drosophila* oocytes', *Journal of Cell Science*, 120(17), pp. 3123–3137. Available at: https://doi.org/10.1242/jcs.009977.

Kitajima, T.S., Kawashima, S.A. and Watanabe, Y. (2004) 'The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis', *Nature*, 427, pp. 510–517.

Kiuchi, T. *et al.* (2014) 'A single female-specific piRNA is the primary determiner of sex in the silkworm', *Nature*, 509(7502), pp. 633–636. Available at: https://doi.org/10.1038/nature13315.

Kleckner, N. (1996) 'Meiosis: how could it work?', *Proceedings of the National Academy of Sciences*, 93(16), pp. 8167–8174. Available at: https://doi.org/10.1073/pnas.93.16.8167.

Kleinman-Ruiz, D. *et al.* (2017) 'Novel efficient genome-wide SNP panels for the conservation of the highly endangered Iberian lynx', *BMC Genomics*, 18(1), p. 556. Available at: https://doi.org/10.1186/s12864-017-3946-5.

Köhler, F. and Deein, G. (2010) 'Hybridisation as potential source of incongruence in the morphological and mitochondrial diversity of a Thai freshwater gastropod (Pachychilidae, Brotia H. Adams, 1866)', *Zoosystematics and Evolution*, 86(2), pp. 301–314. Available at: https://doi.org/10.1002/zoos.201000013.

Köhler, F. and Glaubrecht, M. (2006) 'A systematic revision of the southeast Asian freshwater gastropod *Brotia* (Cerithioidae: Pachychilidae)', *Malacologia*, 48, pp. 159–251.

Kokko, H., Heubel, K.U. and Rankin, D.J. (2008) 'How populations persist when asexuality requires sex: the spatial dynamics of coping with sperm parasites', *Royal Society B: Biological Sciences*, 275(1636), pp. 817–825. Available at: https://doi.org/doi.org/10.1098/rspb.2007.1199.

van der Kooi, C.J., Matthey-Doret, C. and Schwander, T. (2017) 'Evolution and comparative

ecology of parthenogenesis in haplodiploid arthropods', *Evolution Letters*, 1(6), pp. 304–316. Available at: https://doi.org/10.1002/evl3.30.

Kramer, M.G. and Templeton, A.R. (2007) 'Life-history changes that accompany the transition from sexual to parthenogenetic reproduction in *Drosophila mercatorum*', *Evolution*, 55(4), pp. 748–761. Available at: https://doi.org/10.1111/j.0014-3820.2001.tb00811.x.

Lagos, D. *et al.* (2007) 'The transformer gene in *Bactrocera oleae*: the genetic switch that determines its sex fate', *Insect Molecular Biology*, 16(2), pp. 221–230. Available at: https://doi.org/10.1111/j.1365-2583.2006.00717.x.

Lamatsch, D.K. *et al.* (2000) 'Noninvasive determination of genome size and ploidy level in fishes by flow cytometry: detection of triploid *Poecilia formosa*', *Cytometry*, 39(2), pp. 91–95. Available at: https://doi.org/10.1002/(SICI)1097-0320(20000201)39:2<91::AID-CYTO1>3.0.CO;2-4.

Lamatsch, D.K. *et al.* (2011) 'Isolation of a cancer-associated microchromosome in the sperm-dependent parthenogen *Poecilia formosa*', *Cytogenetic and Genome Research*, 135(2), pp. 135–142. Available at: https://doi.org/10.1159/000331271.

Lamatsch, D.K. and Stöck, M. (2009) 'Sperm-Dependent Parthenogenesis and Hybridogenesis in Teleost Fishes', in I. Schön, K. Martens, and P. Dijk (eds) *Lost Sex*. Dordrecht: Springer Netherlands, pp. 399–432. Available at: https://doi.org/10.1007/978-90-481-2770-2_19.

Lampert, K.P. (2008) 'Facultative parthenogenesis in vertebrates: Reproductive error or chance?', *Sexual Development*, 2(6), pp. 290–301. Available at: https://doi.org/10.1159/000195678.

Langley, A.R. *et al.* (2014) 'New insights into the maternal to zygotic transition', *Development*, 141(20), pp. 3834–3841. Available at: https://doi.org/10.1242/dev.102368.

Lehtonen, J. *et al.* (2013) 'Evolutionary and ecological implications of sexual parasitism', *Trends in Ecology & Evolution*, 28(5), pp. 297–306. Available at: https://doi.org/10.1016/j.tree.2012.12.006.

Lehtonen, J., Jennions, M.D. and Kokko, H. (2012) 'The many costs of sex', *Trends in Ecology*

& Evolution, 27(3), pp. 172–178. Available at: https://doi.org/10.1016/j.tree.2011.09.016.

Leung, C. and Angers, B. (2018) 'Imitating the cost of males: A hypothesis for coexistence of all-female sperm-dependent species and their sexual host', *Ecology and Evolution*, 8(1), pp. 266–272. Available at: https://doi.org/10.1002/ece3.3681.

Lin, C.-J. *et al.* (2018) 'The hpRNA/RNAi pathway is essential to resolve intragenomic conflict in the *Drosophila* male germline', *Developmental Cell*, 46(3), pp. 316-326.e5. Available at: https://doi.org/10.1016/j.devcel.2018.07.004.

Linnen, C.R. and Farrell, B.D. (2007) 'Mitonuclear discordance is caused by rampant mitochondrial introgression in *Neodiprion* (Hymenoptera: Piprionidae) sawflies', *Evolution*, 61(6), pp. 1417–1438. Available at: https://doi.org/10.1111/j.1558-5646.2007.00114.x.

Loppin, B. *et al.* (2005) 'The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus', *Nature*, 437(7063), pp. 1386–1390. Available at: https://doi.org/10.1038/nature04059.

Lucchesi, J.C. (1973) 'Dosage compensation in *Drosophila*', *Annual Review of Genetics*, 7(1), pp. 225–237. Available at: https://doi.org/10.1146/annurev.ge.07.120173.001301.

Lynch, M. and Gabriel, W. (1990) 'Mutation load and the survival of small populations', *Evolution*, 44(7), pp. 1725–1737. Available at: https://doi.org/10.1111/j.1558-5646.1990.tb05244.x.

Macgregor, H.C. and Uzzell, T.M. (1964) 'Gynogenesis in salamanders related to *Ambystoma jeffersonianum*', *Science*, 143(3610), pp. 1043–1045. Available at: https://doi.org/10.1126/science.143.3610.1043.

Malik, H.S. and Bayes, J.J. (2006) 'Genetic conflicts during meiosis and the evolutionary origins of centromere complexity', *Biochemical Society Transactions*, 34(4), pp. 569–573. Available at: https://doi.org/10.1042/BST0340569.

Mallet, J. (2005) 'Hybridization as an invasion of the genome', *Trends in Ecology & Evolution*, 20(5), pp. 229–237. Available at: https://doi.org/10.1016/j.tree.2005.02.010.

Masui, Y. and Markert, C.L. (1971) 'Cytoplasmic control of nuclear behavior during meiotic

maturation of frog oocytes', *Journal of Experimental Zoology*, 177(2), pp. 129–145. Available at: https://doi.org/10.1002/jez.1401770202.

Maynard Smith, J. (1978) 'The evolution of sex', *Genetical Research*, 32(3), pp. b1–b9. Available at: https://doi.org/10.1017/S0016672300018693.

Miller, R.R. and Schultz, R.J. (1959) 'All-female strains of the teleost fishes of the genus *Poeciliopsis*', *Science*, 130(3389), pp. 1656–1657. Available at: https://doi.org/10.1126/science.130.3389.1656.b.

Miller, W.J., Ehrman, L. and Schneider, D. (2010) 'Infectious speciation revisited: Impact of symbiont-depletion on female fitness and mating behavior of *Drosophila paulistorum*', *PLoS Pathogens*. Edited by C. Parrish, 6(12), p. e1001214. Available at: https://doi.org/10.1371/journal.ppat.1001214.

Mims, M.C. *et al.* (2010) 'Geography disentangles introgression from ancestral polymorphism in Lake Malawi cichlids', *Molecular Ecology*, 19(5), pp. 940–951. Available at: https://doi.org/10.1111/j.1365-294X.2010.04529.x.

Mirzaghaderi, G. and Hörandl, E. (2016) 'The evolution of meiotic sex and its alternatives', *Proceedings of the Royal Society B: Biological Sciences*, 283(1838), p. 20161221. Available at: https://doi.org/10.1098/rspb.2016.1221.

Mishmar, D. *et al.* (2003) 'Natural selection shaped regional mtDNA variation in humans', *Proceedings of the National Academy of Sciences*, 100(1), pp. 171–176. Available at: https://doi.org/10.1073/pnas.0136972100.

Miyata, M. *et al.* (2017) '*Wolbachia* -induced meiotic drive and feminization is associated with an independent occurrence of selective mitochondrial sweep in a butterfly', *Biology Letters*, 13(5), p. 20170153. Available at: https://doi.org/10.1098/rsbl.2017.0153.

Mogie, M. (1986) 'Automixis: its distribution and status', *Biological Journal of the Linnean Society*, 28(3), pp. 321–329. Available at: https://doi.org/10.1111/j.1095-8312.1986.tb01761.x.

Mohr, K. (1966) 'Chrysomelidae', in *Die Käfer Mitteleuropas*. Spektrum Akademischer Verlag.

Moore, B.P., Woodroffe, G.E. and Sanderson, A.R. (1956) 'Polymorphism and parthenogenesis in a ptinid beetle', *Nature*, 177, pp. 847–848.

Moran, N.A., McCutcheon, J.P. and Nakabachi, A. (2008) 'Genomics and evolution of heritable bacterial symbionts', *Annual Review of Genetics*, 42(1), pp. 165–190. Available at: https://doi.org/10.1146/annurev.genet.41.110306.130119.

Muller, H.J. (1964) 'The relation of recombination to mutational advance', *Mutation Research*, 1, pp. 2–9.

Nachman, M.W. *et al.* (1996) 'Nonneutral mitochondrial DNA variation in humans and chimpanzees', *Genetics*, 142(3), pp. 953–963. Available at: https://doi.org/10.1093/genetics/142.3.953.

Nakanishi, K. *et al.* (2008) 'Novel RNA sequences associated with late male killing in *Homona magnanima*', *Proceedings of the Royal Society B: Biological Sciences*, 275(1640), pp. 1249–1254. Available at: https://doi.org/10.1098/rspb.2008.0013.

Nanda, I. *et al.* (1995) 'Chromosomal evidence for laboratory synthesis of a triploid hybrid between the gynogenetic teleost *Poecilia formosa* and its host species', *Journal of Fish Biology*, 47(4), pp. 619–623. Available at: https://doi.org/10.1111/j.1095-8649.1995.tb01928.x.

Nasmyth, K. (2001) 'Disseminating the genome: Joining, resolving, and separating sister chromatids during mitosis and meiosis', *Annual Review of Genetics*, 35(1), pp. 673–745. Available at: https://doi.org/10.1146/annurev.genet.35.102401.091334.

Neaves, W.B. and Baumann, P. (2011) 'Unisexual reproduction among vertebrates', *Trends in Genetics*, 27(3), pp. 81–88. Available at: https://doi.org/10.1016/j.tig.2010.12.002.

Negri, I. *et al.* (2010) 'Sex and stripping: The key to the intimate relationship between *Wolbachia* and host?', *Communicative & Integrative Biology*, 3(2), pp. 110–115. Available at: https://doi.org/10.4161/cib.3.2.10520.

Newton, M.E., Wood, R.J. and Southern, D.I. (1976) 'A cytogenetic analysis of meiotic drive in the mosquito, *Aedes aegypti* (L.)', *Genetica*, 46(3), pp. 297–318. Available at:

https://doi.org/10.1007/BF00055473.

Nissen, I., Müller, M. and Beye, M. (2012) 'The *Am-tra2* gene Is an essential regulator of female splice regulation at two levels of the sex determination hierarchy of the honeybee', *Genetics*, 192(3), pp. 1015–1026. Available at: https://doi.org/10.1534/genetics.112.143925.

Niu, B.-L. *et al.* (2005) 'Cloning and alternative splicing analysis of *Bombyx mori Transformer-2* gene using silkworm EST database', *Acta Biochimica et Biophysica Sinica*, 37(11), pp. 728–736. Available at: https://doi.org/10.1111/j.1745-7270.2005.00106.x.

Nunes, M.D.S., Nolte, V. and Schlötterer, C. (2008) 'Nonrandom *Wolbachia* infection status of *Drosophila melanogaster* strains with different mtDNA haplotypes', *Molecular Biology and Evolution*, 25(11), pp. 2493–2498. Available at: https://doi.org/10.1093/molbev/msn199.

Olsen, M. and Marsden, S. (1954) 'Natural parthenogenetisis in turkey eggs', *Science*, (120), pp. 545–546.

Page, S.L. and Hawley, R.S. (2005) 'The *Drosophila* meiotic mutant *mei-352* Is an allele of *klp3A* and reveals a role for a kinesin-like protein in crossover distribution', *Genetics*, 170(4), pp. 1797–1807. Available at: https://doi.org/10.1534/genetics.105.041194.

Palermo, G. (1992) 'Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte', *The Lancet*, 340(8810), pp. 17–18. Available at: https://doi.org/10.1016/0140-6736(92)92425-F.

Pane, A. *et al.* (2002) 'The *transformer* gene in *Ceratitis capitata* provides a genetic basis for selecting and remembering the sexual fate', *Development*, 129(15), pp. 3715–3725. Available at: https://doi.org/10.1242/dev.129.15.3715.

Park, S.-W. *et al.* (2010) 'MSL cis-spreading from *roX* gene up-regulates the neighboring genes', *Biochemical and Biophysical Research Communications*, 399(2), pp. 227–231. Available at: https://doi.org/10.1016/j.bbrc.2010.07.059.

Perlman, S.J. *et al.* (2015) 'Maternal transmission, sex ratio distortion, and mitochondria', *Proceedings of the National Academy of Sciences*, 112(33), pp. 10162–10168. Available at: https://doi.org/10.1073/pnas.1421391112.

Perlman, S.J., Hunter, M.S. and Zchori-Fein, E. (2006) 'The emerging diversity of *Rickettsia*', *Proceedings of the Royal Society B: Biological Sciences*, 273(1598), pp. 2097–2106. Available at: https://doi.org/10.1098/rspb.2006.3541.

Peterson, B.K. *et al.* (2012) 'Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species', *PLoS ONE*. Edited by L. Orlando, 7(5), p. e37135. Available at: https://doi.org/10.1371/journal.pone.0037135.

Pfender, W.F. *et al.* (2011) 'Mapping with RAD (restriction-site associated DNA) markers to rapidly identify QTL for stem rust resistance in Lolium perenne', *Theoretical and Applied Genetics*, 122(8), pp. 1467–1480. Available at: https://doi.org/10.1007/s00122-011-1546-3.

Phillips, W.M. (1979) 'A contribution to the study of species relations within the chrysomelid genus *Altica* Müller in Britain', *Zoological Journal of the Linnean Society*, 66(3), pp. 289–308. Available at: https://doi.org/10.1111/j.1096-3642.1979.tb01911.x.

Poinsot, D., Charlat, S. and Merçot, H. (2003) 'On the mechanism of *Wolbachia* -induced cytoplasmic incompatibility: Confronting the models with the facts: Problems and paradigms', *BioEssays*, 25(3), pp. 259–265. Available at: https://doi.org/10.1002/bies.10234.

Pollard, D.A. *et al.* (2006) 'Widespread Discordance of Gene Trees with Species Tree in *Drosophila*: Evidence for Incomplete Lineage Sorting', *PLoS Genetics*. Edited by B.F. McAllister, 2(10), p. e173. Available at: https://doi.org/10.1371/journal.pgen.0020173.

Pompanon, F. *et al.* (2005) 'Genotyping errors: causes, consequences and solutions', *Nature Reviews Genetics*, 6(11), pp. 847–859. Available at: https://doi.org/10.1038/nrg1707.

Potok, M.E. *et al.* (2013) 'Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern', *Cell*, 153(4), pp. 759–772. Available at: https://doi.org/10.1016/j.cell.2013.04.030.

Presgraves, D.C. (2000) 'A genetic test of the mechanism of *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila*', *Genetics*, 154(2), pp. 771–776. Available at: https://doi.org/10.1093/genetics/154.2.771.

Prestel, M. *et al.* (2010) 'The activation potential of MOF is constrained for dosage

compensation', *Molecular Cell*, 38(6), pp. 815–826. Available at: https://doi.org/10.1016/j.molcel.2010.05.022.

Puritz, J.B. *et al.* (2014) 'Demystifying the RAD fad', *Molecular Ecology*, 23(24), pp. 5937–5942. Available at: https://doi.org/10.1111/mec.12965.

Quattro, J.M., Avise, J.C. and Vrijenhoek, R.C. (1992) 'An ancient clonal lineage in the fish genus *Poeciliopsis* (Atheriniformes: Poeciliidae).', *Proceedings of the National Academy of Sciences*, 89(1), pp. 348–352. Available at: https://doi.org/10.1073/pnas.89.1.348.

Rand, D.M., Clark, A.G. and Kann, L.M. (2001) 'Sexually antagonistic cytonuclear fitness interactions in *Drosophila melanogaster*', *Genetics*, 159(1), pp. 173–187. Available at: https://doi.org/10.1093/genetics/159.1.173.

Rasch, E.M., Monaco, P.J. and Balsano, J.S. (1982) 'Cytophotometric and autoradiographic evidence for functional apomixis in a gynogenetic fish, *Poecilia formosa* and its related, triploid unisexuals', *Histochemistry*, 73(4), pp. 515–533. Available at: https://doi.org/10.1007/BF00493366.

Reed, K.M. and Werren, J.H. (1995) 'Induction of paternal genome loss by the paternal-sex-ratio chromosome and cytoplasmic incompatibility bacteria (*Wolbachia*): A comparative study of early embryonic events', *Molecular Reproduction and Development*, 40(4), pp. 408–418. Available at: https://doi.org/10.1002/mrd.1080400404.

Rice, W.R. (2013) 'Nothing in genetics makes sense except in light of genomic conflict', *Annual Review of Ecology, Evolution, and Systematics*, 44(1), pp. 217–237. Available at: https://doi.org/10.1146/annurev-ecolsys-110411-160242.

Rigaud, T., Juchault, P. and Mocquard, J.-P. (1997) 'The evolution of sex determination in isopod crustaceans', *BioEssays*, 19(5), pp. 409–416. Available at: https://doi.org/10.1002/bies.950190508.

Riparbelli, M.G., Gottardo, M. and Callaini, G. (2017) 'Parthenogenesis in Insects: The Centriole Renaissance', in M. Kloc (ed.) *Oocytes*. Cham: Springer International Publishing (Results and Problems in Cell Differentiation), pp. 435–479. Available at: https://doi.org/10.1007/978-3-319-60855-6_19.

Rohlfing, K. *et al.* (2023) 'When does the female bias arise? Insights from the sex determination cascade of a flea beetle with a strongly skewed sex ratio', *Functional & Integrative Genomics*, 23(2), p. 112. Available at: https://doi.org/10.1007/s10142-023-01023-1.

Ross, L., Shuker, D.M. and Pen, I. (2011) 'The evolution and suppression of male suicide under paternal genome elimination', *Evolution*, 65(2), pp. 554–563. Available at: https://doi.org/10.1111/j.1558-5646.2010.01148.x.

Ruiz-Pesini, E. *et al.* (2004) 'Effects of purifying and adaptive selection on regional variation in human mtDNA', *Science*, 303(5655), pp. 223–226. Available at: https://doi.org/10.1126/science.1088434.

Runemark, A. *et al.* (2018) 'Hybridization, sex-specific genomic architecture and local adaptation', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1757), p. 20170419. Available at: https://doi.org/10.1098/rstb.2017.0419.

Ryan, S.L. and Saul, G.B. (1968) 'Post-fertilization effect of incompatibility factors in *Mormoniella*', *Molecular and General Genetics MGG*, 103(1), pp. 29–36. Available at: https://doi.org/10.1007/BF00271154.

Salvemini, M. *et al.* (2009) '*Ceratitis capitata* transformer-2 gene is required to establish and maintain the autoregulation of *Cctra*, the master gene for female sex determination', *The International Journal of Developmental Biology*, 53(1), pp. 109–120. Available at: https://doi.org/10.1387/ijdb.082681ms.

Sanchez, L. (2008) 'Sex-determining mechanisms in insects', *The International Journal of Developmental Biology*, 52(7), pp. 837–856. Available at: https://doi.org/10.1387/ijdb.072396ls.

Sanderson, A.R. (1960) 'The cytology of a diploid bisexual spider beetle, *Ptinus clavipes* Panzer and its triploid gynogenetic form *mobilis* Moore', *Proceedings of the Royal Society of Edinburgh. Section B. Biology*, 67(4), pp. 333–350. Available at: https://doi.org/10.1017/S0080455X00000801.

Saridaki, A. and Bourtzis, K. (2010) '*Wolbachia*: more than just a bug in insects genitals',

Current Opinion in Microbiology, 13(1), pp. 67–72. Available at: https://doi.org/10.1016/j.mib.2009.11.005.

Sasai, K. *et al.* (1996) 'Chromosome spreading techniques for primary gastrointestinal tumors', *Journal of Gastroenterology*, 31(4), pp. 505–511. Available at: https://doi.org/10.1007/BF02355049.

Saura, A., Lokki, J. and Suomalainen, E. (1993) 'Origin of polyploidy in parthenogenetic weevils', *Journal of Theoretical Biology*, 163(4), pp. 449–456. Available at: https://doi.org/10.1006/jtbi.1993.1130.

Schartl, M., Wilde, B., *et al.* (1995) 'Evolutionary origin of a parthenoform, the Amazon molly *Poecila Formosa*, on the basis of a molecular genealogy', *Evolution*, 49(5), pp. 827–835. Available at: https://doi.org/10.1111/j.1558-5646.1995.tb02319.x.

Schartl, M., Nanda, I., *et al.* (1995) 'Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish', *Nature*, 373, pp. 68–71.

Schlupp, I. (2005) 'The evolutionary ecology of gynogenesis', *Annual Review of Ecology, Evolution, and Systematics*, 36(1), pp. 399–417. Available at: https://doi.org/10.1146/annurev.ecolsys.36.102003.152629.

Schmidt, B.C. and Sperling, F.A.H. (2008) 'Widespread decoupling of mtDNA variation and species integrity in *Grammia tiger* moths (Lepidoptera: Noctuidae)', *Systematic Entomology*, 33(4), pp. 613–634. Available at: https://doi.org/10.1111/j.1365-3113.2008.00433.x.

Schmidt, T.R. *et al.* (2001) 'Evolution of nuclear- and mitochondrial-encoded subunit interaction in cytochrome c oxidase', *Molecular Biology and Evolution*, 18(4), pp. 563–569. Available at: https://doi.org/10.1093/oxfordjournals.molbev.a003836.

Schultz, R.J. (1961) 'Reproductive mechanism of unisexual and bisexual strains of the viviparous fish *Poeciliopsis*', *Evolution*, 15(3), p. 302. Available at: https://doi.org/10.2307/2406230.

Schultz, R.J. (1966) 'Hybridization experiments with an all-female fish of the genus *Poeciliopsis*', *The Biological Bulletin*, 130(3), pp. 415–429. Available at:

https://doi.org/10.2307/1539747.

Schultz, R.J. (1969) 'Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates', *The American Naturalist*, 103(934), pp. 605–619. Available at: https://doi.org/10.1086/282629.

Schweyen, H., Rozenberg, A. and Leese, F. (2014) 'Detection and removal of PCR duplicates in population genomics ddRAD studies by addition of a degenerate base region (DBR) in sequencing adapters', *The Biological Bulletin*, 227(2), pp. 146–160.

Seehausen, O. (2004) 'Hybridization and adaptive radiation', *Trends in Ecology & Evolution*, 19(4), pp. 198–207. Available at: https://doi.org/10.1016/j.tree.2004.01.003.

Segarra, C. and Petitpierre, E. (1982) 'Preliminary data on the chromosomes of European Alticinae (Coleoptery, Chrysomelidae)', *Spixiana Supplement*, 7, pp. 29–37.

Semiatizki, A. *et al.* (2020) 'Effects, interactions, and localization of *Rickettsia* and *Wolbachia* in the house fly parasitoid, *Spalangia endius*', *Microbial Ecology*, 80(3), pp. 718–728. Available at: https://doi.org/10.1007/s00248-020-01520-x.

Serbus, L.R. *et al.* (2008) 'The genetics and cell biology of *Wolbachia*-host interactions', *Annual Review of Genetics*, 42(1), pp. 683–707. Available at: https://doi.org/10.1146/annurev.genet.41.110306.130354.

Shoemaker, D.D. *et al.* (2004) 'Decreased diversity but increased substitution rate in host mtDNA as a consequence of *Wolbachia* endosymbiont infection', *Genetics*, 168(4), pp. 2049–2058. Available at: https://doi.org/10.1534/genetics.104.030890.

Shukla, J.N. and Palli, S.R. (2012) 'Sex determination in beetles: Production of all male progeny by parental RNAi knockdown of transformer', *Scientific Reports*, 2(1), p. 602. Available at: https://doi.org/10.1038/srep00602.

Shukla, J.N. and Palli, S.R. (2013) '*Tribolium castaneum* Transformer-2 regulates sex determination and development in both males and females', *Insect Biochemistry and Molecular Biology*, 43(12), pp. 1125–1132. Available at: https://doi.org/10.1016/j.ibmb.2013.08.010.

Shukla, J.N. and Palli, S.R. (2014) 'Production of all female progeny: an evidence for the presence of male sex determination factor on Y chromosome', *Journal of Experimental Biology*, p. jeb.100438. Available at: https://doi.org/10.1242/jeb.100438.

Siede, D. (1998) 'Bestimmungshilfe für die mitteleuropäischen *Altica*-Arten (Coleoptera, Chrysomelidae)', *Entomologische Blätter*, 94, pp. 77–90.

Simões, M.V.P. (2012) 'Male and female reproductive systems of *Stolas conspersa* (Germar) (Coleoptera, Chrysomelidae, Cassidinae)', *Revista Brasileira de Entomologia*, 56(1), pp. 19–22. Available at: https://doi.org/10.1590/S0085-56262012005000007.

Simon, J.-C. *et al.* (2003) 'Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals: ROUTES TO PARTHENOGENESIS IN ANIMALS', *Biological Journal of the Linnean Society*, 79(1), pp. 151–163. Available at: https://doi.org/10.1046/j.1095-8312.2003.00175.x.

Simpson, S.J. and Douglas, A.E. (eds) (2013) *The Insects: Structure and Function*. 5th edn. Cambridge University Press.

Spolsky, C.M., Phillips, C.A. and Uzzell, T. (1992) 'Antiquity of clonal salamander lineages revealed by mitochondrial DNA', *Nature*, 356(6371), pp. 706–708. Available at: https://doi.org/10.1038/356706a0.

Stahlhut, J.K. *et al.* (2010) 'The mushroom habitat as an ecological arena for global exchange of *Wolbachia*', *Molecular Ecology*, 19(9), pp. 1940–1952. Available at: https://doi.org/10.1111/j.1365-294X.2010.04572.x.

Stalker, H.D. (1954) 'Parthenogenesis in *Drosophila*', *Genetics*, 39(1), pp. 4–34. Available at: https://doi.org/10.1093/genetics/39.1.4.

Stetz, J.B. *et al.* (2016) 'Discovery of 20,000 RAD–SNPs and development of a 52-SNP array for monitoring river otters', *Conservation Genetics Resources*, 8(3), pp. 299–302. Available at: https://doi.org/10.1007/s12686-016-0558-3.

Stouthamer, R., Breeuwer, J.A.J. and Hurst, G.D.D. (1999) '*Wolbachia pipientis* : Microbial manipulator of arthropod reproduction', *Annual Review of Microbiology*, 53(1), pp. 71–102.

Available at: https://doi.org/10.1146/annurev.micro.53.1.71.

Straub, T. *et al.* (2005) 'The *Drosophila* MSL complex activates the transcription of target genes', *Genes & Development*, 19(19), pp. 2284–2288. Available at: https://doi.org/10.1101/gad.1343105.

Sugimoto, T.N. *et al.* (2010) 'Expression of a doublesex homologue is altered in sexual mosaics of *Ostrinia scapulalis* moths infected with *Wolbachia*', *Insect Biochemistry and Molecular Biology*, 40(12), pp. 847–854. Available at: https://doi.org/10.1016/j.ibmb.2010.08.004.

Sugimoto, T.N. and Ishikawa, Y. (2012) 'A male-killing *Wolbachia* carries a feminizing factor and is associated with degradation of the sex-determining system of its host', *Biology Letters*, 8(3), pp. 412–415. Available at: https://doi.org/10.1098/rsbl.2011.1114.

Suomalainen, E. (1969) 'On the sex chromosome trivalent in some Lepidoptera females', *Chromosoma*, 28(3). Available at: https://doi.org/10.1007/BF00284928.

Suomalainen, E. and Saura, A. (1973) 'Genetic polymorphism and evolution in parthenogenetic animals. I. Polyploid Curculionidae', *Genetics*, 74(3), pp. 489–508. Available at: https://doi.org/10.1093/genetics/74.3.489.

Suzuki, K. (1988) 'Comparative morphology of the internal reproductive system of the Chrysomelidae (Coleoptera)', in P. Jolivet, E. Petitpierre, and T.H. Hsiao (eds) *Biology of Chrysomelidae*. Dordrecht: Springer Netherlands, pp. 317–355. Available at: https://doi.org/10.1007/978-94-009-3105-3_19.

Suzuki, M.G. *et al.* (2012) 'Effect of RNAi-mediated knockdown of the *Bombyx mori transformer-2* gene on the sex-specific splicing of Bmdsx pre-mRNA', *The International Journal of Developmental Biology*, 56(9), pp. 693–699. Available at: https://doi.org/10.1387/ijdb.120049ms.

Suzuki, S. *et al.* (2020) 'Unisexual hybrids break through an evolutionary dead end by two-way backcrossing', *Evolution*, 74(2), pp. 392–403. Available at: https://doi.org/10.1111/evo.13903.

Takahata, N. (1984) 'A model of extranuclear genomes and the substitution rate under within-

generation selection', *Genetical Research*, 44(1), pp. 109–115. Available at: https://doi.org/10.1017/S0016672300026288.

Tao, Y., Masly, J.P., *et al.* (2007) 'A sex-ratio meiotic drive system in *Drosophila simulans*. I: An autosomal suppressor', *PLoS Biology*. Edited by D. Barbash, 5(11), p. e292. Available at: https://doi.org/10.1371/journal.pbio.0050292.

Tao, Y., Araripe, L., *et al.* (2007) 'A sex-ratio meiotic drive system in *Drosophila simulans*. II: An X-linked distorter', *PLoS Biology*. Edited by D. Barbash, 5(11), p. e293. Available at: https://doi.org/10.1371/journal.pbio.0050293.

Thrasher, D.J. *et al.* (2018) 'Double-digest RAD sequencing outperforms microsatellite loci at assigning paternity and estimating relatedness: A proof of concept in a highly promiscuous bird', *Molecular Ecology Resources*, 18(5), pp. 953–965. Available at: https://doi.org/10.1111/1755-0998.12771.

Tin, M.M.Y. *et al.* (2015) 'Degenerate adaptor sequences for detecting PCR duplicates in reduced representation sequencing data improve genotype calling accuracy', *Molecular Ecology Resources*, 15(2), pp. 329–336. Available at: https://doi.org/10.1111/1755-0998.12314.

Tomiuk, J. and Loeschcke, V. (1992) 'Evolution of parthenogenesis in the *Otiorhynchus scaber* complex', *Heredity*, 68(5), pp. 391–397. Available at: https://doi.org/10.1038/hdy.1992.58.

Tram, U. and Sullivan, W. (2002) 'Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia* -induced cytoplasmic incompatibility', *Science*, 296(5570), pp. 1124–1126. Available at: https://doi.org/10.1126/science.1070536.

Turelli, M. and Hoffmann, A.A. (1991) 'Rapid spread of an inherited incompatibility factor in California *Drosophila*', *Nature*, 353(6343), pp. 440–442. Available at: https://doi.org/10.1038/353440a0.

Turelli, M., Hoffmann, A.A. and McKechnie, S.W. (1992) 'Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations', *Genetics*, 132(3), pp. 713–723. Available at: https://doi.org/10.1093/genetics/132.3.713.

Turner, B.J. *et al.* (1980) 'Evolutionary genetics of a gynogenetic fish, *Poecilia formosa*, the Amazon molly', *Evolution*, 34(2), p. 246. Available at: https://doi.org/10.2307/2407389.

Turner, B.J. *et al.* (1983) 'Clonal diversity and evolutionary dynamics in a diploid-triploid breeding complex of unisexual fishes (Poecilia)', *Evolution*, 37(4), p. 798. Available at: https://doi.org/10.2307/2407920.

Uzzell, T. (1970) 'Meiotic mechanisms of naturally occurring unisexual vertebrates', *The American Naturalist*, 104(939), pp. 433–445. Available at: https://doi.org/10.1086/282678.

Vavre, F. *et al.* (2001) 'Within-species diversity of *Wolbachia*-induced cytoplasmic incompatibility in haplodiploid insects', *Evolution*, 55(8), pp. 1710–1714. Available at: https://doi.org/10.1111/j.0014-3820.2001.tb00691.x.

Vavre, F. *et al.* (2002) 'Infection polymorphism and cytoplasmic incompatibility in Hymenoptera-*Wolbachia* associations', *Heredity*, 88(5), pp. 361–365. Available at: https://doi.org/10.1038/sj.hdy.6800063.

Veneti, Z. *et al.* (2005) 'A functional dosage compensation complex required for male killing in *Drosophila*', *Science*, 307(5714), pp. 1461–1463. Available at: https://doi.org/10.1126/science.1107182.

Verhulst, E.C., Beukeboom, L.W. and van de Zande, L. (2010) 'Maternal control of haplodiploid sex determination in the wasp *Nasonia*', *Science*, 328(5978), pp. 620–623. Available at: https://doi.org/10.1126/science.1185805.

Verhulst, E.C., van de Zande, L. and Beukeboom, L.W. (2010) 'Insect sex determination: it all evolves around *transformer*', *Current Opinion in Genetics & Development*, 20(4), pp. 376–383. Available at: https://doi.org/10.1016/j.gde.2010.05.001.

Vershinina, A.O. and Kuznetsova, V.G. (2016) 'Parthenogenesis in Hexapoda: *Entognatha* and non-holometabolous insects', *Journal of Zoological Systematics and Evolutionary Research*, 54(4), pp. 257–268. Available at: https://doi.org/10.1111/jzs.12141.

Virkki, N. (1970) 'Sex chromosomes and karyotypes of the Alticidae (Coleoptera)', *Hereditas*, 64(2), pp. 267–282. Available at: https://doi.org/10.1111/j.1601-5223.1970.tb02300.x.

Vrijenhoek, R., C. (1989) 'Genetic and ecological constraints on the origins and establishment of unisexual vertebrates', *Evolution and ecology of unisexual vertebrates*, 466, pp. 24–31.

Watts, P.C. *et al.* (2006) 'Parthenogenesis in Komodo dragons', *Nature*, 444(7122), pp. 1021–1022. Available at: https://doi.org/10.1038/4441021a.

Weeks, A.R., Velten, R. and Stouthamer, R. (2003) 'Incidence of a new sex–ratio–distorting endosymbiotic bacterium among arthropods', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1526), pp. 1857–1865. Available at: https://doi.org/10.1098/rspb.2003.2425.

Wei, J. *et al.* (2020) 'High prevalence of *Wolbachia* infection does not explain unidirectional cytoplasmic incompatibility of *Altica* flea beetles', *Ecological Entomology*, 45(1), pp. 67–78. Available at: https://doi.org/10.1111/een.12774.

Werren, J.H. (1997) 'Biology of *Wolbachia*', *Annual Review of Entomology*, 42(1), pp. 587–609. Available at: https://doi.org/10.1146/annurev.ento.42.1.587.

Werren, J.H., Baldo, L. and Clark, M.E. (2008) '*Wolbachia*: master manipulators of invertebrate biology', *Nature Reviews Microbiology*, 6(10), pp. 741–751. Available at: https://doi.org/10.1038/nrmicro1969.

Werren, J.H. and Windsor, D.M. (2000) '*Wolbachia* infection frequencies in insects: evidence of a global equilibrium?', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1450), pp. 1277–1285. Available at: https://doi.org/10.1098/rspb.2000.1139.

White, M.A. *et al.* (2009) 'Fine-scale phylogenetic discordance across the house mouse genome', *PLoS Genetics*. Edited by M.H. Schierup, 5(11), p. e1000729. Available at: https://doi.org/10.1371/journal.pgen.1000729.

Wickham, H. (2016) *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.

Wolff, J.N. *et al.* (2014) 'Mitonuclear interactions: evolutionary consequences over multiple biological scales', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1646), p. 20130443. Available at: https://doi.org/10.1098/rstb.2013.0443.

Woodroffe, G.E. (1958) 'The mode of reproduction of *Ptinus clavipes* Panzer form *mobilis*

Moore (=*P. latro* auct.) (Coleoptera: Ptinidae)', *Proceedings of the Royal Entomological Society of London. Series A, General Entomology*, 33(1–3), pp. 25–30. Available at: https://doi.org/10.1111/j.1365-3032.1958.tb00390.x.

Xu, J. and Hausdorf, B. (2021) 'Repeated hybridization increased diversity in the door snail complex Charpentieria itala in the Southern Alps', *Molecular Phylogenetics and Evolution*, 155, p. 106982. Available at: https://doi.org/10.1016/j.ympev.2020.106982.

Yusuf, M. and Turner, B. (2004) 'Characterisation of *Wolbachia*-like bacteria isolated from the parthenogenetic stored-product pest psocid *Liposcelis bostrychophila* (Badonnel) (Psocoptera)', *Journal of Stored Products Research*, 40(2), pp. 207–225. Available at: https://doi.org/10.1016/S0022-474X(02)00098-X.

Zug, R. and Hammerstein, P. (2012) 'Still a host of hosts for *Wolbachia*: Analysis of recent data suggests that 40% of terrestrial arthropod species are infected', *PLoS ONE*. Edited by R. Cordaux, 7(6), p. e38544. Available at: https://doi.org/10.1371/journal.pone.0038544.

11 Acknowledgements

First and foremost, I would like to thank my supervisor Susanne Dobler for giving me the opportunity to join her work group and work on my doctorate. I have had the pleasure of meeting the work group during my apprenticeship in 2012 and was delighted when I got the opportunity to come back and work on a flea beetle that had a rather peculiar reproduction phenotype, which fascinated me from the first day onward. I would like to thank her for the patience and feedback I got whenever I needed it. She always had an open ear for any problems I might have faced and always had suggestions on how to improve. The discussions we had led to ideas on how to address a certain issue I had and that always helped in the long run. She encouraged me to try new methods and made sure that I knew that she trusted that I would be able to do it. I would also like to thank her for giving me the opportunity to take part in conferences, in Hamburg, online, and in Prague. All of them have been an experience I look back on fondly. Furthermore, I would like to thank Susanne Dobler for guiding all of us through the difficult time of the pandemic, in which the laboratories were closed, which, in turn, led to anxiety on our, the PhD students', side. Throughout those months, she has always made it clear that she is on our side and tries her best to ensure that we can continue with our studies.

Secondly, I would like to thank Bernhard Hausdorf for agreeing to be my second supervisor and for always being willing to discuss anything 'ddRAD'. There have been multiple paper clubs and discussion meetings with him and Jie Xu, all of which have helped a lot. Working with a new method that I have never used before is not easy, but Bernhard and Susanne made sure that I was comfortable enough to tackle anything thrown my way.

I would also like to thank Kim Rohlfing for her unrelenting support and the countless discussion sessions we had. The advice she gave me, the questions she asked, the kind words of support she said all helped me through the ups and downs of this dissertation. She always made sure I knew what I was doing, that I was comfortable with what I was doing, and that I knew I could always come to her should I ever need help. It was not just the discussion sessions we had that helped me immensely, but also the fun lunch outings we had. We could share laughter and joy whilst still managing to squeeze in some beetle topics.

Furthermore, I would like to thank Cen Zeng for everything she did. She not only showed me how ddRAD works, both in the lab and on the computer, but also made sure I understood the

theory behind it. Whenever I had a question, she was always more than willing to help, which was more than I could have asked for. We had multiple discussions, not just about my data but about hers as well, and I can only hope that I have managed to help her at least a little bit as well. Sharing an office with her was always fun and I was always looking forward to the random conversations we would have throughout the day.

I would also like to thank Bharat Parthasarathy for lending me his time and energy to solve the statistics issue. He put so much work into helping me figure out what way was the best to address the complex data set that I had and to show that the results were indeed significant. We met multiple times, discussed ideas, and in the end made it work. For that, I would also like to thank Jutta Schneider for pointing me into the right direction and for advising me to ask Bharat for help. Without them, I would probably still be looking for a way to do the statistics.

A special thank you goes to Frank Friedrich, who was always more than willing to help me with my chromosome spreads. It didn't matter whether that was allowing me to use his lab to prepare them, use his microscope to get an idea of what I was looking at, or just general advice and discussions that helped to point me in the right direction. It was always a joy to talk to him.

I would also like to thank all of those who are and were a part of the hybrid consortium. Our trips to Pevestorf and Sylt were not only very fun (and included a lot of good food) but also helped to gather ideas and motivation. Talking to everyone who was also working on hybrids was enlightening and to see their progress and struggles helped me with mine.

Furthermore, I would like to thank Shabnam Mohammadi, who was always willing to read anything I had written to check for grammar and language issues. She always gave me valuable advice on what I had written, on how to improve, and generally always had an open ear. We had a lot of fun in Prague in 2022, listening to talks, strolling through the city, and eating a lot of amazing food. That conference trip will always stay with me and I will always remember it fondly. It was a lot of fun.

Thank you to Cäcilia Plate, who I talked to a lot of times about how to proceed. We were often in similar situations, so we understood what the other was going through, and that helped immensely to gather new motivation and ideas to continue. Thank you for all of the comic relief moments in your office and for the joy you spread in the work group. And thank you to

Marlena Herbertz, whose calm demeanour helped to ground me whenever I felt like I was drowning.

Thank you to Sebastian Franke, who I have spent countless hours talking about *Altica* and what was and was not possible. What he does in his studies is highly intriguing and I was always excited to hear about what result he had gotten out of his experiments. Lennart Yue, who worked as a student assistant in our group, also deserves a very big thank you for all of the samples he extracted for me. I know it was a lot of work, but he helped me to not lose my head in the mountains of samples. We had countless chats while we were busily working our way through the extractions and haplotyping, and all I can say is that he lights up a room whenever he enters. I am very thankful to have met him.

Furthermore, I would like to thank Mario Röpcke. We had some very entertaining chats ever since he started his PhD in 'AG Dobler', and amongst those we had a lot of discussions about not only my topic but his as well. It was very nice being able to maybe help him as he has helped me. Even if that only includes cheering each other up. That is just as important as helping with scientific problems. I would also like to thank Vera Wagschal for always knowing where the BSA stock is, and for keeping an eye on everyone and everything. She is full of knowledge and does not hesitate to share that with anyone. I enjoyed our chats during incubation times a lot. I would also like to thank Christian Lohr for being patient with me. The first time I asked for his help, I did not know what to expect of my chromosome spreads and what to look out for, but no matter how often I bothered him, he always stayed calm and willing to help.

A very big thank you goes to my parents. Without them, without their never-ending support and love, I would not have managed to finish this dissertation. They have been there when I needed to let off some steam, when I needed to hear words of encouragement, and when I needed to just sit back and relax. They have been supportive of me throughout my entire life and have always made sure that I knew that I could achieve anything if I only wanted to. They made sure I knew what I was capable of. I cannot thank you two enough for that!

Another big thank you goes to my brother, whose unexpected and always hilarious comments functioned as a sort of comic relief. No matter what was going on, he would always point out something that was directly in front of my nose as if it was the most logical and obvious thing on earth and that I just could not see amongst the mountain of data, pages to write, and

consequences to think of. You and Nadine have always been kind and fun to be around, and I want to thank you for that. Thank you for being an amazing brother!

Furthermore, I'd like to thank my grandparents, both on earth and beyond. All four of you have been so supportive of me going my way, and have always been excited to hear about what was going on in my life the next time I saw you. Because of one small comment from my grandfather, I decided to just go for it and do my PhD Thank you for being the way you are and for being patient with me. I know, all of those moments in which I tried to explain what I was doing were rather confusing.

I have learnt a lot during those last four, nearly four and a half, years. Not only methods and ways to problem-solve, but also how it is to work with people who I enjoy being around. I have really enjoyed my time as part of the 'AG Dobler' and will miss you all dearly.

12 Supplemental

12.1 Beetle crosses in quaddRAD analysis

Table 5 - Names, mtDNA HTs, and sex of the offspring of the individuals that went into the quaddRAD analysis. f = female, m = male, ? = could not be determined. 19 = caught in 2019, 21 = caught in 2021. Bü = Büchen, Gü = Güster, We = Wedel, Pev = Pevestorf.

Mother	HT mother	Father	HT father	Sex offspring
19_Bü_171	HT1	19_Bü_2	HT2	1 – f 2 – f 3 – f 4 – f 5 – f
19_Bü_134	HT1	19_Bü_240	HT2	1 – f 2 – f 3 – f 4 – f 5 – f
19_Bü_62	HT1	19_Bü_27	HT2	1 – f 2 – f 3 – f 4 – f 5 – f
19_Bü_114	HT1	19_Bü_170	HT2	1 – f 2 – f 3 – f 4 – f 5 – f

Mother	HT mother	Father	HT father	Sex offspring
19_Gü_207	HT1	19_Bü_41	HT2	1 – f 2 – f 3 – f 4 – f 5 – f
19_Bü_31	HT1	19_We_15	HT2	1 – f 2 – f 3 – ? 4 – f 5 – f
21_Gü_24	HT2	21_Bü_12	HT2	1 – m 2 – m 3 – f 4 – m 5 – m
21_Gü_98	HT2	21_Bü_263	HT2	1 – ? 2 – ? 3 – ? 4 – ? 5 – ?
21_Bü_45	HT2	21_Bü_74	HT2	1 – f 2 – f 3 – m 4 – f 5 – m
21_Bü_60	HT2	21_Bü_89	HT2	1 – f 2 – f 3 – f 4 – m 5 – f

Mother	HT mother	Father	HT father	Sex offspring
19_Bü_38	HT2	19_Bü_129	HT2	1 – f 2 – m 3 – f 4 – m 5 – f
19_Bü_45	HT2	19_Bü_4	HT2	1 – m 2 – f 3 – m 4 – m 5 – m
19_Bü_87	HT2	19_Bü_91	HT2	1 – m 2 – m 3 – m 4 – m 5 – f
19_Pev_66	HT3	19_Pev_108	?	1 – f 2 – m 3 – f 4 – m 5 – f

12.2 Adapters and primers used for quaddRAD

Table 6 – Adapters and their sequences that I used for the paternity analysis. Developed by Franchini *et al.*, 2017.

Adapter	Adapter name	Adapter sequence
quaddRAD-i5	quaddRAD-i5-top_#01_AACCCG	5'-CGCTCTTCCGATCTNNNNAACCCGTGCA-PHOS -3'
	quaddRAD-i5-bottom_#01_AACCCG	5'-PHOS-CGGGTTNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
	quaddRAD-i5-top_#02_AAGGGA	5'-CGCTCTTCCGATCTNNNNAAGGGATGCA-PHOS -3'
	quaddRAD-i5-bottom_#02_AAGGGA	5'-PHOS-TCCCTTNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
	quaddRAD-i5-top_#03_CAACTA	5'-CGCTCTTCCGATCTNNNNCAACTATGCA-PHOS -3'
	quaddRAD-i5-bottom_#03_CAACTA	5'-PHOS-TAGTTGNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
quaddRAD-i7	quaddRAD-i7-top_#01_AGTCAT	5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNAGTCAT-3'
	quaddRAD-i7-bottom_#01_AGTCAT	5'-CGATGACTNNNNAGATCGGAAGAGCA-3'
	quaddRAD-i7-top_#02_GATCGT	5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGATCGT-3'
	quaddRAD-i7-bottom_#02_GATCGT	5'-CGACGATCNNNNAGATCGGAAGAGCA-3'
	quaddRAD-i7-top_#03_GCATTG	5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGCATTG-3'
	quaddRAD-i7-bottom_#03_GCATTG	5'-CGCAATGCNNNNAGATCGGAAGAGCA-3'
	quaddRAD-i7-top_#04_TTAATG	5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNTTAATG-3'
	quaddRAD-i7-bottom_#04_TTAATG	5'-CGCATTAANNNNAGATCGGAAGAGCA-3'

Table 7 – Primers and their sequences that I used for the paternity analysis. Developed by Franchini *et al.*, 2017.

Primer	Primer name	Primer sequence
quaddRAD-i5XX	quaddRAD-i501_TATAGCCT	5'-AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGAC*G-3'
	quaddRAD-i502_ATAGAGGC	5'-AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCCTACACGAC*G-3'
	quaddRAD-i503_CCTATCCT	5'-AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCCTACACGAC*G-3'
	quaddRAD-i504_GGCTCTGA	5'-AATGATACGGCGACCACCGAGATCTACACGGCTCTGAACACTCTTTCCCTACACGAC*G-3'
quaddRAD-i7XX	quaddRAD-i701_ATTACTCG	5'-CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTGGAGTTCAGACGTGTGC*T-3'
	quaddRAD-i702_TCCGGAGA	5'-CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGACGTGTGC*T-3'
	quaddRAD-i703_CGCTCATT	5'-CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAGACGTGTGC*T-3'
	quaddRAD-i704_GAGATTCC	5'-CAAGCAGAAGACGGCATACGAGATGGAATCTCGTGACTGGAGTTCAGACGTGTGC*T-3'

12.3 Stacks commands

To be used in the console. The user changes according to the user profile that is logged in. PoolFile = for example, P0101_FKDL192534341-1a-D701-AK1680_HTLLCDSXX_L2_1/_2; files needed for CloneFilter from pool P0101. From ustacks onward, family 19_Bü_114 x 19_Bü_170 is used as an example, the family with the particular locus ID 2393.

12.3.1 CloneFilter

/Users/alinasanken/Desktop/Stacks\ V2.41/clone_filter -1 PoolFile_1.fq.gz -2 PoolFile_2.fq.gz -i gzfastq -o ./filtered/ --inline_inline --oligo_len_1 4

12.3.2 Process_radtags

/Users/alinasanken/Desktop/Stacks\ V2.41/process_radtags -P -1 ./filtered/PoolFile_1.1.fq.gz -2 ./filtered/PoolFile_2.2.fq.gz -i gzfastq -o ./prtags/ -b Barcodes_Pool.txt --renz_1 pstI --renz_2 mspI --inline_inline -s 20 -c -q -r

12.3.3 Ustacks

/Users/alinasanken/Desktop/Stacks\ V2.41/ustacks -f ./prtags/114x170_1.1.fq.gz -o ./ustacksdata/ --name 114x170_1 -i 1 -m 10 -M 3

12.3.4 Cstacks

/Users/alinasanken/Desktop/Stacks\ V2.41/cstacks -P ./ustacksdata/ -M ./PopulationMap_Family114x170.txt -n 1

12.3.5 Sstacks

/Users/alinasanken/Desktop/Stacks\ V2.41/sstacks -P ./ustacksdata/ -M ./PopulationMap.txt

12.3.6 Tsv2bam

/Users/alinasanken/Desktop/Stacks\ V2.41/tsv2bam -P ./ustacksdata/ -M ./PopulationMap.txt -R ./prtags/

12.3.7 Gstacks

/Users/alinasanken/Desktop/Stacks\ V2.41/gstacks -P ./ustacksdata/ -M ./PopulationMap.txt

12.3.8 Populations

/Users/alinasanken/Desktop/Stacks_V2.41/populations -P ./ustacksdata/ -O ./populationsdata_family114x170/ -M ./PopulationMap_114x170.txt -p 2 -r 0.8 --hwe --min-mac 2 --structure --vcf --genepop --hzar --phylip --phylip-var --treemix --fasta-samples --fasta-samples-raw

12.4 Python 3 scripts

12.4.1 Extract_paternity

First Python3 script to filter out and compare SNPs to determine the inheritance type. Written by C. Zeng.

```
from tempfile import mkstemp
from shutil import move
from os import remove, close
import os.path
import re
import argparse
import glob
import pdb
import gzip

#commands example:
#python3 extract_paternity_SNPs-f_simpified.py -i ./4stacks_m4M3-all/paternity-Famiy1/populations.snps.vcf -o family62x27-fs.out

def parse_input():  #read filenames from input
	parser = argparse.ArgumentParser(description='Given a directory containing the vcf file of one family in paternity analysis and the script will select potential SNPs or loci providing traces of allele inheritance')
	parser.add_argument("-i", "--inputfile", required=True, help="input vcf file name") #.matches.tsv.gz
	parser.add_argument("-o", "--outputfile", required=True, help="output file name.")

	args = parser.parse_args()

	inputfile = args.inputfile
	outputfile = args.outputfile

	return inputfile,outputfile

def inheritage(m_allele, f_allele, p_allele, currentSNP):
	currentSNP1 = reverse_slicing(currentSNP)
	typemarker = 1
	if (currentSNP in m_allele) or (currentSNP1 in m_allele):
		if (currentSNP in p_allele) or (currentSNP1 in p_allele):
			typemarker+=1
			if (currentSNP in f_allele) or (currentSNP1 in f_allele): typemarker+=1
	else:
		typemarker = 6
```

```
                if (currentSNP in f_allele) or (currentSNP1 in f_allele):
                        typemarker-=1
                        if (currentSNP in p_allele) or (currentSNP1 in p_allele): typemarker-=1
                elif (currentSNP in p_allele) or (currentSNP1 in p_allele): typemarker = 7
        return typemarker

def gamete(list1,list2):
        gamete_temp = [list1]
        for i in list1:
                for j in list2:
                        a_temp1 = i+j
                        a_temp2 = j+i
                        if not(a_temp1 in gamete_temp or a_temp2 in gamete_temp): gamete_temp.append(a_temp1)
        return gamete_temp

def reverse_slicing(s):
        return s[::-1]

def printSNP(seq): #print lines to output file
        with open(outputfile, 'a+') as f_o:
                for j in seq:
                        f_o.write('\t'.join(i for i in j))
                        f_o.write('\n')

def printheader(header1): #print the header
        with open(outputfile, 'w+') as f_o:
                f_o.write('\t'.join(i for i in header1))
                f_o.write('\n')

def readsample(sp_path): #read vcf files line by line
        with open(sp_path,'r') as f_in:
                count = 1
                header_info = ['Locus_ID','SNP_location']
                SNP_info = []
                for line in f_in:
                        #print(line)
                        if (count < 15): #no information in first 14 lines, skip it
                                count += 1
                                continue
                        line1 = line.strip('\n')
                        if not line1: continue  #avoid reading empty lines
                        lineinfo = line1.split('\t')
                        if count== 15 : #line no.15 is the header
                                header_info = header_info + lineinfo[9:]+['inheritance','possibilities','%'] #store the sample names in following order: mother, father, offsprings
                                printheader(header_info)
```

```
                        count += 1
                else:
                        motherSNP = lineinfo[9].split(':')[0] #extract the SNP within the
line
                        fatherSNP = lineinfo[10].split(':')[0]
                        if (not motherSNP == fatherSNP) and (not (motherSNP == './.' or
fatherSNP == './.')) : #select SNPs with difference in parents and no missing data
                                templine = [lineinfo[0],lineinfo[1]] #information of SNP
location
                                tempSNP = [lineinfo[3]]          #different SNPs at this
position
                                tempSNP += lineinfo[4].split(',')
                                missingsample = 0    #used in counting missing data in
offsprings
                                 #estimate inheritance
                                inheritance_temp = []
                                inheritance_count = [0,0,0,0,0,0,0,0,0]
                                for i in range(9,9+par_nr+off_nr):  #extract SNPs for each
sample
                                        temp0 = lineinfo[i].split(':')[0].split('/')
                                        temp1 = ""
                                        for j in temp0:
                                                if j == '.':
                                                        temp1 += '0'
                                                else:
                                                        temp1 += tempSNP[int(j)]
                                        if i==9:
                                                m_alleles = gamete(temp1,temp1)
                                        elif i == 10:
                                                f_alleles = [temp1]
                                                p_alleles = gamete(templine[2],temp1)
                                        else:
                                                inher_type                              =
inheritage(m_alleles,f_alleles,p_alleles,temp1)
                                                inheritance_temp.append(inher_type)
                                                if inher_type == 6:
                                                        missingsample+=1

                                                elif             inher_type             ==3:
inheritance_count[2]+=1
                                                else: inheritance_count[inher_type]+=1
                                        templine.append(temp1)
                                #print(templine)

                                ### drop SNPs with only one alleles
                                tt = sorted(set(templine[2:2+par_nr+off_nr]), key =
templine[2:2+par_nr+off_nr].index)
                                if (len(tt)==1):
```

```
                                        continue
                                elif (len(tt)==2) and ('00' in tt): continue

                                ### drop SNPs of missing data of all offsprings
                                tt = tt[2:]
                                if (len(tt)==1) and ('00' in tt): continue

                                templine.append(','.join(str(e) for e in
inheritance_temp))
                                if inheritance_count[7]>1:

        inheritance_count[7]=inheritance_count[7]+inheritance_count[2]+inheritance_coun
t[4]
                                        inheritance_count[2] = 0
                                        inheritance_count[4] = 0
                                elif (inheritance_count[2]>0) and
(inheritance_count[4]>0):

        inheritance_count[7]=inheritance_count[7]+inheritance_count[2]+inheritance_coun
t[4]
                                        inheritance_count[2] = 0
                                        inheritance_count[4] = 0
                                possibilities = []
                                poss_percent = []
                                for i in range(len(inheritance_count)):
                                        if inheritance_count[i]>0:
                                                possibilities.append(inheritance[i])

        poss_percent.append(str(int(inheritance_count[i]*100/off_nr))+'%')
                                templine.append(','.join(possibilities))
                                templine.append(','.join(poss_percent))
                                #print(missingsample)
                                #if missingsample<=missing_tolerance:
                                SNP_info.append(templine) #drop this SNP when
missing data is too high
                                if len(SNP_info) > 200 : #print SNPs to file
                                        #print(SNP_info)
                                        printSNP(SNP_info)
                                        SNP_info = []
                #print(SNP_info)
                if len(SNP_info) >0 : printSNP(SNP_info) #print left SNPs in temperary storage
to file

if __name__ == "__main__":
        inheritance                                                              =
['','Maternal_Only','Maternal_or_Sexual','','Sexual_or_Paternal','Paternal_Only','','Sexual_Re
production']
        par_nr = 2
```

```
        off_nr = 5
        missing_tolerance = 2
        inputfile,outputfile = parse_input()
        readsample(inputfile)

### the meaning of inheritance number from 1 to 7
### 1: only produced by maternal gametes
### 2: produced by maternal gametes or sexual reproduction
### 3: produced by maternal gametes, paternal gemetes or sexual reproduction (count as 'Maternal_or_Sexual')
### 4: produced by paternal gametes or sexual reproduction (count as 'Sexual_or_Paternal')
### 5: only produced by paternal gametes
### 6: missing data
### 7: only produced by sexual reproduction (count as 'Sexual_Reproduction')
```

12.4.2 Sum_paternity

Second Python3 script to summarise the results from ‘Extract_paternity’ into percentages.

Written by C. Zeng.

```
from tempfile import mkstemp
from shutil import move
from os import remove, close
import os.path
import re
import argparse
import glob
import pdb
import gzip

#command example:
#python3 sum_paternity_off.py -i family31xWe15-2.out -o sum_family31xWe15.out

def parse_input():
        parser = argparse.ArgumentParser(description='Given a directory containing locus sequences for all diploid individuals, the number of individuals and an output directory, merge sequences from one allele into single fasta sequence line and write new fasta in output directory.')
        parser.add_argument("-i", "--inputgroups", required=True, help="input folder of samples and their popmap for this catalog.")
        parser.add_argument("-o", "--outputdir", required=True, help="output file directory for all group summary.")

        args = parser.parse_args()

        inputdir = args.inputgroups
        outputdir = args.outputdir
```

```
        return inputdir,outputdir

def printsum(sum_count):
        with open(outputdir, 'w+') as f_o:
                f_o.write('Offspring'+'\t'+'PaternityTypes'+'\t'+'%PaternityTypes'+'\n')
                offID = 1
                f_type_sum = [0]*7
                f_per_sum = [0.0]*7
                for i in sum_off:
                        p_type = ''
                        p_percent = ''
                        for j in range(0,7):
                                p_type += str(j+1)+':'+str(i[j])+';'
                                f = i[j]*100/sum_count
                                p_percent += str(j+1)+':'+'%.2f'%f+'%'+';'
                                f_per_sum[j]+=f
                                f_type_sum[j]+=i[j]
                        p_type = p_type[:-1]
                        p_percent = p_percent[:-1]
                        f_o.write(str(offID)+'\t'+p_type+'\t'+p_percent+'\n')
                        offID +=1
                p_percent_sum = ''
                p_type_sum = ''
                for j in range(7):
                        f = f_type_sum[j]/off_nr
                        #print(k)
                        p_type_sum += str(j+1)+':'+'%.1f'%f+';'
                        f = f_per_sum[j]/off_nr
                        #print(f)
                        p_percent_sum += str(j+1)+':'+'%.2f'%f+'%'+';'
                p_type_sum = p_type_sum[:-1]
                p_percent_sum = p_percent_sum[:-1]
                f_o.write('averange:'+'\t'+p_type_sum+'\t'+p_percent_sum+'\n')
                f_o.write('total loci:'+str(sum_count))

def readFile(inputpath):
        with open(inputpath, 'r') as f_in:
                count = 0
                for line in f_in:
                        count +=1
                        if count == 1 : continue
                        if (line[0] == '\n'): continue
                        types = line.strip('\n').split('\t')[9]
                        typelist = types.split(',')
                        #print(typelist)
                        for i in range(0,off_nr):
```

```
                        sum_off[i][int(typelist[i])-1] +=1
                        #print(sum_off)
            #print(count)
            printsum(count-1)

if __name__ == "__main__":

        inputdir,outputdir = parse_input()
        off_nr = 5
        sum_off = [[0]*7 for i in range(off_nr)]
        readFile(inputdir)
```

12.5 R script for Bayesian modelling

Written by Bharat Parthasarathy.

```
#Preparing the data and loading it in R
getwd()
dat<-read.csv("File.csv")
str(dat)

dat<-read_xlsx("File1.xlsx")

#Doing isometric log-ratio (ilr) transformation of the compositional predictor variables containing 0 values
a<-data.frame(dat$Maternal,dat$Paternal,dat$Sexual.Reproduction,dat$Unknown)

#Before going to the next step ("require" function), make sure the package 'compositions' is installed in your computer
require(compositions)
ilrdat<-ilr(a)
ilrdat
dat<-data.frame(dat,ilrdat)
str(dat)

#So the variables V1, V2 and V3 are the transformed variables which we will use as predictors for the Bayesian regression model
require(rstanarm)
mod1<-
stan_glmer(HT~V1+V2+V3+(1|Family),data=dat,family=binomial,iter=6000,chains=4)
launch_shinystan(mod1)
```

13 List of abbreviations

-m	minimum number of raw reads required to form a stack (putative allele)
-M	number of mismatches allowed between stacks (putative alleles) to merge them into a putative locus
-n	number of mismatches allowed between putative loci during the construction of the catalogue
-p	minimum number of samples a locus must be present in to process the locus
-r	minimum of percentage of individuals in a population required to process a locus
°C	degrees Celsius
A	adenine
bp	base pairs
cDNA	complementary DNA
CI	cytoplasmic incompatibility
CID	centromere identifier
COI	cytochrome oxidase I
d	days
DAPI	4',6-diamidino-2-phenylindole
DCC	dosage compensation components
ddRAD (seq)	double digest restriction-site DNA sequencing
DNA	desoxyribonucleic acid
dsx	*doublesex* gene
DSX^F	*doublesex* female splice variant in *D. melanogaster*
DSX^M	*doublesex* male splice variant in *D. melanogaster*
dsxf1	*doublesex* female splice variant 1 in *A. lythri*
dsxf2	*doublesex* female splice variant 2 in *A. lythri*
dsxm	*doublesex* male splice variant in *A. lythri*
EDTA	ethylenediaminetetraacetic acid
FDR	first division restitution
FISH	fluorescence in situ hybridisation
g	gram
G	guanine
gDNA	genomic DNA
h	hours
HPLC	high performance liquid chromatography
HT	haplotype
KCl	potassium chloride
KOH	caustic potash/ potassium hydrogen
KH_2PO_4	potassium dihydrogen phosphate
M	molar
max. g	maximum centrifugal acceleration
min	minutes
mL	millilitre
mM	millimolar

mm	millimetre
mtDNA	mitochondrial DNA
Na_2HPO_4	sodium hydrogen phosphate
NaCl	sodium chloride
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PFA	paraformaldehyde
PGE	paternal genome elimination
quaddRAD (seq)	quadruple barcode double digest restriction-site DNA sequencing
RAD (seq)	restriction-site DNA sequencing
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
s	seconds
SD	standard deviation
SDR	second division restitution
*Sf*9	*Spodoptera frugiperda*-derived
SNP	single nucleotide polymorphism
SR	serine/arginine-rich
Sxl	*Sexlethal* gene
SXL	Sexlethal protein
SXL^E	early Sexlethal protein
SxlPe	early *Sexlethal* promotor
SxlPm	late *Sexlethal* promotor
T	thymine
TAE	Tris base, acetic acid, EDTA buffer
TBE	Tris base, boric acid, EDTA buffer
TBS	Tris-buffered saline
Tra	*Transformer* gene
TRA/Tra	Transformer protein
Tra^F	female Tra protein
U/µL	units per microlitre
V	Volt
µL	microlitre
µM	micromolar
µm	micrometre

14 Eidesstattliche Versicherung – Declaration of oath

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, den Unterschrift