Evolutionary genetics and dynamics of transitions in sex determination

Schenkel, Martijn

DOI:
10.33612/diss.166344703

CHAPTER VII:

DISCUSSION

Martijn A. Schenkel

Evolution of sex determination mechanisms

In this thesis, I investigated the evolutionary genetics and dynamics of transitions in sex determination. Despite representing an essential component of the development of sexually reproducing organisms, the regulation of sex determination (SD) itself is highly labile from an evolutionary perspective. Control over the SD process can be achieved in myriad ways and different cues, both genetic and environmental, can impinge on this process (Bachtrog et al., 2014; Beukeboom & Perrin, 2014). The result is an astounding variation between species, and in some cases within species, in the mechanisms regulating sexual differentiation, as well as the genes involved. This variation may arise through adaptive evolution when selection favours the spread of novel SD systems (i.e. genes, chromosomes, or mechanisms). Previous work on evolutionary transitions in SD has sought to uncover the selective pressures acting on SD and the conditions under which SD transitions may take place (reviewed in (van Doorn, 2014)). Transitions in SD may be driven by selective pressures arising from phenomena such as parent-offspring conflict (e.g. Kozielska *et al.*, 2006; Kuijper & Pen, 2014), sex ratio distortion (reviewed in (Uller et al., 2007)), and linkage to nearby sexually antagonistic genes (van Doorn & Kirkpatrick, 2007, 2010; Muralidhar & Veller, 2018).

Previous work on the evolution of SD mechanisms has resulted in a solid theoretical framework of how variation in SD may arise and how this incites further evolution. These models generally assume that SD is (1) a stand-alone and straightforward process where sex is a monogenic trait encoding a simple phenotype, rather than being a complex process involving many genes that is integrated into the overall developmental programme of an organism, or (2) a simplistic discrete switch which can be set to either a male and female state, rather than assuming the underlying regulation is more continuous and can be affected by many factors. A drawback of such simplistic models is that they do not adequately represent the complexity of SD. SD systems may involve a mixture of environmental (ESD) and genetic (GSD) cues, which is particularly evident in the skink *Niveoscincus ocellatus* (Pen et al., 2010) in which highland populations have GSD but lowland populations have ESD. Other complex SD systems may involve mixtures of different GSD mechanisms, such as in the European common frog *Rana temporaria* (Ma et al., 2016) which features an XY male heterogamety system in northern European

populations and a yet uncharacterized XY male heterogamety system in southern populations. Research on SD continues to find diversity, such as in Dipteran insects where we know of four different genes that can lead to male development (Hall et al., 2015; Krzywinska et al., 2016; Sharma et al., 2017; Meccariello et al., 2019). The current theoretical framework for the evolution of SD is largely incapable of explaining polymorphic SD, where multiple genes may control sex, let alone predicting when they occur. Polymorphic SD is generally considered a transitory state between two different monomorphic systems (Rice, 1986), despite polymorphic SD persisting over extended periods of time in some species (e.g. (Kozielska et al., 2008; Ma et al., 2016)). This is because in most existing models of SD evolution, transitions in SD are generally complete, so that only a single SD gene will remain after sufficient time has passed. Stable polymorphic SD is only observed under a restricted range of parameter values in the limited cases where it is observed at all (e.g. van Doorn & Kirkpatrick, 2007). There is thus an urgent need for models on the evolution of SD that account for the underlying complexity and the further biology of the organism. SD genes manifest their effect through interactions with many other genes that effectuate sexual differentiation. The link between the SD gene and the function for which it is selected (i.e. determining the sex phenotype) is more complex than currently considered.

In this thesis, I have modelled several complex scenarios of SD evolution. In Chapter 4, evolution of the genes targeted by an SD gene can contribute substantially to the evolution of novel SD mechanisms. Here, environmental influences on the target gene cause it to supersede the ancestral SD gene. Interactions that do not strictly involve SD may also affect the evolution of SD. In Chapter 3 I have shown that epistatic interactions between an autosomal gene and a gene linked to a (novel) SD gene affect fitness. Epistasis modifies the selection acting on the linked gene, which leads to altered selection of the co-adapted gene complex that is formed by the interacting non-SD gene and the SD gene. This affects the stability or invasibility of respectively ancestral and novel SD genes, and thereby can both promote or inhibit transitions between SD systems. Taken together, it is apparent that SD genes evolve in the context of a genomic architecture of a multitude of other genes spread across different chromosomes.

Sex determination and sexual function

Although principally defined by the capacity to produce different types of gametes (Parker et al., 1972), males and females differ in more aspects. That is because anisogamy comprises the basis that will give rise to further sexual dimorphism, which eventually leads to behavioural, morphological, physiological and even genetic (sex chromosomal) differences between males and females. Sexual identity, however, remains rooted in anisogamy, and what it entails ought to be considered first and foremost in this context. As outlined in Chapter 2, sexual dimorphism may arise in response to differences in reproductive capacity and the social context in terms of which compatible mates are available and with which individuals one must compete. These differences result in a divergence of selective pressures acting on males and females, and adaptive evolution can then give rise to sexual dimorphism as males and females both evolve in response to the selective pressures experienced by them.

Given that SD allows for further sexual dimorphism to evolve (beyond the difference in gamete size), sexual selection has played a large role in the evolution of SD (van Doorn, 2014). Indeed, SD does not comprise a simple binary switch between gamete types, but rather a complicated developmental program that affects many aspects of individual biology (e.g. (Aryan et al., 2020)). This consideration has been used to explain the evolution of the *Drosophila* SD pathway (Pomiankowski et al., 2004). There, the SD cascade is postulated to evolve to provide sexually dimorphic expression of SD genes, whose action differently affects fitness in males and females. Maleness, for example, is not solely defined by the production of the smaller gametes, but rather by a capacity to reproduce, and hence gain fitness, through these reduced gametes. Canalization of the SD process ensures that the expression of genes involved in sexual differentiation is uniformly set, which promotes specialization of an individual's reproductive capacity as either a male or a female.

When considering SD as a complex developmental program rather than a simple binary switch, it becomes apparent that many genes may be involved in its execution. SD mechanisms are often represented as a regulatory cascade in which a single upstream gene regulates one or more downstream components, which in turn also regulate one or more components even further downstream, and so forth. The result is that in moving down the cascade, an increasing number of genes

becomes involved in regulating sexual differentiation. The entirety of sex-specific development is subject to regulation by a single regulatory network which is spearheaded by the master SD gene at the top of the cascade. However, in some species not all sex-specific functions are necessarily under direct control of the master SD gene (a term that is therefore effectively obsolete, but which I will continue to use for the sake of simplicity). For example, Y-chromosomes generally contain many genes that have functions in male-specific processes such as spermatogenesis (Lahn & Page, 1997; Skaletsky et al., 2003), and in species like *Drosophila melanogaster* the Y-chromosome is not required for maleness in the broad sense, but contains genes conferring essential male-related functions (Bridges, 1916a; b). Individuals carrying a Y-chromosome with a non-functional mutant master SD gene may still exhibit some male-specific functions, and similarly misexpression of the perceived male-determining gene in (e.g. via transgenesis in non-Y-bearing individuals) may not be sufficient to achieve full male function (Aryan et al., 2020). Therefore, instead of SD being a simple developmental program controlled by a single gene, SD consists of several modules, each of which may be regulated a specific gene (or set of genes). Taken together, when all modules are set in a congruent manner, the SD program in its entirety is carried out uniformly so that the individual commits entirely to being either female or male.

We may consider the genes involved with sexual differentiation as being subject to sexually antagonistic selection, i.e. they represent loci under intralocus sexual conflict (IASC; see Chapter 2). They have a beneficial effect when expressed in conjunction with other developmental processes that promote the production of spermatozoa c.q oocytes, or more simply put when the carrier is male c.q. female. However, they have a detrimental effect when they are expressed along with developmental processes which would promote the production of oocytes over spermatozoa, i.e. in otherwise female individuals or c.q spermatozoa over oocytes in otherwise male individuals. IASC loci are predicted to evolve so that conflict becomes resolved either by becoming sex-specifically expressed, or by becoming linked to a sex-determining gene (Bonduriansky & Chenoweth, 2009; Parsch & Ellegren, 2013). In systems with polygenic SD, the genes involved are predicted to become linked to each other, e.g. to prevent fertility issues (Charlesworth et al., 2005). Here, the regulation of SA genes and SD genes exhibit similar patterns. It remains unclear under which conditions IASC loci are more likely to evolve to become

sex-linked versus when they become sex-specifically expressed. Similarly, some sex-specific functions may be less likely to be assimilated into the regulatory network controlled by the master SD gene for yet unknown reasons, and instead they may evolve to be uniformly regulated along with other SD genes simply by becoming linked to these other SD genes. A possible explanation here may be that full monogenic control of SD is infeasible as the SD program is carried out by a still too variable set of genes.

Evolution of polymorphic sex determination mechanisms

Spatial heterogeneity in SD may arise by local rather than global transitions in SD mechanisms. Any mechanisms capable of driving a transition in SD may then be capable of causing such heterogeneity, provided that their action is restricted to certain geographical localities. For example, selection by direct benefits (Bull & Charnov, 1977) may favour a given SD gene in one location, but when its beneficial effects are context-dependent, this may result in it being disfavoured elsewhere. A more complicated example would be when meiotic drive sex chromosomes (cf. (Jaenike, 2001; Kozielska et al., 2010)) are unable to spread due to incompatibilities with other (e.g. autosomal) genes in some but not all populations (Verspoor et al., 2018). Similarly, when genetic variants at SA loci are differently selected upon in different environments (García-Roa et al., 2020), their benefit or cost to carriers may differ accordingly, so that the evolution of an SD gene near these loci becomes less favourable (cf. (van Doorn & Kirkpatrick, 2007, 2010)).

The model presented in Chapter 3 shows that established sex chromosomes may experience increased stability owing to interactions with autosomal genes. The fitness effect of the autosomal gene was modelled as neutral on its own, and had a relatively simple effect on individual fitness via epistatic interactions. This model results in a straightforward genetic network underlying fitness. Fitness under natural conditions is however construed by a vastly more complicated genetic architecture. Under natural conditions, this network may be exposed to different selective pressures in different habitats, and as a result is likely to diverge between populations to some extent. When local adaptation affects those components of the network that are also involved in autosome-sex chromosome interactions, the stabilizing effect of these interactions on the SD system may be affected, so that in

different populations the stability of the SD system may vary accordingly. Such a scenario may apply to the different male-determining M-factors found in the housefly *Musca domestica*. In this species, males bearing an M-factor on autosome III (III^M) had a fitness benefit over males bearing the 'standard' Y-chromosomal M-factor (Y^M) in one experiment (Hamm et al., 2009), though a similar experiment using different strains found that Y^M bearing males had a fitness benefit over III^M males (Hamm & Scott, 2008). This suggests that the fitness of Y^M and/or III^M males is affected by their genetic background. To test this more rigorously, it is necessary to introgress different M-factors into different backgrounds (similar to the baby-sex chromosome procedure in Chapter 5) followed by assessing their capacity to invade into a population or inversely to withstand invasion by another M-factor.

Polymorphic SD systems may however not have evolved due to SD drivers acting only locally. In Chapter 4, I showed that environmental influences on SD can enable transitions in SD, but may not necessarily be the driving force behind these transitions. Instead, environmental influences can allow for SD genes to evolve to interact differently with other genes within the regulatory network of SD. Mutations in SD genes resulting in altered interactions may occur anywhere, but are likely to be strongly counterselected when the resulting altered interactions are not accommodated under local conditions. When such mutations occur under favourable conditions, it may invade as driven by other drivers of SD transition (e.g. sex ratio selection), but only in those parts of the populations where conditions are similarly favourable. The effect of environmental influences on SD may however be much more straightforward. The model in Chapter 4 assumes a positive effect of an environmental cue such as temperature on the expression level of an SD gene, but negative effects are also possible. For example, increased temperature may increase the degradation rate of a specific SD gene's product so that it cannot perform its function. A different gene which is insensitive (or less sensitive) may evolve to function as a substitute. Altogether, polymorphism in SD mechanisms need not be caused by local modulation of a driver of SD transitions but rather by modulation of the regulatory network underlying SD. The manner in and extent to which such effects occur are likely to be underreported, and may provide fruitful options for future research on SD systems.

Transitions in sex determination and the evolution of sex chromosomes

Transitions in SD require genomic reorganisations and may set off further evolutionary change. The evolution of a new SD gene on a former autosome represents the first step in the evolution of sex chromosomes (reviewed in (Charlesworth et al., 2005; Schenkel & Beukeboom, 2016)). The sex chromosomes represent specialized genomic niches, because they are differently transmitted from and to males and females (Patten et al., 2013). Sex-chromosomal genes may therefore evolve differently from their autosomal counterparts, for example by increased selection for male-beneficial variants on the Y-chromosome (Rice, 1998). A striking feature of differentiated sex chromosomes is their specialized gene content, which is characterized by an excess of regulatory genes and/or genes with sex-specific functions compared to autosomes (e.g. (Lahn & Page, 1997; Bellott et al., 2014)). Interactions between sex-chromosomal and autosomal genes may be abundant, resulting in a pivotal role for the sex chromosomes in individual fitness. Sex chromosome evolution is characterized by being initiated by small-scale changes on the level of individual genes, and culminating in chromosome-wide differentiation of both the X- and the Y-chromosome. In some cases, the process may continue further and lead to the loss of the sex-determining chromosome (i.e. the male-determining Y in XY systems or female-determining W in ZW systems), which represents another SD transition from an XY or ZW to an X0 or Z0 system (Graves, 2006; Bachtrog, 2013).

Although late-stage sex chromosomes have been thoroughly studied across different species, early-stage sex chromosomes have been studied on a much smaller scale. In Chapter 5, I demonstrated the utility of *M. domestica* as a model system for studying the initial phases of sex chromosome evolution. By exploiting the variation in SD mechanisms in this species, I was able to establish strains in which formerly-autosomal male-determining genes (M-factors) were crossed out of a genomic background with a dominant female-determining gene tra^{D}. In presence of tra^{D}, M-factors can be transmitted through males and females, but when crossed out of this background M-factors induce masculinization in all carriers. This mimics the *de novo* evolution of a male-determining gene and the evolution of a novel Y-chromosome. Aside from being straightforward to perform, this methodology has three key conceptual advantages over other commonly-used approaches to studying

early-stage sex chromosome evolution. Firstly, early-stage sex chromosome evolution can be studied from the absolute onset and in real time. Other studies have used a retrospective approach aimed at inferring past evolution of sex chromosomes systems by studying systems of varying age. Secondly, comparative analyses can be performed within a single species, rather than between sex chromosome systems in different species (e.g. (Bracewell & Bachtrog, 2020)). Thirdly, this approach is highly repeatable as it can be used to generate different replicates, and thereby additionally allows for novel sex chromosomes to be maintained under different conditions to assess the importance of different evolutionary phenomena in driving the early stages of sex chromosome evolution. The possibility to generate new sex chromosome systems in *M. domestica* (and possibly other species harbouring similar variation in SD mechanisms) therefore provides a powerful tool to study sex chromosome evolution with unprecedented rigour.

The utility of baby-sex chromosomes for studying early sex chromosome evolution however requires a methodology for assessing fitness in *M. domestica*. In Chapter 6, I discussed how fitness may be measured in a sex-specific manner in *M. domestica*, and how proxies for male and female fitness may be developed in this species. In females, fitness is primarily determined by fecundity, with increased egg production being strongly associated with increased offspring numbers. However, a female's fitness may also be influenced by her ability to discern low- and high-quality mates, and to prevent low-quality mates from mating with her. In males, fitness is primarily determined by mating success, as females are thought to exhibit low to no remating owing to males transferring a seminal product which inhibits this behaviour (Leopold, 1970; Leopold, Terranova, Thorson, et al., 1971).

I however assumed that female promiscuity is strongly inhibited by males so that females only mate once. This might not be true. Males that mate repeatedly become less efficient in inhibiting remating in later mates (Leopold, Terranova, & Swilley, 1971). The seminal compound which inhibits remating is transferred during later stages of

copulation (Riemann et al., 1967; Arnqvist & Andrés, 2006), and females exhibit higher remating rates when mating is prematurely disrupted. Remating inhibition may be less effective if mating is often disrupted in natural conditions or if males may mate sequentially with different females. Female promiscuity and by extension

postcopulatory sexual selection may then be much more prominent than documented here (Birkhead & Pizzari, 2002; Pitnick & Hosken, 2002). Female promiscuity would favour adaptations in males that act after mating to enhance their fitness (e.g. via sperm precedence). Female fitness may also be affected, as promiscuity can have both direct (i.e. increased fecundity) as well as indirect (e.g. good genes, genetic diversity) benefits to the female. Additionally, female promiscuity extends the scope for interlocus sexual conflict to occur: males no longer control the paternity over the offspring generated by the females they have mated, and females may actively mediate paternity instead via e.g. cryptic female choice. Taken together, the extent to which females are promiscuous, and the impact thereof on how fitness is accrued by males and females in *M. domestica*, requires further investigation for a definitive fitness assessment methodology may be established. Such fitness assays are crucial for determining the selective effects of baby-sex chromosomes at different evolutionary stages, which will reveal how these chromosomes evolve during their early stages.

Conclusion

The diversity of SD mechanisms has in the past been explained primarily by models that utilize a simplified view of sex determination and, in a sense, the outcome of the SD process. This has led to the identification of processes that may affect the evolution of SD mechanisms, but in line with their simplistic origin, their explanatory power with regard to complex SD systems is limited. The notion that complex SD systems are evolutionary instable (e.g. (Rice, 1986)) is at odds with the reality that such systems can persist for extended periods of time, suggesting that these systems have in fact been shaped and are maintained by adaptive benefits. Understanding what these benefits are requires an integrative consideration of how different processes may interact to affect SD genes (Figure 1). Here, the evolution of SD genes is affected not only by the selective pressures acting on SD genes or loci to which they are linked (as commonly seen in models of SD turnover), but may also be affected by environmental factors as well as genetic interactions with other genes or chromosomes. A less simplistic perspective such as the one proposed here, where SD is considered as a more complex developmental process involving many genes and as being sensitive to perturbation by genetic and non-genetic factors, may

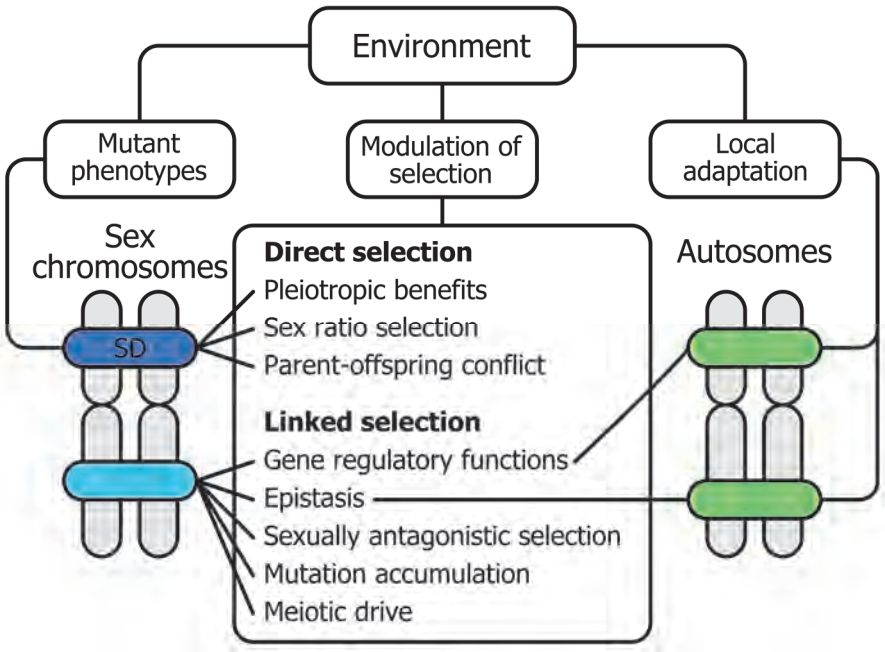

Figure 1: Evolution of sex determination mechanisms in an integrative perspective. A given sex-determining gene (SD, dark blue) can evolve in response to numerous factors. Selection can act directly on SD either via its sex-determining function or via some other pleiotropic function. Linkage to other genes (light blue) results in linked (or indirect) selection from affecting it. Through this, it may also be affected by the remainder of the genome as the linked gene interacts with other autosomal genes (green). Overlaying the spectrum of selective pressures acting on SD and the chromosome pair on which it is located, environmental dependencies can affect SD in various ways. Context-dependence of mutant phenotypes means that some SD variants may exert different functions under different conditions. Environmental influences may bias the various selective pressures potentially acting on SD via direct or linked selection. Third, local adaptation to environment may alter the genetic composition of autosomal loci with which the sex chromosome interacts, or alternatively the loci on the sex chromosomes itself (not shown). Altogether, SD is affected by a myriad of phenomena that may affect its evolution.

provide novel insights into complex SD systems in particular as well as SD systems in general. The diversity of SD mechanisms has often been hailed as being in stark contrast with its simple binary outcome. This perspective must be cast away, and instead we must regard the diversity of SD mechanisms as being in line with the complexity of ecological and evolutionary processes with which it is so strongly intertwined.

BIBLIOGRAPHY

Abbott JK, Nordén AK & Hansson B (2017). Sex chromosome evolution: historical insights and future perspectives. *Proceedings of the Royal Society of London Series B-Biological Sciences* **284**:20162806.

Ågren JA, Munasinghe M & Clark AG (2019). Sexual conflict through mother's curse and father's curse. *Theoretical Population Biology* **9**:1–9.

Alho JS, Matsuba C & Merilä J (2010). Sex reversal and primary sex ratios in the common frog (*Rana temporaria*). *Molecular Ecology* **19**:1763–1773.

Andrés JA & Arnqvist G (2001). Genetic divergence of the seminal signal-receptor system in houseflies: the footprints of sexually antagonistic coevolution? *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**:399–405.

Arak A & Enquist M (1993). Hidden preferences and the evolution of signals. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **340**:207–213.

Archer CR, Stephens RM, Sharma MD & Hosken DJ (2017). The *Drosophila simulans* Y chromosome interacts with the autosomes to influence male fitness. *Journal of Evolutionary Biology* **30**:1821–1825.

Arnqvist G & Andrés JA (2006). The effects of experimentally induced polyandry on female reproduction in a monandrous mating system. *Ethology* **112**:748–756.

Arnqvist G & Nilsson T (2000). The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour* **60**:145–164.

Arnqvist G & Rowe L (2005). Sexual conflict. Princeton University Press, Princeton, NJ, USA.

Aryan A, Anderson MAE, Biedler JK, Qi Y, Overcash JM, Naumenko AN, Sharakhova M V., Mao C, Adelman ZN & Tu Z (2020). *Nix* alone is sufficient to convert female *Aedes aegypti* into fertile males and *myo-sex* is needed for male flight. *Proceedings of the National Academy of Sciences of the United States of America* **117**:17702–17709.

Bachtrog D (2008). The temporal dynamics of processes underlying Y chromosome degeneration. *Genetics* **179**:1513–1525.

Bachtrog D (2013). Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. *Nature Reviews Genetics* **14**:113–124.

Bachtrog D, Kirkpatrick M, Mank JE, McDaniel SF, Pires JC, Rice WR & Valenzuela N (2011). Are all sex chromosomes created equal? *Trends in Genetics* **27**:350–

357.

Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman TL, Hahn MW, Kitano J, Mayrose I, Ming R, Perrin N, Ross L, Valenzuela N, Vamosi JC & The Tree of Sex Consortium (2014). Sex determination: why so many ways of doing it? *PLoS Biology* **12**:e1001899.

Baldwin FT & Bryant EH (1981). Effect of size upon mating performance within geographic strains of the housefly, *Musca domestica* L. *Evolution* **35**:1134–1141.

Bateman AJ (1948). Intra-sexual selection in *Drosophila*. *Heredity* **2**:349–368.

Becker RA, Wilks AR, Brownrigg R, Minka TP & Deckmyn A (2018). maps: draw geographical maps. https://cran.r-project.org/package=maps

Bedhomme S, Bernasconi G, Koene JM, Lankinen A, Arathi HS, Michiels NK & Anthes N (2009). How does breeding system variation modulate sexual antagonism? *Biology Letters* **5**:717–720.

Beekman M, Nieuwenhuis B, Ortiz-Barrientos D, Evans JP & Beekman M (2016). Sexual selection in hermaphrodites, sperm and broadcast spawners, plants and fungi. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **371**:20150541.

Bellott DW, Hughes JF, Skaletsky H, Brown LG, Pyntikova T, Cho TJ, Koutseva N, Zaghlul S, Graves T, Rock S, Kremitzki C, Fulton RS, Dugan S, Ding Y, Morton D, Khan Z, Lewis L, Buhay C, Wang Q, Watt J, Holder M, Lee S, Nazareth L, Rozen S, Muzny DM, Warren WC, Gibbs RA, Wilson RK & Page DC (2014). Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. *Nature* **508**:494–499.

Berta P, Hawkins JR, Sinclair AH, Taylor A, Griffiths BL, Goodfellow PN & Fellous M (1990). Genetic evidence equating *SRY* and the testis-determining factor. *Nature* **348**:448–450.

Betrán E, Thornton K & Long M (2002). Retroposed new genes out of the X in *Drosophila*. *Genome Research* **12**:1854–1859.

Beukeboom LW & Perrin N (2014). The Evolution of Sex Determination. Oxford University Press, Oxford, United Kingdom.

Billeter J-C, Jagadeesh S, Stepek N, Azanchi R & Levine JD (2012). *Drosophila melanogaster* females change mating behaviour and offspring production based on social context. *Proceedings of the Royal Society of London Series B-*

Biological Sciences **279**:2417–25.

Billeter J-C, Villella A, Allendorfer JB, Dornan AJ, Richardson M, Gailey DA & Goodwin SF (2006). Isoform-specific control of male neuronal differentiation and behavior in *Drosophila* by the *fruitless* gene. *Current Biology* **16**:1063–1076.

Birkhead TR & Pizzari T (2002). Postcopulatory sexual selection. *Nature Reviews Genetics* **3**:262–273.

Birkhead TR, Pizzari T, Pitnick S, Hosken DJ, Birkhead TR & Pizzari T (2002). Postcopulatory sexual selection. Evolutionary Behavioral Ecology. (ed by CW Fox & DF Westneat) Oxford University Press, pp 262–273.

Black IV WC & Krafsur ES (1987). Fecundity and size in the housefly: investigations of some environmental sources and genetic correlates of variation. *Medical and Veterinary Entomology* **1**:369–382.

Bluhm CK & Gowaty PA (2004). Social constraints on female mate preferences in mallards, *Anas platyrhynchos*, decrease offspring viability and mother productivity. *Animal Behaviour* **68**:977–983.

Bonduriansky R & Chenoweth SF (2009). Intralocus sexual conflict. *Trends in Ecology & Evolution* **24**:280–288.

Bopp D (2010). About females and males: continuity and discontinuity in flies. *Journal of Genetics* **89**:315–323.

Bopp D, Saccone G & Beye M (2014). Sex determination in insects: variations on a common theme. *Sexual Development* **8**:20–28.

Bordenstein SR & Theis KR (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLoS Biology* **13**:1–23.

Bracewell R & Bachtrog D (2020). Complex evolutionary history of the Y chromosome in flies of the *Drosophila obscura* species group. *Genome Biology & Evolution* **12**:494–505.

Bridges CB (1916a). Non-disjunction as proof of tile chromosome theory of heredity. *Genetics* **1**:1–52.

Bridges CB (1916b). Non-disjunction as proof of tile chromosome theory of heredity (concluded). *Genetics* **1**:107–163.Brockhurst MA, Chapman T, King KC, Mank JE, Paterson S & Hurst GDD (2014). Running with the Red Queen: the role of biotic conflicts in evolution. *Proceedings of the Royal Society of London Series B-Biological Sciences* **281**:20141382.

Brommer JE (2000). The evolution of fitness in life-history theory. *Biological Reviews*

75:377–404.

Brooks R & Endler JA (2001). Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* **55**:1002–1015.

Bryant EH (1980). Geographic variation in components of mating success of the housefly, *Musca domestica* L., in the United States. *American Naturalist* **116**:655–669.

Bugrov AG, Jetybayev IE, Karagyan GH & Rubtsov NB (2016). Sex chromosome diversity in Armenian toad grasshoppers (Orthoptera, Acridoidea, Pamphagidae). *Comparative Cytogenetics* **10**:45–59.

Bull JJ & Charnov EL (1977). Changes in the heterogametic mechanism of sex determination. *Heredity* **39**:1–14.

Burghardt G, Hediger M, Siegenthaler C, Moser M, Dübendorfer A & Bopp D (2005). The *transformer2* gene in *Musca domestica* is required for selecting and maintaining the female pathway of development. *Development Genes and Evolution* **215**:165–176.

Bürkner PC (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software* **80**:1-28.

Burtis KC & Baker BS (1989). *Drosophila doublesex* gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides. *Cell* **56**:997–1010.

Çakır Ş & Kence A (1996). The distribution of males having XY and XX chromosomes in housefly populations (Diptera: Muscidae) of Turkey. *Genetica* **98**:205–210.

Çakır Ş & Kence A (2000). Polymorphism of *M* factors in populations of the housefly, *Musca domestica* L., in Turkey. *Genetical Research, Cambridge* **76**:19–25.

Calsbeek R, Duryea MC, Goedert D, Bergeron P & Cox RM (2015). Intralocus sexual conflict, adaptive sex allocation, and the heritability of fitness. *Journal of Evolutionary Biology* **28**:1975–1985.

Carrillo J, Danielson-François A, Siemann E & Meffert L (2012). Male-biased sex ratio increases female egg laying and fitness in the housefly, *Musca domestica*. *Journal of Ethology* **30**:247–254.

Carvalho AB, Vicoso B, Russo CAM, Swenor B & Clark AG (2015). Birth of a new gene on the Y chromosome of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **112**:12450–12455.

Chapman T, Arnqvist G, Bangham J & Rowe L (2003). Sexual conflict. *Trends in Ecology & Evolution* **18**:41–47.

Chapman T, Liddle LF, Kalb JM, Wolfner MF & Partridge L (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**:241–244.

Charlesworth B (1978). Model for evolution of Y chromosomes and dosage compensation. *Proceedings of the National Academy of Sciences of the United States of America* **75**:5618–5622.

Charlesworth B (1980). Evolution in Age-Structured Populations. Cambridge University Press, Cambridge, United Kingdom.

Charlesworth D (2018). The guppy sex chromosome system and the sexually antagonistic polymorphism hypothesis for Y chromosome recombination suppression. *Genes* **9**:264.

Charlesworth B & Charlesworth D (1978). A model for the evolution of dioecy and gynodioecy. *American Naturalist* **112**:975–997.

Charlesworth B & Charlesworth D (2000). The degeneration of Y chromosomes. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **355**:1563–1572.

Charlesworth D, Charlesworth B & Marais G (2005). Steps in the evolution of heteromorphic sex chromosomes. *Heredity* **95**:118–128.

Charlesworth B, Jordan CY & Charlesworth D (2014). The evolutionary dynamics of sexually antagonistic mutations in pseudoautosomal regions of sex chromosomes. *Evolution* **68**:1339–1350.

Charlesworth B, Morgan MT & Charlesworth D (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**:1289–1303.

Charnov EL (1979). Simultaneous hermaphroditism and sexual selection. *Proceedings of the National Academy of Sciences of the United States of America* **76**:2480–2484.

Charnov EL & Bull JJ (1977). When is sex environmentally determined? *Nature* **266**:828–832.

Chow CY, Wolfner MF & Clark AG (2010). The genetic basis for male × female interactions underlying variation in reproductive phenotypes of *Drosophila*. *Genetics* **186**:1355–1365.

Clark NL, Gasper J, Sekino M, Springer SA, Aquadro CF & Swanson WJ (2009).

Coevolution of interacting fertilization proteins. *PLoS Genetics* **5**:e1000570.

Clutton-Brock TH & Parker GA (1995). Sexual coercion in animal societies. *Animal Behaviour* **49**:1345–1365.

Colwell AE & Shorey HH (1975). The courtship behavior of the house fly, *Musca domestica* (Diptera: Muscidae). *Annals of the Entomological Society of America* **68**:152–156.

Connallon T (2015). The geography of sex-specific selection, local adaptation, and sexual dimorphism. *Evolution* **69**:2333–2344.

Connallon T & Clark AG (2011). The resolution of sexual antagonism by gene duplication. *Genetics* **187**:919–937.

Connallon T & Clark AG (2014). Evolutionary inevitability of sexual antagonism. *Proceedings of the Royal Society of London Series B-Biological Sciences* **281**:20132123.

Cox RM & Calsbeek R (2009). Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *American Naturalist* **173**:176–187.

Darwin C (1859). On the Origin of Species by Means of Natural Selection, or, the Preservation of Favoured Races in the Struggle for Life. John Murray, London, England.

Dawkins R (1976). The Selfish Gene. Oxford University Press, Oxford, United Kingdom.

Demir E & Dickson BJ (2005). *fruitless* splicing specifies male courtship behavior in *Drosophila*. *Cell* **121**:785–794.

Demuth JP, Flanagan RJ & Delph LF (2014). Genetic architecture of isolation between two species of *Silene* with sex chromosomes and Haldane's rule. *Evolution* **68**:332–342.

Denholm I, Franco MG, Rubini PG & Vecchi M (1985). Geographical variation in house-fly (*Musca domestica* L.) sex determinants within the British Isles. *Genetical Research, Cambridge* **47**:19–27.

Devlin RH & Nagahama Y (2002). Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* **208**:191–364.

van Doorn GS (2009). Intralocus sexual conflict. Year in Evolutionary Biology 2009. pp 52–71.

van Doorn GS (2014). Evolutionary transitions between sex-determining mechanisms: a review of theory. *Sexual Development* **8**:7–19.

van Doorn GS & Kirkpatrick M (2007). Turnover of sex chromosomes induced by sexual conflict. *Nature* **449**:909–912.

van Doorn GS & Kirkpatrick M (2010). Transitions between male and female heterogamety caused by sex-antagonistic selection. *Genetics* **186**:629–645.

Doums C, Bremond P, Delay B & Jarne P (1996). The genetical and environmental determination of phally polymorphism in the freshwater snail *Bulinus truncatus*. *Genetics* **142**:217–225.

Dübendorfer A & Hediger M (1998). The female-determining gene *F* of the housefly, *Musca domestica*, acts maternally to regulate its own zygotic activity. *Genetics* **150**:221–226.

Dübendorfer A, Hediger M, Burghardt G & Bopp D (2002). *Musca domestica*, a window on the evolution of sex-determining mechanisms in insects. *International Journal of Developmental Biology* **46**:75–79.

Ellegren H & Parsch J (2007). The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics* **8**:689–698.

Emerson JJ, Kaessmann H, Betrán E & Long M (2004). Extensive gene traffic on the mammalian X chromosome. *Science* **303**:537–540.

Engen S, Lande R, Sæther BE & Stephen Dobson F (2009). Reproductive value and the stochastic demography of age-structured populations. *American Naturalist* **174**:795–804.

Ezaz T, Sarre SD, O'Meally D, Marshall Graves JA & Georges A (2009). Sex chromosome evolution in lizards: independent origins and rapid transitions. *Cytogenetic and Genome Research* **127**:249–260.

Fabig G, Müller-Reichert T & Paliulis L V. (2016). Back to the roots: segregation of univalent sex chromosomes in meiosis. *Chromosoma* **125**:277–286.

Feldmeyer B (2009). The Effect of Temperature on Sex Determination. PhD thesis, University of Groningen.

Feldmeyer B, Kozielska M, Weissing FJ, Beukeboom LW & Pen I (2008). Climatic variation and the geographical distribution of sex determination mechanisms in the housefly. *Evolutionary Ecology Research* **10**:797–809.

Fisher RA (1930). The Genetical Theory of Natural Selection. Oxford University Press, Oxford, United Kingdom, United Kingdom.

Fisher RA (1931). The evolution of dominance. *Biological Reviews* **6**:345–368.

Foerster K, Coulson T, Sheldon BC, Pemberton JM, Clutton-Brock TH & Kruuk LEB (2007). Sexually antagonistic genetic variation for fitness in red deer. *Nature* **447**:1107–1110.

Franco MG, Rubini PG & Vecchi M (1982). Sex-determinants and their distribution in various populations of *Musca domestica* L. of Western Europe. *Genetical Research, Cambridge* **40**:279–293.

Francuski L, Jansen W & Beukeboom LW (2020). Effect of temperature on egg production in the common housefly. *Entomologia Experimentalis et Applicata*.

Gailey DA, Billeter J-C, Liu JH, Bauzon F, Allendorfer JB & Goodwin SF (2006). Functional conservation of the *fruitless* male sex-determination gene across 250 Myr of insect evolution. *Molecular Biology and Evolution* **23**:633–643.

Garbaczewska M, Billeter JC & Levine JD (2013). *Drosophila melanogaster* males increase the number of sperm in their ejaculate when perceiving rival males. *Journal of Insect Physiology* **59**:306–310.

García-Roa R, Garcia-Gonzalez F, Noble DWA & Carazo P (2020). Temperature as a modulator of sexual selection. *Biological Reviews* **3**:.

Garnier S (2018). Default color maps from "matplotlib." *https://cran.r-project.org/package=viridis*.

Ge C, Ye J, Weber C, Sun W, Zhang H, Zhou Y, Cai C, Qian G & Capel B (2018). The histone demethylase KDM6B regulates temperature-dependent sex determination in a turtle species. *Science* **360**:645–648.

Gelman A, Goodrich B, Gabry J & Vehtari A (2019). R-squared for Bayesian Regression Models. *American Statistician* **73**:307–309.

Geuverink E & Beukeboom LW (2014). Phylogenetic distribution and evolutionary dynamics of the sex determination genes *doublesex* and *transformer* in insects. *Sexual Development* **8**:38–49.

Godin JGJ & McDonough HE (2003). Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behavioral Ecology* **14**:194–200.

Goodfellow PN & Lovell-Badge R (1993). *SRY* and sex determination in mammals. *Annual Review of Genetics* **27**:71–92.

Goulson D, Bristow L, Elderfield E, Brinklow K, Parry-Jones B & Chapman JW (1999). Size, symmetry, and sexual selection in the housefly, *Musca domestica*.

Evolution **53**:527–534.

Gowaty PA (2012). The evolution of multiple mating. *Fly* **6**:3–11.

Gowaty PA, Kim YK & Anderson WW (2012). No evidence of sexual selection in a repetition of Bateman's classic study of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **109**:11740–11745.

Grafen A (2020). The Price equation and reproductive value. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**:0–2.

Graves JAM (2006). Sex chromosome specialization and degeneration in mammals. *Cell* **124**:901–914.

Green MM (1980). Transposable elements in *Drosophila* and other Diptera. *Annual Review of Genetics* **14**:109–120.

Gromko MH & Pyle DW (1978). Sperm competition, male fitness, and repeated mating by female *Drosophila melanogaster*. *Evolution* **32**:588–593.

Haerty W, Jagadeeshan S, Kulathinal RJ, Wong A, Ram KR, Sirot LK, Levesque L, Artieri CG, Wolfner MF, Civetta A & Singh RS (2007). Evolution in the fast lane: rapidly evolving sex-related genes in *Drosophila*. *Genetics* **177**:1321–1335.

Haig D, Úbeda F & Patten MM (2014). Specialists and generalists: the sexual ecology of the genome. *Cold Spring Harbor Perspectives in Biology* **6**:a017525.

Haldane JBS (1922). Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics* **12**:101–109.

Hall AB, Basu S, Jiang X, Qi Y, Timoshevskiy VA, Biedler JK, Sharakhova M V, Elahi R, Anderson MAE, Chen X, Sharakhov I V, Adelman ZN & Tu Z (2015). A male-determining factor in the mosquito *Aedes aegypti*. *Science* **348**:1268–1270.

Hall MD, Lailvaux SP, Blows MW & Brooks RC (2010). Sexual conflict and the maintenance of multivariate genetic variation. *Evolution* **64**:1697–1703.

Hamilton WD (1964). The genetical evolution of social behavior. I. *Journal of Theoretical Biology* **7**:1–16.

Hamilton WD (1967). Extraordinary sex ratios. *Science* **156**:477–488.

Hamm RL, Gao J-R, Lin GG-H & Scott JG (2009). Selective advantage for III^M males over Y^M males in cage competition, mating competition, and pupal emergence in *Musca domestica* L. (Diptera: Muscidae). *Environmental Entomology* **38**:499–504.

Hamm RL, Meisel RP & Scott JG (2015). The evolving puzzle of autosomal versus Y-

linked male determination in *Musca domestica*. *G3-Genes Genomes Genetics* **5**:371–384.

Hamm RL & Scott JG (2008). Changes in the frequency of Y^M versus III^M in the housefly, *Musca domestica* L., under field and laboratory conditions. *Genetics Research, Cambridge* **90**:493–498.

Hamm RL & Scott JG (2009). A high frequency of male determining factors in male *Musca domestica* (Diptera: Muscidae) from Ipswich, Australia. *Journal of Medical Entomology* **46**:169–172.

Hamm RL, Shono T & Scott JG (2005). A cline in frequency of autosomal males is not associated with insecticide resistance in house fly (Diptera: Muscidae). *Journal of Economic Entomology* **98**:171–176.

Handley LJL, Ceplitis H & Ellegren H (2004). Evolutionary strata on the chicken Z chromosome: implications for sex chromosome evolution. *Genetics* **167**:367–376.

Hawkins JR, Taylor A, Berta P, Levilliers J, Van der Auwera B & Goodfellow PN (1992). Mutational analysis of *SRY*: nonsense and missense mutations in XY sex reversal. *Human Genetics* **88**:471–474.

Hediger M, Burghardt G, Siegenthaler C, Buser N, Hilfiker-Kleiner D, Dübendorfer A & Bopp D (2004). Sex determination in *Drosophila melanogaster* and *Musca domestica* converges at the level of the terminal regulator *doublesex*. *Development Genes and Evolution* **214**:29–42.

Hediger M, Henggeler C, Meier N, Perez R, Saccone G & Bopp D (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**:155–170.

Hediger M, Minet AD, Niessen M, Schmidt R, Hilfiker-Kleiner D, Çakır Ş, Nöthiger R & Dübendorfer A (1998). The male-determining activity on the *Y* chromosome of the housefly (*Musca domestica* L.) consists of separable elements. *Genetics* **150**:651–661.

Herpin A, Adolfi MC, Nicol B, Hinzmann M, Schmidt C, Klughammer J, Engel M, Tanaka M, Guiguen Y & Schartl M (2013). Divergent expression regulation of gonad development genes in medaka shows incomplete conservation of the downstream regulatory network of vertebrate sex determination. *Molecular Biology and Evolution* **30**:2328–2346.

Hill WG & Robertson A (1966). The effect of linkage on limits to artificial selection. *Genetics* **8**:269–294.

Hiroyoshi T (1977). Some new mutants and revised linkage maps of the housefly, *Musca domestica* L. *Japanese Journal of Genetics* **52**:275–288.

Hoban S, Kelley JL, Lotterhos KE, Antolin MF, Bradburd G, Lowry DB, Poss ML, Reed LK, Storfer A & Whitlock MC (2016). Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *American Naturalist* **188**:379–397.

Højland DH, Scott JG, Vagn Jensen KM & Kristensen M (2014). Autosomal male determination in a spinosad-resistant housefly strain from Denmark. *Pest Management Science* **70**:1114–1117.

Holland B & Rice WR (1998). Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* **52**:1–7.

Holleley CE, O'Meally D, Sarre SD, Marshall Graves JA, Ezaz T, Matsubara K, Azad B, Zhang X & Georges A (2015). Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. *Nature* **523**:79–82.

Hunter DC, Pemberton JM, Pilkington JG & Morrissey MB (2019). Pedigree-based estimation of reproductive value. *Journal of Heredity* **110**:433–444.

Innocenti P & Morrow EH (2010). The sexually antagonistic genes of *Drosophila melanogaster*. *PLoS Biology* **8**:e1000335.

Inoue H, Fukumori Y & Hiroyoshi T (1983). Mapping of autosomal male-determining factors of the housefly, *Musca domestica* L., by means of sex-reversal. *Japanese Journal of Genetics* **58**:451–461.

Inoue H & Hiroyoshi T (1986). A maternal-effect sex-transformation mutant of the housefly, *Musca domestica* L. *Genetics* **112**:469–482.

Ito H, Fujitani K, Usui K, Shimizu-Nishikawa K, Tanaka S & Yamamoto D (1996). Sexual orientation in *Drosophila* is altered by the *satori* mutation in the sex-determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. *Proceedings of the National Academy of Sciences of the United States of America* **93**:9687–9692.

Jaenike J (2001). Sex chromosome meiotic drive. *Annual Review of Ecology and Systematics* **32**:25–49.

Janzen FJ (1994). Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences of the United States*

of America **91**:7487–7490.

Janzen FJ & Paukstis GL (1991). Environmental sex determination in reptiles: ecology, evolution, and experimental design. *Quarterly Review of Biology* **66**:149–179.

Jiang PP, Hartl DL & Lemos B (2010). Y not a dead end: Epistatic interactions between Y-linked regulatory polymorphisms and genetic background affect global gene expression in *Drosophila melanogaster*. *Genetics* **186**:109–118.

Jordan CY & Charlesworth D (2012). The potential for sexually antagonistic polymorphism in different genome regions. *Evolution* **66**:505–516.

Jordan CY & Connallon T (2014). Sexually antagonistic polymorphism in simultaneous hermaphrodites. *Evolution* **68**:3555–3569.

Kaiser VB, Zhou Q & Bachtrog D (2011). Nonrandom gene loss from the *Drosophila miranda* neo-Y chromosome. *Genome Biology & Evolution* **3**:1329–1337.

Kawecki TJ, Lenski RE, Ebert D, Hollis B, Olivieri I & Whitlock MC (2012). Experimental evolution. *Trends in Ecology and Evolution* **27**:547–560.

Kelly WG, Xu S, Montgomery MK & Fire A (1997). Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. *Genetics* **146**:227–238.

Kikuchi K & Hamaguchi S (2013). Novel sex-determining genes in fish and sex chromosome evolution. *Developmental Dynamics* **242**:339–353.

Kim Y-J, Bartalska K, Audsley N, Yamanaka N, Yapici N, Lee J-Y, Kim Y-C, Markovic M, Isaac E, Tanaka Y & Dickson BJ (2010). MIPs are ancestral ligands for the sex peptide receptor. *Proceedings of the National Academy of Sciences of the United States of America* **107**:6520–6525.

Kirkpatrick M & Hall DW (2004). Sexual selection and sex linkage. *Evolution* **58**:683–691.

Kitano J & Peichel CL (2012). Turnover of sex chromosomes and speciation in fishes. *Environmental Biology of Fishes* **94**:549–558.

Kopp A (2012). *Dmrt* genes in the development and evolution of sexual dimorphism. *Trends in Genetics* **28**:175–184.

Kozielska M (2008). Evolutionary Dynamics of Sex Determination. PhD thesis, University of Groningen.

Kozielska M, Feldmeyer B, Pen I, Weissing FJ & Beukeboom LW (2008). Are autosomal sex-determining factors of the housefly (*Musca domestica*)

spreading north? *Genetics Research, Cambridge* **90**:157–165.

Kozielska M, Pen I, Beukeboom LW & Weissing FJ (2006). Sex ratio selection and multi-factorial sex determination in the housefly: A dynamic model. *Journal of Evolutionary Biology* **19**:879–888.

Kozielska M, Weissing FJ, Beukeboom LW & Pen I (2010). Segregation distortion and the evolution of sex-determining mechanisms. *Heredity* **104**:100–112.

Krupp JJ, Kent C, Billeter JC, Azanchi R, So AKC, Schonfeld JA, Smith BP, Lucas C & Levine JD (2008). Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. *Current Biology* **18**:1373–1383.

Kruuk LEB (2004). Estimating genetic parameters in natural populations using the "animal model." *Philosophical Transactions of the Royal Society B: Biological Sciences* **359**:873–890.

Krzywinska E, Dennison NJ, Lycett GJ & Krzywinski J (2016). A maleness gene in the malaria mosquito *Anopheles gambiae*. *Science* **353**:67–69.

Kuijper B & Pen I (2014). Conflict over condition-dependent sex allocation can lead to mixed sex-determination systems. *Evolution* **68**:3229–3247.

Kuijper B, Pen I & Weissing FJ (2012). A guide to sexual selection theory. *Annual Review of Ecology, Evolution, and Systematics* **43**:287–311.

Kuijper B, Stewart AD & Rice WR (2006). The cost of mating rises nonlinearly with copulation frequency in a laboratory population of *Drosophila melanogaster*. *Journal of Evolutionary Biology* **19**:1795–1802.

Lachance J, Johnson NA & True JR (2011). The population genetics of X-autosome synthetic lethals and steriles. *Genetics* **189**:1011–1027.

Lahn BT & Page DC (1997). Functional coherence of the human Y chromosome. *Science* **278**:675–680.

Lahn BT & Page DC (1999). Four evolutionary strata on the human X chromosome. *Science* **286**:964–967.

Lande R (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**:292–305.

Laturney M & Billeter J-C (2016). *Drosophila melanogaster* females restore their attractiveness after mating by removing male anti-aphrodisiac pheromones. *Nature Communications* **7**:12322.

Laturney M, van Eijk R & Billeter J-C (2018). Last male sperm precedence is

modulated by female remating rate in *Drosophila melanogaster*. *Evolution Letters* **2**:180–189.

Lemos B, Araripe LO & Hartl DL (2008). Polymorphic Y chromosomes harbor cryptic variation with manifold functional consequences. *Science* 91–94.

Lenormand T, Fyon F, Sun E & Roze D (2020). Sex chromosome degeneration by regulatory evolution. *Current Biology* **30**:3001-3006.e5.

Leopold RA (1970). Cytological and cytochemical studies on the ejaculatory duct and accessory secretion in *Musca domestica*. *Journal of Insect Physiology* **16**:1859–1872.

Leopold RA (1976). The role of male accessory glands in insect reproduction. *Annual Review of Entomology* **21**:199–221.

Leopold RA, Terranova AC & Swilley EM (1971). Mating refusal in *Musca domestica*: effects of repeated mating and decerebration upon frequency and duration of copulation. *Journal of Experimental Zoology* **176**:353–359.

Leopold RA, Terranova AC, Thorson BJ & Degrugillier ME (1971). The biosynthesis of the male housefly accessory secretion and its fate in the mated female. *Journal of Insect Physiology* **17**:987–1003.

Liu H & Kubli E (2003). Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **100**:9929–9933.

Lüpold S, Pitnick S, Berben KS, Blengini CS, Belote JM & Manier MK (2013). Female mediation of competitive fertilization success in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **110**:10693–10698.

Lyttle TW (1981). Experimental population genetics of meiotic drive systems. III. Neutralization of sex-ratio distortion in *Drosophila* through sex-chromosome aneuploidy. *Genetics*317–334.

Ma W-J, Rodrigues N, Sermier R, Brelsford A & Perrin N (2016). *Dmrt1* polymorphism covaries with sex-determination patterns in *Rana temporaria*. *Ecology and Evolution* **6**:5107–5117.

Mahajan S, Wei KH, Nalley MJ, Gibilisco L & Bachtrog D (2018). De novo assembly of a young Drosophila Y chromosome using single-molecule sequencing and chromatin conformation capture. 1–28.

Manier MK, Belote JM, Berben KS, Novikov D, Stuart WT & Pitnick S (2010).

Resolving mechanisms of competitive fertilization success in *Drosophila melanogaster*. *Science* **328**:354–357.

Mank JE (2009). Sex chromosomes and the evolution of sexual dimorphism: lessons from the genome. *American Naturalist* **173**:141–150.

Mank JE (2013). Sex chromosome dosage compensation: definitely not for everyone. *Trends in Genetics* **29**:677–683.

Mank JE (2017). Population genetics of sexual conflict in the genomic era. *Nature Reviews Genetics* **18**:721–730.

Marie-Orleach L, Vogt-Burri N, Mouginot P, Schlatter A, Vizoso DB, Bailey NW & Schärer L (2017). Indirect genetic effects and sexual conflicts: partner genotype influences multiple morphological and behavioral reproductive traits in a flatworm. *Evolution* **71**:1232–1245.

Marin I & Baker BS (1998). The evolutionary dynamics of sex determination. *Science* **281**:1990–1994.

McDonald IC, Evenson P, Nickel CA & Johnson OA (1978). House fly genetics: isolation of a female determining factor on chromosome 4. *Annals of the Entomological Society of America* **71**:692–694.

McGraw JB & Caswell H (1996). Estimation of individual fitness from life-history data. *American Naturalist* **147**:47–64.

McIlroy D, Brownrigg R, Minka TP & Bivand R (2020). mapproj: map projections. https://cran.r-project.org/package=mapproj.

McIntyre LM, Bono LM, Genissel A, Westerman R, Junk D, Telonis-Scott M, Harshman L, Wayne ML, Kopp A & Nuzhdin S V (2006). Sex-specific expression of alternative transcripts in *Drosophila*. *Genome Biology* **7**:R79.

Meccariello A, Salvemini M, Primo P, Hall B, Koskinioti P, Dalíková M, Gravina A, Gucciardino MA, Forlenza F, Gregoriou ME, Ippolito D, Monti SM, Petrella V, Perrotta MM, Schmeing S, Ruggiero A, Scolari F, Giordano E, Tsoumani KT, Marec F, Windbichler N, Arunkumar KP, Bourtzis K, Mathiopoulos KD, Ragoussis J, Vitagliano L, Tu Z, Papathanos PA, Robinson MD & Saccone G (2019). *Maleness-on-the-Y* (*MoY*) orchestrates male sex determination in major agricultural fruit fly pests. *Science* **365**:1457–1460.

Meffert LM & Bryant EH (1992). Divergent ambulatory and grooming behavior in serially bottlenecked lines of the housefly. *Evolution* **46**:1399.

Meffert LM & Hagenbuch KL (2005). The genetic architecture of house fly mating

behavior. *Current Topics in Developmental Biology* **66**:189–213.

Meier N, Käppeli SC, Hediger Niessen M, Billeter J-C, Goodwin SF & Bopp D (2013). Genetic control of courtship behavior in the housefly: evidence for a conserved bifurcation of the sex-determining pathway. *PLoS ONE* **8**:e62476.

Meisel RP (2020). Evolution of sex determination and sex chromosomes: a novel alternative paradigm. *BioEssays* **42**:1–11.

Metz JAJ, Geritz SAH & Nisbet RM (1992). How should we define "fitness" for general ecological scenarios? *Trends in Ecology & Evolution* **7**:198–202.

Miller CW & Svensson EI (2014). Sexual selection in complex environments. *Annual Review of Entomology* **59**:427–445.

Moore AJ & Pizzari T (2005). Quantitative genetic models of sexual conflict based on interacting phenotypes. *American Naturalist* **165**:S88–S97.

Moyer JT & Nakazono A (1978). Protandrous hermaphroditism in six species of the anemonefish genus Amphiprion in Japan. *Japanese Journal of Ichthyology* **25**:101–106.

Muller HJ (1918). Genetic variability, twin hybrids and constant hybrids, in a case of balanced lethal mutations. *Genetics* **3**:422–499.

Muller HJ (1964). The relation of recombination to mutational advance. *Mutation Research* **1**:2–9.

Muralidhar P & Veller C (2018). Sexual antagonism and the instability of environmental sex determination. *Nature Ecology & Evolution* **2**:343–351.

Murvosh CM, Fye RL & Labrecque GC (1964). Studies on the mating behavior of the housefly, *Musca domestica* L. *Ohio Journal of Science* **64**:264–271.

Mylius SD & Diekmann O (1995). On evolutionarily stable life histories, optimization and the need to be specific about density dependence. *Oikos* **74**:218–224.

Nakadera Y, Swart EM, Hoffer JNA, Boon O Den, Ellers J & Koene JM (2014). Receipt of seminal fluid proteins causes reduction of male investment in a simultaneous hermaphrodite. *Current Biology* **24**:859–862.

Nigro RG, Campos MCC & Perondini ALP (2007). Temperature and the progeny sex-ratio in *Sciara ocellaris* (Diptera, Sciaridae). *Genetics and Molecular Biology* **30**:152–158.

Noorman N & Den Otter CJ (2002). Effects of relative humidity, temperature, and population density on production of cuticular hydrocarbons in housefly *Musca domestica* L. *Journal of Chemical Ecology* **28**:1819–1829.

Ohno S (1967). Sex chromosomes and sex-linked genes. Springer-Verlag, Berlin, Germany.

Olito C (2016). Consequences of genetic linkage for the maintenance of sexually antagonistic polymorphism in hermaphrodites. *Evolution* **71**:458–464.

Orzack SH, Sohn JJ, Kallman KD, Levin SA & Johnston R (1980). Maintenance of the three sex chromosome polymorphism in the platyfish, *Xiphophorus maculatus*. *Evolution* **34**:633–672.

Page DC, Mosher R, Simpson EM, Fisher EM, Mardon G, Pollack J, McGillivray B, de la Chapelle A & Brown LG (1987). The sex-determining region of the human Y chromosome encodes a finger protein. *Cell* **51**:1091–1104.

Palusa SG, Ali GS & Reddy ASN (2007). Alternative splicing of pre-mRNAs of Arabidopsis serine/arginine-rich proteins: regulation by hormones and stresses. *Plant Journal* **49**:1091–1107.

Pan Q, Feron R, Yano A, Guyomard R, Jouanno E, Vigouroux E, Wen M, Busne JM, Bobe J, Concordet JP, Parrinello H, Journot L, Klopp C, Lluch J, Roques C, Postlethwait J, Schartl M, Herpin A & Guiguen Y (2019). Identification of the master sex determining gene in Northern pike (*Esox lucius*) reveals restricted sex chromosome differentiation.

Parker GA (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews* **45**:525–567.

Parker GA (1979). Sexual selection and sexual conflict. Sexual Selection and Reproductive Competition in Insects. (ed by MS Blum & NA Blum) Academic Press, New York City, pp 123–166.

Parker GA (2006). Sexual conflict over mating and fertilization: an overview. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **361**:235–259.

Parker GA, Baker RR & Smith VGF (1972). The origin and evolution of gamete dimorphism and the male-female phenomenon. *Journal of Theoretical Biology* **36**:529–553.

Parker LM, O'Connor WA, Byrne M, Dove M, Coleman RA, Pörtner HO, Scanes E, Virtue P, Gibbs M & Ross PM (2018). Ocean acidification but not warming alters sex determination in the Sydney rock oyster, *Saccostrea glomerata*. *Proceedings of the Royal Society B: Biological Sciences* **285**:.

Parker GA & Partridge L (1998). Sexual conflict and speciation. *Philosophical*

Transactions of the Royal Society of London Series B-Biological Sciences **353**:261–274.

Parsch J & Ellegren H (2013). The evolutionary causes and consequences of sex-biased gene expression. *Nature Reviews Genetics* **14**:83–87.

Patten MM, Úbeda F & Haig D (2013). Sexual and parental antagonism shape genomic architecture. *Proceedings of the Royal Society of London Series B-Biological Sciences* **280**:20131795.

Pease JB & Hahn MW (2012). Sex chromosomes evolved from independent ancestral linkage groups in winged insects. *Molecular Biology and Evolution* **29**:1645–1653.

Peck JR (1994). A ruby in the rubbish: beneficial mutations, deleterious mutations and the evolution of sex. *Genetics* **137**:597–606.

Pedersen EJ, Miller DL, Simpson GL & Ross N (2019). Hierarchical generalized additive models in ecology: An introduction with mgcv. *PeerJ* **7**:e6876.

Peichel CL, Ross JA, Matson CK, Dickson M, Grimwood J, Schmutz J, Myers RM, Mori S, Schluther D & Kingsley DM (2004). The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. *Current Biology* **14**:1416–1424.

Pen I (2006). When boys want to be girls: effects of mating system and dispersal on parent-offspring sex ratio conflict. *Evolutionary Ecology Research* **8**:103–113.

Pen I, Uller T, Feldmeyer B, Harts A, While GM & Wapstra E (2010). Climate-driven population divergence in sex-determining systems. *Nature* **468**:436–438.

Pennell TM, de Haas FJH, Morrow EH & van Doorn GS (2016). Contrasting effects of intralocus sexual conflict on sexually antagonistic coevolution. *Proceedings of the National Academy of Sciences of the United States of America* **113**:E978–E986.

Pennell TM & Morrow EH (2013). Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. *Ecology and Evolution* **3**:1819–1834.

Pischedda A, Stewart AD, Little MK & Rice WR (2011). Male genotype influences female reproductive investment in *Drosophila melanogaster*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **278**:2165–2172.

Pitnick S & Hosken DJ (2002). Postcopulatory sexual selection. Evolutionary Behavioral Ecology. (ed by CW Fox & DF Westneat) Oxford University Press, Oxford, United Kingdom, pp 379–399.

Poissant J, Morrissey MB, Gosler AG, Slate J & Sheldon BC (2016). Multivariate selection and intersexual genetic constraints in a wild bird population. *Journal of Evolutionary Biology* **29**:2022–2035.

Pokorná MJ & Kratochvíl L (2016). What was the ancestral sex-determining mechanism in amniote vertebrates? *Biological Reviews of the Cambridge Philosophical Society* **91**:1–12.

Pomiankowski A, Nöthiger R & Wilkins A (2004). The evolution of the *Drosophila* sex-determination pathway. *Genetics* **166**:1761–1773.

Prasad NG, Bedhomme S, Day T & Chippindale AK (2007). An evolutionary cost of separate genders revealed by male-limited evolution. *American Naturalist* **169**:29–37.

R Development Core Team (2020). A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing.* https://www.R-project.org.

Ragland SS & Sohal RS (1973). Mating behavior, physical activity and aging in the housefly, *Musca domestica*. *Experimental Gerontology* **8**:135–145.

Reed DH & Bryant EH (2004). Phenotypic correlations among fitness and its components in a population of the housefly. *Journal of Evolutionary Biology* **17**:919–923.

Rens W, Grützner F, O'Brien PCM, Fairclough H, Graves JAM & Ferguson-Smith MA (2004). Resolution and evolution of the duck-billed platypus karyotype with an $X_1Y_1X_2Y_2X_3Y_3X_4Y_4X_5Y_5$ male sex chromosome constitution. *Proceedings of the National Academy of Sciences of the United States of America* **101**:16257–16261.

Rice WR (1984). Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**:735–742.

Rice WR (1986). On the instability of polygenic sex determination: the effect of sex-specific selection. *Evolution* **40**:633–639.

Rice WR (1987a). The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. *Evolution* **41**:911–914.

Rice WR (1987b). Genetic hitchhiking and the evolution of reduced genetic activity of the *Y* sex chromosome. *Genetics* **116**:161–167.

Rice WR (1988). The effect of sex chromosomes on the rate of evolution. *Trends in*

Ecology & Evolution **3**:2–3.

Rice WR (1992). Sexually antagonistic genes: experimental evidence. *Science* **256**:1436–1439.

Rice WR (1994). Degeneration of a nonrecombining chromosome. *Science* **263**:230–232.

Rice WR (1996a). Evolution of the Y sex chromosome in animals. *BioScience* **46**:331–343.

Rice WR (1996b). Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**:232–234.

Rice WR (1998). Male fitness increases when females are eliminated from gene pool: implications for the Y chromosome. *Proceedings of the National Academy of Sciences of the United States of America* **95**:6217–6221.

Rice WR (1999). Genetic polarization: unifying theories for the adaptive significance of recombination. *Journal of Evolutionary Biology* **12**:1047–1049.

Rice WR (2000). Dangerous liaisons. *Proceedings of the National Academy of Sciences of the United States of America* **97**:12953–12955.

Rice WR & Chippindale AK (2001). Intersexual ontogenetic conflict. *Journal of Evolutionary Biology* **14**:685–693.

Rice WR & Holland B (1997). The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behavioral Ecology and Sociobiology* **41**:1–10.

Rideout EJ, Billeter JC & Goodwin SF (2007). The sex-determination genes *fruitless* and *doublesex* specify a neural substrate required for courtship song. *Current Biology* **17**:1473–1478.

Riemann JG, Moen DJ & Thorson BJ (1967). Female monogamy and its control in houseflies. *Journal of Insect Physiology* **13**:407–418.

Riemann JG & Thorson BJ (1969). Effect of male accessory material on oviposition and mating by female house flies. *Annals of the Entomological Society of America* **62**:828–834.

Rowe L, Cameron E & Day T (2003). Detecting sexually antagonistic coevolution with population crosses. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**:2009–2016.

Rowe L, Cameron E & Day T (2005). Escalation, retreat, and female indifference as alternative outcomes of sexually antagonistic coevolution. *American Naturalist*

165:S5–S18.

Roze D & Otto SP (2011). Differential selection between the sexes and selection for sex. *Evolution* **66**:558–574.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Ryner LC, Goodwin SF, Castrillon DH, Anand A, Villella A, Baker BS, Hall JC, Taylor BJ & Wasserman SA (1996). Control of male sexual behavior and sexual orientation in *Drosophila* by the *fruitless* gene. *Cell* **87**:1079–1089.

Santidrián Tomillo P & Spotila JR (2020). Temperature-dependent sex determination in sea turtles in the context of climate change: uncovering the adaptive significance. *BioEssays* **42**:1–6.

Schärer L (2017). The varied ways of being male and female. *Molecular Reproduction & Development* **84**:94–104.

Schärer L & Janicke T (2009). Sex allocation and sexual conflict in simultaneously hermaphroditic animals. *Biology Letters* **5**:705–708.

Schärer L, Janicke T & Ramm SA (2014). Sexual conflict in hermaphrodites. *Cold Spring Harbor Perspectives in Biology* 1–26.

Schärer L & Pen I (2013). Sex allocation and investment into pre- and post-copulatory traits in simultaneous hermaphrodites: the role of polyandry and local sperm competition. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **368**:20120052.

Schenkel MA & Beukeboom LW (2016). Sex chromosome evolution: birth, maturation, decay, and rebirth. Encyclopedia of Evolutionary Biology. pp 72–80.

Schlein Y & Galun R (1984). Male housefly (*Musca domestica* L.) genital system as a source of mating pheromone. *Journal of Insect Physiology* **30**:175–177.

Schmidt R, Hediger M, Nöthiger R & Dübendorfer A (1997). The mutation *masculinizer* (*man*) defines a sex-determining gene with maternal and zygotic functions in *Musca domestica* L. *Genetics* **145**:173–183.

Schmidt R, Hediger M, Roth S, Nöthiger R & Dübendorfer A (1997). The *Y*-chromosomal and autosomal male-determining *M* factors of *Musca domestica* are equivalent. *Genetics* **147**:271–280.

Schneider J, Atallah J & Levine JD (2017). Social structure and indirect genetic effects: genetics of social behaviour. *Biological Reviews of the Cambridge*

Philosophical Society **92**:1027–1038.

Schwanz LE, Georges A, Holleley CE & Sarre SD (2020). Climate change, sex reversal and lability of sex-determining systems. *Journal of Evolutionary Biology* **33**:270–281.

Scott JG, Warren WC, Beukeboom LW, Bopp D, Clark AG, Giers SD, Hediger M, Jones AK, Kasai S, Leichter CA, Li M, Meisel RP, Minx P, Murphy TD, Nelson DR, Reid WR, Rinkevich FD, Robertson HM, Sackton TB, Sattelle DB, Thibaud-Nissen F, Tomlinson C, van de Zande L, Walden K, Wilson RK & Liu N (2014). Genome of the house fly, *Musca domestica* L., a global vector of diseases with adaptations to a septic environment. *Genome Biology* **15**:466.

Sharma A (2018). Identification and characterization of the male-determining gene in the housefly, *Musca domestica*. PhD thesis, University of Groningen.

Sharma A, Heinze SD, Wu Y, Kohlbrenner T, Morilla I, Brunner C, Wimmer EA, van de Zande L, Robinson MD, Beukeboom LW & Bopp D (2017). Male sex in houseflies is determined by *Mdmd*, a paralog of the generic splice factor gene *CWC22*. *Science* **356**:642–645.

Shearman DCA (2002). The evolution of sex determination systems in dipteran insects other than *Drosophila*. *Genetica* **116**:25–43.

Silhacek DL, Carlson DA, Mayer MS & James JD (1972). Composition and sex attractancy of cuticular hydrocarbons from houseflies: Effects of age, sex, and mating. *Journal of Insect Physiology* **18**:347–354.

Sinclair AH, Berta P, Palmer M, Hawkins JR, Griffiths BL, Smith JM, Foster JW, Frischauf A-M, Lovell-Badge R & Goodfellow PN (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* **346**:240–244.

Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T, Chinwalla A, Delehaunty A, Delehaunty K, Du H, Fewell G, Fulton L, Fulton R, Graves T, Hou S-F, Latrielle P, Leonard S, Mardis E, Maupin R, McPherson J, Miner T, Nash W, Nguyen C, Ozersky P, Pepin K, Rock S, Rohlfing T, Scott K, Schultz B, Strong C, Tin-Wollam A, Yang S-P, Waterston RH, Wilson RK, Rozen S & Page DC (2003). The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* **423**:825–837.

Steinemann M & Steinemann S (1992). Degenerating Y chromosome of *Drosophila*

miranda: a trap for retrotransposons. *Proceedings of the National Academy of Sciences of the United States of America* **89**:7591–7595.

Steinemann M & Steinemann S (1998). Enigma of *Y* chromosome degeneration: *neo-Y* and *neo-X* chromosomes of *Drosophila miranda* a model for sex chromosome evolution. *Genetica* **102/103**:409–420.

Stewart AD, Pischedda A & Rice WR (2010). Resolving intralocus sexual conflict: genetic mechanisms and time frame. *Journal of Heredity* **101**:S94–S99.

Stutt AD & Siva-Jothy MT (2001). Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. *Proceedings of the National Academy of Sciences of the United States of America* **98**:5683–5687.

Swanson WJ & Vacquier VD (2002). The rapid evolution of reproductive proteins. *Nature Reviews Genetics* **3**:137–144.

Tennessen JA, Wei N, Straub SCK, Govindarajulu R, Liston A & Ashman TL (2018). Repeated translocation of a gene cassette drives sex-chromosome turnover in strawberries. *PLoS Biology* **16**:e2006062.

Tomita T & Wada Y (1989). Multifactorial sex determination in natural populations of the housefly (*Musca domestica*) in Japan. *Japanese Journal of Genetics* **64**:373–382.

Townsend DS (1989). Sexual selection, natural selection, and a fitness trade-off in a tropical frog with male parental care. *American Naturalist* **133**:266–272.

Traut W (2010). New Y chromosomes and early stages of sex chromosome differentiation: Sex determination in *Megaselia*. *Journal of Genetics* **89**:307–313.

Tregenza T, Wedell N & Chapman T (2006). Introduction. Sexual conflict: a new paradigm? *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **361**:229–235.

Trivers RL (1972). Parental investment and sexual selection. Sexual Selection and the Descent of Man 1871-1971. pp 136–207.

Uller T & Helanterä H (2011). From the origin of sex-determining factors to the evolution of sex-determining systems. *Quarterly Review of Biology* **86**:163–180.

Uller T, Pen I, Wapstra E, Beukeboom LW & Komdeur J (2007). The evolution of sex ratios and sex-determining systems. *Trends in Ecology and Evolution* **22**:292–297.

VanKuren NW & Long M (2018). Gene duplicates resolving sexual conflict rapidly evolved essential gametogenesis functions. *Nature Ecology & Evolution* **2**:705–712.

Vanossi Este S & Rovati C (1982). Inheritance of the arrhenogenic factor *Ag* of *Musca domestica* L. *Bolletino di Zoologia* **49**:269–278.

Vehtari A, Gelman A & Gabry J (2017). Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Statistics and Computing* **27**:1413–1432.

Veller C, Muralidhar P, Constable GWA & Nowak MA (2017). Drift-induced selection between male and female heterogamety. *Genetics* **207**:711–727.

Velzen E Van, Schärer L & Pen I (2009). The effect of cryptic female choice on sex allocation in simultaneous hermaphrodites. *Proceedings of the Royal Society B-Biological Sciences* **276**:3123–3131.

Verhulst EC, van de Zande L & Beukeboom LW (2010). Insect sex determination: it all evolves around *transformer*. *Current Opinion in Genetics & Development* **20**:376–383.

Verspoor RL, Smith JML, Mannion NLM, Hurst GDD & Price TAR (2018). Strong hybrid male incompatibilities impede the spread of a selfish chromosome between populations of a fly. *Evolution Letters* **2**:169–179.

Vibranovski MD, Lopes HF, Karr TL & Long M (2009). Stage-specific expression profiling of *Drosophila* spermatogenesis suggests that meiotic sex chromosome inactivation drives genomic relocation of testis-expressed genes. *PLoS Genetics* **5**:e1000371.

Vicoso B (2019). Molecular and evolutionary dynamics of animal sex-chromosome turnover. *Nature Ecology & Evolution* **3**:1632–1641.

Vicoso B & Bachtrog D (2015). Numerous transitions of sex chromosomes in Diptera. *PLoS Biology* **13**:1–22.

Wagoner DE (1969). Presence of male determining factors found on three autosomes in the house fly, *Musca domestica*. *Nature* **223**:187–188.

Warner DA & Shine R (2008). The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* **451**:566–568.

Wei N, Govindarajulu R, Tennessen JA, Liston A, Ashman TL & Sayres MW (2017). Genetic mapping and phylogenetic analysis reveal intraspecific variation in sex chromosomes of the Virginian strawberry. *Journal of Heredity* **108**:731–739.

Werren JH & Beukeboom LW (1998). Sex determination, sex ratios, and genetic conflict. *Annual Review of Ecology and Systematics* **29**:233–261.

Werren JH, Hatcher MJ & Godfray HCJ (2002). Maternal-offspring conflict leads to the evolution of dominant zygotic sex determination. *Heredity* **88**:102–111.

Wickham H, Averick M, Bryan J, Chang W, D'Agostino McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Lin Pedersen T, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H, D' L, Mcgowan A, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Lin Pedersen T, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K & Yutani H (2019). Welcome to the Tidyverse. *Journal of Open Source Software* **4**:1686.

Wilke CO (2019). cowplot: streamlined plot theme and plot annotations for "ggplot2." https://cran.r-project.org/package=cowplot.

Wilkins AS (1995). Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determination pathway. *BioEssays* **17**:71–77.

Wood SN (2017). Generalized Additive Models: An Introduction with R. Chapman & Hall/CRC, Boca Raton, FL, USA.

Wright AE, Darolti I, Bloch NI, Oostra V, Sandkam B, Buechel SD, Kolm N, Breden F, Vicoso B & Mank JE (2017). Convergent recombination suppression suggests role of sexual selection in guppy sex chromosome formation. *Nature Communications* **8**:.

Wright AE, Harrison PW, Montgomery SH, Pointer MA & Mank JE (2014). Independent stratum formation on the avian sex chromosomes reveals inter-chromosomal gene conversion and predominance of purifying selection on the W chromosome. *Evolution* **68**:3281–3295.

Wu Y (2018). Characterisation of the *M*-locus and functional analysis of the male-determining gene in the housefly. PhD thesis, University of Groningen.

Wu CI & Xu EY (2003). Sexual antagonism and X inactivation - The SAXI hypothesis. *Trends in Genetics* **19**:243–247.

Yapici N, Kim Y-J, Ribeiro C & Dickson BJ (2008). A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour. *Nature* **451**:33–37.

Zawistowski S & Richmond RC (1986). Inhibition of courtship and mating of *Drosophila melanogaster* by the male-produced lipid, cis-vaccenyl acetate.

Journal of Insect Physiology **32**:189–192.

Zhou Q & Bachtrog D (2012a). Sex-specific adaptation drives early sex chromosome evolution in *Drosophila*. *Science* **337**:341–345.

Zhou Q & Bachtrog D (2012b). Chromosome-wide gene silencing initiates Y degeneration in *Drosophila*. *Current Biology* **22**:522–525.

SUMMARY

Sex determination (SD) is the process by which individuals commit to being either male or female during their development. SD must be properly executed to ensure that the sexual development occurs in a consistent manner, which in turn is required to ensure that the individual is capable of reproducing. Improper execution of the SD program results in incomplete female or male development and such intersexuality is generally associated with (severely) impaired reproductive capacity. Thus, the process of SD is subject to strong selection. In stark contrast to this, the mechanisms controlling SD are highly variable, as different cues both genetic and non-genetic may impinge on the process.

Past (mostly theoretical) work has sought to determine how different evolutionary phenomena contribute to the diversification of SD mechanisms. Different models predict that SD mechanisms can evolve either by neutral evolution or adaptive evolution. Additionally, various biological conflicts can contribute to SD evolution, such as conflicts between parents and their offspring, between different genetic elements (e.g. nuclear versus cytoplasmic) or between males and females (sexual conflict). Despite these advances in our understanding of how different factors may affect SD evolution, several unresolved issues remain, of which two are most relevant here. Firstly, most species exhibit a single SD mechanism (monomorphic SD), whereas in others multiple different SD mechanisms (polymorphic SD) may persist, e.g. when different SD mechanisms occur in different populations. Secondly, SD in some species is controlled solely by genetic factors (GSD) whereas in others it is controlled by environmental factors (ESD). In many species SD may however be affected by a combination of genetic and environmental factors, but it is unclear which stable SD mechanisms may be formed under such mixed control. Extending the existing body of work on SD evolution is necessary to increase our understanding of more complex SD systems.

Aside from the open questions with regard to the cause of transitions in SD, others remain with regard to their consequences. The evolution of a novel SD gene is thought to set off the evolution of a former autosome into a sex chromosome (proto-sex chromosome). Theory predicts that during this process, the sex-biased transmission pattern of the SD gene-bearing chromosome favours its enrichment with sexually antagonistic (SA) genes, which have a positive effect in one sex but a negative effect in the other. For example, a male-determining gene would thereby become associated with genetic variants that enhance fitness in male bearers, but

decrease fitness in female bearers. Testing this theory has proven difficult, owing in large part to the lack of suitable model systems.

In this thesis, I have studied the evolutionary genetics and dynamics of transitions in sex determination, with the twofold aim of increasing our understanding of both the causes of SD evolution as well as its consequences. I was inspired by and made use of the housefly *Musca domestica*, in which various SD systems are found. This species harbours different male-determining genes on both sex chromosomes and all autosomes, and a dominant female-determining gene that overrules the action of the male-determining genes. In nature, the frequencies of these genes vary between populations along latitudinal gradients. The work presented in this thesis is based on this complex SD system, and seeks to contribute to an increased understanding of how it may have evolved and how it may be exploited to study the evolution of SD and sex chromosomes.

In Chapter II, I have first reviewed past work on sexual conflict, which encompasses two distinct subforms: intralocus (IASC) and interlocus sexual conflict (IRSC). IASC is defined by the occurrence of conflicting selective pressures acting on a shared gene in males and females, so that the optimal genotype for males is not the same as that for females. Under IASC, sex-specific adaptative evolution is impeded as this would require maladaptive evolution in the opposite sex. IASC can be resolved by the evolution of sex-specific genetic architectures for males and females, after which the underlying loci may then differentiate between the two sexes.

IRSC is defined as the conflict between (potential) reproductive partners over the outcome of interactions between them, where the optimal outcome for the male and female is different. Both parties are independently affected by selection to maximize their own fitness by achieving their preferred outcome, even if at the expense of their mates. Evolutionary change under IRSC occurs in response to the social environment, which is formed by an individual's pool of compatible mates. When an adaptation arises in one sex that enhances the capacity of individuals of this sex to achieve their preferred outcome when interacting with potential mates, individuals of the other sex become less likely to achieve their preferred outcome. This then leads to enhanced selection in the maladapted sex to evolve counteradaptations. IRSC is therefore characterized by reiterated bouts of

adaptation-counteradaptation, and may give rise to fast and continuous evolutionary change.

In chapter III, I investigated the effect of interchromosomal interactions on the stability of existing sex chromosome systems and the invasibility of novel sex chromosomes. Sex chromosomes often harbour a number of genes, aside from the SD gene, which interact with other autosomal genes to affect individual fitness. Such interactions can give rise to epistasis, where the combined effect of both genes differs from the sum of their terms. Here, I show that such epistatic interactions can have both stabilizing and destabilizing effects on an ancestral SD system. Epistasis can also enhance the invasibility of novel sex chromosomes, but these effects are generally weaker. This difference is likely caused by the less extensive differentiation of novel sex chromosomes, due to which epistasis would occur less frequently. Once established, even young sex chromosomes may already have undergone substantial genetic differentiation at a limited number of loci. If these are involved in epistasis, it can strongly enhance the stability of sex chromosomes even if only established recently.

Sex can be determined genetically or environmentally, but in many species both types of cues may affect the SD process. How this affects the evolution of the genetic component remains unresolved. In Chapter IV, I investigated the evolution of a genetic SD mechanism in which the expression of one sex determination gene is positively affected by temperature. I showed that different SD systems may be evolutionary stable depending on the activity level of this gene, which in turn depends on the local temperature. Variation in temperature between different locations (i.e. subpopulations) then enables the establishment of different SD systems between them.

I applied this temperature model to the housefly *M. domestica* to determine if it can explain the existence of its multiple SD systems and their latitudinal distribution. I found that the reported allelic frequency gradient of its female-determining gene (*transformer*) may be explained by local invasion of a dominant variant in high-temperature environments. From this, gradients in male-determining genes may arise as well. Male-determining genes that have a benefit in heterozygotes but a cost in homozygotes may then be favoured in locations where the dominant female-determining variant is not present (and therefore male-determining genes can only be heterozygous). If a dominant female-determining

gene is however present, the costs of this particular male-determining gene in homozygous individuals cause it to be counterselected against, and instead a different male-determining gene may arise in these populations. This can thus generate frequency gradients in at least three SD genes as found in *M. domestica*, and thereby provides the first adaptive evolutionary hypothesis for the existence of this complex SD system.

The existence of different SD systems in *M. domestica* not only posed an evolutionary conundrum, but also provides opportunities for solving one. Male-determining genes are expected to induce Y-chromosome evolution on the chromosomes that harbour them. In presence of a dominant female-determining gene, this process is halted, and instead these chromosomes remain in a regular autosomal state. In Chapter V, I used interpopulation crosses to introgress autosomal male-determining genes from a population with a dominant female-determining gene into a genomic background without it. This process mimics the evolution of a new SD gene on an autosome, and establishes a pair of so-called baby-sex chromosomes. These baby-sex chromosomes can be used to study the very first stages of sex chromosome evolution, which in naturally-occurring systems is generally infeasible due to the degenerative nature of (late-stage) Y-chromosome evolution. I furthermore discussed how this can be used to test the "sexual antagonism" hypothesis of sex chromosome evolution, which is canonically accepted but for which empirical evidence remains sparse.

Exploiting the housefly as a model system for evolutionary biology in general and SD evolution in particular requires an understanding of how fitness is determined in this species, and how it can accurately be estimated. In Chapter VI, I investigated how female and male fitness may be assessed in *M. domestica*. For females, fitness is in large part determined by their own fecundity, whereas for males fitness is determined by the capacity to achieve mating success and thereby access to females' eggs for fertilization. However, current knowledge on housefly mating behaviour is limited, and the impact of female promiscuity on both female and male fitness is unknown. Under female promiscuity, the scope for selection extends to cover the postcopulatory phase, which can lead to the evolution of a variety of traits in males and females that can affect individual fitness, e.g. via IRSC. To develop a definitive fitness assessment methodology, it will be necessary to consider to what extent female remating occurs in this species, and how this influences males and females.

The evolutionary variability of SD systems has long been considered to be at odds with its conserved function, which must be executed carefully to avoid severe fitness costs to the individual. It has nonetheless become clear that many evolutionary phenomena can contribute to the diversification of SD. In light of this, it may be necessary to reconsider the paradigm for SD evolution: rather than being constrained by its critical function, we must consider this function as being integrated into the biology of the organism as a whole. Through this, selection can affect it in many ways, and adaptations in the underlying system arise accordingly to yield an astonishing variety of SD systems.

SAMENVATTING

Geslachtsbepaling is het proces waarbij individuen zich ontwikkelen als vrouw of man. Geslachtsbepaling moet zorgvuldig uitgevoerd worden zodat de seksuele ontwikkeling op een consistente manier plaatsvindt, wat weer nodig is om ervoor te zorgen dat het individu zich kan voortplanten. Wanneer de geslachtsbepaling niet correct verloopt leidt dit tot onvolledige mannelijke of vrouwelijke ontwikkeling. Zulke interseksualiteit gaat vaak gepaard met een (sterk) verminderde reproductief vermogen. De uitkomst van het geslachtsbepalingsproces is dus onderhevig aan sterke selectie. In tegenstelling hierop zijn de mechanismen die de geslachtsbepaling instrueren zeer variabel, en kunnen er zowel tussen soorten als tussen populaties van een soort verschillende geslachtsbepalingsmechanismen bestaan, waarbij zowel genetische als niet-genetische factoren het geslachtsbepalingsproces kunnen beïnvloeden.

Eerder (voornamelijk theoretisch) onderzoek heeft de rol van verscheidene evolutionaire processen in de diversificatie van geslachtsbepalingsmechanismen geprobeerd aan te tonen. Verschillende modellen voorspellen dat geslachtsbepalingsmechanismen kunnen evolueren door neutrale of adaptieve evolutie. Daarnaast kunnen biologische conflicten bijdragen aan de evolutionaire diversificatie van geslachtsbepalingsmechanismen, zoals conflicten tussen ouders en hun nakomelingen, tussen verschillende genetische elementen (bijvoorbeeld nucleaire versus cytoplasmatische elementen), of tussen vrouwen en mannen (seksueel conflict). Ondanks de belangrijke bijdrage van deze modellen aan ons begrip van de evolutie van geslachtsbepalingsmechanismen kunnen enkele aspecten daarvan met deze modellen nog niet verklaard worden, waarvan twee hier het meest relevant zijn. Ten eerste, hoewel de meeste soorten een enkel geslachtsbepalingsmechanisme hebben, bestaan er ook soorten met meerdere mechanismen, zowel binnen een enkele populatie als tussen verschillende populaties. Ten tweede, geslachtsbepaling vindt in sommige soorten plaats enkel op basis van genetische verschillen tussen mannen en vrouwen, terwijl in andere soorten het plaats vindt op basis van omgevingsfactoren zoals temperatuur. In veel soorten kan geslachtsbepaling echter door zowel genetische als omgevingsfactoren beïnvloedt worden, maar welke stabiele geslachtsbepalingssystemen (waarin vrouwen en mannen in vergelijkbare proporties voorkomen en interseksualiteit zeldzaam of zelfs compleet afwezig is) dan gevormd kunnen worden is nog onduidelijk. Het is derhalve nodig dat we onze kennis van de evolutie van

geslachtsbepalingsmechanismen verder ontwikkelen om dergelijke systemen beter te begrijpen.

Naast de open vragen met betrekking tot de oorzaak van evolutionaire transities in geslachtsbepalingsmechanismen bestaan er ook onbeantwoorde vragen met betrekking tot de gevolgen daarvan. De evolutie van een nieuw geslachtsbepalingsgen wordt geacht ertoe te leiden dat het chromosoom waarop het zich bevindt zich ontwikkelt van een normaal autosoom in een geslachtschromosoom. Theoretische modellen voorspellen dat tijdens dit proces dit chromosoom geslachtsspecifieke adaptatie ondergaat als gevolg van de verschillen in overerving van en naar vrouwen en mannen. Hierbij zullen seksueel-antagonistische varianten, die een positief effect in dragers van het ene geslacht hebben maar een negatief effect in dragers van het andere geslacht, toenemen in frequentie. Een gen voor mannelijkheid zou bijvoorbeeld gekoppeld kunnen raken aan genetische varianten die de fitness van mannetjes verbetert, maar die in vrouwtjes juist een verlaging van de fitness zouden veroorzaken. Het testen van deze theorie is moeilijk gebleken, mede doordat er nog geen geschikte modelsystemen voor bestaan.

In dit proefschrift heb ik de evolutionaire genetica en dynamica van transities in geslachtsbepalingsmechanismen onderzocht, met het tweeledige doel om zowel ons begrip van de oorzaken als de gevolgen van dergelijke transities te vergroten. Ik ben hierbij geïnspireerd door de geslachtsbepaling van de huisvlieg *Musca domestica*, waarin verscheidene geslachtsbepalingsmechanismen voorkomen. In deze soort komen mannelijkheidsgenen voor op beide geslachtschromosomen en op alle autosomen, evenals een vrouwelijkheidsgen dat dominant is over alle mannelijkheidsgenen. In de natuur varieert de frequentie van al deze genen tussen populaties langs een gradiënt die van een hoge breedtegraad naar een lage breedtegraad loopt. Het werk in dit proefschrift is gebaseerd op dit gecompliceerde geslachtsbepalingsmechanisme, en er op gericht om te ontrafelen hoe dit systeem geëvolueerd kan zijn en hoe het gebruikt kan worden voor toekomstig onderzoek aan de evolutie van geslachtsbepalingsmechanismen en geslachtschromosomen.

In Hoofdstuk II bespreek ik eerst voorgaand onderzoek op het gebied van seksueel conflict, waarin twee vormen onderscheiden worden: intralocus (IASC) en interlocus seksueel conflict (IRSC). IASC wordt gedefinieerd als de situatie waarin

tegenstrijdige selectiedrukken in vrouwen en mannen een gen beïnvloeden dat in beide geslachten voorkomt, zodat het optimale genotype voor mannen en vrouwen niet hetzelfde is. In dit geval wordt geslachtsspecifieke adaptatie verhinderd doordat dit vereist dat het andere geslacht maladaptie ondergaat. Dit conflict kan opgelost worden wanneer de genetische basis voor vrouwen en mannenvan elkaar gescheiden wordt, waarna de betrokken loci vervolgens gedifferentieerd kunnen raken tussen de twee geslachten.

IRSC wordt op haar beurt gedefinieerd als het conflict tussen (potentiële) partners over de uitkomst van interacties tussen hen; hierbij is de optimale uitkomst voor de vrouw anders dan die voor de man. Selectie beïnvloedt beiden afzonderlijk om hun eigen fitness te maximaliseren, zelfs als dit ten koste gaat van de fitness van hun partner(s). Evolutie als gevolg van IRSC vindt plaats in de sociale context die gevormd wordt door de (potentiële) partners van het individu. Wanneer een adaptatie ontstaat in het ene geslacht waardoor individuen van dat geslacht beter in staat zijn om hun voorkeursuitkomst te bewerkstelligen, heeft dit als noodzakelijk gevolg dat individuen van het andere geslacht minder kans maken om hun voorkeursuitkomst te bereiken. Hierdoor ervaren deze individuen een hogere selectiedruk om tegenadaptaties te evolueren. IRSC wordt derhalve gekenmerkt door herhalende rondes van adaptie-tegenadaptatie, en kan daardoor leiden tot snelle en continue evolutionaire verandering.

In Hoofdstuk III onderzoek ik het effect van interchromosomale interacties op de stabiliteit van bestaande geslachtschromosomen en de invasieve capaciteit van nieuwe geslachtschromosomen. Geslachtschromosomen bevatten naast het geslachtsbepalingsgen een aantal andere genen die betrokken zijn bij interacties met autosomale genen en daardoor de fitness van de drager beïnvloeden. Zulke interacties kunnen leiden tot epistasie, waarbij het cumulatieve effect van beide genen afwijkt van de som van hun individuele effecten. Hier toon ik aan dat dergelijke epistatische interacties zowel een stabiliserend als een destabiliserend effect kunnen hebben op bestaande geslachtschromosomen. Epistasie kan tevens de invasie van nieuwe geslachtschromosomen bevorderen, maar deze effecten zijn doorgaans wel zwakker. Zodra een geslachtschromosoompaar zich verspreid heeft kan er op een select aantal loci al genetische differentiatie tussen de beide chromosomen opgetreden zijn, zelfs als dit paar recent geëvolueerd is. Indien deze loci betrokken zijn bij epistatische interacties kan dit de stabiliteit van dit

geslachtschromosoompaar versterken, waarbij dit geldt voor zowel oude als recent-geëvolueerde geslachtschromosomen.

Geslachtsbepaling kan zowel door genetische als omgevingsfactoren plaatsvinden, maar in veel soorten kunnen beide typen factoren een invloed hebben. Het was nog onduidelijk hoe dit de evolutie van de genetische component kan beïnvloeden. In Hoofdstuk IV onderzoek ik de evolutie van een genetisch geslachtsbepalingsmechanisme waarin de expressie van één van de betrokken genen positief beïnvloed wordt door temperatuur. Ik toon aan dat de activiteit van dit gen bepaalt welke geslachtsbepalingsmechanismen evolutionair stabiel zijn. Omdat de activiteit beïnvloed werd door temperatuur is het daarnaast mogelijk dat temperatuurverschillen tussen populaties ertoe leiden dat in deze populaties verschillende geslachtsbepalingsmechanismen evolueren.

Dit model paste ik vervolgens toe op de huisvlieg *M. domestica* om te bepalen of het bestaan van verschillende geslachtsbepalingsmechanismen en de geografische verspreiding van deze mechanismen hiermee verklaard kan worden. Ik ontdekte dat de allelfrequenties op het vrouwelijkheidsgen (*transformer*) in de natuur verklaart kunnen worden door de lokale invasie van een dominante variant in locaties met een hoge temperatuur. Als gevolg hiervan kunnen tevens gradiënten in de frequenties van mannelijkheidsgenen ontstaan. Mannelijkheidsgenen die geassocieerd zijn met een fitnessvoordeel in heterozygoten, maar een fitnessnadeel in homozygoten kunnen dan selectief voordeel hebben in locaties met een lage temperatuur, waar het dominante vrouwelijkheidsgen afwezig is en deze mannelijkheidsgenen dus enkel in heterozygoten voorkomen. Daarentegen hebben deze mannelijksgenen een selectief nadeel in warmere locaties waar dit vrouwelijkheidsgen wel aanwezig is, en ze dus ook in homozygoten kunnen voorkomen en tot een fitnessnadeel kunnen leiden. Deze mannelijkheidsgenen kunnen dan verdrongen worden door andere mannelijkheidsgenen die niet geassocieerd zijn met fitnessnadelen in homozygoten. Hierdoor kunnen dus tenminste drie gradiënten in allelfrequenties ontstaan zoals die aangetroffen zijn in *M. domestica*, en wordt dus een adaptief-evolutionaire verklaring gegeven voor het bestaan van dit gecompliceerde geslachtsbepalingssysteem.

Het bestaan van meerdere geslachtsbepalingsmechanismen in *M. domestica* is niet alleen een evolutionair vraagstuk, maar is tevens een middel om een ander vraagstuk te beantwoorden. De aanwezigheid van een mannelijkheidsgen op een

chromosoom wordt geacht ertoe te leiden dat dit chromosoom evolueert als een Y-chromosoom. Als er tevens een dominant vrouwelijkheidsgen aanwezig is wordt dit proces echter voorkomen, en zullen de chromosomen met mannelijkheidsgenen voortbestaan als reguliere autosomen. In Hoofdstuk V gebruik ik kruisingen tussen individuen van verschillende populaties om mannelijkheidsgenen uit een populatie met een dominant vrouwelijkheidsgen in een genomische achtergrond te kruizen waar dit vrouwelijkheidsgen afwezig is. Dit proces imiteert de evolutie van een nieuw mannelijkheidsgen op een (voormalig) autosoom, en leidt daardoor tot de formatie van een nieuw geslachtschromosoompaar, die we baby-geslachtschromosomen noemen. Deze baby-geslachtschromosomen kunnen gebruikt worden om de eerste stappen in de evolutie van geslachtschromosomen te bestuderen. Dit wordt normaliter verhinderd doordat onder natuurlijke omstandigheden het Y-chromosoom onderhevig is aan genetische degeneratie. Ik bespreek daarnaast hoe deze baby-geslachtschromosomen gebruikt kunnen worden om de "seksueel antagonisme"-hypothese van geslachtschromosoomevolutie te testen; deze theorie wordt door velen geaccepteerd, maar het empirisch bewijs ervoor is nog altijd schaars.

Om de huisvlieg te gebruiken als modelsysteem voor evolutionair-biologisch onderzoek in het algemeen en specifiek de evolutie van geslachtsbepalingsmechanismen is een grondigere kennis van hoe fitness wordt bepaald in deze soort vereist, en hoe nauwkeurige schattingen van fitness gemaakt kunnen worden. In Hoofdstuk VI onderzoek ik hoe vrouwelijke en mannelijke fitness vastgesteld kan worden in *M. domestica*. Voor vrouwtjes geldt dat fitness voornamelijk bepaald wordt door hun eigen fecunditeit, d.w.z. de capaciteit om bevruchte eieren te produceren. Voor mannetjes geldt daarentegen dat fitness bepaald wordt door hun paringssucces en de daaruit voortvloeiende toegang tot eitjes van hun vrouwelijke partners. Echter, de huidige kennis van het paringsgedrag van huisvliegen is ontoereikend, en de rol van vrouwelijke promiscuïteit op de fitness van zowel vrouwtjes als mannetjes is nog onbekend. Indien vrouwtjes promiscuïteit vertonen kan dit leiden tot post-copulatieve selectie, wat weer kan leiden tot de evolutie van nieuwe eigenschappen in mannetjes en vrouwtjes ter bevordering van hun fitness, bijvoorbeeld als gevolg van IRSC. Om een definitieve methodologie voor het vaststellen van fitness te ontwikkelen is het nodig om de rol van vrouwelijke promiscuïteit in deze soort vast te stellen, en tevens wat voor gevolgen dit heeft voor hoe fitness in mannetjes en vrouwtjes kan worden bewerkstelligd.

De evolutionaire variabiliteit van geslachtsbepalingsmechanismen is lange tijd geacht in strijd te zijn met haar sterk geconserveerde functie, die nauwkeurig uitgevoerd moet worden om ernstige fitnessnadelen voor het individu te voorkomen. Desalniettemin is het nu duidelijk dat vele evolutionaire processen kunnen bijdragen aan de diversificatie van geslachtsbepalingsmechanismen. Derhalve is het wellicht nodig om de heersende paradigma te heroverwegen: in plaats van dat geslachtsbepaling beperkt wordt door sterke selectie op de bijbehorende functie, moeten we deze functie beschouwen als een onderdeel dat geïntegreerd is in de algehele biologie van het organisme. Hierdoor kan het door en via allerlei factoren beïnvloed worden, met als gevolg dat adaptaties in het onderliggende systeem ook op allerlei manieren kunnen ontstaan – uiteindelijk resulterend in een verbijsterende verscheidenheid aan geslachtsbepalingsmechanismen.

AUTHOR INFORMATION

Martijn A. Schenkel

EGDB / TRÊS
Groningen Institute for Evolutionary Life Sciences
University of Groningen
maschenkel@gmail.com

Leo W. Beukeboom

EGDB
Groningen Institute for Evolutionary Life Sciences
University of Groningen
l.w.beukeboom@rug.nl

Jean-Christophe Billeter

Neurobiology
Groningen Institute for Evolutionary Life Sciences
University of Groningen
j.c.billeter@rug.nl

Julia I. Camacho

SPHERE
Department of Biology
University of Antwerp
julia.camachogarcia@uantwerpen.be

Tomás Cunha

Department of Animal Biology
Faculty of Sciences
University of Lisbon
trochac@gmail.com

Ido Pen

TRÊS
Groningen Institute for Evolutionary Life Sciences
University of Groningen
i.r.pen@rug.nl

CURRICULUM VITAE

Martijn Schenkel was born on November 4th, 1990 in Groningen, The Netherlands. He started his BSc Biology at the University of Groningen in 2009, and graduated in 2013 with a double major in Ecology & Evolution and Marine Biology. After this, he started the MSc Top Program Evolutionary Biology at the University of Groningen. During this time, he carried out research projects on housefly sex determination genes with prof. dr. Leo Beukeboom and dr. Louis van de Zande at the University of Groningen, and on post-host shift performance of the reproductive parasite *Arsenophonus nasoniae* with Greg Hurst at the Institute for Integrative Biology of the University of Liverpool. He graduated with honours (*cum laude*) in 2015, after which he started working a dual position as a research assistant in the Evolutionary Genetics, Development & Behaviour group at the University of Groningen, and as a copy editor for the journal Entomologia Experimentalis et Applicata. In 2016, he started his PhD with prof. Leo Beukeboom, prof. Ido Pen and prof. Jean-Christophe Billeter as part of the Adaptive Life program of the Groningen Institute for Evolutionary Life Sciences at the University of Groningen.

Publications

MA Schenkel, J-C Billeter, LW Beukeboom* & I Pen* (in prep). Divergent evolution of genetic sex determination mechanisms along environmental gradients.

MA Schenkel, LW Beukeboom & I Pen (in prep). Epistatic interactions between the sex chromosomes and autosomes can affect the stability of sex determination systems.

F Chen, **MA Schenkel**, L van de Zande & Beukeboom LW (in prep). Inbreeding shows absence of complementary sex determination in two *Leptopilina* species (Figitidae, Hymenoptera).

X Li, S Visser, D Bopp, E Wimmer, **MA Schenkel**, E Geuverink, F Marec, L van de Zande & LW Beukeboom (in prep). Genomic characterization and localization of the transposable male determining loci in the housefly (*Musca domestica*).

MA Schenkel, I Pen, LW Beukeboom & J-C Billeter (2018). Making sense of intralocus and interlocus sexual conflict. *Ecology & Evolution* **8:** 13035-13050.

SR Parratt, CL Frost, **MA Schenkel**, A Rice, GDD Hurst & KC King (2016). Superparasitism drives heritable symbiont dynamics and host sex ratio in a wasp. *PLoS Pathogens* **12(6):** e1005629.

MA Schenkel & LW Beukeboom (2016). Sex chromosome evolution: birth, maturation, decay and rebirth. In: *Encyclopedia of Evolutionary Biology* (edited by RM Kliman), vol. 4, pp. 72-80. Oxford: Academic Press.

ACKNOWLEDGEMENTS

Since starting my PhD in 2016, I have met and worked with tons of great people that have made the journey all the more pleasant. In fact, counting flies and fixing bugs is pretty strenuous and boring, and really all the fun bits involve others. For this, I would like to thank a number of people, though honestly it would be impossible to address each of them in the manner they deserve – but I will try.

Knowing how difficult I can be, I'd like to extend my deep gratitude towards my supervisors Leo Beukeboom, Ido Pen, and Jean-Christophe Billeter, who endured my antics for over four long years – quite an impressive feat to say the least. I am thankful for your faith in me, particularly when I struck out from being overworked in the first year of my PhD. Moreover, you have all had to put up with the verbose ramblings that made up the drafts for the chapters in this thesis (and maybe not just the drafts). I only hope that, despite my resistance to brevity, you can live with the final product.

As part of my Master's degree, I joined Leo's lab in 2014 to work on a project on housefly sex determination (after which I swore never to work on houseflies again...). Since then, I have worked with Leo on various projects, culminating in this PhD thesis. Leo, I would like to thank you for all your efforts and support since starting that first project. When I graduated in 2015, you gave me the opportunity to write a proposal for a PhD position within the Adaptive Life program and arranged a temporary position as a research assistant. We first set out to do a project on *Wolbachia*, but unfortunately things didn't turn out as intended and I ended up declining this position. Despite this, you still were willing to hire me for this project and even managed to negotiate an employee position (rather than a bursary position) on my behalf. You have given me some very free reign over this project, and have involved me in many other fun ones over the years that have really helped me develop my own views on science in general, and evolution in particular. And not to forget, you have been a very good sport over all the jokes I have made at your expense.

Next, I would like to thank Ido. As the initial plans for this PhD project went down the drain and instead we went for a more theoretical approach, your efforts became (even more) essential to my success. You've helped me hone my skills in R and C++, and have made me a lot more math- and stats-savvy than I ever would have hoped to be at the onset of my PhD. You brought a lot of (dark/twisted) humour to the progress meetings and our model meetings were filled with similar jokes.

However, you also know when to be critical of what I do, and often managed to hit just the right buttons to make sure I would take your feedback to heart.

Kindly, I would also like to thank Jean-Christophe, for he has been nothing but kind over these years. Your unbridled enthusiasm combined with your thoughtfulness regarding my wellbeing have made it a joy to work with you. Your (often extensive) feedback on my writing has been essential to make this thesis at least somewhat comprehensible. Although the project turned out a bit less empirical than initially envisioned, you never shied away from being involved, and were always ready to help work out the different experiments and models.

With my verbosity in mind, I would like to thank my thesis assessment committee Franjo Weissing, Hanna Kokko, and Nina Wedell for taking the time to read and evaluate my thesis. Similarly, I would like to thank the defense committee for their efforts.

After about 7 years in EvolGen (later EGDB), I can safely say my time here has been enjoyable. I'd like to thank everyone that has been part of our not-so-little group. This especially goes out to my office mates over the years; it is with good reason that our office is known as the "party office", and three of my past office mates in particular have contributed to this nickname. Yuan (Yuyu), you have been the victim of numerous pranks involving your unusual napping habits, diet, cleanliness, and of course kiwi eggs. Despite this, you persevered. It's been great fun to have been around for it all and to have been your paranymph. Xuan (Xuxu), you have been a constant source of funny videos, terrible music, and perhaps-a-bit-too-inappropriate jokes – but aren't we all guilty on that one? Oddly enough, we never really did any experiments together, but our work has overlapped in so many other ways – and I'm glad it did. In light of all those good moments shared and all the helpful scientific discussions, I'm also very happy to have you as a paranymph. Peter (Пётр), you were always willing to chat about all sorts of topics, whether it be in-depth discussions of scientific concepts, updates on the hares on the roof, plain old juicy gossip, or simply relentless criticism of other people, always thoroughly deserved of course. Aside from my office mates, I would also like to thank some of my other colleagues in particular. Ljubinka, together with Xuan and me you formed the core of Team Musca for a long time. Your warmheartedness is unsurpassed, which is best exemplified by all those times where you helped me out with culture work issues or virgin collections. Louis ("Ha Louis!"), ever since we were in the

faculty council together all the way back in 2012, you have been around to provide guidance and as a partner-in-crime for many, many terrible puns that have made the going all the more easier. Sander, you have only recently rejoined our lab, but of course we spent some time together already as we carried out other Master's projects in 2014. Although you went away to the Czech Republic for your own PhD, we still kept in touch, and our long email exchanges were very welcome breaks. I'd also like to take a moment to appreciate the members (past and present) of Team Musca, the best team: Akash, Anna, Anna, Fangfei, Ljubinka, Julia, Kim, Maaike, Marloes, Marloes, Marrit, Sander, Tomás, Weihong, Xuan, and Yanli, as well as the numerous students that at one point helped out along the way. I also want to thank Rogier and Peter for their support with the housefly culturing.

Supervising and teaching students is one of the nicest parts of doing a PhD. To my BSc students, Geert-Jan, Jaron, Laura, and Rozemarijn, thank you for your efforts on characterizing M-factors and for a first exploratory analysis of the temperature model. Additionally, I've had the joy of supervising two wonderful MSc students. Julia, you joined us for a project shortly after I started my PhD, and shortly before I got ill. As a result, you had to carry out your project with a level of independence that could not within reason be expected – yet you did so anyway. At first you were "so confused" with the whole topic, but as time progressed you showed true mastery, and the fruits of your labour speak for themselves. It was obvious then already that I wanted you to be my paranymph, and I am happy that you accepted. Tomás, you came to me down on your luck after your first project in our group went sour and you were forced to quit. Rather than avoiding any blame, you took full responsibility and promised me things would go differently this time around. You've faithfully kept this promise, and even after you had to leave the Netherlands and return to Portugal you were still keeping my informed on your efforts to finish the project and your degree. You did a great job overcoming these setbacks, and showed great resolve in doing so.

Houseflies – can't live with 'em, can't live without 'em? At least the first part I know to be true, and since they are so hard to get rid of the second part is at the very least plausible. Despite being an absolute pain, the nifty characteristics of these small critters and their sex determination mechanisms have been a source of continued inspiration. They have thus been fundamental to this thesis. A heartfelt thanks is in place for all those flies that have been sacrificed to make my work

possible – in particular the logistics of the introgression experiment were accompanied with a true muscicide. On a more positive note, I hope you all liked the boys and girls I tried to set you up with.

Even though doing a PhD is often a blast, it can easily eat up all your time and energy, and has a creepy habit of staying on the mind at all times. Because of this, sometimes your work may benefit most from not working at all. Finding a way to shut off and keep your brain running idly can be quite challenging, but for me has proven essential. One of the things that helped me most was to swim, or perhaps more accurately to swim a lot. I've met a bunch of great people through this hobby of mine, and would like to extend my thanks to all of them. Thanks for all the advice, chats, and shared grievance over other people that quite frankly make for terrible swimmers at best and terrible people at worst. I suppose even a nuisance can be a good way to take your mind of things, particularly when shared with others that feel the same way.

Rather similarly, I'd also like to thank my long-time friends for all fun we've had together, and of course for all the fun still to be had. Alex, Alex, Alie, Elise, Ella, Leo, Lisa, Maik, Mathanja, Merijn, Robbert, Roeland, and Sterre, together we've had such good times in numerous different ways. Thanks for the many parties, beers shared (especially the foul ones), games played (especially the klaverjas tournaments), football matches watched and associated activities (especially the Eredivisievoorspellingen), jokes made (especially at each other's expense), advice given (especially the ones no one in their right mind would follow), and patience you've had with me.

Except for all those mentioned above, I have been also been lucky to have had the unwavering support of my family. Thanks to my aunts and uncles, for their continued interest in my work and encouragement, either in person or through the many cards you have sent me over the years. To my brother Michiel, I'd like to thank you for all the support, always. Funnily enough, although we are similar in many ways, we differ strongly in others. As a result, you have been an excellent and often much-needed counterbalance to who I am. Finally, I want to thank my parents, Marcel and Stella. You have stood with me through all the highs and lows no matter what. In light of your eternal support, for which I cannot put my gratitude into words, I dedicate this thesis to you – with love.