Evolutionary Dynamics of Sex Determination

Mechanistic Theory and Empirical Investigations

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Evolutionary Dynamics of Sex Determination

Mechanistic Theory and Empirical Investigations

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CHAPTER 1

General introduction and thesis overview

Variety of sex determining mechanisms

Sex determination is a fundamental developmental process. Proper sexual differentiation includes not only the development of gonads, but also of the central nervous system responsible for proper sexual behaviour, as well as other secondary characters specific for a given sex (e.g. plumage in birds, horns in some mammals and beetles). Mutations interfering with these developmental processes are expected to have strong negative effects on fitness. Yet, sex determining (SD) mechanisms vary greatly between different taxonomic groups or even between closely related species (Bull 1983; Volff & Schartl 2001; Kraak & Pen 2002; Saccone *et al.* 2002; Janzen & Phillips 2006; Mank *et al.* 2006; Takehana *et al.* 2007; Fig 1.1). The most common SD systems are male heterogamety, female heterogamety, haplodiploidy and environmental sex determination, but there is also a variety of other mechanisms (Box 1.1). Moreover, even in species with seemingly similar SD mechanisms, e.g. male heterogamety, the actual genetic mechanism may be different (Box 1.1). The diversity of SD mechanisms among closely related species suggest that they can evolve rapidly (Marin & Baker 1998; Werren & Beukeboom 1998). Sex determination may even be variable within one species. An example is the housefly, *Musca domestica*, in which strains with male heterogamety, female heterogamety, monogeny and environmental effects are known (Box 1.2, Fig 1.3).

Thanks to the development of molecular techniques, we are improving our understanding of the genetic basis of sex determination. It has recently become evident that different sex determining mechanisms in such phylogenetically diverse groups as insects, nematodes, mammals, and even cnidarians share some molecular patterns (Cline & Meyer 1996; Marin & Baker 1998; Yi & Zarkower 1999; Zarkower 2001; Miller *et al.* 2003). Typically, SD pathways consist of multiple regulatory genes arranged in a linear cascade, where the expression of genes on one level of the cascade regulates the genes at one level below, all the way down to a bi-functional switch gene at the bottom of the cascade (Fig 1.2). The product of the switch gene at the bottom of the cascade is differentially spliced in both sexes and it controls a number of genes responsible for proper female or male development (Goldman & Arbeitman 2007). Comparative studies show that the genes at the bottom of the cascade are conserved, whereas genes higher up tend to diverge (Raymond *et al.* 1998; Zarkower 2001; Saccone *et al.* 2002; Shearman 2002; DiNapoli & Capel 2008; Fig 1.2). In many species maternal products influence the expression of SD genes in the offspring and are necessary for proper development (Ahringer *et al.* 1992; Dübendorfer & Hediger 1998; Schütt & Nöthiger 2000; Fig 1.2).

Theoretically, any gene which takes control of the expression of the top gene in the cascade may lead to a new SD mechanism (Marin & Baker 1998). In addition, mutations in more downstream genes, for example rendering them insensitive to the gene up in the hierarchy, can lead to a new SD system (Nöthinger & Steinmann-Zwicky 1985). This situation happened in the housefly in which female heterogamety

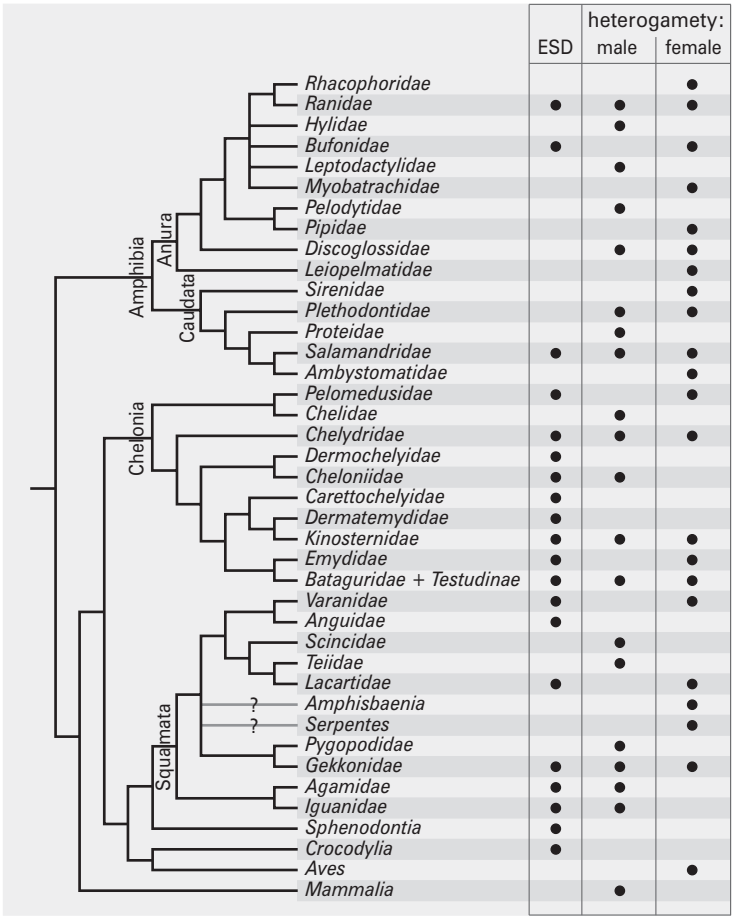

Figure 1.1. Variety of SD mechanisms in the Tetropoda. Note that SD mechanism (ESD – environmental sex determination or male or female heterogamety) may differ between closely related families or even within a family. For explanation of different SD mechanisms see Box 1.1. Figure after Kraak & Pen 2002.

was obtained by a dominant mutation in a female-determining factor, making it insensitive to the male-determining factor, which is higher in the hierarchy and controls sex determination in standard strains (Dübendorfer *et al.* 2002; Box 1.2). Additionally, mutations in genes which are not sex-specifically expressed, but are necessary for the proper action of sex specific genes in the SD pathway, may lead to a new SD mechanism (Schütt & Nöthiger 2000). The ease of creating new SD mechanisms has, for example, been shown in the roundworm *Caenorhabditis elegans* for which 18 different lab strains were created by different mutations in seven genes involved in SD determination (Hodgkin 2002). In most of the strains a single gene

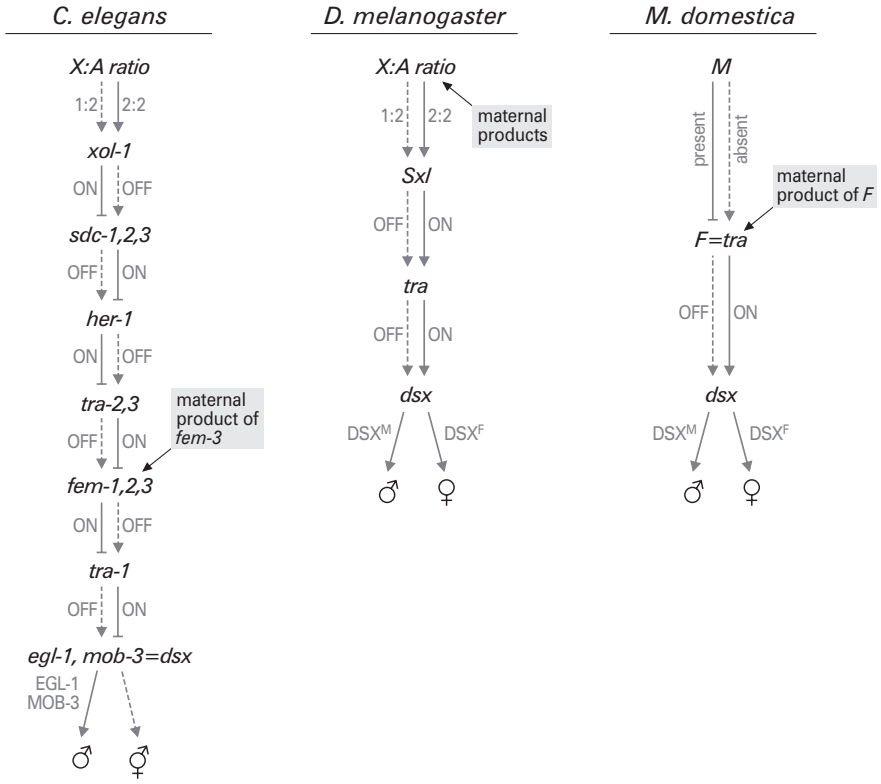

Figure 1.2. Sex determining cascades in the roundworm, *Caenorhabditis elegans*; the fruitfly, *Drosophila melanogaster* and the housefly, *Musca domestica*. Multiple genes are involved in sex determination. The expression of genes on one level of the cascade regulates the genes at one level below. Arrows indicate positive regulation, solid - in the presence of the product of the gene above, dashed - in its absence. Blunt arrows depict negative regulation. For example, in *D. melanogaster* the primary SD signal consists of the ratio of number of X chromosomes to number of sets of autosomes (but see Erickson & Quintero 2007 for alternative hypothesis). Multiple offspring genes (not shown) as well as maternal products are involved in establishing this ratio. If this ratio is 2:2, an active product of *Sxl* is produced. It leads to the production of active product of *tra*, which in turn assures that *dsx* is spliced to the female specific form. *Dsx* controls many genes (not shown) eventually leading to female development. If the X:A ratio equals 1:2, no active product of *Sxl* is present, therefore no active product of *tra*. *Dsx* is spliced in male-specific manner leading to male development. Note that in all three species genes at the bottom of the cascade are homologous. Homology between other genes decreases with increased phylogenetic distance between species and higher position in the SD cascade. *Sxl* is not involved in sex determination in *M. domestica* (Meise *et al.* 1998). More details on the SD cascades presented here can be found e.g. in Cline & Meyer 1996; Dübendorfer *et al.* 2002.

became a major factor in sex determination leading to male and female heterogamety, monogeny and even temperature dependent SD. Interestingly, an SD gene could encode a transcription factor, a trans-membrane factor, an extracellular protein, a cytoplasmic protein, a phosphatase or a tRNA. This shows that a wide

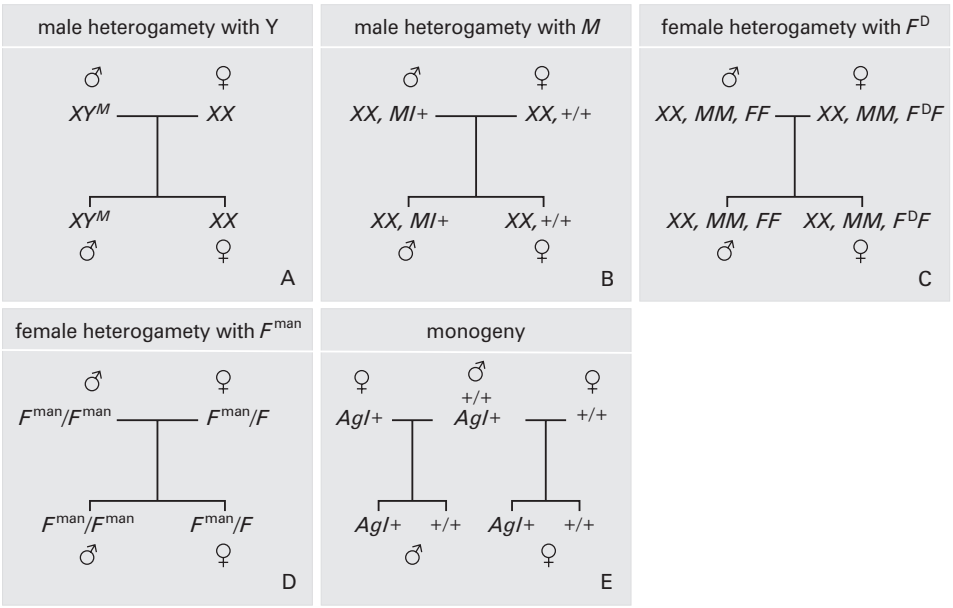

Figure 1.3. Examples of SD mechanisms found in the housefly. (A) Male heterogamety with a dominant male determining factor (*M*) located on the Y chromosome. (B) Male heterogamety with a dominant male determining factor (*M*) located on an autosome. (C) Female heterogamety with the female determining factor F^D insensitive to *M*. (D) Female heterogamety with the recessive masculinizing mutation F^{man}. (E) Monogeny with the maternal-effect mutation *Ag*. More details on the SD mechanisms in the housefly in Box 2.2. Figure adjusted from Dübendorfer *et al.* 2002.

range of molecules may exert an SD function (Hodgkin 2002). Similarly, in the housefly different mutations in SD genes lead to strains with male or female heterogamety or monogeny (Fig 1.3, Box 1.2). In some strains temperature also influences sex determination (Schmidt *et al.* 1997a). Sex reversal caused by mutations of genes in the SD cascade is also known in mammals (DiNapoli & Capel 2008).

Evolution of sex determining mechanisms

Comparative studies lend support to the hypothesis that SD regulatory pathways have evolved from the bottom up (Wilkins 1995). Recurrent recruitment of new elements at the top of the cascade but also new mutations in genes already involved in sex determination have probably led to the large variety of extant SD systems (Nöthinger & Steinmann-Zwicky 1985; Kraak & Pen 2002; Shearman 2002; Mank *et al.* 2006).

However, while steady empirical progress is being made in unravelling the genetics of sex determining mechanisms and their evolutionary history, there is still little understanding of why and how new genes are added to the SD cascades leading to changes in the SD system. Although the evolution of sex determination has been studied for almost a century now (summarised e.g. in Bull 1983; Werren & Beukeboom 1998; Uller *et al*. 2007) and various selective forces have been proposed to play a role in this process, many questions still remind unresolved.

In this section I briefly discuss the most important evolutionary forces that are involved in the evolution of SD mechanisms. I start with factors of more general importance. Then, I discuss selective forces which are specific for genes involved in sex determination, i.e. sex ratio selection. Next, I discuss genetic conflict and what its consequences can be for the evolution of new SD systems. I then show that the establishment of a new SD mechanism may have implications for the evolution of differentiated sex chromosomes. This in turn may either trigger further changes in the SD mechanism or hamper them. Throughout, I point out that the different processes involved in the evolution of SD mechanisms are closely intertwined. Changes brought about by one selective force may pave the way for other selective processes and further changes in SD mechanisms.

No direct selection

New SD genes can readily arise in laboratory and wild populations. But how do they spread, leading to a shift in SD mechanism? The simplest answer is that genetic drift in finite populations may lead to the fixation of a new SD mutation and a corresponding change in the SD system. It has been shown (Bull & Charnov 1977) that, in cases where individuals differing in SD factors do not differ in fitness, there is an infinite number of neutrally stable equilibria at which multiple SD factors can coexist. In finite populations, random fluctuations in the frequencies of different SD factors will eventually lead to the fixation of one of them (Bull & Charnov 1977; Jayakar 1987). However, the assumption of equal fitness of all genotypes with different SD factors will often be unrealistic and new rare mutations have a great chance of being lost from the population. Therefore, pure genetic drift probably plays only a minor role in the evolution of SD mechanisms.

As any other mutation, new SD factors can spread due to their linkage with genes under positive selection (hitchhiking effect). In this case, an SD gene is not a direct target of selection, but it may spread nevertheless, since individuals in which it resides have higher fitness (Bull & Charnov 1977; Jayakar 1987; Shearman 2002). Linkage with genes under positive selection has been proposed as an explanation for the presence of multiple SD factors in a few species. For example, linkage with DDT resistance genes may be partially responsible for the spread of autosomal male-determining factors in the housefly (Kerr 1970; Franco *et al*. 1982). In the platyfish, *Xiphophorus maculatus*, a new female-determining factor may have spread because of its linkage to pigment alleles beneficial to females (Bull & Charnov 1977;

Orzack *et al.* 1980). However, it should be noted that recombination tends to break linkage between the SD factor and the allele under positive selection. This may prevent a complete switch to a new SD mechanism (Rice 1986; Lande *et al.* 2001). However, in some species recombination is restricted to one sex (e.g. achiasmatic meiosis, in many insects; Traut 1999). Once can imagine, that this could facilitate the hitchhiking of new SD genes with other mutations, but the formal models are lacking. It could be interesting to check if there is more variation in SD mechanisms (or more specifically, location of the SD gene) in taxonomic groups with achiasmatic meiosis.

Viability and fecundity selection

SD genes can also be the direct target of viability or fertility selection. Sex determination is a fundamental developmental process and as such can have a direct effect on an individual's fitness. New mutations in genes at the bottom of the SD cascade which directly control genes responsible for the development of the proper sexual phenotype will probably often lead to reduced fertility or even the development of sterile intersexes (individuals with a mixture of male and female features). This may often prevent the spread of new mutations in these SD genes and be responsible for the high conservation of SD genes at the bottom of SD cascades in a variety of taxa (Marin & Baker 1998; Raymond *et al.* 1998; Yi & Zarkower 1999; Zarkower 2001; Miller *et al.* 2003).

Mutations in genes higher up in the SD cascade do not seem to have such a strong effect on individual fitness (Hodgkin 2002). However, it is known that some mutations in genes in the SD cascade lead to unreliable developmental cues (production of both male and female specific products) and the development of individuals with decreased fecundity (Hodgkin 2002; DiNapoli & Capel 2008). Such mutations may often be selected against, but this is not always the case. Pomiankowski *et al.* (2004) proposed a model for the evolution of the SD cascade in *D. melanogaster* (see Fig 1.2) driven by viability and fertility selection. They started with the assumption that initially only one (switch) gene was responsible for sex determination, and that this gene was heterozygous in males. Because of this, both male and female specific transcripts were produced in males, which, as a consequence, were partly feminized and had decreased fitness. New mutations were selected to increase the reliability of the developmental cue and fitness in males, even though they decrease female fitness. That, in turn, selected for new mutations increasing the reliability of developmental cue in females. In this way Pomiankowski and colleagues reconstructed the evolution of the *D. melanogaster* SD cascade, showing that new genes (or mutations of already existing genes) can spread in this system and take over sex determination. Their model requires specific assumptions on the fitness effects of different mutations and on the genetic details of *Drosophila* SD cascades. However, selection for reliable developmental cues is probably an important force in the evolution of any SD system.

To my knowledge, the model of Pomiankowski and colleagues (2004) is the only one addressing the switch to a new SD system, which is driven by fecundity of viability disadvantages associated with the previous system. Yet, it is easily conceivable that many natural SD systems may have negative effects on fitness (e.g. reduced fertility) at least in individuals of one sex. I present three examples. First, some of the mutations in SD genes, although leading to suboptimal phenotypes (e.g. not fully fertile), may become fixed due to genetic drift (or hitchhiking). Second, in a number of species SD genes have been shown to be temperature sensitive. Extreme temperatures may interfere with the proper expression of such genes and lead to the production of sterile individuals (Belote & Baker 1982; Schmidt *et al.* 1997a; Wallace *et al.* 1999; Schütt & Nöthiger 2000). This may not be a problem under normal environmental conditions. If, however, environmental conditions change (e.g. due to global warming), this may lead to increased production of sterile individuals. Third, due to the degeneration of the chromosome carrying the dominant sex determiner (Rice 1996a; see also below), a sex determining gene may become embodied in a largely heterochromatic non-functional environment. This may lead to the underexpression of SD genes and the improper control of sexual development (Hodgkin 1992; Hediger *et al.* 1998). In all these cases, a new SD factor may be selected for if it increases the reliability of developmental cues and prevents the production of suboptimal phenotypes. However, formal theoretical studies of these scenarios are lacking.

Sex ratio selection

Mutations in SD genes or acquisition of new genes at the top of the SD hierarchy may also lead to complete sex-reversal, with negligible effects on fertility or viability (Dübendorfer *et al.* 2002; Hodgkin 2002). However, such mutations are not selectively neutral, since they will often lead to a change in offspring sex ratio. Accordingly, sex ratio selection may be the most important selection pressure influencing the evolution of SD mechanisms.

In many cases the evolutionarily stable sex ratio in a population is expected to be 1:1 (Fisher 1930). A simple heterogametic SD system (XY or ZW) assures this ratio under Mendelian segregation and new SD factors leading to biased sex ratios are expected to be selected against (Eshel 1975). However, there are also situations where a 1:1 sex ratio is not evolutionarily stable. For example, Fisher's equal allocation principle (Fisher 1930; Box 1.3) states that mothers should invest equally in sons and daughters, which often leads to an evolutionarily stable sex ratio deviating from 1:1. If the costs of producing a son are not equal to the costs of producing a daughter, a sex ratio biased towards the "cheaper" sex is favoured (Fisher 1930; Trivers 1974; Box 1.3). There is an extensive theory on biased evolutionarily stable sex ratios under many circumstances (see e.g. Karlin & Lessard 1986; Hardy 2002). For example, it has been shown that selection favours a sex ratio bias under inbreeding, or under local mate or resource competition (Hamilton 1967; Charnov 1975, 1982; Werren & Taylor 1984; Reinhold 1996; Werren & Hatcher 2000; Werren

et al. 2002; Wade *et al.* 2003; Pen 2006). Under these circumstances SD systems like male or female heterogamety may constrain the level of adaptation. Sex ratio adjustment, e.g. due to the preferential abortion of offspring of the "wrong" sex, may be costly and difficult to achieve (but see Pen & Weissing 2002; West *et al.* 2002; West & Sheldon 2002; Rutkowska & Badyaev 2007). Theoretically, sex ratio adjustment could also be achieved by differential segregation of sex chromosomes in meiosis (Rutkowska & Badyaev 2007), but such sex chromosomal segregation distortion is susceptible to be "taken-over" by selfish genetic elements (see below).

In the case of selection for a biased sex ratio in a population with a heterogametic SD system one should therefore expect that new SD genes, leading to the production of the favoured sex, can invade the population. However, formal models substantiating this are scarce. The few existing models do indeed show that a new SD factor can invade leading either to a switch to a new heterogametic system or to a multi-factorial one (Bull 1983; Vandeputte *et al.* 2007). However, even in systems with multiple SD factors, the evolutionarily stable sex ratio was never achieved in these models.

Sex ratio adjustment is probably easiest to obtain in species with a haplodiploid SD system, where the proportion of males often corresponds to the proportion of unfertilized eggs. Typically, the sex ratios observed in hymenopteran species with local mate competition do indeed closely correspond to theoretical expectations (Charnov 1982; Werren 1983; Shuker & West 2004; Shuker *et al.* 2006). It has even been proposed that the flexibility to adjust sex ratios is one of the forces maintaining the haplodiploid system of sex determination (Hamilton 1967).

Even if the population's sex ratio is expected to be close to 1:1, selection may favour biased sex ratios in individual families. If the reproductive value of sons and daughters is differentially affected by environmental conditions (or the condition of their parents), then strongly female-biased sex ratios should be produced under conditions beneficial for females, while strongly male-biased sex ratios should be produced in alternative conditions (Trivers & Willard 1973; Charnov & Bull 1977). Only few genetic SD mechanisms are sufficiently flexible to produce such condition-dependent evolutionarily stable sex ratios. Accordingly, selection may favour environmental sex determination (ESD), where the sex of an individual is determined in response to the environmental conditions under which it is reared (Charnov & Bull 1977). In many species with ESD, males do indeed develop under conditions favourable for males and females under conditions favourable for females (for examples see e.g. Bull 1983, 1985; but see Janzen & Phillips 2006).

However, the above advantage of ESD may turn into a disadvantage when environmental conditions change rapidly or in unpredictable ways. For example, if the temperature rises due to global warming, species with temperature dependent sex determination may suffer from strongly biased population sex ratios. This may select for a switch from ESD to GSD. It has been shown experimentally that in silversides (*Menidia menidia*) a change in environmental conditions leading to a strongly biased sex ratio, can rapidly lead to the elimination of ESD and the restoration of equal sex

ratios (Conover & Vanvoorhees 1990; Conover *et al.* 1992). Theory predicts that large fluctuations in environmental conditions between generations, and subsequently fluctuations in populations sex ratio, will also select for the production of equal sex ratios in all environmental conditions and a switch to GSD (Bull 1981). However, under some circumstances with spatial and temporal variation, species with ESD may outcompete species with GSD because of a colonisation advantage in new environments, which is due to the overproduction of females (Freedberg & Taylor 2007).

Genetic conflict

In sexually reproducing species the associations between different parts of the genome (different chromosomes, cytoplasmic factors) are temporary. Therefore, they may be subject to selection in opposite directions, which leads to genetic conflict (Partridge & Hurst 1998). Genetic conflict is believed to be an important evolutionary force leading, for example, to uniparental inheritance of cytoplasmic genes, genomic imprinting, the degeneration of Y chromosomes, many features of sexual behaviour and changes in SD mechanisms (for reviews see Hurst *et al.* 1996; Partridge & Hurst 1998; Werren & Beukeboom 1998; Burt & Trivers 2006). It has even been argued that "genetic conflict is the most likely general explanation for the diversity of sex-determining mechanisms" (Werren & Beukeboom 1998). Genetic conflict can act on different levels of organisation: intergenomic conflict (between genes in different individuals), intragenomic conflict (between different genes within one individual) and intralocus conflict (between different alleles at one locus). Conflict on all these levels may lead to changes in SD mechanisms.

Intergenomic conflict

An example of intergenomic conflict potentially having a strong influence on the evolution of SD mechanisms is maternal-offspring conflict over the sex ratio. Maternal genes are selected to maximize the mother's inclusive fitness and genes expressed in the offspring are selected to maximize the offspring's inclusive fitness (Pen 2006). It has been shown that under many circumstances where biased sex ratios are favoured (see above) the optimal sex ratio from the point of view of the mother differs from the optimal sex ratio from the point of view of the offspring (Fisher 1930; Trivers 1974; Werren & Hatcher 2000; Werren *et al.* 2002; Pen 2006; Box 1.3). Typically the optimal sex ratio from an offspring's point of view is less biased than the maternal sex ratio, but this is not a general rule (Pen 2006).

This discrepancy between optimal sex ratios leads to conflict between maternal and offspring genes and may lead to changes in sex determination. When the sex ratio is at the maternal optimum, dominant offspring genes increasing production of the underrepresented (from the point of view of offspring) sex can invade the population (Werren & Hatcher 2000; Werren *et al.* 2002; Pen 2006). Similarly, when the sex ratio is at the offspring optimum, a maternal gene producing a sex ratio biased in the direction of the maternal optimum can invade (Werren & Hatcher 2000; Werren

et al. 2002). When an invading offspring gene has a fully masculinizing or feminizing effect, conflict between maternal and offspring genes can lead to evolution of male or female heterogamety, respectively (Werren *et al.* 2002). As a consequence, the population sex ratio will eventually be equal to 1:1 even though this is neither favoured by the mother nor the offspring. When the invading maternal gene has a fully feminizing effect, genetic conflict can lead to the evolution of monogeny (Werren *et al.* 2002).

Maternally expressed genes are known to affect sexual development in, for example, *C. elegans* (Ahringer *et al.* 1992), *D. melanogaster* (Schütt & Nöthiger 2000) and *M. domestica* (Schmidt *et al.* 1997a; Dübendorfer & Hediger 1998; Fig 1.2). In the blowfly, *Chrysomya rufifacies*, SD is completely under the control of the maternal genotype (Ullerich 1984). Maternal products placed in eggs interact with SD genes of the developing individual and are necessary for its proper sexual differentiation. Mutations have been found in maternally expressed genes that led to laboratory strains in which the sex of the offspring depends on the maternal genotype, for example in the housefly (Vanossi Este & Rovati 1982; Inoue & Hiroyoshi 1986) and *C. elegans* (Hodgkin 2002). Therefore, there is scope for maternal-offspring conflict over sex determination to arise. However, in most species with GSD sex is determined by offspring genes, and maternal control seems to be restricted to a few species (Bull 1983). The reason for this paucity of maternal control may be that the products of maternally expressed genes are present in the developing zygote only during the first cell cycles. They are later degraded, while offspring genes are expressed during the rest of development (Schier 2007).

Maternal control over sex ratio seems to be much greater in species with haplodiploidy (mother can differentially utilize sperm) or ESD (maternal nest choice). In these systems offspring control over sex ratio may be limited (Bulmer & Bull 1982), but conflict can be potentially strong. However, theoretical models investigating the effect of maternal-offspring conflict on the evolution of sex determination in haplodiploids or species with ESD are, to my knowledge, lacking.

Optimal sex ratios can also differ between the two parents and between the father and the offspring (Fisher 1930; Trivers 1974; Pen & Weissing 2002). This would also lead to conflict and theoretically could influence the evolution of SD mechanisms, but I do not know of any formal analysis. Paternal control over the sex of the offspring is probably limited. In species with a male heterogametic system autosomal genes causing over-production of Y or X sperm could be favoured under some circumstances (Bull & Bulmer 1981). However, I do not know of any study showing adaptive (from the individual point of view) adjustments in the strength and direction of sex chromosome drive (but see Bulmer 1988).

Intragenomic conflict

In sexually reproducing species different parts of the genome are transmitted differentially through the two sexes. In diploid species, the autosomes are transmitted equally through males and females and genes on the autosomes will usually benefit

from equal sex ratios in the population (Fisher 1930). In male heterozygous species the Y chromosome is transmitted only through males. Genes on the Y chromosome would, therefore, favour a strongly male-biased sex ratio. In contrast genes on the X chromosome reside twice as often in females than in males, and would favour a female biased sex ratio. A similar argument applies to female heterogamety where W is transmitted only by females and Z twice as often by males than females. Cytoplasmic elements (mitochondria, endosymbiotic bacteria etc.) are usually transmitted only by females and not through males and will, therefore, benefit from female biased sex ratios. Because of their different transmission patterns, different parts of the genome will be in conflict over sex ratio, which may lead to changes in SD mechanism (Cosmides & Tooby 1981; Partridge & Hurst 1998; Werren & Beukeboom 1998).

Sex chromosomes, due to their largely reduced recombination in the heterogametic sex (see below), are especially prone to accumulating selfish genetic elements invreasing their own transmission in the heterozygous sex (see Jaenike 2001 for a review). These so-called segregation distorters or meiotic drive genes often eliminate gametes harbouring the homologous chromosome leading to biased sex ratios in the offspring. They will spread in the population, even at the expense of individual fitness (Haig & Bergstrom 1995; Weissing & van Boven 2001). Segregation distorters may lead to the extinction of the population, due to strongly biased sex ratios (Hamilton 1967). Biased sex ratios lead to selection for suppressors, both on the sex chromosome against which the distorter segregates, and on autosomes which often favour equal sex ratios. Suppressors of drive have been found in most of the species possessing segregation distorters (e.g. Jaenike 2001; Burt & Trivers 2006). However, biased sex ratios lead also to sex ratio selection and are expected to select for changes in SD mechanisms. In male biased populations new feminizing factors are expected to spread and in female biased populations new masculinizing factors should invade (Bull & Charnov 1977; Cosmides & Tooby 1981; Werren & Beukeboom 1998; Burt & Trivers 2006).

Segregation distorters have been found in a number of taxa, ranging from fungi, to plants and animals (for reviews see e.g. Jaenike 2001; Burt & Trivers 2006). Due to their widespread occurrence, sex linked segregation distorters may be an important force in the evolution of SD mechanisms. Theoretical models show that meiotic drive could be (or have been) a driving force behind a change in the sex determining mechanism of the wood lemming, *Myopus schisticolor* (Bengtsson 1977), the mole, *Talpa occidentalis* (McVean & Hurst 1996), the creeping vole, *Microtus oregoni* (Charlesworth & Dempsey 2001), the housefly, *Musca domestica* (Clark 1999), the sciarid fly, *Sciara coprophila* (Haig 1993b) and scale insects, Neococcoidea (Haig 1993a).

Sex ratio distorters have also been found among cytoplasmic factors (mitochondria, intracellular bacteria and other microorganisms). Due to their transmission only (or mainly) through females, cytoplasmic factors distorting the sex ratio towards females or converting males into females will spread in the population (Bull

1983; Werren 1987). Male-killing microbes are known from a variety of insect species. Bacteria transforming genetic males into females are known in crustaceans. In Hymenoptera *Wolbachia* bacteria can induce parthenogenesis (for more details on cytoplasmic sex ratio distorters see Bull 1983; Werren & Beukeboom 1998).

The spread of cytoplasmic sex ratio distorters inducing female biased sex ratios will in turn select for nuclear genes restoring equal sex ratios. For example, a dominant autosomal masculinizer can invade a population with a feminizing cytoplasmic element, leading to changes in the SD mechanism (Caubet *et al.* 2000). However, in the model of Caubet *et al.* (2000) masculinizing genes never reached fixation, but a polymorphic SD mechanism evolved. Alternatively, co-evolution between parental genes and the cytoplasmic sex ratio distorter may lead to the evolution of monogeny – some females producing only females (under cytoplasmic control) and some females producing only males (under control of maternal autosomal genes; Werren 1987). It has also been proposed that cytoplasmic male-killers may facilitate the evolution of haplodiploidy, due to selection for increased viability of haploid males (Engelstädter & Hurst 2006).

Empirical evidence for the influence of cytoplasmic sex ratio distorters on sex determination comes, for example, from the isopod *Armadillidium vulgare*. In this species many populations harbour one or two feminizing elements: a Wolbachia-like bacterium (*F*) and/or another factor (*f*) which may be a segment of the bacterial genome unstably integrated into the host genome (Juchault & Mocquard 1993; Rigaud & Juchault 1993). In some populations feminizing factors completely took over the original SD mechanisms of female heterogamety resulting in all individuals being genotypically males (ZZ) and all females having the cytoplasmic factor (Juchault & Mocquard 1993). Additionally, in populations harbouring feminizing factors, but not in the populations without them, masculinizing gene (*M*) has been found on an autosome, supporting theoretical expectations (Taylor 1990). However, *M* can only override the feminizing effect of *f*, but not *F*, leading to complex dynamics between different SD factors (Rigaud & Juchault 1993).

Intralocus conflict

Genetic conflict leading to changes in SD mechanisms can also occur within one locus. The conflict stems from the fact that a gene can be subject to selection in the opposite direction when expressed in males compared to when expressed in females. For example, if males have higher optimal body weight than females, alleles increasing weight will be beneficial for males, but detrimental for females, and vice versa for alleles decreasing weight. Alleles whose fitness effects in one sex are negatively related to their fitness effects in the other sex are called sexually antagonistic (SA) alleles (Rice 1992). Sexual antagonism may result not only from sex-specific optima, but also from sex-specific pleiotropy (Rice 1987).

The presence of SA genes in the vicinity of SD genes is believed to have a strong effect on the evolution of differentiated sex chromosomes (Charlesworth 1991; Rice

1996a). Differentiation of sex chromosomes has a strong indirect influence on the evolution of SD mechanisms (see below). However, the presence of SA alleles in the genome can also have more direct effects on sex determination. Rice (1986) showed that linkage with an SA locus may lead to the spread of a new sex determining factor and a switch from polygenic sex determination to a one-locus SD system (Rice 1986). Recently the idea of SA genes influencing the evolution of sex determination has further been studied theoretically. Van Doorn and Kirkpatric (2007) showed that SA variation on autosomes can facilitate the spread of a new (autosomal) sex determining factor, leading to a change from an XY system to an autosomal system. In contrast, SA fitness variation on the sex chromosomes acts against changes in SD mechanism.

There is increasing experimental evidence that SA genes are common in genomes of a number of species (Forsman 1995; Vieira *et al.* 2000; Chippindale *et al.* 2001; Rice & Chippindale 2001; Gibson *et al.* 2002; Fedorka & Mousseau 2004; Kozielska *et al.* 2004). Therefore, they are potentially of considerable importance for the evolution of SD mechanisms. However, the theory in this direction is only starting to be developed (Van Doorn & Kirkpatrick 2007) and empirical evidence is lacking.

Sex chromosome differentiation and the evolution of SD mechanisms

The vast literature on the evolution of heteromorphic sex chromosomes has been reviewed a number of times (Rice 1987; Charlesworth 1991, 1996; Rice 1996a; Charlesworth *et al.* 2005). Therefore, I will summarise it only briefly. Differentiation of sex chromosomes concerns mainly species with male or female heterogamety. Since this mode of sex determination seems to be most common (Bull 1983) it is worth considering in more detail how differentiation of sex chromosomes influences changes in SD mechanisms. For simplicity, I will focus on the evolution of the X and Y chromosomes in a male heterogametic system, but the same reasoning applies to the Z and W chromosomes in female heterogametic systems.

The first step in the differentiation of sex chromosomes is the reduction in recombination between two homologous sex chromosomes (X and Y). There are several reasons why selection would favour reduced recombination. For example, if sex is determined by multiple SD genes located on a single chromosome, recombination between SD loci may lead to intermediate genotypes and the development of sterile intersexes. In that case reduced recombination between SD loci would provide a selective advantage (see Charlesworth 1996; Rice 1996a).

Accumulation of SA variation on sex chromosomes may also favour reduced recombination. Consider, for example, a dominant male determining allele (similar reasoning applies when sex is determined by a recessive female determining allele). By definition such an allele is present only in males. Accordingly, closely linked alleles beneficial for males but detrimental to females (SA alleles) will hitchhike with the male determining gene. Since recombination may break the linkage, causing the SA allele to be expressed also in females (where it has a negative effect on fitness) there will be selection to decrease the recombination rate in the vicinity of the SD

locus (Rice 1987). This will lead to the accumulation of new SA alleles and selection for decreased recombination even further away from the SD locus. Consequently, recombination between homologous sex chromosomes will cease along large parts of the chromosome.

A lack of recombination between X and Y chromosomes leads to the degeneration of the Y chromosome. There are a number of processes which may be responsible for this: the accumulation of deleterious mutations through genetic drift, genetic hitchhiking and background selection (for details see e.g. Rice 1996a; Charlesworth *et al.* 2005; Bachtrog 2006). Moreover, the presence of segregation distorters on the Y chromosome may also select for autosomal genes silencing the sex chromosome (Hamilton 1967).

Due to the degeneration of the Y chromosome, eventually many genes will be present only on the X chromosome. As a result, the two sexes will differ in the dosage of these genes. This may have detrimental effects on fitness and lead to the evolution of dosage compensation (Charlesworth 1996). Dosage compensation can be achieved by either increasing the level of expression of genes in the heterogametic sex (as in *Drosophila*), or decreasing the expression in the homogametic sex (as in mammals and *C. elegans*).

Differentiation of sex chromosomes may influence future changes in SD mechanisms. For example, degeneration of the chromosome carrying the dominant SD gene, may lead to the under-expression of the gene due to it being surrounded by a heterochromatic, non-functional environment (Hodgkin 1992). Under-expression of the SD gene may lead to a non-reliable SD signal and production of infertile individuals (Hodgkin 1992; Hediger *et al.* 1998) selecting for evolution of new SD genes.

Reduced recombination between homologous sex chromosomes is believed to facilitate the accumulation of segregation distorters, since their action usually demands a close linkage between a distorter allele and an insensitive responder allele (Lyttle 1991; Jaenike 2001; Burt & Trivers 2006). Segregation distorters on sex chromosomes lead to biased sex ratios and may facilitate the spread of new SD genes. Moreover, segregation distorters on the Y chromosome may also select for autosomal genes silencing the sex chromosome and further increase its degeneration (Hamilton 1967).

Differentiation of sex chromosomes may, however, also prevent changes in the SD mechanism. Even if a new SD gene is selectively favoured it may be prevented from spreading (or fixation) if it leads to the production of YY genotypes which lack essential genes that are present only on the X chromosome or if Y possesses recessive lethal mutations (Jayakar 1987; Lande *et al.* 2001). Alternatively, XX males may be sterile if the Y chromosome possesses genes necessary for spermatogenesis. Although in many species Y chromosomes are largely degenerated, they may contain genes necessary for male fertility (Roldan & Gomendio 1999). If this is the case, a new masculinizing gene will be prevented from spreading, since it will lead to production of infertile XX males.

In species in which dosage compensation is controlled by the same genes as somatic development, like in *D. melanogaster*, novel changes in sex determination may often lead to unviable offspring due to improper dosage compensation (Schütt & Nöthiger 2000). More generally, in species in which SD genes have pleiotropic effects, changes in SD mechanisms may be more difficult (Marin & Baker 1998).

In conclusion, differentiation of sex chromosomes may on the one hand facilitate changes in SD mechanisms, but on the other hand hamper them. Taking into account level of differentiation of sex chromosomes may be necessary to properly estimate possibilities of changes in SD mechanism.

A niche for this thesis

As shown above, multiple selective forces may be involved in the evolution of SD mechanisms. They include the hitchhiking effect, viability and fertility selection, sex ratio selection and different forms of genetic conflict. There is a vast amount of theoretical models and empirical data increasing our understanding of this process, but there are still many unanswered questions.

Need for new theory

Multiple selective forces may be involved, but little is known about their interaction. Only few theoretical models consider such interactions and virtually all of these models focus on the detrimental fitness effect of sex chromosomal segregation distorters. The existing models exemplify the importance of studying different selective forces in concert. For example, Charlesworth & Dempsey (2001) showed that the invasion probability of a new distorter chromosome and the change in SD mechanism depends on the inbreeding level in the population. In a model for the SD system of field mice (genus *Akodon*) Hoekstra & Hoekstra (2001) showed that a single selective force is not adequate to explain the frequency of XY females seen in nature. Only the combination of segregation distortion in males and females and the increased fecundity of XY females explains the natural patterns. I expect that, more generally, new insights in the evolution of SD mechanisms will be gained from models incorporating different selective forces.

More mechanistic models are needed. Thanks to molecular studies, our knowledge about the molecular basis of sex determination has increased dramatically. This information allows making realistic assumptions on how different SD genes may interact with each other. Already in 1985 Nöthinger & Steinmann-Zwicky proposed that simple mutations in genes that are part of SD cascades may lead to a variety of SD mechanisms, including male and female heterogamety, maternal control and environmental sex determination. However, explicit models investigating what selection pressures might lead to the spread of a given mutation are rare. One of the few existing mechanistic models was developed by Pomiankowski *et al.* (2004) in order

to explain the evolution of the SD cascade in *D. melanogaster* (discussed in more detail above). This model makes many assumptions specific for *Drosophila* and, as such, cannot be used to answer general questions. However, it demonstrates the importance of the interaction between genes and their expression patterns in the evolution of SD cascades. I strongly believe that taking into account properties of SD cascades into models of evolution of SD mechanisms will bring novel and often surprising insights.

Models on sex ratio selection and maternal-offspring conflict often assume the existence of genes coding for arbitrary sex ratios (Werren & Hatcher 2000; Werren *et al.* 2002; Pen 2006). Many genetic systems are not able to achieve these ratios. For example, theoretical studies of the three allele SD system of platyfish under sex ratio selection (Bull 1983) show that the optimal sex ratio is never achieved. The incorporation of mechanistic constraints is, therefore, necessary to understand such real-world systems.

Need for empirical model systems

Mechanistic models need to be based on our knowledge of the SD mechanisms seen in nature. On the other hand, our understanding of SD mechanisms in natural systems profits from theoretical insights. Therefore, combining a theoretical and empirical approach is crucial for understanding the evolution of SD mechanisms.

Comparative studies shed some light on the evolution of sex determination, but they give little information about the evolutionary dynamics of SD mechanisms. It is often impossible to infer selective forces in the past from present variation, since multiple selective forces and evolutionary routes can lead to the same outcome (McVean & Hurst 1996).

The best way to test theoretical models empirically would be to perform controlled experiments with species with multiple SD mechanisms. One way would be to use laboratory lines of a species for which mutants of SD genes are available (like *C. elegans*; Hodgkin 2002). Another way would be to use a species which shows natural variation for SD mechanisms, like lemmings, the platyfish or the housefly (Bull 1983). Although very few species show natural variation of SD mechanisms, studying them allows us to both study of the selective pressures responsible for the polymorphism in SD mechanisms in nature, and perform controlled experiments designed to test theoretical models.

The housefly is one of the few known species in which multiple SD mechanisms exist in natural populations (Box 1.2; Fig 1.3). They range from male and female heterogamety to monogeny and temperature dependence. Many molecular details are known about the genes in the SD cascade and how different mutations in these genes lead to the observed variety in sex determination, but there are still many unknowns. In particular, little is known about the *M* factors (see Box 1.2). Molecular studies are needed to establish whether different *M* factors are the same gene or different genes and how they exert its masculinizing function.

Little is also known about the selective forces responsible for the spread and maintenance of the different SD factors in the housefly. It seems that the XY system with the *M* factor located on the Y chromosome (Fig 1.3A) is the ancestral state, since it is also the most common in closely related species (Boyes *et al.* 1964). Moreover, the X and Y chromosome are morphologically differentiated suggesting that they evolved a long time ago (Charlesworth *et al.* 2005). Accordingly, the first reports on autosomal SD factors (autosomal *M* and F^D) appeared only around 1960 (reviewed by Franco *et al.* 1982).

It has been proposed that autosomal *M* factors have spread due to their linkage with insecticide resistance genes (Kerr 1970; Franco *et al.* 1982), since the isolation of autosomal *M* factors coincided with the appearance of insecticide resistance in natural populations of the housefly (Tomita & Wada 1989b). An additional factor appears to be temperature, since the geographical distribution of different SD factors follow latitudinal and altitudinal clines on most continents studied so far (Europe - Franco *et al.* 1982; North America - Hamm *et al.* 2005; Japan - Tomita & Wada 1989b; Turkey - Çakir & Kence 1996). The "standard" XY system, with an *M* factor located on the Y chromosome, prevails at higher latitudes and altitudes. At lower latitudes and altitudes autosomal *M* and F^D factors are usually present. Also linkage with segregation distorters has been proposed as a force leading to the spread of autosomal *M* factors (Clark 1999). However, evidence supporting these different hypotheses is still not convincing (discussed in more details in this thesis). Other forces, like maternal-offspring conflict over the sex ratio (Werren *et al.* 2002) can also not be excluded.

The existence of multiple SD factors in natural housefly populations is an interesting problem by itself. A better understanding of this variety and dynamics may allow the verification of already existing theories on the evolution of sex determination. If existing theories are not satisfactory, new models need to be created, possibly also with application to other SD systems. Also, controlled laboratory experiments can be performed in order to test different evolutionary theories.

Aim of this thesis

The aim of this research was to combine both theoretical and empirical approaches to gain more insights in the evolution of sex determination. I built mechanistic models based on the knowledge on the SD mechanisms found in nature. Some of these models were inspired by the SD system of the housefly. To better understand the evolutionary forces shaping the SD system of the housefly, I collected field data and I performed controlled experiments in the laboratory.

Thesis overview

The following eight chapters of this thesis are arranged in two parts. Part I (Chapters 2-5) concerns theoretical models of the evolution of SD mechanisms; Part II (Chapters 6-9) presents field and experimental data on the diversity and evolution of SD mechanisms in the housefly.

Part I: Theory.

Chapter 2: To better understand the constrains imposed by real-world SD systems on the evolution of the sex ratio, the dynamics of multi-factorial SD mechanisms under selection for biased sex ratios are investigated. The important questions are: can sex ratio bias be achieved by systems consisting of multiple SD loci? What are the frequencies of different SD factors at equilibrium and can they explain the patterns observed in natural populations of the housefly? Inspired by the SD system of the housefly, we consider two independent loci with dominant male determining factors and one locus with a female determining factor, insensitive to male determining genes. The dynamics of different SD factors and their equilibrium frequencies are studied under different strengths and direction of sex ratio selection (different costs of male and female production).

Chapter 3: The aim of this chapter is to develop a general mechanistic model for the evolution of genetic SD systems, explicitly incorporating key insights emerging from recent empirical work on the genetics of SD mechanisms. The question is: can fertility selection together with sex ratio selection lead to the evolution of simple SD cascades and the variety of SD systems seen in nature? To this end, we developed a model for the evolution of regulatory genes that do not act in a simple switch-like manner but in a more quantitative way. The regulatory genes lead to the production of a feminizing product. To trigger female development, the amount of the product must surpass a noisy threshold level. Otherwise males are produced, or sterile intersexes if the amount of product is too close to the threshold. In addition to fertility selection, evolution of the SD system is affected by sex ratio selection. By letting both maternal genes and offspring genes affect the level of the feminizing product in the developing offspring, we can study how the evolution of SD regulation is affected by maternal-offspring conflict over the sex ratio.

Chapter 4: This chapter investigates the effect of segregation distortion on the evolution of SD mechanisms. All previous models on this topic were specifically tailored to a particular species. To derive more general conclusions, we analyze a more generic model. Three scenarios are considered: a driving X chromosome, a driving Y chromosome, and a driving autosome with a male determining factor. The invasion prospects of a new male- or female-determining factor are investigated, depending on the strength of distortion and the fitness effects of the distorter allele.

Chapter 5: It is well known that intralocus genetic conflict can influence the evolution of SD mechanisms, but little theoretical work has been devoted to this

topic. To gein more insights into this process, we study the conditions under which new SD factors can spread in response to the accumulation of sexually antagonistic (SA) variation on the original sex chromosomes. Additionally, we investigate the effect of sex chromosome differentiation, dominance effect pattern of different SA alleles, and the linkage of newly arising SD factors with the SA loci on the outcome of the evolutionary dynamics.

Part II: Empirical data.

Chapter 6: In the northern hemisphere, the frequency of autosomal SD factors in natural housefly populations increases with decreasing latitude. Is the same pattern seen in the southern hemisphere? Can temperature explain the global patterns in the distribution of SD mechanisms in the housefly? To answer these questions, we study the distribution of SD factors in natural populations of the housefly in South Africa and Tanzania. In combination with compiled literature data, the new data are subjected to a statistical analysis, in order to investigate whether temperature or latitude is a better predictor of the frequency of different SD factors in the natural populations of the housefly.

Chapter 7: It has been often postulated that autosomal SD factors of the housefly are spreading north, replacing the standard XY system. However, this has never been systematically investigated in the field. We therefore investigated the distribution of different SD factors in European populations of the housefly along a north-south transect. This current distribution is then compared with the distribution reported 25 years ago.

Chapter 8: The distribution of SD factors in natural populations of the housefly suggests that autosomal factors have a fitness advantage over the standard XY system under high temperatures and a disadvantage under low temperature. To test this hypothesis, we performed temperature-controlled laboratory experiments that allowed us to quantify the effects of temperature on the fitness of flies with different SD factors. For two different autosomal *M* factors, invasion experiments were performed under two different temperatures in order to see if they could invade a population with a standard XY system and whether the invasion prospects were temperature-dependent. For females, we investigated under various temperatures whether, and to what extent, females with the *F* and the F^D factor differed in lifespan and lifetime reproductive success.

Chapter 9: In North American populations of the housefly, segregation distortion linked to autosomal *M* factors has been reported. As I have described above, segregation distortion may strongly affect the evolutionary dynamics of sex determination. Therefore, we studied the prevalence of sex-linked segregation distortion in European populations of the housefly. To this end, we investigated the sex ratio produced by males with different autosomal *M* factors and Y chromosomes.

Chapter 10 presents the final summarizing discussion.

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Box 1.1. An overview of the variety of sex determining mechanisms

There exists a variety of sex determining (SD) mechanisms. The mechanisms can be categorized in several ways. I present on one way of categorization of the different SD mechanisms, which is consistent with the scope of my thesis. However, as indicated below, there are various alternatives (e.g. Bull 1983; Werren & Beukeboom 1998).

Sex determining mechanisms are often classified into two main categories: genetic (or genotypic) sex determination and environmental sex determination.

Environmental sex determination (ESD): Sex is determined according to environmental cues, independent of an individual's genotype. The same individual develops into a male under some environmental conditions, but into a female under other conditions. The environmental cue for sexual differentiation can be, for example, nutritional status (in Mermithidae nematodes), photoperiod (the amphipod *Gammarus duebeni*) or the social environment (the marine worm *Bonellia viridis*; Bull 1983, 1985). Probably the most widespread environmental factor affecting sex determination is temperature, leading to so-called **temperature dependent sex determination** (TSD). TSD is known, for example, from many reptiles and some fish species (Bull 1983; Janzen & Phillips 2006).

Genetic sex determination (GSD): The primary signal in sexual differentiation is based on genetic cues. There is a large variety of GSD systems and they can be classified along two independent axes:
(a) maternal vs. offspring control;
(b) presence of sex-specific alleles vs. dosage effect.

1. Presence of sex-specific alleles. Sex may be determined by sex-specific allele(s) at one or more primary SD loci or by the presence of sex-specific SD genes. The sex-specific alleles may either be present in the offspring or in their mother. Systems where sex is determined by offspring genotype are usually associated with heterogamety, while sex determination via the maternal genotype often results in monogeny.

Heterogamety: One of the sexes is heterozygous at one or more SD loci and the other sex is homozygous at all SD loci. Both male and female heterogamety occurs in nature. Chromosomes bearing loci with a primary SD signal are called sex chromosomes. Homologous sex chromosomes may differ only at the SD locus (heterogamety in the broad sense). However, sex chromosomes are often differentiated across larger regions that are not involved in sex determination, or one of the homologues may even be missing (as in an XO system; Bull 1983). Such cases correspond to heterogamety in the narrow sense.

Under **male heterogamety (XY system)**, males are heterozygous at one or more SD loci. The sex chromosome specific to males is typically denoted by Y, and the homologous chromosome is denoted by X. Hence, males are XY and females are XX. Sex determination can be achieved, for example, by a dominant male determining gene located on the Y chromosome (e.g. in mammals, most insects). Male heterogamety refers also to systems in which sex determination occurs by a balance between X chromosomes and autosomes (X:A ratio; e.g. in *Caenorhabditis elegans* and possibly *Drosophila melanogaster*; but see Erickson & Quintero 2007).

Under **female heterogamety (ZW system)**, females are the heterogametic sex and males are the homogametic sex. The sex chromosomes are denoted by Z and W; females are ZW and males are ZZ. Female heterogamety is present, for example, in birds, butterflies and snakes (Bull 1983).

Monogeny: The sex of offspring depends entirely on the maternal genotype. Some females produce only male offspring while others produce only female offspring. This is found in a few insect species, e.g. *Sciara coprophila* and *Chrysomya rufifacies* (Bull 1983).

2. Dosage effects. GSD is not mediated by male- or female-specific alleles or genes. Instead, sex depends on the combination or dosage of alleles at one or more SD loci. This form of sex determination is usually related to ploidy level.
Haplodiploidy: Males usually develop from unfertilized eggs and are haploid, while females develop from fertilized eggs and are diploid. The actual genetics of sex determination may vary. Some species possess so-called complementary sex determination, where individuals heterozygous at a SD locus develop into females and homozygous or hemizygous individuals develop into males (most Hymenoptera). In other species (e.g. *Nasonia* wasps) maternal imprinting of the offspring SD gene is probably involved and sex depends on the presence (females) or absence (males) of unimprinted alleles (Beukeboom *et al.* 2007).

Any classification of SD mechanisms is only a simplification, since often a number of factors can be involved. Below are examples of SD mechanisms at different levels of classification that fail to fall into the above categories.

A. ESD vs. GSD. There are species in which a mixture between GSD and ESD exists. In species with GSD, genetic factors can sometimes be overridden by extreme environmental conditions leading to sex-reversal. Similarly, genetic factors can be important in species with ESD. As an example, one might think of a species where sex is determined by the temperature of the nest, but nest temperature is in turn affected by maternal behaviour and, hence, by the maternal genotype. Mixture of GSD and ESD has been found, for example, in some fish species (Conover et al. 1992; Sato *et al.* 2005).

B. Sex-specific alleles vs. dosage effect. SD systems in which sex is determined by the X:A ratio (or dose of X chromosomes) are usually referred to as heterogamety (see above). However, in this system sex determination is based on the dose of X-linked genes and can even be viewed as haplodiploidy for the sex chromosomes. This may also apply to many XO (or ZO) systems where the Y (or W) chromosome is lacking, but the genetics of sex determination are not known yet.

C. Maternal vs. offspring genes. In some amphipods sex is partly determined by cytoplasmic factors (e.g. intracellular bacteria; Bull 1983; Werren & Beukeboom 1998). They feminize offspring with an otherwise male genotype. Therefore, the sex of the offspring is dependent on its own nuclear genotype and the presence of cytoplasmic factors. But the cytoplasmic factors in the offspring are inherited from the mother making sex determination dependent on maternal (cytoplasmic) genotype.

Additionally, in many species both offspring genes and maternal products of nuclear genes are involved in sex determination (Fig 1.2). Accordingly, it is sometimes difficult to distinguish between maternal and offspring control (Box 1.2).

D. Male vs female heterogamety. In some species multiple alleles or genes on different chromosomes may be involved in sex determination leading to a mixture of male and female heterogamety (e.g. in *Musca domestica*; Dübendorfer *et al.* 2002; Box 1.2).

Box 1.2. Sex determination in the housefly

Multiple SD mechanisms have been described in natural and laboratory populations of the housefly, *Musca domestica*. All of them seem to be variations of a common sex determining cascade (Fig 1.2). As in *D. melanogaster*, at the bottom of the SD cascade in the housefly is a switch gene – the *Musca domestica* homologue of *doublesex* (*Md-dsx*; Hediger *et al.* 2004). Alternative splicing produce either a male-specific protein variant, inducing male development, or a female-specific variant, leading to female development. Sex-specific splicing of *Md-dsx* is regulated by the *F* gene which is a homologue of the *Drosophila transformer* (*tra*) gene (M. Hediger and D. Bopp, personal communication). The presence of the *F* gene product leads to female-specific splicing of *Md-dsx* and its absence to male-specific splicing. *F* is activated in early embryos by maternal product of *F* deposited in the egg. Hence, *F* is auto-regulated and the expression of *F* during the whole of development (from early embryogenesis till metamorphosis) is necessary to ascertain female development (Hilfiker-Kleiner *et al.* 1993). For auto-regulation of *F* and female splicing of *Md-dsx* constant expression of another gene, the *transformer2* (*tra2*) homologue, is necessary, although it is equally expressed in both sexes (Burghardt *et al.* 2005).

In males *F* is suppressed by the so-called *M* factor. Activity of the *M* factor at any time between early embryogenesis and metamorphosis breaks the auto-regulatory loop of *F* and leads to male development (Hilfiker-Kleiner *et al.* 1993). The *M* factor can be located on each chromosome of the housefly: Y, X and all of the five autosomes (Denholm *et al.* 1983; Tomita & Wada 1989b). Nothing is known about the *M* factor at the molecular level. *M* factors on different chromosomes have the same masculinizing effect on development (Schmidt *et al.* 1997b), although if they are located in a heterochromatic region, masculinisation is not complete, resulting in the production of intersexes (Hediger *et al.* 1998). Therefore, *M* factors located on different chromosomes can be one and the same gene located on a transposable element, as is known from *Megaselia scalaris* (Traut & Willhoeft 1990), or they can represent different genes on each chromosome. Theoretically, different genes could exert a masculinizing effect by breaking the auto-regulatory *F* loop. For example, *M* factors could exert their function by binding with the product of *F* interfering with its splicing function. Alternatively, *M* could be a loss of function mutation in the *tra2* gene (or another yet unknown gene) which is necessary for maintaining the auto-regulatory *F* loop (Schütt & Nöthiger 2000; Burghardt *et al.* 2005).

In many populations with autosomal *M* factors a dominant mutation of *F* is known, called F^D, which is no longer blocked by the *M* factor (independent of

its location) and therefore its presence in the zygote leads to female development, even if there are several *M* factors present (Dübendorfer *et al.* 2002). Therefore, in populations with F^D individuals with multiple *M* factors can often be found. F^D is always expressed and does not need the maternal *F* product for auto-regulation. More detailed molecular data on how F^D avoids suppression by *M* are lacking.

Additional variation in SD mechanisms has been found in laboratory populations of the housefly. In one strain, a recessive mutation *masculinizer* (*man*) has been described, which is probably a loss of function mutation of *F* (Schmidt *et al.* 1997a). Homozygous individuals (F^{man}/F^{man}) do not produce the *F* product and develop as males. In heterozygous individuals (F^{man}/F) the wild type allele of *F* is active and they mostly develop as females. However, a single *F* allele in mothers is not always sufficient to activate the *F* in their offspring. Therefore F^{man}/F mothers produce also intersexes and fertile males among their genotypically female progeny (XX; F^+/F^+ or XX; F^{man}/F^+). This effect is dependent on the mothernal age and the temperature experienced before oviposition. With increasing age and temperature, F^{man}/F female produces more males and intersexes (Schmidt *et al.* 1997a).

Another laboratory strain shows an even stronger maternal effect (Vanossi Este & Rovati 1982). The *Ag* factor (*Arrhenogenic*) in that strain is a maternal effect sex determiner. In strains without *M* factors, heterozygous females (*Ag*/+) produce mostly sons and intersexes whereas the wildtype females produce exclusively daughters. The genotype of the father or the zygote itself does not affect its sex. *Ag* is probably lethal in homozygous state as *Ag/Ag* flies have never been found. The *Ag* factor is probably a variant of the *M* factor located on autosome I, which lost its somatic function. As a consequence, it does not block the *F* factor in the developing zygote, but prevents activity of *F* in the female germ line (Hilfiker-Kleiner *et al.* 1994). Therefore, females having the *Ag* factor do not place *F* product in the zygote, which thus develops as a male. The effect of *Ag* is temperature sensitive: at higher temperatures fewer males and intersexes are produced in this strain (Schmidt *et al.* 1997b).

Box 1.3. Maternal-offspring conflict over the sex ratio

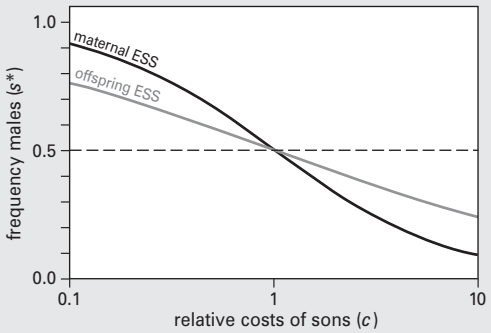
There are many circumstances under which selection favours biased sex ratios (see e.g. Hardy 2002). In many such cases the evolutionarily stable sex ratio of maternal genes differs from the ESS sex ratio of offspring genes. Typically offspring genes favour less biased sex ratios than maternal genes (but see Pen 2006). This stems from the fact that in populations with a sex ratio bias, individuals of the minority sex typically have a higher reproductive value. Accordingly, offspring genes may get a selective advantage if they tend to end up in individuals belonging to the minority sex.

One of the conditions under which maternal genes favour different sex ratios than offspring genes is when the maternal cost of producing a daughter differs from the cost of producing a son. In a fundamental contribution, Fisher (1930) showed that, at an evolutionarily stable equilibrium, parents should allocate equal amounts of resources into male and female offspring: $n_M C_M = n_F C_F$, where n_M and n_F are the expected number of sons and daughters, and C_M and C_F are the costs of a producing a son or a daughter, respectively. If we quantify the sex ratio by the proportion of sons, $s = n_M/(n_M + n_F)$, and if we denote the relative cost of sons by $c = C_M/C_F$, Fisher's "equal allocation principle" can be reformulated as:

$$s^* = \frac{1}{1+c}$$

As explained above, the evolutionarily stable sex ratios of offspring will be less biased. Trivers (1974) showed that, in case of full-sibling families, the offspring ESS is given by:

$$s^* = \frac{1}{1+\sqrt{c}}$$



The discrepancy between the maternal and the offspring sex ratios can potentially lead to conflict over sex determination and changes in the SD mechanism (see main text).

PART I

Theory

CHAPTER 2

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Sex ratio selection and multi-factorial sex determination in the housefly: A dynamic model

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Abstract

Sex determining mechanisms are highly variable between different taxonomic groups and appear to change relatively quickly during evolution. Sex ratio selection could be a dominant force causing such changes. We investigate theoretically the effect of sex ratio selection on the dynamics of a multi-factorial sex determining system. The system considered resembles the naturally occurring three-locus system of the housefly which allows for male heterogamety, female heterogamety and a variety of other mechanisms. Sex ratio selection is modeled by assuming cost differences in the production of sons and daughters, a scenario leading to a strong sex ratio bias in the absence of constraints imposed by the mechanism of sex determination. We show that, despite of the presumed flexibility of the sex determining system considered, equilibrium sex ratios never deviate strongly from 1:1. Even if daughters are very costly, a male-biased sex ratio can never evolve. If sons are more costly, the sex ratio can be slightly female biased but even in case of large cost differences the bias is very small (<10% from 1:1). Sex ratio selection can lead to a shift in the sex determining mechanism, but cannot be the sole cause of complete switches from one sex determining system to another. In fact, more than one locus remains polymorphic at equilibrium. We discuss our results in the context of evolution of the variable sex determining mechanism found in natural housefly populations.

Introduction

Sex determination is a fundamental developmental process in animals and plants and one might therefore expect the underlying mechanisms to be conserved. Yet the opposite is true: sex determining (SD) mechanisms vary considerably between closely related taxonomic groups and evolutionary transitions from one system to another seem to occur frequently (Bull 1983; Marin & Baker 1998; Werren & Beukeboom 1998; Kraak & Pen 2002). Common SD mechanisms are male heterogamety (males *XY* and females *XX*, such as in nearly all mammals and many insect groups), female heterogamety (females *ZW* and males *ZZ*, such as in birds, lepidopterans and snakes), haplodiploidy (females diploid and males haploid, such as in hymenopterans) and environmental sex determination (such as in some reptiles and fish), but there exist a variety of other mechanisms (Bull 1983).

It is still far from clear why SD mechanisms are so evolutionarily unstable and what forces are responsible for their rapid turnover rate. Genetic conflict and sex ratio selection might play an important role (Eberhard 1980; Werren & Beukeboom 1998). For example, models have been proposed that show how conflicting selection pressures on autosomal genes and cytoplasmic factors may induce transitions from female heterogamety to male heterogamety (Caubet *et al.* 2000). Despite such theoretical advances, not much empirical progress has been made. In particular, little experimental work has been done (but see Conover & Vanvoorhees 1990; Conover *et al.* 1992; Basolo 1994; Carvalho *et al.* 1998; Basolo 2001). One reason for the lack of experiments is presumably that SD mechanisms are usually fixed (or thought to be so) in individual species, although some exceptions are known (Bull 1983).

The housefly (*Musca domestica*) is such an exception. In this species, several different SD mechanisms have been found to co-exist in field populations (Fig. 2.1; Franco *et al.* 1982; Denholm *et al.* 1985; Tomita & Wada 1989b). In the so-called standard *XY* strains, a male-determining factor (*M*) is located on the *Y* chromosome and males are *XY* and females *XX*. The *M* factor blocks the action of an autosomal *F* which is necessary for female development. In addition to the standard *XY* system, field populations have been discovered in which an *M* factor is located on one or several of the five autosomes, or even on an X chromosome. These autosomal (more precisely, non-*Y*) *M* factors seem to have appeared relatively recently and may be spreading, replacing the standard *XY* system in many locations (see Franco *et al.* 1982; Tomita & Wada 1989a). Intriguingly, the frequency of autosomal *M* factors seems to decrease with latitude and altitude, northern and high altitude populations are usually dominated by the standard *XY* system. Such geographical clines have been found in Europe (Franco *et al.* 1982), Japan (Tomita & Wada 1989b), Turkey (Çakir & Kence 1996) and the USA (Hamm *et al.* 2005). In most populations with autosomal *M* factors, an additional epistatic factor F^{D} ($F^{Dominant}$) occurs, dictating female development, even in the presence of up to three *M* factors (see McDonald *et al.* 1978; Franco *et al.* 1982). Presumably F^{D} evolved after the invasion of autosomal

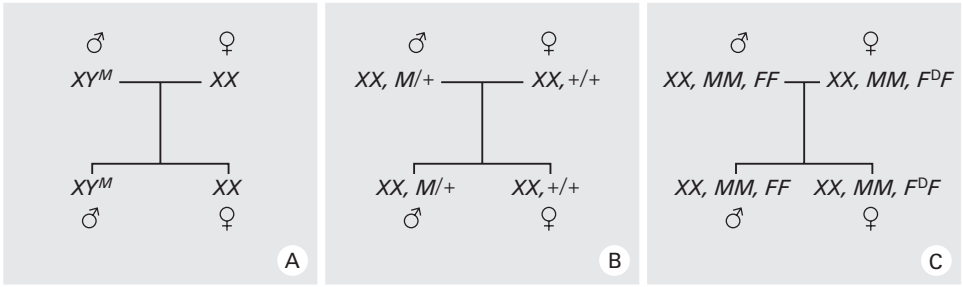

Figure 2.1. Common sex determining mechanisms in natural populations of *Musca domestica*. (A) The standard *XY* system – male determining factor (*M*) present on the *Y* chromosome. (B) Autosomal system with male heterogamety – *M* present on one of the autosomes, males and females homozygous for *X*. (C) Autosomal system with female heterogamety – males and females are homozygous for *X* and autosomal *M*, sex is determined by presence (females) or absence (males) of the epistatic factor F^D. Figure adjusted from Dübendorfer *et al.* 2002.

M factors, instead of vice versa, since populations with F^D always have autosomal *M* factors but not the other way round. Some populations with F^D appear to be fixed for an autosomal *M*, and in such populations most flies have two *X* chromosomes, *YY* genotypes being rare (Franco *et al.* 1982; Denholm *et al.* 1983, 1985; Denholm *et al.* 1990). This has been taken to suggest that *YY* genotypes may have lower viability, but direct evidence for this is lacking. In addition to SD systems comprising *M* factors and F^D, several other mechanisms have been discovered in the laboratory, including a mechanism that induces monogeny (Dübendorfer *et al.* 2002).

Whatever the causes for the variability and distribution of SD mechanisms in the housefly (more about this in the Discussion), this organism is potentially very suitable for conducting experimental studies on the evolution of sex determination, and we are currently embarking on such studies. However, in addition to carrying out experiments, it is useful to obtain more theoretical insight into the dynamical behavior of the housefly system. Therefore we present here a study of a three-locus model, with an XY "locus", an autosomal *M* locus and an autosomal F^D locus. We extend and earlier analysis of Jayakar (1987) who studied a similar model but focused on a number of two-locus sub-models, mixing either *XY* with F^D (or, mathematically equivalently, *M* and F^D) or mixing *XY* and autosomal *M*. In contrast to Jayakar (1987), who mainly considered the potential effect of meiotic drive, we here investigate the effect of sex ratio selection on the dynamics of the three-locus system. The reason is that the selection for or against biased sex ratios in thought to be, at least theoretically, an important contributing factor in evolutionary transitions between SD systems (Bull 1983; Wilkins 1995; Werren & Beukeboom 1998; Werren & Hatcher 2000; Kraak & Pen 2002; Werren *et al.* 2002). There are various scenarios how natural selection might lead to bias in the primary sex ratio (Hamilton 1967;

Charnov 1975, 1982; Werren & Taylor 1984; Reinhold 1996; Werren & Hatcher 2000; Beukeboom *et al.* 2001; Werren *et al.* 2002; Wade *et al.* 2003). Here we focus on the most basic mechanism where sons and daughters differ in how much they "cost" to produce by the parents. Selection will then act on genes affecting the sex ratio to favor overproduction of the "cheaper" sex (Fisher 1930; Trivers 1974).

We aim to achieve three goals with this study. Firstly, our study might contribute to understanding to what extent real-world sex determining systems constrain the evolution of the sex ratio. This is important because most models of sex ratio evolution assume that the sex ratio is a continuous variable and that any sex ratio is feasible by the underlying genetic system (Pen & Weissing 2002). Secondly, we hope that our model sheds some more light on the frequencies of SD factors and sex ratios that have been observed in field populations of the housefly. And last but not least, we hope that our results will be useful in designing and interpreting future laboratory experiments that will be carried out with houseflies and other organisms.

The model

We model the dynamics of a sex determination system consisting of three gene loci on three different chromosomes, each locus having two possible alleles. The first locus corresponds to the standard *XY* sex determination system, having an *X* "allele" and a *Y* (male-determining) "allele". The second locus has a male-determining *M* allele and a neutral "+" allele. The third locus has an epistatic female-determining F^D allele and a standard *F* allele (we call *Y, M* and F^D the "focal" alleles at their loci). The total number of possible genotypes is therefore $3^3 = 27$, but we focus on a subset of 18 genotypes, since the 9 genotypes with two F^D alleles are not feasible because males never have F^D alleles (Table 2.1) and hence females are never homozygous for F^D.

A genotype is encoded by a triplet $\mathbf{i} = (i_1, i_2, i_3) = (\#Y, \#M, \#F^D)$, tracking the number of focal alleles at each locus. The sexual phenotype determined by genotype $\mathbf{i}$ is encoded as a binary variable: $s(\mathbf{i})=0$ for females and $s(\mathbf{i})=1$ for males. The frequencies of genotype $\mathbf{i}$ among adult females and adult males are written as $p_f(\mathbf{i})$ and $p_m(\mathbf{i})$ ($\sum p_f(\mathbf{i}) = \sum p_m(\mathbf{i}) = 1$). Note that for each $\mathbf{i}$ either $p_f(\mathbf{i})$ or $p_m(\mathbf{i})$ must be zero because the genotype $\mathbf{i}$ uniquely determines sex.

The conditional distribution of genotype $\mathbf{k}$ among the offspring of parents with genotypes $\mathbf{i}$ and $\mathbf{j}$ is denoted by $T(\mathbf{k}|\mathbf{ij})$. Assuming independent assortment of chromosomes, $T(\mathbf{k}|\mathbf{ij})$ can be written as:

$$T(\mathbf{k}|\mathbf{ij}) = P(k_1|i_1 j_1)P(k_2|i_2 j_2)P(k_3|i_3 j_3)\,, \qquad (1)$$

where $P(k_n|i_n j_n)$ is the probability that an offspring receives k_n copies of a focal allele at locus n, given that the parents have i_n and j_n copies of that allele. Observe that for

Table 2.1. All possible genotypes and their representation in the model.

Females				Males			
Genotype			Code (**i**)	Genotype			Code (**i**)
XX	++	*FF*	(0,0,0)	*XY*	++	*FF*	(1,0,0)
XX	++	FF^D	(0,0,1)	*XY*	*M*+	*FF*	(1,1,0)
XX	*M*+	FF^D	(0,1,1)	*XY*	*MM*	*FF*	(1,2,0)
XX	*MM*	FF^D	(0,2,1)	*XX*	*M*+	*FF*	(0,1,0)
XY	++	FF^D	(1,0,1)	*XX*	*MM*	*FF*	(0,2,0)
XY	*M*+	FF^D	(1,1,1)	*YY*	++	*FF*	(2,0,0)
XY	*MM*	FF^D	(1,2,1)	*YY*	*M*+	*FF*	(2,1,0)
YY	++	FF^D	(2,0,1)	*YY*	*MM*	*FF*	(2,2,0)
YY	*M*+	FF^D	(2,1,1)				
YY	*MM*	FF^D	(2,2,1)				

all n $\sum_{k_n} P(k_n | i_n j_n) = 1$. A parent with i_n copies transmits either 0 or 1 copy, with expected value $i_n/2$, assuming "honest" Mendelian inheritance. The number of copies received by an offspring is therefore distributed according to

$$\begin{aligned} P(k_n = 0 | i_n j_n) &= (1 - \tfrac{1}{2} i_n)(1 - \tfrac{1}{2} j_n) \\ P(k_n = 1 | i_n j_n) &= \tfrac{1}{2} i_n (1 - \tfrac{1}{2} j_n) + \tfrac{1}{2} j_n (1 - \tfrac{1}{2} i_n) \\ P(k_n = 2 | i_n j_n) &= \tfrac{1}{4} i_n j_n \end{aligned} \qquad (2)$$

The number and viability of offspring may depend on the genotypes of the parents and the genotype of the offspring. In particular, the number of offspring produced by a genotype pair **ij** is denoted by $u(\mathbf{ij})$ and the viability of an offspring with genotype **k** by $v(\mathbf{k})$. We shall use the notation $w(\mathbf{ij},\mathbf{k})$ as shorthand for $u(\mathbf{ij})v(\mathbf{k})$.

Under random mating, the probability that an **i**-female mates with a **j**-male is given by the product of their frequencies, $p_f(\mathbf{i})\ p_m(\mathbf{j})$. Assuming discrete and non-overlapping generations, the sex-specific genotype frequencies $p'_f(\mathbf{k})$ and $p'_m(\mathbf{k})$ after one round of reproduction and selection are given by the recursions

$$\begin{aligned} S_2\, p'_m(\mathbf{k}) &= \frac{1}{\bar{w}} \sum_{\mathbf{ij}} p_f(\mathbf{i})\ p_m(\mathbf{j})\ T(\mathbf{k}|\mathbf{ij})\ s(\mathbf{k})\ w(\mathbf{ij},\mathbf{k}) \\ (1-S_2)\, p'_f(\mathbf{k}) &= \frac{1}{\bar{w}} \sum_{\mathbf{ij}} p_f(\mathbf{i})\ p_m(\mathbf{j})\ T(\mathbf{k}|\mathbf{ij})[1-s(\mathbf{k})]\ w(\mathbf{ij},\mathbf{k}) \end{aligned} \qquad (3)$$

where

$$\bar{w} = \sum_{\mathbf{k}} \sum_{\mathbf{ij}} p_f(\mathbf{i})\ p_m(\mathbf{j})\ T(\mathbf{k}|\mathbf{ij})\ w(\mathbf{ij},\mathbf{k}) \qquad (4)$$

is the mean number of surviving offspring, averaged over all pairs, and

$$S_2 = \frac{1}{\bar{w}} \sum_{\mathbf{k}} \sum_{\mathbf{ij}} p_f(\mathbf{i})\, p_m(\mathbf{j})\, T(\mathbf{k}|\mathbf{ij}) s(\mathbf{k})\, w(\mathbf{ij},\mathbf{k}) \tag{5}$$

is the sex ratio (proportion males) after viability selection (the *secondary* sex ratio). The *primary* sex ratio (before viability selection) is given by

$$S_1 = \frac{1}{\bar{u}} \sum_{\mathbf{k}} \sum_{\mathbf{ij}} p_f(\mathbf{i})\, p_m(\mathbf{j})\, T(\mathbf{k}|\mathbf{ij}) s(\mathbf{k})\, u(\mathbf{ij}) \tag{6}$$

where $\bar{u}$ is the mean family size.

Where possible, we used analytical methods to analyze (3), but in most cases we had to use numerical iterations. To investigate dependence on initial conditions, for each parameter combination 200 random initial genotype frequencies were sampled.

Sex ratio selection

To incorporate sex ratio selection in the model, we give all parents the same amount of resources and we let a son cost $0 < c < \infty$ times the (fixed) resource requirements of a daughter. The average cost per offspring is then proportional to $s(\mathbf{ij})c + 1 - s(\mathbf{ij})$, where $s(\mathbf{ij}) = \sum_k T(\mathbf{k}|\mathbf{ij}) s(\mathbf{k})$ is the family sex ratio produced by an **ij** pair. Hence, up to a constant of proportionality, the number of offspring produced by a pair is given by

$$s(\mathbf{ij}) = \frac{1}{s(\mathbf{ij})c + 1 - s(\mathbf{ij})} \tag{7}$$

If sons are more costly than daughters ($c > 1$), a female-biased sex ratio is selectively favored. The opposite holds true if daughters are more costly ($c < 1$). Under perfect parental control of the family sex ratio, selection unconstrained by the SD mechanism favors equal allocation of resources (Fisher 1930), which corresponds to a primary sex ratio of $1/(1+c)$. We use the Fisherian sex ratio as one of the benchmarks for the sex ratios predicted by our model. In our model, there is no direct parental control of the sex ratio, but rather the genotypes of the offspring determine the sex ratio. Therefore, as a second benchmark we use the optimal sex ratio from the offspring's point of view, when the sex ratio is unconstrained by the SD mechanism. We call this the Triversian sex ratio, since Trivers (1974) first showed that it is given by $1/(1+\sqrt{c})$ when the relatedness between offspring with the same mother is $1/2$. Note that Triversian sex ratios are less biased than Fisherian sex ratios.

Results

No sex ratio selection

As a "null model" we studied what happens when there are no cost differences between sons and daughters and no survival differences between genotypes (i.e. $w(\mathbf{ij},\mathbf{k})$ = constant). It can be shown analytically (see Appendix) that all equilibria of the system (3) have an even sex ratio, i.e. $S^*_1 = S^*_2 = 1/2$. Numerical iterations showed that the equilibria are reached quite fast, usually within 10 generations (Fig. 2.2). When introduced at low frequency, F^D and M always persist but never reach appreciable frequencies. Jayakar (1987) studied a model where F^D was introduced into an XY population (without additional autosomal M) and found that F^D always disappears. Apparently, the presence of M is necessary to allow the F^D factor to persist. When M and F^D are introduced at higher frequencies, they can persist at relatively high frequencies, as long as the initial sex ratio does not depart too much from 50:50.

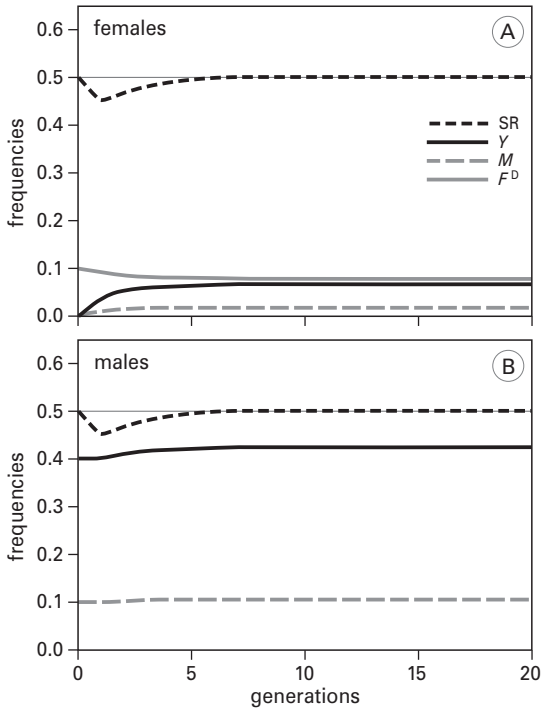

Figure 2.2. Example of dynamics of the sex-specific frequencies of the Y chromosome, the autosomal M factor, F^D, and the sex ratio (proportion of sons; SR). Sons and daughters are assumed to be equally costly (c =1). Note that the sex ratio converges to 0.5.

Sex ratio selection

***Daughters more costly than sons (c<1)*:** Under this scenario, male-biased sex ratios are selectively favored, but, somewhat surprisingly, the equilibrium primary sex ratio was always even. The time required for the system to reach equilibrium depends on the initial genotype frequencies and the strength of selection and may be as long as hundreds of generations when selection is weak (the same applies when $c>1$, see below). The F^D factor is always removed from the population, regardless of the frequency at which it is introduced (Fig. 2.3). The logic behind this appears to be that females with an F^D factor always produce at most 50% sons (see Table 2.2), whereas females without an F^D factor produce at least 50% sons. Since selection favors a male-biased sex ratio, the wild type *F* allele never has a selective disadvantage (unless the population sex ratio happens to be strongly male-biased, which is at most a transient state) and ultimately goes to fixation. When this happens, the system reduces to a population with a mixture of *X, Y* and *M*. It may appear counter-intuitive at first sight that such a system cannot produce male-biased sex ratios at equilibrium, since all males with at least two male-determining factors are capable of

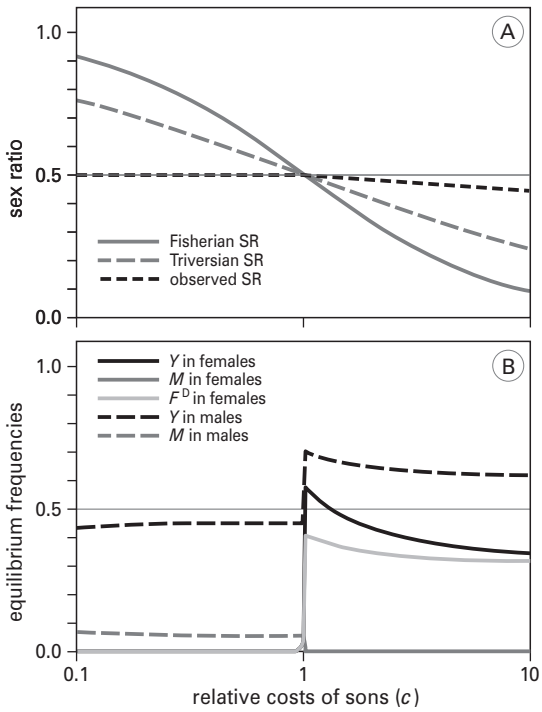

Figure 2.3. Equilibrium sex ratio compared to Fisherian and Triversian expectations (A) and equilibrium frequencies of sex determining factors (B) as a function of the relative cost of sons (c). The outcome depends partly on initial frequencies (see Results), which were here: $p(Y)=0.225$, $p(M)=0.025$, $p(F^D)=0.025$.

Table 2.2. Family sex ratios (proportion sons) as a function of maternal (rows) and paternal (columns) genotype. Note that sex ratios produced by mothers with F^D are at most 1/2, and those of mothers without F^D at least 1/2.

	XY ++ *FF*	*XY* *M*+ *FF*	*XY* *MM* *FF*	*XX* *M*+ *FF*	*XX* *MM* *FF*	*YY* ++ *FF*	*YY* *M*+ *FF*	*YY* *MM* *FF*
XX, ++, *FF*	1/2	3/4	1	1/2	1	1	1	1
XX, ++, FF^D	1/4	3/8	1/2	1/4	1/2	1/2	1/2	1/2
XX, *M*+, FF^D	3/8	7/16	1/2	3/8	1/2	1/2	1/2	1/2
XX, *MM*, FF^D	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
XY, ++, FF^D	3/8	3/8	1/2	3/8	1/2	1/2	1/2	1/2
XY, *M*+, FF^D	7/16	15/32	1/2	7/16	1/2	1/2	1/2	1/2
XY, *MM*, FF^D	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
YY, ++, FF^D	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
YY, *M*+, FF^D	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
YY, *MM*, FF^D	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2

producing male-biased sex ratios when mated to females without F^D (Table 2.2). However, in the absence of F^D, *YY* males are never produced and the same holds true for *MM* males. A simple argument shows that *XY/M*+ males also disappear quickly: *XY/M*+ males produce 75% sons and therefore a family size of

$$\frac{1}{3/4\,c + 1/4} = \frac{4}{3c + 1} \tag{8}$$

XY/++ and *XX/M*+ males produce 50% sons and a family size of

$$\frac{1}{1/2\,c + 1/2} = \frac{4}{2c + 2} \tag{9}$$

Therefore, in term of family size (see equation (7)), *XY/M*+ males have a relative advantage to the tune of

$$\frac{2c + 2}{3c + 1} \tag{10}$$

For $c<1$, this advantage is between 1 and 2. On the other hand, the *XY/M*+ males have the disadvantage that only a quarter of their offspring also have the XY/M+ genotype. The family size advantage cannot compensate for this and as a consequence the frequency of *XY/M*+ decays at a geometric rate. Thus, the only male genotypes remaining are *XY/*++ and *XX/M*+, their ultimate frequencies lying on a curve of neutral equilibria (Bull & Charnov 1977; Jayakar 1987).

Sons more costly than daughters (c>1): Now female-biased sex ratios are expected to be selectively favored, and this is indeed what we found. The equilibrium sex ratio is always biased towards females and the bias increases with the relative cost of sons, *c*. For a given *c*, the equilibrium sex ratio is independent of initial conditions. However, the magnitude of the sex ratio bias is relatively small (<10% from 1:1) compared to Fisherian and Triversian optimal sex ratios, even in situations where sons are much more expensive to produce than daughters (Fig. 2.3).

Surprisingly, only a single male-determining factor can remain in the population. If *M* is introduced at low frequency, it will ultimately disappear. Conversely, if *M* is initially present at a higher frequency than *Y*, then the latter will disappear. For a given $c>1$, the equilibrium frequencies of F^D and the remaining male-determining factor are independent of the initial conditions. F^D never reaches a frequency of 0.5 among females, hence a fully female heterogametic system does not evolve. In fact, with increasing *c*, the equilibrium F^D frequency decreases somewhat (Fig. 2.3). The explanation seems to be that sex ratio selection maintains polymorphism at the locus with the remaining male-determining factor, due to the fact that heterozygous males produce more daughters than homozygous males (Table 2.2.).

Selection against *YY*

We investigated what happens when *YY* genotypes have lower survival than other genotypes, which has been offered as an explanation for the scarcity of *Y* in populations harboring an F^D allele (Franco *et al.* 1982), although direct evidence for lower viability of *YY* is lacking. The typical outcome of numerical iterations for $c>1$ is that the *Y* chromosome disappears from the population and is replaced by autosomal *M*. When sex ratio selection in sufficiently week (*c* close to 1) and *YY* genotypes have sufficiently low survival or for $c<1$, F^D disappears and a stable coexistence of *Y* and *M* results (Fig. 2.4).

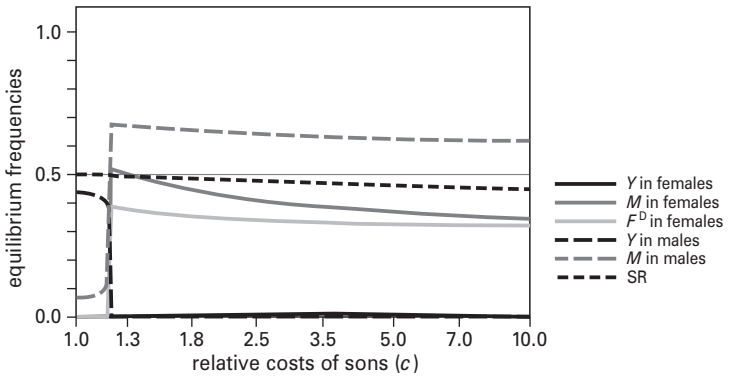

Figure 2.4. Equilibrium sex ratio (SR) and equilibrium frequencies of sex determining factors as a function of the relative cost of sons (*c*) when relative viability of *YY* genotypes equals 0.8. Initial allele frequencies: $p(Y)=0.225$, $p(M)=0.025$, $p(F^D)=0.025$.

Discussion

Sex ratio evolution and constraints on adaptation

We have shown that in the absence of viability differences and cost differences between sons and daughters, the basic three-locus SD system of the housefly always has an even sex ratio at equilibrium (see Appendix). In fact, the analysis shows that under the same assumptions, the result continues to hold true for any number of unlinked SD loci with any number of alleles per locus. Therefore, the result also applies to populations with *M* factors on multiple autosomes (as have been observed; Wagoner 1969; Franco *et al.* 1982; Tomita & Wada 1989b; personal observations).

Our numerical analysis shows that even when males are "cheaper" than females, male-biased sex ratios cannot be achieved in equilibrium by the housefly system. Female-biased equilibrium sex ratios are possible, when daughters cost less than sons to produce, but the magnitude of the bias is much smaller than predicted under perfect maternal or offspring control (Fig. 2.3A). We found this somewhat surprising, since mixtures of genotypes that create strongly biased sex ratios are possible for the housefly system (Table 2.2) but apparently not stable. A similar lack of flexibility of a genetic SD system in producing biased sex ratios was found by Bull (1983), who studied a one-locus three-allele model, designed to mimic a platyfish SD system, allowing for cost-differences between sons and daughters. Equilibrium sex ratios for this model were biased, but only very weakly so. These results highlight the potential importance of the constraints imposed by genetic mechanisms on the precision and magnitude of adaptation (Shuker & West 2004).

Offspring sex ratios in natural populations of the housefly have not been studied much, but two studies of several Turkish housefly populations (Çakir & Kence 1996; Çakir 1999) found that the vast majority of populations have sex ratios that do not differ significantly from 1:1, the few exceptions having slightly male-biased or female-biased sex ratios. Male-biased sex ratios are not predicted by our model, however, it should be noted that very large samples are required to detect weakly biased sex ratios, so more and larger studies are needed to get a reliable picture of housefly sex ratios in the wild.

Maternal-zygote conflict

Werren *et al.* (2002) presented a model that shows how sex ratio selection induces an evolutionary conflict between mothers and their offspring which in turn may lead to a shift in the SD system. In this model selection for male-biased sex ratios leads to the evolution of female heterogamety by means of a dominant female-determining factor that acts in the zygote, and vice versa that selection for female-biased sex ratios promotes the establishment of a male heterogametic SD system. To some extent this contradicts our results. Although in our model a fully male heterogametic or female heterogametic system never evolves, selection for female-biased sex ratios

leads to a system where a large majority of females are heterozygous FF^D, whereas males are all homozygous *FF*, which is close in some sense to a female heterogametic system. The main difference between the two models is that in our model all genes act in the zygote whereas the model of Werren *et al.* also allows for maternally acting genes to affect the sex of the mother's offspring. In the absence of zygotic SD genes, the maternal genes in Werren *et al.*'s model determine the sex ratio among the mother's offspring, and the result is that the sex ratio evolves towards a Fisherian equilibrium. Since the sex ratio from the offspring's point of view is "too biased" in this equilibrium (Trivers 1974), a rare dominant zygotic determiner of the minority sex can invade such a population and in effect establish a new heterogametic SD system. This result is of course limited to situations where the maternal ability to manipulate the sex ratio is sufficiently unconstrained. If genetic or physiological constraints limit this ability (our model; Pen & Weissing 2002), then selection may not be able in the long run to produce a sex ratio more biased than the Triversian optimum, in which case a rare dominant zygotic determiner of the rare sex no longer has a selective advantage. Of course one could also argue the other way around and interpret Werren *et al.*'s analysis as providing an evolutionary reason why genetic constraints (e.g. dominant zygotic sex determining factors) prevent full maternal control of the sex ratio. Interestingly, in the housefly there is clear evidence that maternal genes can affect or even completely determine the sex of the mother's offspring (Vanossi Este & Rovati 1982; Inoue & Hiroyoshi 1986; Hilfiker-Kleiner *et al.* 1994; Schmidt *et al.* 1997a; Dübendorfer & Hediger 1998), although the latter extreme has only been observed in a laboratory population (Vanossi Este & Rovati 1982). In flies with the standard *XY* system, input of maternally produced *F* factor is a necessary condition for female development. It is conceivable that variation in maternally produced *F* can have a quantitative effect on the offspring sex ratio. To determine how this interplay between maternally acting genes and zygotically acting genes affects the co-evolutionary dynamics of SD mechanisms in the housefly remains a theoretical and experimental challenge.

Explaining variability between natural housefly populations

Is sex ratio selection alone sufficient to explain the observed frequencies of *M* and F^D in natural housefly populations? In view of our results this seems unlikely. In most populations with non-standard SD systems *M* and F^D co-occur, both at high frequencies (Tomita & Wada 1989b). According to our model (Fig. 2.3 and 2.4) this should only occur if sons are more costly than daughters and if either *YY* genotypes are selected against or *M* has a high initial frequency. We already mentioned that there is some evidence that individuals homozygous for *Y* might have lower fitness (Franco *et al.* 1982). Occurrence of *M* at high initial frequencies requires, however, presence of additional mechanisms (see below). Most importantly, how likely is it that sons are more costly than daughters in houseflies? Unfortunately, this question is hard to answer at this point due to lack of data. However, since adult females are

larger than adult male houseflies (Goulson *et al.* 1999) and presumably need more food, it seems more likely that daughters, rather than sons, adversely affect family survival, which would make sons the "cheaper" sex. On the other hand, cost differences are not the only causes of selection for biased sex ratios. Female-biased sex ratios can also be selected for under conditions of inbreeding (Hamilton 1967) or when females have a greater dispersal tendency than males (Bulmer 1986; Frank 1986). We have studied stochastic individual-based simulations of subdivided populations where female-biased sex ratios are selectively favored (results not shown), and they yielded very similar results as the much simpler cost-based model above, in the sense that male-biased sex ratios never occur at equilibrium and female-biased sex ratios deviate at most only slightly from 50:50. There is some evidence that in houseflies local populations might sometimes be small enough to experience some inbreeding (Black & Krafsur 1986a), thus favoring female-biased sex ratios. Variation in local population structure might occur geographically for climatological reasons. Although all this suggests that in the wild the prerequisites might be met to let sex ratio evolution be responsible for the co-occurrence of *M* and F^D at high frequencies, our model cannot explain how initially rare autosomal *M* factors can reach high frequencies in the absence of F^D, as has been observed in several Japanese populations (Tomita & Wada 1989b), although it is of course possible that frequencies in natural populations are not at equilibrium.

A number of other hypotheses, not mutually exclusive, have been proposed to account for the observed variation in sex determining systems in field populations of the housefly. The earliest explanations for the emergence of autosomal *M* factors in housefly populations propose that *M* factors "hitchhike" with genes conferring a fitness benefit. Theoretical models (Bull & Charnov 1977; Jayakar 1987) have shown that such hitchhiking may cause transitions between SD mechanisms. Indeed, the first isolation of autosomal *M* factors coincided with the appearance of insecticide resistance in natural populations, as noted by Tomita and Wada (1989a). In some populations, DDT resistance has been shown to be linked with M^{II} or M^{III} (*M* located on the second and third chromosome, respectively; Kerr 1970; Franco *et al.* 1982). Geographical clines in *M* frequencies might then be attributed to regional variation in DDT application. However, recent findings shed doubt on the general validity of this hitchhiking hypothesis, since in North American populations no correlation was found between insecticide resistance and the distribution of autosomal *M* factors (Hamm *et al.* 2005). In addition, the spread of M^X in England (Denholm *et al.* 1985) is also unlikely to be accounted for by coupling to resistance genes.

Meiotic drive has also been invoked as an explanation for the spread of *M* and F^D. Jayakar (1987) showed with population genetic models that under certain conditions a standard *XY* system can be replaced by an *XX/M+* male-heterogametic system if a driving *M* factor is introduced into the ancestral *XY* population. The *XX/M+* populations would have male-biased sex ratios allowing the subsequent spread of an F^DF^D factor, ultimately leading to a system with female heterogamety. This explanation

cannot be ruled out entirely at the moment, since there is some weak evidence that autosomal *M* factors can sometimes show meiotic drive (Clark 1999; own observations). However, it is not clear how drive can explain the observed geographical clines.

In our model, we did not consider the interaction between sex ratio selection and another selective forces such as hitchhiking and meiotic drive. Where sex ratio selection alone fails to induce a full shift between different heterogametic SD systems, it seems likely that sex ratio selection in conjunction with other selective forces may easily cause such shifts. A full theoretical analysis of the interaction between sex ratio selection and all possible genotype-specific viability differences in the housefly system would be quite complex. Until more is known about genotype-specific viabilities in the housefly, such analysis is best left to the future. In the mean time, our results including lower fitness of *YY* genotypes suggest that, even thought detrimental genotypes are removed (as expected: see Bull & Charnov 1977), final genotype frequencies are affected by the strength of sex ratio selection (Fig. 2.4).

At the moment it is therefore hard to judge whether sex ratio selection has been an important cause of the remarkable variation in housefly SD mechanisms. However, the housefly can still serve as a useful model organism for experiments on the evolution of sex determination. Our model and future theoretical work will be important for designing and understanding the experiments.

Appendix

Here we show that without fitness differences ($w(\mathbf{ij},\mathbf{k})$ = constant), all equilibria of the system (3) produce an even sex ratio. The argument is quite general and holds for SD systems with any number of unlinked loci and any number of alleles per locus.

First we introduce some new notation. Let the sex-specific allele frequencies (of the focal allele) at locus n be denoted by $p_f(n)$ and $p_m(n)$. They are easily calculated from the sex-specific genotype frequencies. Genotype $\mathbf{i}$ has i_n copies of the focal allele at locus n, hence $i_n/2$ is the relative frequency of the focal allele at locus n for genotype $\mathbf{i}$. The frequency of the allele among all females is therefore simply given by

$$p_f(n) = \sum_{\mathbf{i}} p_f(\mathbf{i}) \frac{i_n}{2}. \qquad \text{(A1)}$$

Allele frequencies in males are calculated similarly.

Let p^*_f and p^*_m denote equilibrium frequencies in females and males. Adding the two equations in (3) yields the equilibrium condition

$$S^* p^*_m(\mathbf{k}) + (1-S^*) p^*_f(\mathbf{k}) = \sum_{\mathbf{ij}} p^*_f(\mathbf{i}) p^*_m(\mathbf{j})\, T(\mathbf{k}|\mathbf{ij}) , \qquad \text{(A2)}$$

where $S^* = S^*_1 = S^*_2$ is the equilibrium sex ratio. Now sum both sides of (A1) over all **k**, weighing each term by $k_n/2$, where k_n is the number of focal alleles at locus n. In view of (A1), this operation transforms the genotype frequencies on the left-hand side of (A2) into the frequencies of the focal allele at locus n:

$$S^* p^*_m(n) + (1-S^*) p^*_f(n) = \sum_{ij} p^*_f(\mathbf{i}) p^*_m(\mathbf{j}) \sum_k \frac{k_n}{2} T(\mathbf{k}|\mathbf{ij}) \; . \tag{A3}$$

Let us first give a heuristic argument why (A3) implies that the equilibrium sex ratio is ½. The right-hand side of (A3) is the frequency of the focal allele in the offspring produced by all parents. This ought to be the same as the arithmetic mean of the frequencies in males and females, if mating is at random and segregation is unbiased. In other words: we expect the right-hand side of (A3) to equal $\frac{1}{2} p^*_m(n) + \frac{1}{2} p^*_f(n)$. If this is true, it follows that in equilibrium either $p^*_m(n) = p^*_f(n)$ or $S^* = \frac{1}{2}$. For a genetic system of sex determination, it is not plausible (although theoretically possible, see Karlin & Lessard 1986) that the frequency of sex determining factors is, at all loci, the same in both sexes. In fact, we are not aware of any genetic SD system where $p^*_m(n) = p^*_f(n)$ can hold for all alleles at all loci. Accordingly, the sex ratio at equilibrium must always be even in such systems. For example, in the housefly, the frequency of the epistatic F^D allele cannot be the same for females and males, unless the frequency is zero. But if the F^D frequency is zero, then the frequency of *M* factors cannot be identical in males and females.

Now we shall prove that these heuristic arguments are correct. The rules of Mendelian segregation, as embodied in (1) and (2), imply that

$$\begin{aligned}
\sum_k \frac{k_n}{2} T(\mathbf{k}|\mathbf{ij}) &= \sum_k \frac{k_n}{2} \prod_l P(k_l|i_l j_l) \\
&= \sum_k \frac{k_n}{2} P(k_n|i_n j_n) \prod_{l\neq n} P(k_l|i_l j_l) \\
&= \sum_{k_n} \frac{k_n}{2} P(k_n|i_n j_n) \prod_{l\neq n} \sum_{k_l} P(k_l|i_l j_l) \\
&= \sum_{k_n} \frac{k_n}{2} P(k_n|i_n j_n) \\
&= \tfrac{1}{2} P(k_n=1|i_n j_n) + P(k_n=2|i_n j_n) \\
&= \tfrac{1}{4} i_n + \tfrac{1}{4} j_n \; .
\end{aligned} \tag{A4}$$

The last step follows directly from (2). As a result, the right-hand side of (A3) reduces to

$$\begin{aligned}
\sum_{ij} p^*_f(\mathbf{i}) p^*_m(\mathbf{j}) \sum_k \frac{k_n}{2} T(\mathbf{k}|\mathbf{ij}) &= \tfrac{1}{2} \sum_i p^*_f(\mathbf{i}) \frac{i_n}{2} + \tfrac{1}{2} \sum_j p^*_m(\mathbf{i}) \frac{j_n}{2} \\
&= \tfrac{1}{2} p^*_f(n) + \tfrac{1}{2} p^*_m(n) \; ,
\end{aligned} \tag{A5}$$

as expected.

CHAPTER 3

Manuscript in preparation

Towards a mechanistic evolutionary theory of sex determination: the importance of selection against intersexes

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Abstract

We present a mechanistic model for the evolution of sex determining systems based on recent insights from molecular studies. Specifically, we use individual-based simulations to model the gradual evolution of regulatory genes with a quantitative effect on the amount of a feminizing product. The amount of product must surpass a noisy threshold level to trigger female development, otherwise males are produced, or sterile intersexes if the amount of product is too close to the threshold. We impose sex ratio selection by assuming cost differences in the production of sons and daughters. By letting both maternal genes and offspring genes affect the level of feminizing product in the developing offspring, maternal-offspring sex ratio conflict drives the evolution of the regulatory genes. Selection against intersexes leads to dimorphism of either offspring genes or maternal genes, but not both. When a dimorphism evolves in offspring genes, either a female-heterogametic or a male-heterogametic sex determining system is the outcome, and the sex ratio stabilizes at equality. By contrast, when maternal genes evolve to a dimorphic state, monogeny evolves; that is, all females produce single-sex families, and the population sex ratio evolves to the maternal optimum. Which system evolves is to some extent random but can be partially predicted by initial conditions and the direction and strength of sex ratio selection. To simulate the growth of sex determining pathways, we perturbed the evolved equilibrium by introducing a new masculinizing gene in the population. The result is a series of rapid switches between sex determining systems, interspersed by long periods of apparent stability. We conclude that our simple mechanistic model is able to capture much of the observed dynamics and variability of extant sex determining mechanisms.

Introduction

While steady empirical progress is being made in unravelling the genetics of sex determining mechanisms and their evolutionary history, theoretical models for the evolution of sex determination (SD) have been lagging behind. The aim of this paper is to develop a general mechanistic model for the evolution of genetic SD systems, explicitly incorporating key insights emerging from recent empirical work, which we will now briefly summarize.

It has become evident that such phylogenetically diverse groups as flies, nematodes, mammals, and even cnidarians share some molecular mechanisms of sex determination (Marin & Baker 1998; Raymond *et al.* 1998; Yi & Zarkower 1999; Zarkower 2001; Miller *et al.* 2003). Typically, SD pathways consist of multiple regulatory genes arranged in a linear cascade, where the expression of genes on one level of the cascade regulates genes from the level below, all the way down to a bi-functional switch gene at the bottom, whose products are differentially spliced in the two sexes and trigger either female or male development. Comparative studies show that the genes at the bottom of the cascade tend to be conserved, whereas genes higher up tend to diverge (Zarkower 2001; Saccone *et al.* 2002; Shearman 2002), lending support to the hypothesis that SD regulatory pathways have evolved from the bottom up (Wilkins 1995). Thus, recurrent recruitment of new elements at the top of the cascade has led to the large variety of SD systems in extant organisms, such as male heterogamety, female heterogamety and monogeny (Nöthinger & Steinmann-Zwicky 1985; Kraak & Pen 2002; Shearman 2002; Mank *et al.* 2006).

Usually SD pathways are considered as cascades of ON and OFF genes, whose products are present in one sex and absent in the other (Nöthinger & Steinmann-Zwicky 1985; Schütt & Nöthiger 2000; Saccone *et al.* 2002). Recent findings indicate that the truth may not be so extreme. Tarone *et al.* (2005) showed that sex-specific products of most genes from the *Drosophila melanogaster* SD cascade are present in both sexes and that there is systematic quantitative variation in the level of gene expression between different strains. Similarly, in the housefly *Musca domestica* the "strength" of feminizing and masculinizing factors can differ between strains, leading to different SD systems but based on the same basic mechanism (Dübendorfer *et al.* 2002). This suggests that SD genes act to a large extend quantitatively and a certain threshold of feminizing (or masculinizing) factors needs to be reached to assure proper development of a female (or a male). Further support for a quantitative basis of sex determination comes from developmental disorders resulting from an ambiguous SD signal when the quantity of SD factors is too close to a threshold separating the two sexual pathways. In *M. domestica*, an insufficient amount of the feminizing factor F may lead to the development of infertile intersexes (Schmidt *et al.* 1997a; Schmidt *et al.* 1997b). In *D. melanogaster* too high levels of females-specific SD genes in males (or male-specific genes in females) can lead to sex-specific mortality through inaccurate dosage compensation (Schütt & Nöthiger 2000). Sex

reversal or intersexuality has also been attributed to different levels of expression of SD genes in the house mouse, *Mus musculus* (Nagamine *et al.* 1999) and in the medaka fish, *Oryzias latipes* (Otake *et al.* 2006).

Not only genes present in the offspring itself determine its sex, maternally expressed genes are also known to affect sexual development. For example in *Caenorhabditis elegans* (Ahringer *et al.* 1992), *D. melanogaster* (Schütt & Nöthiger 2000) and *M. domestica* (Schmidt *et al.* 1997a; Dübendorfer & Hediger 1998) maternal products placed in eggs interact with SD genes of developing individual and are necessary for its proper sexual development. In the blowfly, *Chrysomya rufifacies*, SD is completely under the control of maternal genotype resulting in monogeny (Ullerich 1984): all females produce progeny of one sex, some produce only daughters and others only sons. Maternal effects factors include mainly proteins and RNA placed by a mother in the eggs. The involvement of genes expressed both by mother and the offspring itself may under some circumstances lead to a conflict over sex determination and it is thought to be an important force shaping the SD system (Werren & Beukeboom 1998; Werren *et al.* 2002).

As mentioned above we took into account all these properties of SD in our model. Firstly, we assume that genes involved in SD have quantitative feminizing effects and that a certain threshold has to be reached to assure female development, otherwise males are produced. Secondly, we implemented that ambiguous SD signals (amount of feminizing product to close to the threshold) leads to the development of intersexes. Thirdly, both maternal and offspring genes are involved in SD and can produce feminizing products. Lastly, we allowed for a new masculinizing gene to be acquired into the SD pathway leading to the evolution of genetic cascades.

The last two properties have already been recognized before and incorporated in SD evolution models (e.g. Werren & Hatcher 2000; Werren *et al.* 2002; Pen 2006), but the first two have gained little attention so far. A threshold for SD signals has mainly been used in the models of environmental sex determination (ESD), but not in genetic sex determination models (GSD) (except some polygenetic SD systems; Bulmer & Bull 1982). Selection against ambiguous SD signals resulting in production of less fit phenotypes, e.g. intersexes is largely unexplored (but see Pomiankowski *et al.* 2004). This is remarkable because the costs of improper sex determination may be inherent to systems working on the basis of a genetic switch, e.g. the SD mechanism where the presence or absence of a given signal switches between male and female development.

We used sex ratio selection as a driving force for the evolution of sex determination in our model, since selection for or against biased sex ratios along with maternal-offspring conflict is thought to be an important factor leading to changes in SD systems (Wilkins 1995; Werren & Beukeboom 1998; Kraak & Pen 2002).

We show that selection against intersexes has profound effects on the SD mechanism. Together with sex ratio selection it leads to the evolution of male or female

heterogamety or monogeny. Therefore, our simple mechanistic model is able to capture much of the observed dynamics and variability of extant sex determining mechanisms.

The model

In our model sex is determined by a simple sex determining cascade with a switching device at the bottom. We model the evolution of regulatory genes that determine the strength of the signal going into the device. The switching device responds to the total amount of a feminizing product F, relative to a threshold value T. If the amount of feminizing product is higher than the threshold, the individual develops as a female, otherwise it becomes a male. However, if the amount F is too close to T, the signal is "ambiguous" and an individual becomes a sterile intersex. Thus, the sex of an individual is determined according to the following rules:

- if $F > T + \delta$, an individual becomes a female; (1a)
- if $F < T - \delta$, an individual becomes a male; (1b)
- if $T - \delta \leq F \leq T + \delta$, an individual becomes an intersex. (1c)

In other words, there is an "intersex range" of magnitude 2δ centered at T. To investigate the importance of selection against intersexes for the evolution of sex determination, we will study two versions of our models: without intersexes ($\delta = 0$) and with intersexes ($\delta > 0$).

We consider three scenarios for genetic regulation of F:
- Maternal control – F regulated by a maternal locus F_m;
- Offspring control – F regulated by an offspring locus F_o;
- Joint control – F regulated by additive interaction of F_m and F_o.

Both independently segregating loci are diploid and carry alleles from an infinite set of potential alleles coding for any nonnegative number representing an amount of feminizing product. In case of maternal or offspring control, F is the sum of the two allelic values on F_m or F_o. In case of joint control, F is the sum of the four allelic values on both loci.

We allow for some developmental noise by adding a small, normally distributed quantity with mean zero and standard deviation of 0.025 to T (see Fig 3.1). We checked different values for the standard deviation of the distribution and the results are the same as long as it is considerably lower than T and higher than δ.

We imposed selection for biased sex ratios in our model as the basic driving force for the evolution of sex determination. This will cause genetic conflict between maternal genes and offspring genes over sex determination (Trivers 1974; Bull 1983; Wilkins 1995; Werren and Beukeboom 1998; Werren and Hatcher 2000; Werren *et*

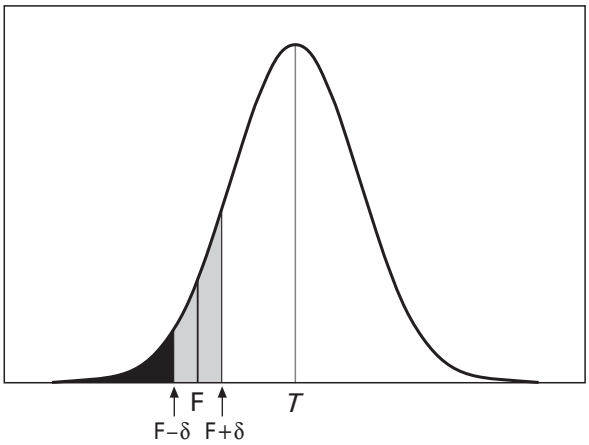

Figure 3.1. Distribution of the variation in the value of threshold T and its effect on sexual development. This variation reflects random effects on developmental sensitivity to clues. It may cause an individual to become a female even if the amount of feminizing product F is below the threshold necessary for female development. If the amount of product F is too close to T infertile intersexes are produced. The black area represents the probability that the individual will develop as a female, grey as an intersex and white as a male. This distribution also reflects the expected frequencies of females, intersexes and males among the progeny of a female with alleles of F_m gene summing up to F (under maternal control over sex determination).

al. 2002; Pen 2006; Uller *et al.* 2007). We assume that mothers have a fixed amount of resources available for reproduction and that a son costs $c > 0$ times as much as a daughter. It is well known (Fisher 1930) that under perfect maternal control over the family sex ratio it will evolve until an equal allocation of resources to sons and daughters is reached. The "Fisherian sex ratio" (proportion sons) or "maternal optimum" s^*_m is biased towards the "cheaper" sex and given by

$$s^*_m = \frac{1}{1+c} \quad . \tag{2}$$

If, on the other hand the offspring have perfect control over their own sex, Trivers (1974) showed by means of an inclusive fitness argument that the equilibrium sex ratio is less biased than the Fisherian sex ratio, and given by

$$s^*_o = \frac{1}{1+\sqrt{c}} \quad . \tag{3}$$

This result holds if offspring from the same family are related by 1/2, in other words if they are diploid full sibs with unrelated diploid parents. We will refer to (3) as the "Triversian sex ratio" or "offspring optimum".

In our model we have intersexes in addition to males and females, and we assume that the cost of an intersex is the mean of a son's cost (c) and a daughter's

cost (1): $(1+c)/2$. If sons, daughters and intersexes occur with frequencies p_s, p_d and p_i (such that $p_s + p_d + p_i = 1$) in a brood, then for a fixed amount of resources the number of offspring n is inversely proportional to the mean offspring cost:

$$n \propto \frac{1}{p_d + p_s c + p_i \frac{1+c}{2}}. \quad (4)$$

We used individual-based simulations to model the evolution of sex determination caused by gradual (co)evolution of the maternal and offspring regulatory genes. We assumed discrete non-overlapping generations and a fixed population size of N = 10,000 individuals. N new individuals were generated each generation using the following algorithm: first we assign one random male to each female in the population and then draw with replacement a female; given her genotype and a genotype of her pre-assigned partner and the level of developmental noise calculate their expected proportions of sons (p_s), daughters (p_d) and intersexes (p_i) (see Fig 3.1); then use equation (4) to scale the relative survival probability of the pair's offspring; draw a random number to decide whether an offspring actually survives; if it does not draw a new parental pair and start again, otherwise continue: create a new genotype by drawing random alleles from both parents; determine the offspring's sex based on its own and/or maternal genotype and random developmental noise; for each allele, decide whether it will mutate (with probability 0.05); if it mutates, add a normal deviate (mean zero, standard deviation 0.001) to the allelic value; add the offspring to the next generation; repeat until N new individuals have been created. We set T at a fixed arbitrary value of 1 and the first generation was genetically monomorphic such that the mean value of F was equal to T, ensuring an even sex ratio in the first generation. Simulations were run for sufficiently many generations until equilibrium appeared to have been reached.

Results

No intersexes

First we consider scenarios without intersexes ($\delta = 0$). Under this assumption our model is equivalent with standard sex ratio models (at least for the cases in which only one SD gene is present) and we will discuss it only shortly as a reference to the results of the model with intersexes.

Maternal control: Only F_m can evolve in this scenario, and its value either increases (when $c>1$) or decreases ($c<1$), as expected. The maternal sex ratio optimum (Equation 2) is reached (Fig 3.2A).

Offspring control: Only F_o can evolve in this scenario, and it either increases (when $c>1$) or decreases ($c<1$), as expected. The offspring sex ratio optimum (Equation 3) is reached (Fig 3.2B).

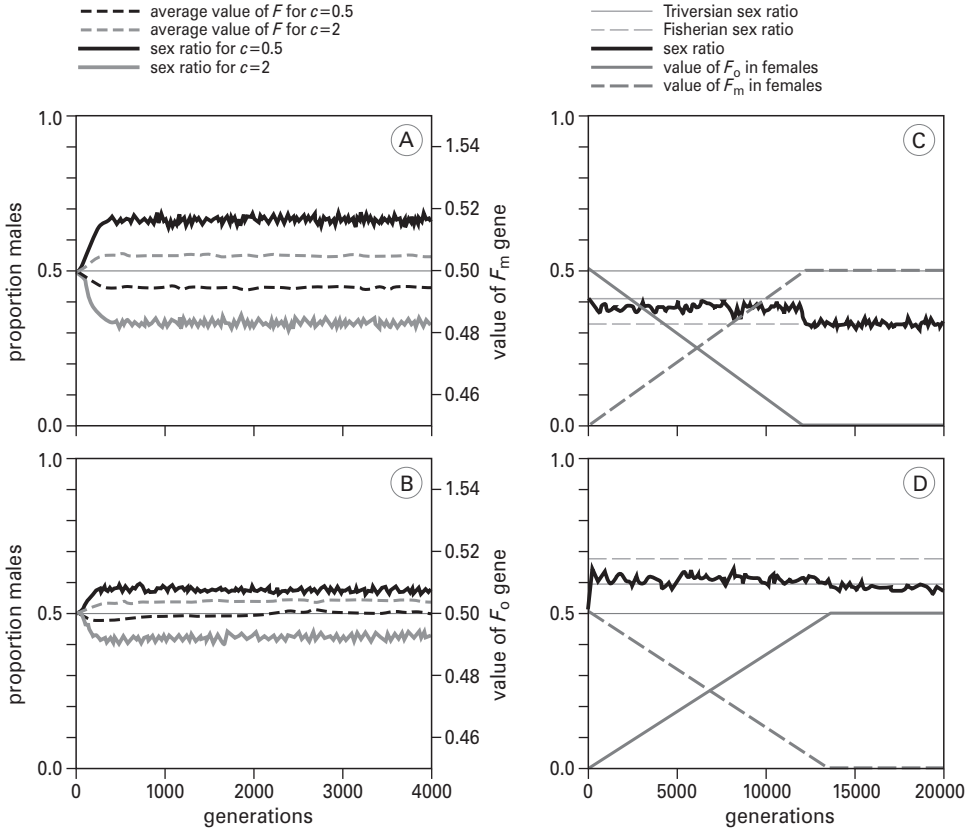

Figure 3.2. Change in sex ratio (proportion males) and average value of SD genes (F_m and/or F_o) over time for different scenarios of the model without intersexes. Only the values of the SD gene(s) in females are given since in males they are only slightly lower. (A) Maternal control over sex determination. Results for $c=0.5$ and $c=2$ are presented. Note the different scale for sex ratio and value of F_m and that the Fisherian sex ratio evolves. (B) Offspring control over sex determination. As for A. but values of F_o are presented and Triversian sex ratio evolves. (C) Joint control over sex determination for $c=2$. Qualitatively the same result (removal of F_o and Fisherian sex ratio) holds for any $c>1$. (D) Joint control over sex determination for $c=0.5$. Again qualitatively the same result (removal of F_m and Triversian sex ratio) holds for any $c<1$. For $c=0.5$ Fisherian sex ratio $s^*_m = 0.67$ and Triversian sex ratio $s^*_o = 0.59$, for $c=2$: $s^*_m = 0.33$ and $s^*_o = 0.41$.

Joint control: For $c<1$ (sons cheaper than daughters), F_m always goes to zero and F_o converges to a value such that the sex ratio approaches the offspring optimum, regardless of the initial values of F_m and F_o (Fig 3.2D). For $c>1$, the opposite happens: now F_o goes to zero and F_m stabilizes at a value such that the sex ratio is near the maternal optimum (Fig 2C). These results are easy to understand: in the first case ($c<1$) mothers "prefer" fewer daughters than the offspring prefer, and in the second scenario ($c>1$), it is the offspring who prefer a less female-biased sex ratio. The party in favour of fewer daughters always "loses" because there is a lower

limit (zero) to the amount of feminizing product that can be produced, but no upper limit. Whenever the sex ratio is between the maternal optimum and the offspring optimum, the party in favour of (relatively) fewer daughters will evolve lower *F*-values, while the other party will increase its production of feminizing product. The ensuing "arms race" will continue until the party in favour of fewer daughters hits rock-bottom and ceases production of feminizing product. At this point, the party in favour of more daughters is "free" to evolve *F* upwards until its optimal sex ratio is reached.

Note that for all three control scenarios without intersexes, the end result is in effect a genetically monomorphic population, with sex being determined solely by chance fluctuations in the level of the threshold *T* (Table 3.1).

Table 3.1. Summary of the results for the different SD scenarios based only on the level of the feminizing product F. F is produced by genes active in the mother (F_m; maternal control) or genes active in the offspring (F_o; offspring control) or both (joint control). *c* is the relative cost of producing a son vs. a daughter. The third and fourth column show the pattern of the evolution of maternal and offspring genes, respectively. The last column shows which SD system evolved under the given condition. The upper part of the table shows results for the model without intersexes and the bottom part with intersexes.

Control over F production	*c*	F_m evolution	F_o evolution	Equilibrium sex ratio	Equilibrium SD system
Without intersexes					
Maternal control	$c<1$	No branching	-	Maternal optimum	Monomorphic
	$c>1$	No branching	-	Maternal optimum	Monomorphic
Offspring control	$c<1$	-	No branching	Offspring optimum	Monomorphic
	$c>1$	-	No branching	Offspring optimum	Monomorphic
Joint control	$c<1$	Decreases to 0	No branching	Offspring optimum	Monomorphic
	$c>1$	No branching	Decreases to 0	Maternal optimum	Monomorphic
With intersexes					
Maternal control	$c<1$	Branching	-	Maternal optimum	Monogeny
	$c>1$	Branching	-	Maternal optimum	Monogeny
Offspring control	$c<1$	-	Branching	0.5	XY or ZW
	$c>1$	-	Branching	0.5	XY or ZW
Joint control*	$c<1$	Branching	No branching	Maternal optimum	Monogeny
		No branching	Branching	0.5	XY (or ZW)
	$c>1$	No branching	Branching	0.5	ZW
		Branching	No branching	Maternal optimum	Monogeny

* – Which SD system evolves depends also on the initial value of F_o and F_m and on the value of *c*. See Fig 3.5 for details.

Model with intersexes

Now we allow for infertile intersexes to be produced when the amount F of feminizing product is too close to the threshold value *T*. We show results for a δ-value of 0.01, but the results are insensitive to variations in δ, as long as it is not too close to zero and sufficiently lower than the variation in threshold *T*.

Maternal control: Due to selection against the production of intersexes, the F_m locus "branches" into two "alleles" – one "high *F*" allele and one "low *F*" allele (Fig 3.3). The resulting SD system is always monogeny, that is, half the females produce only sons and the other half produces only daughters, and no intersexes are produced. Interestingly, even though clearly no individual female produces the Fisherian sex ratio at equilibrium, the population sex ratio does evolve towards the

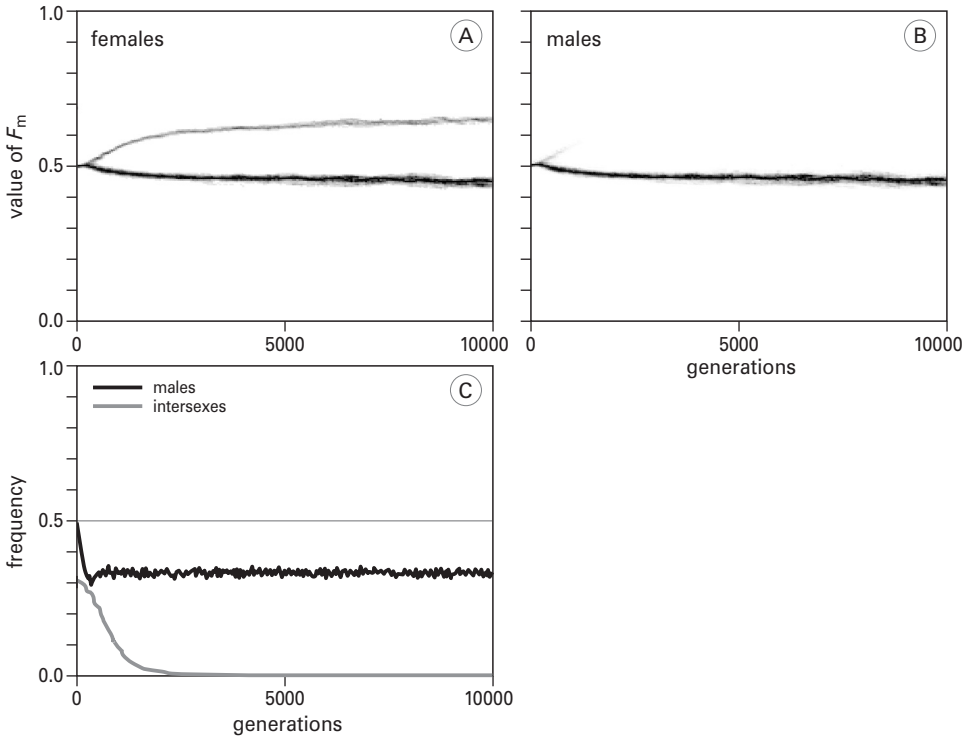

Figure 3.3. Evolution of monogeny under maternal control over SD in the model with intersexes. Distribution of the values of F_m in females (A) and males (B) and changes in the sex ratio (proportion of males among fertile offspring) and the proportion of intersexes over time (C) for $c=2$ is shown. Darker color indicates higher frequencies of a gene with a given value. Branching occurs on the F_m locus with the presence of "high" and "low" alleles of F_m in females (in proportion 1:3) and only "low" allele in males. Two types of female exist in the population, heterozygous females producing only daughters and females homozygous for the lower allele producing only sons. After monogeny is established the sex ratio reaches the maternal optimum ($s^*_m = 0.33$). This is the most common outcome of the evolution of SD under maternal control with intersexes independently of the value of *c* (for exceptions see Results).

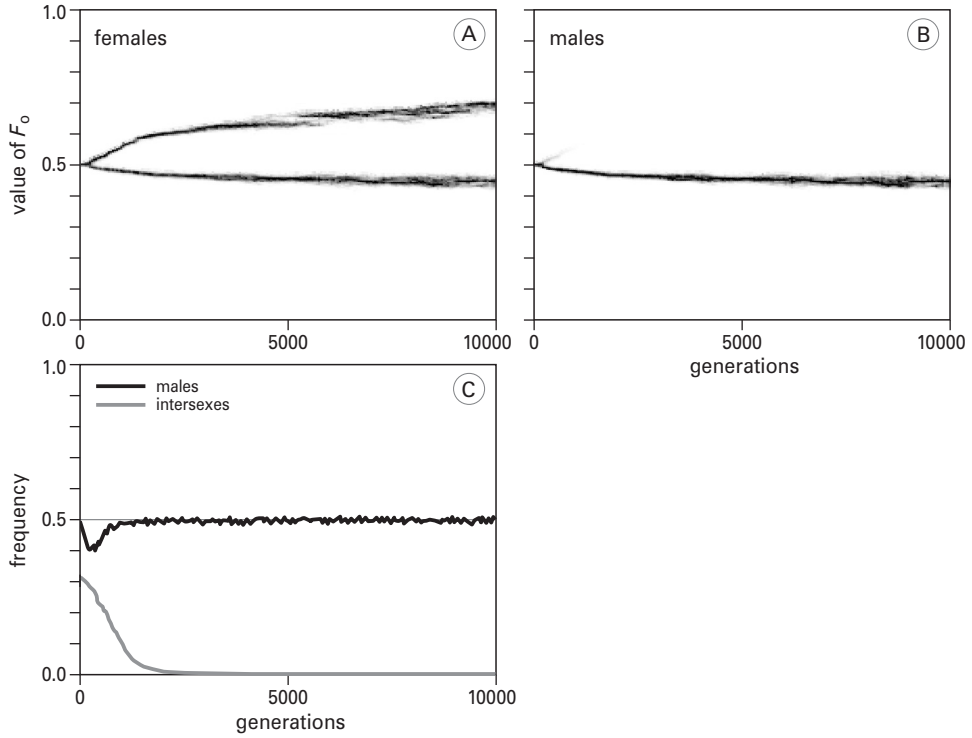

Figure 3.4. Evolution of female heterozygosity under offspring control over SD in the model with intersexes. Distribution of the values of F_o in females (A) and males (B) and changes in the sex ratio (proportion of males among fertile offspring) and the proportion of intersexes over time (C) for $c=2$ are shown. Darker color indicates higher frequencies of a gene with a given value. Branching occurs on the F_o locus with the presence of "high" and "low" allele of F_o in females (in proportion 1:1) and only "low" allele in males, being equivalent with female heterogamety (or a ZW system). Note that this results in 50:50 sex ratios. This is the most common outcome of the evolution of SD under offspring control with intersexes, but branching in males can also occur leading to male heterogamety (XY system; see Results).

maternal optimum. This is necessarily so, as the following argument demonstrates. Females that produce only daughters have a relative family size of 1, compared to a family size of $1/c$ for son-producing females. Since the two types of female are equally frequent, the ratio of daughters to sons at the population level equals c.

At the genetic level there are two possible outcomes. The most common outcome is that the daughter-producing females are heterozygous for a high allele and a low allele, while son-producing females and males are homozygous for a low allele (Fig 3.3). Alternatively and less frequently, the daughter-producing females are homozygous for a high allele and son-producing females heterozygous for a high and a low allele, while males can have any of the three possible genotypes. In the latter outcome high alleles are relatively more frequent, and this outcome was observed more often in our simulations (results not shown) for relatively high values of c.

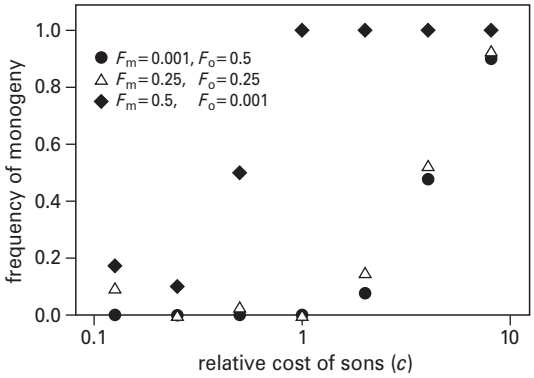

Figure 3.5. Evolution of monogeny under joint control over SD and with intersexes as a function of the relative cost of sons (c). Three cases with different initial values of F_m and F_o genes are presented. Frequencies are based on 40 runs and always either monogeny evolves or zygotic control over SD (with male or female heterozygosity, for $c<1$ and $c>1$, respectively).

This makes sense, since high c-values select for more female-biased sex ratios, which in turn favours high F-values.

Offspring control: Now the F_o locus branches, again leading to a population dimorphic for a low and a high allele. One of the sexes is always homozygous and the opposite sex heterozygous, and in equilibrium every female produces on average an even sex ratio. Again there are two possible outcomes at the genetic level. The first outcome is that females are heterozygous for a low and a high allele, while males are homozygous for a low allele. Therefore, female heterogamety evolves and we call this outcome a ZW system (Fig 3.4). The other outcome – male heterogamety, which we call a XY system – has heterozygous males and females homozygous for a high allele. A ZW system evolves more often than a XY system, especially for high values of c (results not shown), but it is not clear to us why.

Joint control: In contrast to the case without intersexes, the outcome of evolution now depends on the initial values of F_m and F_o, but always one of them branches because of the strong selection against intersexes. Similarly to the case without intersexes, genes that favour a lower sex ratio (higher production of F; F_o for $c<1$ and F_m for $c>1$) are more often in control of sex determination, especially under more extreme c values. Here, sex ratio selection is stronger compared to selection against intersexes, which is largely independent of c (Fig 3.5).

For $c<1$ branching of F_o is the most common outcome leading to the evolution of an XY system. This is because, similarly to the case without intersexes, a difference between maternal and offspring optimal sex ratios will lead to an increase in the value of F_o and a decrease in F_m. Low alleles of F_o can spread only because they decrease the number of intersexes. As a result, the frequency of the lower allele will be smaller and it will be present only in males, leading to the XY system (Fig 3.6). Only if the initial value of F_m is low can a ZW system evolve. Alternatively a high

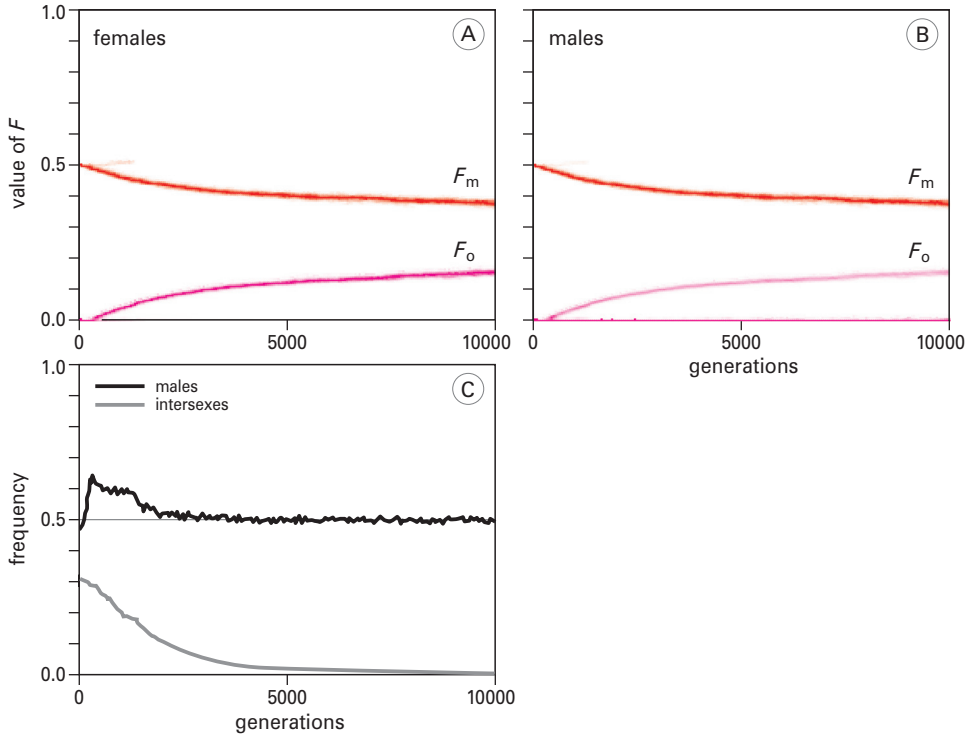

Figure 3.6. Evolution of male heterozygosity for F_o (XY system) under selection for a male biased sex ratio ($c=0.5$) and maternal-offspring conflict between F_m and F_o genes (joint control over SD in the model with intersexes). (A) Distribution of the values of F_m and F_o in females. (B) Distribution of the values of F_m and F_o in males. (C) Sex ratio (proportion of males among fertile offspring) and the proportion of intersexes in the population.

allele of F_m can spread leading to maternal control over SD. This happens more often when the initial value of F_m is much higher than the value of F_o (Fig 3.5).

For $c>1$ the opposite pattern is true: the maternal-offspring conflict over sex ratio leads to an increase in the value of F_m and a decrease in F_o. Now a branching of F_m occurs even if its starts with the value of virtually zero. Such cases are rare for low c, but increase with higher c and with the initial value of F_m (and lower value of F_o). Since higher alleles of F_m are favoured, the low F_m allele is less frequent. Monogeny evolves and females homozygous for the higher allele of F_m produce only daughters and heterozygous females produce only sons. Only if the initial values of F_o are virtually zero can both types of monogeny evolve (see maternal control above). Alternatively branching can occur also on F_o, but since the lower allele of F_o is more favourable in maternal-offspring conflict over the sex ratio, a higher allele can spread only in female leading to ZW system.

In cases where the maternal genes' control over sex determination evolves (branching of F_m) the maternal sex ratio optimum is achieved. When zygotic genes

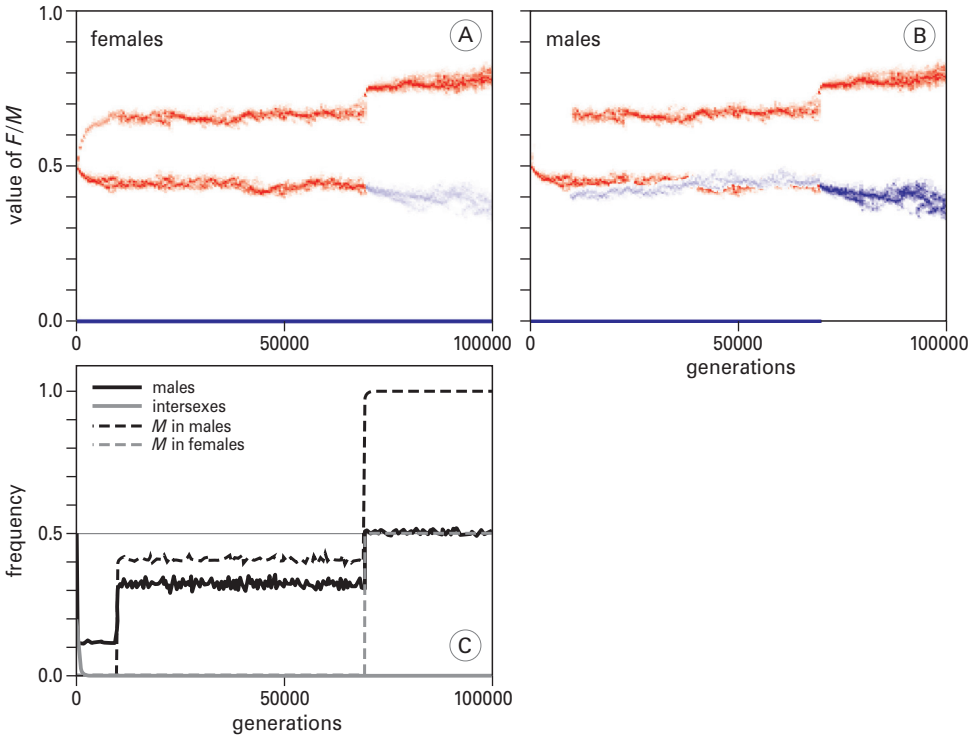

Figure 3.7. Changes in the SD system after the introduction of a strong masculinizing factor *M* in the population with monogeny and selection towards female biased sex ratios ($c=8$). (A) Distribution of the values of F_m (red) and *M* (blue) in females. (B) Distribution of F_m and *M* in males. (C) Changes in sex ratio (proportion of males among fertile offspring), the proportion of intersexes and frequencies of *M* factor in male and females in time. *M* was introduced in generation 10000 with an initial value of 0.4. A two-locus polymorphic SD system is established, sex ratio is above both the maternal ($s^*_m = 0.11$) and the offspring ($s^*_o = 0.26$) optimum. Around generation 70000 F_m reaches high values which make it possible for *M* to appear also in females and the system changes to female heterogamety for *M* with equal sex ratios. Note long periods of apparent stability of the system when sex ratio and frequency of *M* does not change and very sudden switches between SD systems.

control SD (branching of F_o) equal sex ratios are obtained. In some cases with strong sex ratio selection after initial branching on F_o, the value of the lower allele decreases to zero and subsequently branching on F_m can occur. Then, the system changes to monogeny and biased sex ratios.

Table 3.1 summarizes all the results for the evolution of *F* alleles under different scenarios of control over sex determination.

Invasion of masculinizing factor *M*

So far we have only considered the evolution of feminizing factors, but often SD systems consist of cascades of multiple genes with opposite effects. Therefore, we

decided to introduce (at a frequency of 0.025) a new zygotic masculinizing gene *M*, after the system with only feminizing factor(s) reached equilibrium. *M* is expressed in the zygote and its product, M, decreases the amount of F product in an additive manner. Therefore, the functional amount of the F product used in equations (1) is now given by F-M.

M invades only if the population sex ratio is below the Triversian optimum, which can be shown analytically (Appendix). Invasion of *M* when the sex ratio is below the offspring optimum is intuitive since *M* is a factor expressed in the offspring and it increases the family sex ratio, which is favoured when the sex ratio is below the Triversian optimum. A few trials indicate that the frequency of *M* after invasion depends on the previously existing system and the strength of *M* itself. However, a full analysis of the invasion dynamics of *M* would be too complicated and beyond the scope of this article, therefore we only checked a few scenarios of which we will present only one in detail to point out some interesting appearing properties as a starting point for future work.

Figure 3.7 shows what happens when a strong *M* (its presence in offspring always assures that it will become male independent of maternal genotype) is introduced in the system with maternal control and strong sex ratio selection for female biased sex ratio ($c=8$). *M* invades but it does not reach a frequency of 0.5. Both alleles of F_m stay in the population and the population sex ratio remains below 0.5, but is less biased than Triversian sex ratio. If F_m and *M* could not evolve this system would be stable, as can be proved with a population genetic model similar to the one used by Kozielska *et al.* (2006; not shown). In general, the frequency of the *M* factor and the sex ratio stay constant for many generations, but there are cases in which F_m increases to the point where females with two higher alleles can produce females even if the offspring possesses *M*. At this point sudden changes in the system occur. *M* fixates in males and reaches a frequency of 0.5 in females – resulting in a ZW system for M as a recessive male determinant (Figure 3.7). At the same time the lower allele of F_m is lost and the higher increases in value. With further increase of the value of F_m, M can eventually fixate in both males and females. Because of the increase of the intersexes on this transition point branching is favoured, which occurs on either the F_m or the *M* locus.

Discussion

We showed that a relatively simple SD mechanism based on quantitative effects of feminizing and masculinizing factors can lead to the evolution of many SD systems resembling the ones seen in nature. Starting with a homogenous population we could obtain male heterogamety, female heterogamety or monogeny, depending on the selective forces applied.

Quantitative effects in sex determining mechanism and intersexes

Although genes in SD cascades have been usually considered to have only two possible states: ON and OFF, recent findings indicate that this may not always be the case and many SD genes seem to have quantitative effects (Dübendorfer *et al.* 2002; Tarone *et al.* 2005; see Introduction). Quantitative effects underlying discrete morphs traditionally imply the use of threshold models (Roff 1996). Bull (1983) already proposed that a threshold for the male or female developmental pathway may exist in the developing embryo and that the amount of masculinizing or feminizing factors above or below this threshold may determine the sex of the developing organism. This idea has been used in models for ESD, but only to a very limited degree for GSD (e.g. the polygenetic model of Bulmer & Bull 1982). The primary signal in the SD cascade of *D. melanogaster* and *C. elegans* (i.e. the X:A ratio) is a well known example of a dosage dependent effect of multiple genes on SD. However, a quantitative approach seems also appropriate for the mode of action of a single gene, as discussed above. Furthermore, the mode of regulation and action of SD genes allow them to exert quantitative effects. SD genes are often (at least in insects) splicing factors and their amount will influence their effectiveness in assuring male- of female-specific splicing of other target genes and hence the proper sexual development of the individual. Genes in SD cascades with only one level of expression (i. e. 0 or 100%) may arise after the sex determining function was taken over by a factor higher in the hierarchy (see below). The level of expression can be an intrinsic property of the gene itself, but frequently appears to be controlled by other regulatory genes or by the amount of chromatisation of the surrounding chromosomal region (Hediger *et al.* 1998).

In our model we assumed that there is a potential for genetic variation in the amount of F produced by the SD genes. Alternatively we could also allow the threshold, *T*, to evolve, since it is known that sensitivity to clues can also show genetic variation (Roff 1996). However, letting *T* evolve will in principle be equivalent with introducing a dominant masculinizing factor, since an increase of *T* would increase the frequency of males and a decrease to zero would mean that only females are produced. We did a few simulations which confirm this notion and we decided to start with a simpler model with only feminizing factors, but future analysis of the model with an evolving threshold may bring new insights into the evolution of sex determination.

Probably the most important outcome of our study is the profound effect of the cost of an ambiguous signal near the switch point (production of intersexes) for the evolution of SD mechanisms. If the switch between one or the other sex is very sharp (a pure male or female always develops) a homogenous population with an optimal sex ratio evolves. However, if there is a "grey zone" (meaning the presence of intersexes) around the switching point, the outcome of evolution is very different and two different alleles always evolve on the SD locus. Without implementing any dominance relationship between alleles, dominant and recessive SD genes emerge in

the model. Insufficient discrimination between two different pathways if the signal is close to the switching point seems a reasonable assumption since a growing body of evidence shows that in animals inappropriate levels of expression of SD genes leads to production of intersexes (Vanossi Este & Rovati 1982; Schmidt *et al.* 1997a; Nagamine *et al.* 1999; Hodgkin 2002; Otake *et al.* 2006).

To our knowledge this is the first model which explicitly shows the general importance of cost of imperfect SD signals in the evolution of SD mechanism. Pomiankowski *et al.* (2004) recognized that an ambiguous sexual signal during development may be a powerful force in SD evolution. They proposed a chain of events leading to the evolution of the Drosophila SD cascade with "sexual selection as a principle motor for evolutionary change". What they actually meant is that presence of the *doublesex* (the master switch gene) protein which is unspecific for a given sex will decrease its fitness, leading to the evolution of ways to increase the reliability of the SD signal. A drawback of their model is that it is very specific for *D. melanogaster* raising the question whether it has any general importance. Moreover, they required a lot of assumptions about the acquisition of new genes in the SD pathway.

Our model is more general and shows that if the sexual signal cannot be clearly interpreted close to the switching point this has profound effects on the evolution of SD and is responsible for the evolution of distinct male and female alleles of SD genes. This leads to the evolution of male heterozygosity, female heterozygosity and monogeny which are the main SD systems seen in nature, hence, our model reveals that selection against intersexes might have been an important factor in the evolution of sex determination.

Maternal-offspring conflict over sex ratio

Another factor strongly influencing the outcome of evolution is the direction (whether male- or female-bias is favoured) and strength of sex ratio selection, which also determines the strength of maternal-offspring conflict. We observe the general pattern that if SD is under the control of feminizing genes, maternal control over SD is more prone to evolve under the selection for male biased sex ratios and otherwise offspring control evolves. It seems that offspring control is on average a more common outcome of maternal-offspring conflict, which is consistent with the pattern seen in nature, although in our model the frequency of cases in which monogeny evolves is more common than in nature. This may be caused by our assumption that maternal and offspring F products are equivalent, namely maternal and offspring genes are additive and each of them have the potential to determine sex by itself. The situation seems more complicated in the few species in which genetic details of maternal control are known (Ahringer *et al.* 1992; Schmidt *et al.* 1997a; Dübendorfer & Hediger 1998; Schütt & Nöthiger 2000). In these species both maternal and offspring genes are necessary to assure proper sexual development. We used our simple additive model as a first attempt to analyze to interaction between maternal and offspring genes. However, additionally we also made a model

(results not shown) in which maternal and offspring F product were under the control of the same gene (active in mother and offspring, respectively), but an additional gene active in the mother regulated the proportion of F product she places in an egg, analogous to the situation seen in the housefly (Dübendorfer *et al.* 2002). This model gives more complicated results, but similarly to the results of our main model, maternal control is more prone to evolve when sons are more costly and otherwise offspring control evolves, showing that it may be a common property of the SD systems based on feminizing factors. Although more realistic models, taking also into account, that maternal products are active only in the early embryogenesis (Schier 2007), will show whether the pattern reminds the same. But first a better understanding of the interaction between maternal and offspring genes in different species is needed to recognize general patterns (if any) and include them in the models.

There is already vast literature on adaptive sex allocation under different circumstances (see e.g. Godfray & Werren 1996; Hardy 2002; West *et al.* 2002), but little work had been done on how sex ratio selection may influence the evolution of SD systems (for review see Uller *et al.* 2007). Werren *et al.* (2002) were the first to model the evolution of SD system under maternal-offspring conflict over sex ratios. They showed that under selection for female-biased sex ratio a male heterozygosity (an XY system) evolved by means of a masculinizing offspring gene that can override maternal genes. This result is very similar to our result for the model with maternal control and invasion of masculinizing factor M. However, due to polymorphism on the maternal locus induced by selection against intersexes, after the initial invasion of *M* we also often see polymorphism on more than one SD locus. Additionally, if SD genes can evolve in strength, maternal-offspring conflict can lead to a long chain of changes in SD system, not restricted to male heterogamety and not even offspring control over SD. Under conflict between maternal and offspring feminizing genes over the sex ratio, male heterozygosity can evolve when male-biased sex ratios are favoured. However, our model concentrates on the maternal-offspring conflict between feminizing genes and does not assume that offspring genes can override maternal genes or vice versa.

Our previous model (Kozielska *et al.* 2006) considered the evolution of three-locus SD of the housefly, with two independent dominant male determiners (*M*) and another locus with a feminizing factor epistatic to *M* (Dübendorfer *et al.* 2002). Under sex ratio selection we often observed polymorphism on different SD loci, but this never led to extreme sex ratio biases. We concluded that even in this seemingly flexible system very little sex ratio bias is possible and that genetic mechanisms may constrain adaptation. In the present study, similar patterns are seen. When the precise sex ratio control is constrained by the cost of ambiguous SD signals, allelic SD evolves which leads to equal sex ratios under offspring control. Only maternal control over sex ratio is not constrained by allelic SD. It is interesting that conflict between maternal and offspring genes can actually lead to a change from a biased sex

ratio to no bias (situation not favored by any party in the conflict; Figure 3.7). This indicates that SD evolution under sex ratio selection (and maternal-offspring conflict) may actually be the cause of the genetic constraints for sex ratio bias (see also Werren *et al.* 2002). Although, when we let the system evolve further, in some cases full maternal control can be regained and biased sex ratios achieved.

How important is maternal-offspring conflict over sex ratio in nature? Theory predicts that the conflict can occur whenever the mother favours a different sex ratio than the offspring (Pen & Weissing 2002, Werren & Hatcher 2000; Werren *et al.* 2002), like in the scenario of different cost of producing of male and female offspring (Trivers 1974) which we considered. There is little data on the differential costs of sons and daughters, but a few examples from birds and mammals are discussed in Werren *et al.* (2002). However, this scenario is not the only selective force for biased sex ratios. Whenever the family sex ratio influences the fitness of the offspring or the mother, maternal-offspring conflict over the sex ratio will occur (Werren *et al.* 2002). In many animals males and females differ in size, which may implicate a difference in food requirement and therefore a different effect on survival of the two sexes. Inbreeding or different dispersal tendencies between sexes may also lead to the evolution of biased sex ratios and maternal-offspring conflict (Hamilton 1967; Bulmer 1986; Frank 1986; Pen 2006). Therefore, many ecological and life history conditions seen in nature suggest that maternal-offspring conflict over sex ratio may be common. However, direct observation of the conflict is much more difficult (Badyaev 2008). A way to infer the importance of maternal-offspring conflict in the evolution of SD systems is to check whether predictions of the models on maternal-offspring conflict are met in SD systems in nature. For example our model predicts that in organisms in which sex is determined by a feminizing factor, maternal control over sex determination is more common when male-biased sex ratios are expected compared to cases when female-biased sex ratios are expected.

Invasion of a new SD factor

We only shortly presented the acquisition of new SD genes in our model. We showed that a zygotic masculinizing gene can invade if the sex ratio is below the offspring optimum. Generalization of this result seems warranted, namely that a new sex determining factor can invade a population if it biases the sex ratio towards the offspring optimum, if it is expressed in the offspring, or maternal optimum if it is presented in the mother (Pen 2006). This has been confirmed by us with a number of simulations (not shown).

We only presented one example of the evolution of a SD system after acquiring a new gene, but we can already point out some important insights. Firstly, invasion of a new gene does not always mean that it will completely take over sex determination (see also Werren & Hatcher 2000; Werren *et al.* 2002; Kozielska *et al.* 2006). Therefore, multiple-factor systems as seen in nature can be in a (relatively) stable state. Secondly, a stable population sex ratio and stable frequencies of SD factors do

not necessary mean that the system has reached equilibrium and is not evolving anymore (Fig 3.7). Therefore these standard measures may not always be informative for determining whether the system has reached equilibrium and more detailed genetic studies (e.g. gene expression patterns) may be necessary to properly analyze processes shaping the evolution of the SD system. Moreover, changes from one to the other SD system can be very rapid and lead to a loss of the previous variation. This also shows that the recent evolution of new SD factors may obscure past variation and make it difficult to make any inferences about the past stages of SD system.

More detailed genetic studies on the few species which show variation of SD mechanism, such as the housefly (Dübendorfer *et al.* 2002) or platyfish (Volff & Scharlt 2002) combined with well-designed experiments may verify the predictions of mechanistic models on the evolution of SD and make them useful for explaining the evolution of other SD systems seen in nature.

Appendix

Here we show that a strong masculinizing factor *M* can invade a population only if the sex ratio (proportion sons) is lower than the Triversian sex ratio (offspring optimum) $1/(1+\sqrt{c})$.

Let us assume that the sex ratio under maternal or offspring control is given by s. Now introduce an *M* factor strong enough to turn any individual into a male, regardless of the rest of its genotype. The sex ratio of a family with a father heterozygous for *M* is then given by $s_M = {}^1\!/_2 + {}^1\!/_2\, s$. The family size of a regular father is proportional to $n = 1/(sc + 1 - s)$ (equation (4), with $p_m = s$, p_f=1-s and $p_i = 0$) and that of the *M*-father $n_M = 1/(s_M c + 1 - s_M)$. Now suppose that the frequency of heterozygous *M*-males is given by p. Then the frequency of *M*-males in the next generation is given by

$$p' = p\,\frac{{}^1\!/_2 n_M}{(1-p)ns + pn_M s_M} = p\,\frac{1}{s}\,\frac{sc + 1 - s}{sc + 1 - s + c} + O(p^2) \quad .$$

The condition for rare *M*-males to increase in frequency ($p' > p$ for small p) simplifies to

$$s < \frac{1}{1+\sqrt{c}} \quad .$$

In other words, strong *M* factors can invade if the sex ratio is smaller than the Triversian sex ratio (Equation (3)). Conversely, populations with a sex ratio larger than the Triversian sex ratio cannot be invaded by *M*.

CHAPTER 4

Segregation distortion and the evolution of sex determining mechanisms

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Ido Pen
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Abstract

Segregation distorters, alleles able to bias their own segregation and be eventually present in more than 50% of the functional gametes of heterozygous individuals, have been found in many species. Sex chromosomal distorters lead to biased sex ratios, which may select for changes in sex determining systems. Here we present a model in which we analyze the conditions for the spread of new sex determining factors in a system with a driving sex chromosome. We consider three scenarios: a driving X chromosome, a driving Y chromosome, and a driving autosome with a male determining factor. We investigate how the invasion prospects of a new sex determining factor are affected by the strength of distortion and the fitness effect of the distorter allele. We show that in many cases meiotic drive may induce changes in the sex determining mechanism. When the drive leads to female biased sex ratios, a new masculinizing gene can invade leading to male heterogamety at a new locus. When the drive leads to male biased sex ratios, a feminizing factor can invade, leading to a switch to female heterogamety. Although the presence of driving alleles induces the spread of new sex determining factors, the change in the sex determining system may eventually lead to loss of the driving alleles from the population. Therefore, distorter alleles may be present in a population only in a transient state between turnovers of sex determining mechanisms. This shows that it may be impossible to infer the past forces responsible for changes in sex determining systems and the role of meiotic drive in this process may be underestimated.

Introduction

Most chromosomes follow "fair" Mendelian segregation, resulting in each of the homologues being present in (approximately) 50% of gametes. However, some genetic elements are recovered in more than half of the functional gametes of heterozygous individuals showing so-called segregation distortion or meiotic drive. Segregation distortion occurs in a number of taxa, ranging from fungi to plants and animals (for reviews see e.g. Jaenike 2001; Burt & Trivers 2006).

Segregation distortion is advantageous at the gene level, since distorter alleles have transmission advantage and their frequency in the population will increase. Many distorters in nature show almost complete distortion when unsuppressed (the distorter allele is present in almost 100% of functional sperm). However, considerable variation exists between populations and different distorters, and an effective distortion can range from just above 0.5 to almost 1 (e.g. Sturtevant & Dobzhansky 1936; Hickey & Craig 1966; Gileva 1987; Carvalho *et al.* 1989; Jaenike 1996; van Boven & Weissing 1998; Jaenike 1999; Montchamp-Moreau *et al.* 2001; Atlan *et al.* 2003).

However, the presence of a driving chromosome is usually not neutral with respect to individual fitness, both in hetero- and in homozygous condition (e.g. Wallace 1948; Curtsinger & Feldman 1980; Jaenike 1996; Atlan *et al.* 2004). In extreme, but not uncommon, cases homozygosity for a distorter allele may cause sterility in males or even lethality in males and females (for example, in the t-complex of the mouse and the Segregation Distorter of *Drosophila melanogaster*; see Lyttle 1991; Burt & Trivers 2006). Therefore, there will be selection for suppressors of segregation distortion. Suppressors have been found in most of the species possessing different segregation distorters (e.g. Jaenike 2001; Burt & Trivers 2006).

When segregation distorters are located on sex chromosomes, they not only have an effect on individual fitness, but also lead to biased sex ratios in the population (Jaenike 2001). In addition to selection for suppressors, biased sex ratios are also expected to select for changes in sex determining mechanisms (Bull & Charnov 1977; Cosmides & Tooby 1981; Werren & Beukeboom 1998; Burt & Trivers 2006). Theoretical models show that segregation distortion could be (or have been) a driving force behind a change in the sex determining mechanism of the wood lemming, *Myopus schisticolor* (Bengtsson 1977), the mole, *Talpia occidentalis* (McVean & Hurst 1996), the creeping vole, *Microtus oregoni* (Charlesworth & Dempsey 2001), sciarid fly, *Sciara coprophila* (Haig 1993b), the housefly, *Musca domestica* (Clark 1999) and scale insects, Neococcoidea (Haig 1993a).

All previous models of the effect of segregation distortion on the evolution of sex determining systems are specifically tailored to a particular species. In other words, all these models make different, very specific assumptions concerning the genetics and development of the focal species. Therefore, no general conclusions have emerged so far. Here we present a more general model in which we analyze the conditions for the spread of a new sex determining gene in a system with a

distorting (driving) sex chromosome. Throughout, we assume that segregation distortion occurs only in males, as is the case in most systems of drive, where unequal segregation is due to the dysfunction of sperm lacking the driving element (e. g. Lyttle, 1993).

We consider three scenarios, each with a different driving sex chromosome: a driving X chromosome (scenario 1); a driving Y chromosome (scenario 2); and a driving autosome with a male determining factor (scenario 3). Segregation distortion associated with all such chromosomes has been found in natural populations of various species (Clark 1999; Jaenike 2001; Burt & Trivers 2006). The presence of driving chromosomes leads to female biased (scenario 1) or male biased (scenario 2 and 3) sex ratios, presumably promoting the spread of new masculinizing or feminizing factors, respectively.

For each scenario we consider three different fitness schemes: no fitness differences between genotypes, sterility of males homozygous for the driving element, and lethality of individuals which are homozygous for the driving factor, both males and females. Additionally, for each case we consider different levels of distortion (drive strength), from very weak to almost complete.

We made a model consisting of recurrence equations to answer the following questions. How is the invasion prospect of a new SD factor affected by the strength of segregation and the fitness effect of a distorter present in the population? When invasion is possible, will a new factor spread to fixation leading to a switch to a different SD mechanism? How is the frequency of the segregation distorter affected by the invasion of a new SD factor?

The model

We model the evolutionary dynamics of the sex determining system with a set of recurrence equations. We assume an infinite diploid population with random mating and non-overlapping generations. We analyze three different scenarios for the evolution of sex determination: 1) with a driving X chromosome, 2) with a driving Y chromosome and 3) with a driving autosomal male determining factor. First we will present a general model and then introduce modifications specific to different scenarios.

Genotypes and sex determination: Since the number of ways in which sex could be determined is limitless, we decided to base our model on a relatively general model of sex determination. We consider a sex determination system consisting of three independent gene loci (on three different chromosomes). In the absence of segregation distortion, each locus has two basic alleles, but additional alleles can be present in specific models (see below). The first locus corresponds to the standard *XY* system of sex determination with two basic alleles: *X* and *Y* (dominant male-determining). The second locus has a dominant male-determining (autosomal) *M*

allele and a standard *m* allele. The third locus has a female-determining *F* allele and a standard *f* allele. The *F* is dominant over *M* and *Y*, meaning that the presence of *F* always leads to female development, even if both *Y* and *M* are present in homozygous state. If *F* is absent but *Y* and/or *M* are present, an individual becomes a male, otherwise (neither *Y* nor *M* is present) it becomes a female.

We encode genotypes by triplets $\mathbf{i} = (\mathbf{i}_1, \mathbf{i}_2, \mathbf{i}_3)$, where $\mathbf{i}_n$ corresponds to the genotype on locus *n*. At each locus genotypes are unordered, meaning that the heterozygous genotype *AB* is equivalent with *BA*. The sexual phenotype determined by genotype $\mathbf{i}$ is encoded as a binary variable: $s(\mathbf{i})=0$ for females and $s(\mathbf{i})=1$ for males. The frequencies of genotype $\mathbf{i}$ among adult females and adult males are written as $p_f(\mathbf{i})$ and $p_m(\mathbf{i})$ ($\sum_{\mathbf{i}} p_f(\mathbf{i}) = \sum_{\mathbf{i}} p_m(\mathbf{i}) = 1$). Note that for each $\mathbf{i}$ either $p_f(\mathbf{i})$ or $p_m(\mathbf{i})$ must be zero, because the genotype $\mathbf{i}$ uniquely determines sex.

Fitness: Each genotype has its specific viability, $v(\mathbf{i})$, and fertility, $u(\mathbf{i})$. In our model all females have always the same fertility, therefore, $u_f(\mathbf{i})=1$. Male fertility may depend on its genotype and will be denoted $u_m(\mathbf{i})$. We looked at three fitness schemes: a) there is no fitness disadvantage of homozygosity for driving alleles i.e. $v(\mathbf{i})=1$ and $u_m(\mathbf{i})=1$, for all $\mathbf{i}$; b) males homozygous for the driving allele are sterile, i.e. $u_m(\mathbf{i})=0$ if $\mathbf{i}$ is homozygous for driving allele (i.e. X^dX^d, Y^dY^d or M^dM^d, depending on the scenario) and $u_m(\mathbf{i})=1$ for all other genotypes; c) homozygosity for the driving allele is lethal both in males and females, i.e. $v(\mathbf{i})=0$ if $\mathbf{i}$ is homozygous for the driving alleles and $v(\mathbf{i})=1$ for all other genotypes.

Drive: Segregation is random in females and in males that do not posses driving chromosome or are homozygous for it. In males heterozygous for driving chromosome and sensitive chromosomes, *k* denotes the frequency of the driving allele in sperm and hence indicates the strength of the drive. For all the other genotypes segregation is random. We investigate various values of the drive parameter *k*: 0.55, 0.60, 0.70, 0.80, 0.90, 0.99.

Inheritance: The frequency of genotype $\mathbf{x}$ among the offspring of a cross between a female with genotype $\mathbf{i}$ and a male with genotype $\mathbf{j}$ is denoted by $T(\mathbf{x}|\mathbf{ij})$. $T(\mathbf{x}|\mathbf{ij})$ can be decomposed into three per locus components:

$$T(\mathbf{x}|\mathbf{ij}) = P(\mathbf{x}_1|\mathbf{i}_1\mathbf{j}_1)P(\mathbf{x}_2|\mathbf{i}_2\mathbf{j}_2)P(\mathbf{x}_3|\mathbf{i}_3\mathbf{j}_3) \ , \tag{1}$$

where $P(\mathbf{x}_n|\mathbf{i}_n\mathbf{j}_n)$ is the probability that at locus *n* the offspring genotype will be $\mathbf{x}_n$, given that its mother has genotype $\mathbf{i}_n$ and its father has genotype $\mathbf{j}_n$ at this locus.

If the offspring genotype at a given locus *n* is homozygous, consisting of two copies of allele *A*, then

$$P(\mathbf{x}_n = AA|\mathbf{i}_n\mathbf{j}_n) = \tfrac{1}{2}\, q_A(\mathbf{i}_n)k_A(\mathbf{j}_n)q_A(\mathbf{j}_n) \ , \tag{2a}$$

where $q_A(\mathbf{i}_n)$ and $q_A(\mathbf{j}_n)$ is the number of alleles of type *A* in the mother and the father, respectively. $k_A(\mathbf{j}_n)$ is the segregation ratio of the *A* allele in males of genotype

$\mathbf{j}_n$. $k_A(\mathbf{j}_n)=0$ if A is not present in genotype $\mathbf{j}_n$. If $\mathbf{j}_n$ is heterozygous for the driving and sensitive allele, then $k_A(\mathbf{j}_n)= k$ when A is the distorter allele or $k_A(\mathbf{j}_n)=1- k$ if A is the sensitive allele. $k_A(\mathbf{j}_n)= {}^1\!/_2$ in all other cases.

For heterozygous offspring with genotype AB

$$P(\mathbf{x}_n = AB\,|\,\mathbf{i}_n\mathbf{j}_n) = {}^1\!/_2\, q_A(\mathbf{i}_n)k_B(\mathbf{j}_n)q_B(\mathbf{j}_n) + {}^1\!/_2\, q_B(\mathbf{i}_n)k_A(\mathbf{j}_n)q_A(\mathbf{j}_n)\ . \qquad (2b)$$

Evolutionary dynamics: Under random mating, the probability that an **i**-female mates with a **j**-male is given by the product of their frequencies, $p_f(\mathbf{i})\ p_m(\mathbf{j})$. Allowing fertility differences in males, $u_m(\mathbf{i})$, the frequency of offspring with genotype **x** from all parents, before viability selection, equals

$$\overline{T}(\mathbf{x}) = \sum_{\mathbf{ij}} p_f(\mathbf{i})\ p_m(\mathbf{j})\ \frac{u_m(\mathbf{j})}{\overline{u}_m}\ T(\mathbf{x}\,|\,\mathbf{ij})\ \ , \qquad (3)$$

where $\overline{u}_m = \sum_{\mathbf{j}} p_m(\mathbf{j})u_m(\mathbf{j})$.

Assuming discrete and non-overlapping generations, the sex-specific genotype frequencies $p'_f(\mathbf{k})$ and $p'_m(\mathbf{k})$ after one round of reproduction and selection are given by the recursions (Weissing & van Boven 2001)

$$\begin{aligned} p'_m(\mathbf{k}) &= \frac{1}{\overline{\nu}S}\,\nu(\mathbf{x})s(\mathbf{x})\overline{T}(\mathbf{x}) \\ p'_f(\mathbf{k}) &= \frac{1}{\overline{\nu}(1-S)}\,\nu(\mathbf{x})[1-s(\mathbf{x})]\overline{T}(\mathbf{x}) \end{aligned} \qquad (4)$$

where

$$\overline{\nu} = \sum_{\mathbf{x}} \nu(\mathbf{x})\overline{T}(\mathbf{x}) \qquad (5)$$

is the mean number of surviving offspring, and

$$S = \frac{1}{\overline{\nu}}\ \sum_{\mathbf{x}} \nu(\mathbf{x})\overline{T}(\mathbf{x})s(\mathbf{x}) \qquad (6)$$

is the sex ratio (proportion males) after viability selection.

We use numerical iterations to investigate the dynamics of the system. In all cases, we start with the standard XY system (females: *XX*; *mm*; *ff* and males: *XY*; *mm*; *ff*) and introduce a distorter allele (step 1) at low frequency (0.001). Equations (4) are iterated and when the equilibrium is reached a new SD factor is introduced (step 2) at a frequency of 0.001. Again, equations (4) are iterated till the new equilibrium is reached. Dynamics of the system and the final frequencies of different alleles are analyzed.

We consider three scenarios with different distorter alleles:

Scenario 1. Driving X chromosome
In this version of the model the three alleles segregate at the XY locus: standard *Y*, standard *X* and driving *X*. The latter will be denoted X^{d} and it is assumed to drive only against *Y*. Therefore, if the male genotype $\mathbf{j}_1$ at locus 1 is $X^{\mathrm{d}}Y$, then $k_{X^d}(\mathbf{j}_1) = k$ and $k_Y(\mathbf{j}_1) = 1 - k$. For all other male genotypes segregation of alleles is random, i.e. $k_A(\mathbf{j}_1) = 1/2$.

We start with the standard XY system and in step 1 (see above) introduce a driving X^{d} chromosome. This causes female biased sex ratios and in step 2 an *M* allele is introduced in order to regain equal sex ratios.

Scenario 2. Driving Y chromosome
In this version the three alleles segregate at the XY locus: standard *X*, standard *Y* and driving *Y*. The later will be denoted Y^{d} and it is assumed to drive only against *X*. Therefore, if male genotype $\mathbf{j}_1$ at locus 1 is XY^{d}, then $k_{Y^d}(\mathbf{j}_1) = k$ and $k_X(\mathbf{j}_1) = 1 - k$. For all other male genotypes segregation of alleles is random: $k_A(\mathbf{j}_1) = 1/2$.

We start with the standard XY system and in step 1 introduce a driving Y^{d} chromosome. This causes male biased sex ratios and in step 2 an *F* allele is introduced in order to regain unbiased sex ratios.

Scenario 3. Driving *M*
Only two alleles are present at each locus, but we assume that *M* drives against *m*. Accordingly, the driving alleles will be denoted as M^{d}. If male genotype $\mathbf{j}_2$ at locus 2 is $M^{\mathrm{d}}m$, then $k_{M^d}(\mathbf{j}_2) = k$ and $k_m(\mathbf{j}_2) = 1 - k$. For all other male genotypes segregation of alleles is random and $k_A(\mathbf{j}_2) = 1/2$.

We start with the standard XY system and in step 1 introduce a driving M^{d} allele. This causes male biased sex ratios and in step 2 an *F* allele is introduced in order to regain unbiased sex ratios.

For each scenario, we investigate the impact of different strength of drive (k) and different fitness of individuals homozygous for driving alleles (fitness scheme) on the dynamics of the system.

Results

Scenario 1. Driving X chromosome
For all values of k, the driving X chromosome (X^{d}) always invades the population leading to a female-biased sex ratio. When M is introduced it increases production of males and it is selected for, as long as selection against biased sex ratios is not overcome by selection against sterile genotypes. Once *M* invades, it causes the loss of the

Y chromosome from the population and restores the equal sex ratio. Therefore, in most cases there is a switch from an XY system to an XX system where sex is determined by *M*. Males are again the heterogametic sex, therefore, a new system can be seen as a male heterogamety for *M* (Table 4.1). Interestingly, even though the presence of the driving X^d chromosome is often responsible for the switch from XY to an autosomal system, at equilibrium the X^d chromosome may be no longer present in the population (see below).

No fitness differences between genotypes: Due to its transmission advantage and no fitness costs, X^d fixates in the population removing the standard *X* chromosome, independently of the value of *k*. This leads to a female biased sex ratio with a proportion of females equal to the drive strength *k*. This sex ratio bias facilitates the invasion of *M*, which very quickly spreads replacing Y as an SD factor, leading to male heterogamety for *M* (Fig 4.1A). X^d reaches fixation in both males and females. Although driving chromosomes are still present in the population, the sex ratio is unbiased, since the chromosomes sensitive to drive are absent.

Sterility of X^dX^d males: X^d invades the population and replaces the standard X, as above, since males are always X^dY and, therefore, do not have a fertility disadvantage. However, now the introduction of *M* leads to the production of sterile X^dX^d males and *M* cannot invade the population. Only if the standard *X* is reintroduced into the population together with *M* can they both invade, since some males produced by *M* possess the standard *X* and are fertile. These males have an advantage in strongly female-biased populations and both *M* and standard *X* increase in frequency. An increase in the frequency of *M* leads to the production of sterile X^dX^d males and selection against X^d. X^d is eventually lost from the population and replaced by standard *X* (Fig 4.1B). *Y* is also removed from the population and a male heterogametic system for *M* establishes. It should be noted that although the driving *X* chromosome induced changes in the SD mechanism, it is no longer present in the population.

This happens only for strong drive ($k = 0.80$, 0.90 and 0.99), since then the population sex ratio is very female biased and selection against the biased sex ratio is strong enough to overcome selection against infertile males. Therefore, even though the presence of *M* initially leads to the production of many sterile X^dX^d males, if *X* is present in the population, this cost is compensated for by the increased production of males thanks to *M*. For weaker drive, selection against biased sex ratio is not strong enough to overcome selection against sterile genotypes and *M* cannot invade (Table 4.1).

Lethality of X^dX^d genotypes: X^d invades the population but it cannot fixate, since homozygous genotypes are lethal. Therefore, the sex ratio bias induced by X^d is less pronounced than for the scenarios above (Fig 4.1C). The frequency of X^d increases with *k*, from 0.045 for $k=0.55$ to 0.22 for $k=0.90$. A female-biased sex ratio facilitates the invasion of the masculinizing factor *M*, which in turn leads to a decrease in the frequency of *Y* and increased homozygosity for X^d, also in males.

Table 4.1. Overview of changes in the sex determining system induced by a driving X chromosome (X^d). Male and female genotypes are given together with the equilibrium sex ratio (S^*). Columns correspond to the different fitness schemes considered (top row). When the outcome is the same for two fitness schemes, the corresponding columns are merged. Sometimes the outcome depends on the drive strength (k). In that case different outcomes are listed separately; otherwise the outcome is independent of k. The rows correspond to the sequence of events, from the initial state to the equilibrium state after the introduction of driving X^d and the final equilibrium after the introduction of *M*. After the introduction of *M*, in some cases the standard *X* chromosome has an advantage over X^d and it will invade the population when reintroduced. Therefore, at equilibrium it will be present in the population. For details, see text.

	no fitness differences	sterility of X^dX^d males		lethality of X^dX^d individuals
initial state	♂ *XY*; *mm*; *ff* ♀ *XX*; *mm*; *ff* $S^*=0.5$			
after introduction of X^d	♂ X^dY; *mm*; *ff* ♀ X^dX^d; *mm*; *ff* $S^*=1-k$			♂ $X^\bullet Y$; *mm*; *ff* [1] ♀ $X^\bullet X^\bullet$; *mm*; *ff* [1] $1-k<S^*<0.5$
after introduction of *M*	♂ X^dX^d; *Mm*; *ff* ♀ X^dX^d; *mm*; *ff* $S^*=0.5$	$k=0.55-0.7$: ♂ X^dY; *mm*; *ff* ♀ X^dX^d; *mm*; *ff* $S^*=1-k$	$k=0.8-0.99$: ♂ *XX*; *Mm*; *ff* ♀ *XX*; *mm*; *ff* $S^*=0.5$	♂ *XX*; *Mm*; *ff* ♀ *XX*; *mm*; *ff* $S^*=0.5$
resulting SD system	male heterogamety for *M*	male heterogamety for *Y*	male heterogamety for *M*	male heterogamety for *M*

[1] - $X^\bullet$ stands for either standard *X* or driving X^d, since polymorphism is maintained in the population

Since the standard *X* is present in the population at high frequency, the decrease in fitness of *M*-bearing males through the production of rare lethal X^dX^d offspring is outweighed by the advantage from an increased production of males. Eventually X^d is removed from the population because of its detrimental effect in the homozygous state, which is not counterbalanced by a transmission advantage, since X^dY males are rare. A male heterogametic system for *M* establishes (Table 4.1).

Scenario 2. Driving Y chromosome

Independently of the strength of drive k and the fitness of homozygous Y^dY^d individuals, Y^d always spreads in the population replacing standard *Y*, since females never harbour Y^d and, therefore, males can never be homozygous for Y^d. Since all males are XY^d, the sex ratio is biased with a proportion of males equal to the drive

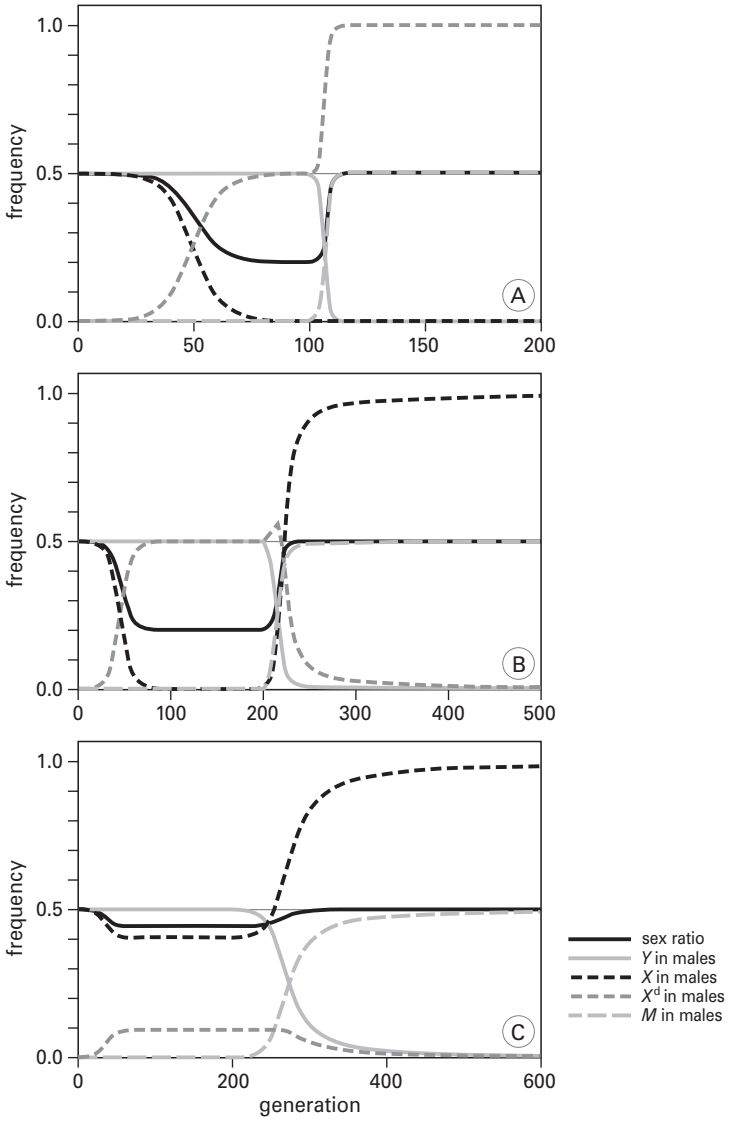

Figure 4.1. Dynamics of the sex determining system in the presence of a driving X^d chromosome. (A) No fitness differences between genotypes. A driving X^d is introduced in generation 0, leading to the loss of standard X and a strongly female biased sex ratio. In generation 100 M is introduced, leading to the restoration of equal sex ratios. Y is removed from the population and a male heterogametic system for M is established. (B) Sterility of X^dX^d males. A driving X^d is introduced in generation 0, leading to the loss of standard X and a strongly female biased sex ratio. In generation 200 M and X are introduced simultaneously, leading to the restoration of an equal sex ratios. X^d and Y disappear resulting in a male heterogametic system for M. Introducing M on its own has no effect, since it is selected against. (C) Lethality of X^dX^d genotypes. After its introduction in generation 0, X^d increases in frequency without replacing standard X. M is introduced in generation 200, leading to male heterogamety for M and removal of X^d and Y (as above). In all panels drive strength k=0.8.

Table 4.2. Overview of changes in the sex determining system induced by a driving *Y* chromosome (Y^d). Data are organized as in Table 4.1. After the introduction of *F*, in some cases the standard, non-driving *Y* chromosome has an advantage over Y^d and it will invade the population when reintroduced. See text for details.

	no fitness differences	sterility of Y^dY^d males	lethality of Y^dY^d individuals	
initial state	♂ *XY*; *mm*; *ff* ♀ *XX*; *mm*; *ff* S^*=0.5			
after introduction of Y^d	♂ XY^d; *mm*; *ff* ♀ *XX*; *mm*; *ff* S^*= *k*			
after introduction of *F*	♂ Y^dY^d; *mm*; *ff* ♀ Y^dY^d; *mm*; *Ff* S^*=0.5	♂ *YY*; *mm*; *ff* ♀ *YY*; *mm*; *Ff* S^*=0.5	*k*=0.55 – 0.6: ♂ XY^d; *mm*; *ff* ♀ *XX*; *mm*; *ff* S^*= *k*	*k*=0.7 – 0.99: ♂ *YY*; *mm*; *ff* ♀ *YY*; *mm*; *Ff* S^*=0.5
resulting SD system	female heterogamety	female heterogamety	female heterogamety	female heterogamety

strength, *k*. If selection against biased sex ratio is strong enough to overcome the selection against unviable or infertile genotypes, *F* is selected for. Once *F* invades the population it usually fixates and the SD mechanism switches to female heterogamety (Table 4.2). Similarly to the scenario 1, a change in the sex determining mechanism may lead to a complete removal of the driving chromosome from the population.

No fitness differences between genotypes: The feminizing *F* allele always spreads in the population leading to fixation of Y^d, and a switch to a female heterogametic SD system. Although Y^d is present in the population, the sex ratio equals 1:1, since the *X* chromosome sensitive to drive is lost.

Sterility of Y^dY^d males: Selection against biased sex ratios facilitates the spread of *F*. It is present only in females, so it does not have direct negative fitness effects. However, *F* females produce some sterile Y^dY^d males, which prevents *F* from fixation. Polymorphism for *X* and Y^d, and *F* and *f* is maintained, but the sex ratio in the population equals 0.5 (Fig 4.2). The frequencies of *F* and Y^d increase with the strength of drive, *k*. At this state, reintroduction of the standard *Y* in the population will lead to its spread, since it does not cause a fitness cost in males. The segregation advantage of Y^d is low, since due to the low frequency of *X*, the frequency of Y^dX males is low. Therefore, the segregation advantage is outweighed by selection against

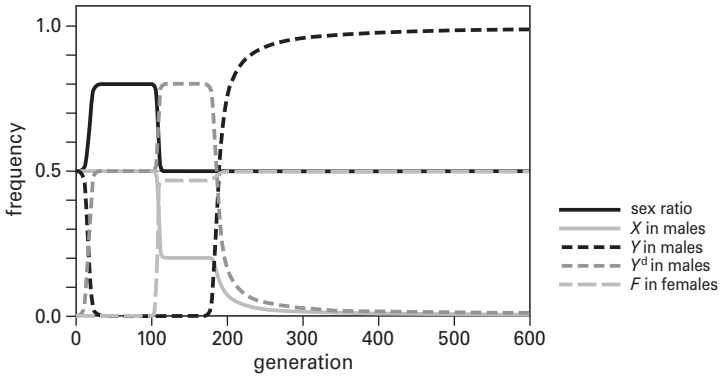

Figure 4.2. Dynamics of the sex determining system with a driving Y^d chromosome for the case that Y^dY^d males are sterile. Y^d is introduced at generation 0, subsequently replacing standard *Y* and leading to a strongly male biased sex ratio. At generation 100 *F* is introduced leading to a polymorphic system with equal sex ratios. If standard *Y* is reintroduced to this system (here in generation 175) it invades the population and fixates, replacing the driving Y^d and leading to a female heterogametic system. Drive strength k=0.8.

sterile Y^dY^d males. The frequency of Y^d decreases and standard *Y* replaces Y^d and spreads to fixation. *X* is removed, *F* is present in all females and a female heterogametic system establishes (Fig 4.2).

Lethality of Y^dY^d genotypes: If drive strength is low, *F* does not invade the population, since selection against a (slightly) biased sex ratio is weaker than selection against the lethal genotypes produced when *F* is present. Therefore, an XY system with the driving Y^d chromosome (i.e. no standard *Y*) and a biased sex ratio is stably obtained for k=0.55 and k=0.60. For higher values of k, the *F* factor always invades the population. Initially a polymorphism on both the *F* and XY locus is maintained, with a population sex ratio that is only slightly male-biased, similarly to the scheme with male sterility. Here, the reintroduction of the standard *Y* also leads to its increase in frequency and eventually removal of Y^d and *X*. *F* reaches a frequency of 0.5 in females and female heterogamety with equal sex ratios is established.

When *F* is present in the population, but before fixation of standard *Y*, in some circumstances autosomal *M* can invade. However, it never reaches fixation, and standard *Y* will fixate in the population once it is reintroduced. Therefore, female heterogamety with the frequency of standard *Y* equal to 1 is an equilibrium state of this system, although polymorphism for *M* and *m* can be present.

Scenario 3. Driving autosomal *M*

Similarly to the case with driving Y^d, driving M^d invades the population independently of drive strength and the fitness of individuals homozygous for M^d, since it is only present in males in heterozygous state. *Y* is removed from the population and

Table 4.3. Overview of changes in the sex determining system induced by a driving autosomal factor M^d. Data organized as in Table 4.1. $M^\bullet$ represents either m or driving M^d. Hence, $M^\bullet M^\bullet$ indicates a polymorphism on this locus and the presence of mm, mM^d, M^dM^d genotypes. F represents either F or f. Hence, $F^\bullet f$ indicates a polymorphism of the genotypes Ff or ff.

	no fitness differences	sterility of M^dM^d males	lethality of M^dM^d individuals		
initial state	♂ *XY*; *mm*; *ff* ♀ *XX*; *mm*; *ff* S^*=0.5				
after introduction of M^d	♂ *XX*; $M^d m$; *ff* ♀ *XX*; *mm*; *ff* $S^* = k$				
after introduction of *F*	♂ *XX*; M^dM^d; *ff* ♀ *XX*; M^dM^d; *Ff* S^*=0.5	♂ *YY*; $M^\bullet M^\bullet$; *ff* ♀ *YY*; $M^\bullet M^\bullet$; *Ff* S^*=0.5	k=0.55 – 0.6: ♂ *XX*; $M^d m$; *ff* ♀ *XX*; *mm*; *ff* $S^* = k$	k=0.7: ♂ *XX*; $M^\bullet M^\bullet m$; *ff* ♀ *XX*; $M^\bullet M^\bullet$; $F^\bullet f$ $0.5 < S^* < k$	k=0.8 – 0.99: ♂ *YY*; $M^\bullet M^\bullet$; *ff* ♀ *YY*; $M^\bullet M^\bullet$; *Ff* S^*=0.5
resulting SD system	female heterogamety	female heterogamety	male heterogamety	polymorphism	female heterogamety

all individuals are *XX*. The population sex ratio is male biased and equal to drive strength, k. Whether F invades depends on the fitness costs of homozygosity for M^d (Table 4.3).

No fitness differences between genotypes: For any drive strength k, selection against male-biased sex ratios facilitates the spread of the feminizing *F* allele. The invasion of *F* leads to fixation of M^d and Y^d, and a switch to a female heterogametic system with 1:1 sex ratio.

Sterility of M^dM^d males: *F* always invades irrespective of drive strength. However, fixation of *F* and M^d is impossible, since then all males would be sterile. Therefore, there is no full switch to female heterogamety, but polymorphism for both M^d and *m*, and *F* and *f* is maintained in the population. Population sex ratio equals 0.5. With increasing k, the equilibrium frequency of M^d and *F* increases. If at any point *Y* is reintroduced in this system, it will reinvade the population, since it assures maleness without fitness costs. Eventually *Y* spreads to fixation and all females become heterozygous for *F*. The frequency of M^d decreases, but it is not removed from the population. Therefore, at the stable equilibrium in effect a female heterogametic system is present, but with a polymorphism at the *M* locus (Fig 4.3).

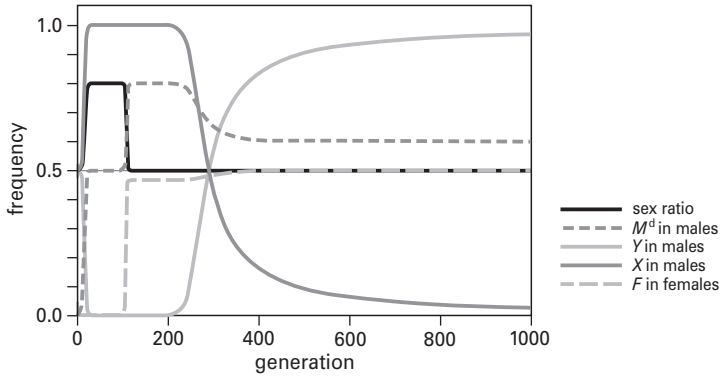

Figure 4.3. Dynamics of the sex determining system with a driving autosomal M^d factor for the case of sterility in M^dM^d males. M^d is introduced in generation 0 leading to the removal of *Y*, the switch to a male heterogametic system for M^d, and a male-biased sex ratio. *F* is introduced in generation 100 and it increases in frequency, but there is no full switch to female heterogamety. When *Y* is reintroduced in generation 200 it increases in frequency, eventually reaching fixation in males and females. A female heterogametic system establishes, but polymorphism on the *M* locus is maintained. Drive strength $k=0.8$.

Lethality of M^dM^d genotypes: If drive strength is weak ($k=0.55$ and $k=0.6$), *F* does not invade the population, since then the population sex ratio is only slightly male-biased and the advantage of producing females by *F* is outweighed by the disadvantage of producing lethal, homozygous M^d individuals. Therefore for weak drive a system with male heterogamety for M^d and a biased sex ratio is stable. For higher *k*, *F* invades, leading to a system polymorphic for *F* and *f*, and M^d and m, with the equilibrium frequency of *F* and M^d increasing with *k* (as it does in the scheme with sterile males). The population sex ratio is slightly male biased. For $k=0.70$ this polymorphic sex determining system is stable and *Y* cannot reinvade the population. For stronger drive ($k=0.80$, $k=0.90$ and 0.99), if *Y* is reintroduced it will spread to fixation, leading to female heterogamety. Although the frequency of M^d decreases, it is not removed from the population and polymorphism at the *M* locus is maintained (similar to the case with sterility of M^dM^d males).

Discussion

We show that in most cases segregation distortion induces changes in the sex determining mechanism, as was to be expected on the basis of verbal arguments and more specific models (Bull & Charnov 1977; Cosmides & Tooby 1981; Werren & Beukeboom 1998; Burt & Trivers 2006). When segregation distortion leads to female biased sex ratios, new masculinizing genes can invade leading to male heterogamety

at a new locus. When segregation distortion leads to male biased sex ratios, new feminizing factors can invade leading to a switch to female heterogamety.

The switch to a new SD system is driven by selection against biased sex ratios. When there are no fitness costs associated with some genotypes, even weak sex ratio selection leads to changes in SD mechanism. However, often sex ratio selection has to be sufficiently strong to overcome selection against infertile or unviable genotypes. Therefore in some cases only strong segregation distorters will lead to changes in SD mechanisms. This suggests that when segregation distortion is weak the evolution of suppressors may be the only way to decrease sex ratio bias. Interestingly, Lyttle (1981) in his model on the evolution of new sex determination through aneuploidy, also concluded that strong drive favours aneuploidy and weak drive favours accumulation of suppressors. We suspect that this is a general phenomenon, since only strong drive will lead to strong sex ratio selection which may outbalance any cost of production of suboptimal genotypes when new sex determining factors are introduced. Moreover, once suppressors start accumulating in the population decreasing the effective drive, this may prevent changes in the sex determining system.

Fitness differences between genotypes influence the invasion prospects of a new SD factor and may prevent its spread. In our model, once a new sex determining factor invades, the final state of the system (male or female heterogamety) is not strongly affected by the fitness differences between genotypes (Tables 4.1-3). We only considered three rather extreme cases of fitness distributions between genotypes, but segregation distorters have been shown to have variable effects on fitness in nature. Cases of decreased fitness of individuals possessing driving alleles, both hetero- and homozygous or even increased fitness of heterozygous females, are known (e.g. Wallace 1948; Curtsinger & Feldman 1980; Jaenike 1996; Atlan *et al.* 2004; Wilkinson *et al.* 2006). The different fitness of different genotypes will influence the balance between sex ratio selection and viability selection acting on new sex determining factors. However, according to our results, once new factors invade, the equilibrium sex determining mechanism is largely independent of the specific fitness effects. This may hold, at least as long as segregation distorters have a negative effect on fitness. When distorter alleles show overdominance in females (Wallace 1948; Curtsinger & Feldman 1980; Wilkinson *et al.* 2006) the outcome of evolution may be different, since both fixation and removal of the distorter allele may not be favoured, although it always occurred in our model. Therefore, more theoretical work is needed to validate whether a complete switch to a new SD system is achieved with different fitness of genotypes.

In some of our models, a complete switch to a new sex determining system could only be achieved if the standard X or Y chromosome was reintroduced in the population. For example in the scenario with driving X chromosome and sterility of homozygous X^dX^d males, new masculinizing factor could invade only if introduced together with the standard X chromosome. This is less implausible than it may

appear at first sight. In our model the standard X or Y chromosome was completely driven out of the population. However, in natural systems a population often consists of many subpopulations with limited gene flow between them. Therefore, one can easily imagine that a driving element will initially appear only in one subpopulation and spread within it, and possibly to other populations. However, if a new sex determining factor appears before the whole population is fixed for a driving allele, migration of standard chromosomes from other subpopulations will lead to its spread also in subpopulations with driving elements. Alternatively, even if the driving allele fixated in the whole population, any mutation restoring vitality or fertility of homozygotes will be favoured and spread in the population, even if it does not have a segregation advantage.

Interestingly, the segregation distorter whose presence initiated the change in the SD system, is often subsequently lost from the population. In fact, it will only be maintained if, in the presence of the new SD factor, its segregation advantage still overcomes its negative fitness effect on higher levels (sterility, lethality, sex ratio bias). It is therefore possible that the presence of a segregation distorter is only a transient state, the traces of which are not longer visible once a new SD system has become established.

Perhaps surprisingly, such an effect has never been found in previous models (Bengtsson 1977; Jayakar 1987; Haig 1993a, 1993b; McVean & Hurst 1996; Charlesworth & Dempsey 2001). In these models a change in the sex determining system usually neutralizes drive, for example, by removing sensitive alleles, but never leads to the complete loss of the driving allele. At the population level, it may be difficult to distinguish between situations where sensitive allele or the driving allele is lost, since in both cases no biased sex ratio is seen anymore. At least in principle, however, such a distinction may be possible using crosses between populations or closely related species where sensitive alleles are still present (Carvalho & Klaczko 1994; Cazemajor *et al.* 1997; Atlan *et al.* 2003). However, once the driving alleles are lost, it may be difficult to accurately demonstrate the role of segregation distortion in the evolution of extant SD mechanisms. Therefore, the role of meiotic drive in evolution of SD mechanisms may be underestimated.

CHAPTER 5

Sexually antagonistic genes and the evolution of sex determining mechanisms

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Abstract

Sexually antagonistic (SA) alleles, beneficial to one sex but detrimental to the other, seem to be common in species in which there were looked for. Theory, supported by experimental data, predicts that SA variation is especially prone to accumulate on sex chromosomes. Accumulation of SA alleles close to sex determining (SD) genes may in turn facilitate reduced recombination and eventually differentiation between sex chromosomes. Although sex determining systems strongly influence the pattern of SA variation little theoretical work has been done on how SA variation can influence the evolution of sex determination. Here, we present a model to investigate the conditions under which new SD factors can spread in response to accumulation of SA variation on the original sex chromosomes. We start with a XY system and let the sex chromosomes accumulate SA variation, and then introduce new male- or female-determining genes to see if they can spread in the population. We investigate the effect of sex chromosome differentiation, dominance effect of different SA alleles and linkage of new SD factors with SA loci on the outcome of the evolutionary dynamics. Our results show that for the system with undifferentiated sex chromosomes (both X and Y chromosome posses homologous SA locus) a new male-determining factor never has a fitness advantage. A new female-determining factor can spread only if it can accumulate SA variation and female-beneficial alleles are dominant or SA alleles show sex-specific dominance. If sex chromosomes are differentiated and only X possesses an SA locus, the conditions under which new SD factors can spread are much less restrictive and new SD factors can spread even if they are not linked with SA alleles, although linkage facilitates their spread. After their initial spread new SD alleles can reach fixation leading to a switch to a new male or female heterogametic SD system. In some cases a new SD factor does not spread to fixation, but a SD system polymorphic on multiple loci is maintained.

Introduction

It is becoming broadly accepted that conflict has a strong impact on male and female co-evolution (Partridge & Hurst 1998; Chapman *et al.* 2003; Arnqvist & Rowe 2005). The two sexes have different roles in reproduction, rooted in anisogamy (Chapman *et al.* 2003). Simplifying, males often increase their fitness by mating with as many females as possible, but females are limited in their reproductive fitness by the number of eggs they lay and usually prefer much fewer matings, since matings may decrease their fitness (for example, due to increased predation rate). Therefore, adaptations increasing the fitness of one sex may lead to a decrease in fitness in the other sex, thus causing sexual conflict. This process has been extensively studied, both theoretically and empirically, although many issues still remain unresolved (Rice 1996b; Cordero & Eberhard 2003; Arnqvist & Rowe 2005).

Many male- and female-beneficial adaptations are located on different loci which results in so-called intragenomic (Rice & Chippindale 2001) or interlocus (Chapman *et al.* 2003) conflict. However, sexual conflict can be present even at a single locus (intralocus conflict; Chapman *et al.* 2003; or intersexual ontogenetic conflict; Rice & Chippindale 2001). For example if males have a higher optimal weight than females, alleles increasing weight will be beneficial for males, but detrimental for females, and the other way round for alleles decreasing weight. Alleles whose fitness effects in one sex are negatively related to their fitness effects in the other sex are called sexually antagonistic (SA) alleles (Rice 1992). Sexual antagonism may result not only from sex-specific optima, but also from sex-specific pleiotropy (Rice 1987).

There is increasing experimental evidence that SA genes are common in genomes of a number of species (Forsman 1995; Vieira *et al.* 2000; Chippindale *et al.* 2001; Rice & Chippindale 2001; Gibson *et al.* 2002; Fedorka & Mousseau 2004; Kozielska *et al.* 2004). Theory predicts that SA genes will be especially prone to accumulate on sex chromosomes. Autosomes are present equally in both sexes and therefore autosomal SA genes can increase and then fixate only if the advantage to one sex overcompensate the disadvantage to the other sex (Rice 1984). In contrast, the segregation of sex chromosomes is biased towards one sex (males for Y chromosome and females for X) facilitating the accumulation of SA genes (Rice 1984, 1987). For example, a recessive male-beneficial SA allele on the X chromosome will spread when rare, since it is expressed in hemizygous males, but not in females (where it initially only occurs in heterozygous state). With the increase in frequency of SA alleles, homozygous females will be produced preventing the allele from fixating and polymorphism will be maintained. A similar rationale applies to a dominant female-beneficial allele, since it will be initially present (and expressed) two times as often in females as in males, leading to its spread when rare (for details see Rice 1984). These theoretical results are supported by the profound sexually antagonistic variation present on the *Drosophila* X chromosome (Gibson *et al.* 2002).

Additionally, a chromosome restricted only to one sex (Y in XY system and W in ZW system) is expected to accumulate SA alleles beneficial to the sex they are present in, even if they are potentially detrimental if expressed in the other sex (e.g. Rice 1996a). This has been confirmed by artificial selection in *Drosophila*, where autosomes were artificially made to segregate in one sex. After a number of generations this sex had higher fitness than controls, but when the autosomes were expressed in the other sex, it resulted in lower fitness (Rice 1992, 1998).

Sex chromosomes not only facilitate the accumulation of SA genes, but their evolution is believed to be strongly influenced by the SA genes themselves. Suppression of recombination is expected to evolve between the sex determining factor and SA genes with alleles beneficial to the heterozygous sex, leading to a gradual increase of Y-(or W-) specific regions and eventually degradation of this chromosome (Charlesworth 1991; Rice 1996a; Charlesworth *et al.* 2005).

Sex determining mechanisms strongly influence the pattern of SA variation that can accumulate, but can SA variation also lead to changes in the sex determining system? Although this idea was already put forward two decades ago (Rice 1986), only very few studies have so far investigated it (Rice 1986; van Doorn & Kirkpatrick 2007). Rice (1986) showed that linkage with a SA locus may lead to the spread of a new sex determining factor and a switch from polygenic sex determination to a one-locus SD system. Van Doorn and Kirkpatric (2007) showed that SA variation on autosomes can facilitate the spread of a new (autosomal) sex determining factor, leading to a change from an XY system to an autosomal system. Both of these studies focused on the scenario in which a new SD factor was linked to a SA locus. They also allowed for only one SA allele per locus.

We take a different approach and concentrate on the case where a new SD factor is not linked with fitness affecting genes. We start with an XY system and let the sex chromosomes accumulate SA variation introduced by mutation. Then we introduce a new autosomal male or female sex determining factor and investigate whether it can spread in the population. For completeness, we also investigate how linkage with a SA locus influences the chance for a new SD factor to spread.

Since it is known that the dominance of SA alleles strongly influences their chance to spread (Rice 1984), we also investigate the effect of dominance of SA alleles on the outcome of the evolutionary dynamics of the SD system. Additionally, we study the effect of sex chromosome differentiation on the dynamics of the system. We consider undifferentiated chromosomes with a SA locus present on both X and Y (Rice 1987; van Doorn & Kirkpatrick 2007), and differentiated chromosomes with a SA locus present only on the X chromosome (Rice 1984; Charlesworth *et al.* 1987).

The model

Sex determination: Since the number of different sex determining (SD) mechanisms seems limitless (Bull 1983), we decided to base our model on a relatively generic sex determining (SD) mode. We consider a sex determining system consisting of three independent gene loci (on three different chromosomes), each locus having two alleles. The first locus corresponds to the standard XY sex determining system with two basic alleles: a male-determining *Y* allele and a sex-neutral *X* allele. We will refer to the chromosomes possessing these alleles as the X and the Y chromosome, respectively, or together as sex chromosomes. The second locus harbours a male-determining *M* allele and a standard *m* allele. The third locus has a female-determining *F* allele and a standard *f* allele. The *F* allele is dominant over *M*, meaning that the presence of *F* always leads to female development, even if both *Y* and *M* are present in homozygous state. If *F* is absent, but at least one male-determining factor, either *Y* or *M*, is present in the genotype, individuals become males, otherwise (no *Y* or *M*) they become females. We arbitrary start with the XY system, as most of the studies on SA variation have been done in species with male heterogamety (Rice & Chippindale 2001; Fedorka & Mousseau 2004). The results of the model will also apply to the ZW systems, assuming that SD factors have opposite effect on sexual differentiation.

Sexually antagonistic (SA) genes: On each chromosome there is also a tightly linked locus that can potentially accumulate sexually antagonistic alleles. We will assume that there is no recombination between the SA gene and the sex determining locus on a given chromosome. For simplicity, we also assume that SA genes directly affect viability and that the positive effect on male viability is equal to the negative effect on female viability. We assume that the value of an allele corresponds to viability in males, meaning that if an allele is expressed in males their fitness is equal to the value of the allele. If an allele is expressed in females, their fitness equals one minus the value of the alleles.

We consider four different dominance scenarios for SA alleles (Table 5.1). a) There is no dominance and viability is dependent on the average of the allele values. b) Male-favouring alleles are dominant, meaning that the allele with the higher value is expressed. c) Female-favouring alleles are dominant, meaning that the allele with the lower value is expressed; d) There is a sex specific dominance, meaning that the allele conferring the higher fitness for a given sex is expressed. This scenario may be interpreted as sex specific expression or sex specific pleiotropy, for example, the situation where the SA alleles interact with both male- and female-specific hormones and a better interaction with male-specific hormones in males results in a worse interaction with female-specific hormones in females. Table 5.1 shows some examples of the fitness of males and females with different genotypes under different dominance scenarios.

Table 5.1. Illustration of the sex-dependent viability effects of different genotypes under four dominance scenarios. For a given genotype male and female viabilities are shown. Here we assume that the values of sexually antagonistic alleles are x_1=0.9 and x_2=0.3. Alleles are encoded by the viability effect they have in males homozygous for this allele. The viability of homozygous females equals one minus the allele values. The viability of heterozygous individuals depends on the dominance scenario for the SA gene (see the model section for details).

	Genotypes					
	x_1x_1		x_1x_2		x_2x_2	
Dominance scenario	male	female	male	female	male	female
1. No dominance (additivity)	0.9	0.1	0.6	0.4	0.3	0.7
2. Male-beneficial alleles dominant	0.9	0.1	0.9	0.1	0.3	0.7
3. Female-beneficial alleles dominant	0.9	0.1	0.3	0.7	0.3	0.7
4. Sex specific dominance	0.9	0.1	0.9	0.7	0.3	0.7

In our model SA alleles can take any value between zero (maximizing female viability) and one (maximizing male viability). At the beginning of the simulation SA genes start at the value of 0.5 (the same fitness in males and females) and every generation with the chance of 0.01 an allele at each locus can mutate. Mutation adds a value from a normal distribution with mean zero and standard deviation 0.005 to the value of the allele. Genes located on different chromosomes act multiplicatively.

X and Y chromosome differentiation: We look at two scenarios for the differentiation between sex chromosomes and the location of SA genes on X and Y chromosomes. First, we assume that there is little differentiation between X and Y and that the SA locus is common for both chromosomes. Accordingly, both males and females are diploid at the SA loci (Rice 1987; van Doorn & Kirkpatrick 2007). Second, we assume strong differentiation between the sex chromosomes, i.e. the Y is degenerated and does not possess SA genes or it is even absent. Therefore, SA genes are located only on the X chromosome (Rice 1984; Charlesworth *et al.* 1987). XX individuals have two alleles and their fitness depends on the dominance scenario (Table 5.1), and XY individuals are hemizygous and the SA allele is always expressed.

Simulation: We use individual-based simulations to model the evolution of the sex determining system. We assume discrete non-overlapping generations and a fixed population size of N = 10,000 diploid individuals.We started each simulation with the standard XY system (all females are *XX*; *mm*; *ff* and all males: *XY*; *mm*; *ff*). SA genes located on each chromosome started with the value of 0.5. *N* new individuals were generated each generation using the following algorithm: first we assign one random male to each female in the population and then draw with replacement a female; given her genotype and a genotype of her pre-assigned partner create an offspring genotype by drawing random chromosomes from both parents; let the SA

genes mutate (equivalent to mutations during gametogenesis; see above for details); then based on the offspring genotype on SA loci determine offspring viability; draw a random number between zero and one to decide whether an offspring actually survives; if it does not (random value above viability value) draw a new mother and start again, otherwise continue: determine the offspring's sex based on its genotype on sex determining loci; add the offspring to the next generation; repeat until *N* new individuals have been created. Simulations are run for sufficiently many generations until equilibrium values of the SA genes appear to have been reached. At this point an *M* allele is introduced in males at the frequency of 0.05. Simulations are run until a new equilibrium is reached and then *F* is introduced in females at the frequency of 0.05. Simulations are run till a new equilibrium is reached. We also examine an alternative scenario in which *F* is introduced before *M*.

For each of the scenarios we look first at the situation where SA genes can evolve only on sex chromosomes, but not on any of the autosomes. We investigate whether autosomal SD factors can invade the system and what the resulting SD mechanism is. Additionally, we compare these results with the situations in which SA genes can evolve on one or both autosomes. For each case we investigate the effect of dominance of SA genes and the order of introducing new SD factors (see above) on the dynamics of the system. For each set of parameters we run 25 duplicate simulations.

Results

We analyze the effect of sex chromosome differentiation, mode of dominance of SA alleles and linkage of new SD factors with SA alleles on the resulting SD system. We categorize the outcomes into five main categories. 1) A new SD factor has a selective disadvantage, does not invade and there is no change in the SD system. 2) A new SD factor always invades, leading to a change in the SD system. For *M* this means that it replaces *Y* (Fig. 5.1A) and for *F* that its frequency in females reaches 0.5 and the SD system switches to female heterogamety (Fig 5.1B). 3) A new SD factor invades in some simulation runs leading to a change in the SD system, but it disappears for others. This suggests a new factor has only a low fitness advantage and it can be lost by drift. 4) A new SD factor appears to be selectively neutral and its frequency seems to be governed by random drift. It can persist in the population for many generations with strong fluctuations in frequency, which may eventually lead to its loss or fixation (Fig 5.1C). In this case the frequency of the factor is highly variable between different simulation runs. 5) A new SD factor invades the population and does not reach fixation, but some intermediate stable frequency. Stable polymorphism is maintained (Fig 5.1D).

A summary of the results for different conditions is given in Table 5.2 and 5.3. In short, switches to new SD systems are easier if SA variation accumulates only on X (differentiated sex chromosomes) and if new SD genes are linked to SA loci.

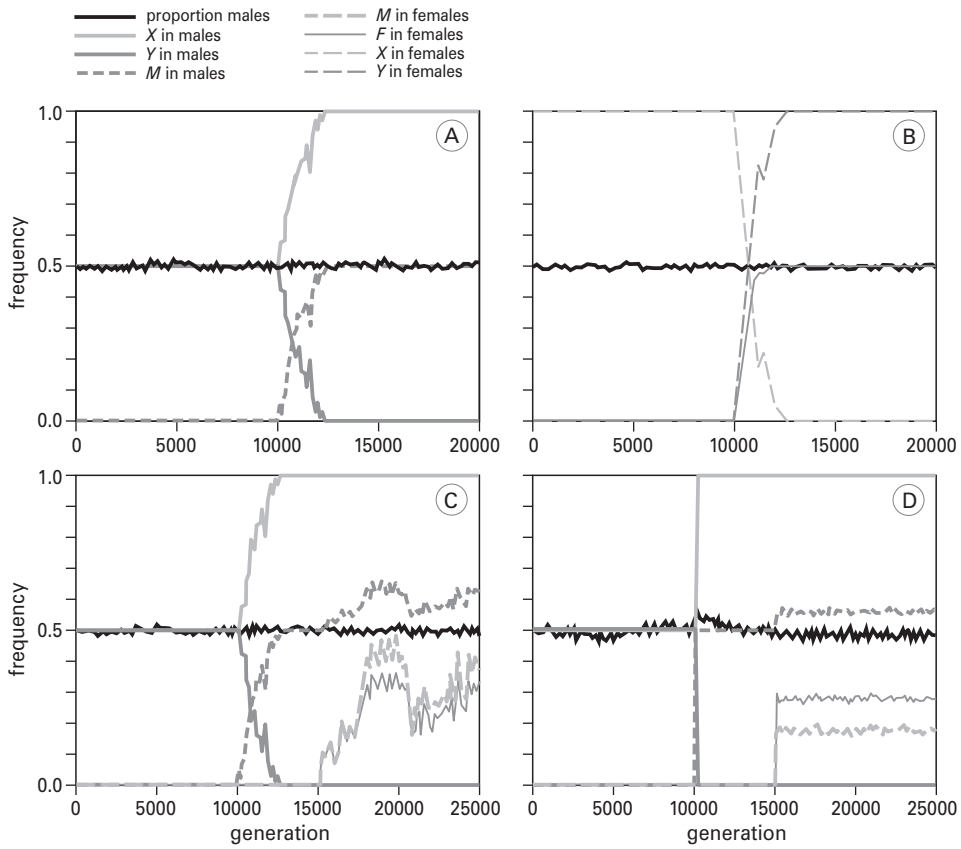

Figure 5.1. Examples of different SD systems evolved after the introduction of new SD factors to a standard XY system. (A) After introduction (generation 10000) *M* invades the population replacing *Y*; the SD system switches from male heterogamety for *Y* to male heterogamety for *M*. (B) *F* invades, leading to a switch to female heterogamety and fixation of *Y*. (C) *F* is neutral when introduced (generation 15000) to the system with male heterogamety for *M* (*M* introduced in generation 15000 replaced *Y*). *F* frequency fluctuates over time and it may be eventually lost or fixate; SD system is in a neutral polymorphism. (D) Protected polymorphism-*F* invades the system with male heterogamety for *M* (*M* introduced in generation 15000 replaces *Y*), but it does not reach fixation. The system polymorphic for *F* and *M* is stable. Results on all panels were obtained with the scenario for differentiated sex chromosomes. The other parameters were as follows: panels A and C – additivity of SA alleles, SA variation only on X chromosome; B – dominance of male-beneficial alleles, SA variation only on X chromosome; D – sex-specific dominance, SA variation on each chromosome.

Below we present some more details on the dynamics of the system for different scenarios. We concentrate on the case in which new SD factors are not linked with a SA locus and only briefly mention other scenarios. We often attempt to explain the observed evolutionary patterns, however, it should be noted that it is often speculative and more detailed analysis is needed to more reliably explain the observed patterns.

Table 5.2. Summary of the results for undifferentiated sex chromosomes (SA locus present both on X and Y chromosome, at homologous locus). The results for the different dominance scenarios are given in the rows, both for the case when *M* was introduced before *F* (*M, F*) and the other way around (*F, M*). In the columns the scenarios for presence of SA genes on different chromosomes are given, as indicated in the upper row: XY – on sex chromosomes; *M* – on autosome with *M/m* locus; *F* – on autosome with *F/f* locus. For each scenario fate of *M* and *F* sex determining factors are given in separate cells and is indicated by different colours: white – a new sex determining factor is selected against; dark grey and white stripes – a new factor invades in less than 50% of cases leading to a switch to a new SD system; light grey – a new factor invades, but there is no full switch to a new system: N – a new factor seems to be neutral, and its frequency is governed by drift; P – protected polymorphism – a new factor reaches a stable frequency. See text and Fig 5.1 for details.

Dominance scenario	Order of introduction	*XY*		*XY,M*		*XY,F*		*XY,M,F*	
		M	*F*	*M*	*F*	*M*	*F*	*M*	*F*
1. No dominance (additivity)	*M,F*								
	F,M								
2. Male-beneficial alleles dominant	*M,F*								
	F,M								
3. Female-beneficial alleles dominant	*M,F*								
	F,M					N		N	
4. Sex specific dominance	*M,F*						P		P
	F,M						P		P

Table 5.3. Summary of the results for differentiated sex chromosomes (SA genes are present only on the X chromosome). The setup and the meaning of the colours is identical to table 5.2 with additional dark grey – a new factor always invades leading to the switch to a new SD system.

Dominance scenario	Order of introduction	*X*		*X,M*		*X,F*		*X,M,F*	
		M	*F*	*M*	*F*	*M*	*F*	*M*	*F*
1. No dominance (additivity)	*M,F*		N						
	F,M	N				N		N	
2. Male-beneficial alleles dominant	*M,F*		N						
	F,M	N		N		N		N	
3. Female-beneficial alleles dominant	*M,F*								
	F,M					N		N	
4. Sex specific dominance	*M,F*		N						P
	F,M					N			

1. Undifferentiated sex chromosomes: SA genes on a homologous locus on both X and Y chromosome. The results are summarised in Table 5.2.

a) Additivity of SA alleles: The value of SA alleles located on the Y chromosome increases to one, maximizing male fitness. The value of alleles located on X decreases to almost zero (maximizing female fitness). Females have higher fitness

and the sex ratio in the population is female biased. However, the masculinizing factor *M* cannot invade since it would lead to the creation of XX males which have very low viability. *F* does not invade either, since it would initially lead to an even more female biased sex ratio and the production of low viability XY females.

b) Dominance of male-beneficial alleles: As above alleles on Y increase to one and alleles on X decrease to zero. However, since male-favouring alleles are dominant, males and females both have maximal fitness and the sex ratio is equal to 0.5. New sex determining factors do not invade, since they would lead to suboptimal, in terms of fitness, genotypes (as above).

c) Dominance of female-beneficial alleles: The value of SA alleles on Y increases to one, but there is great variation of SA alleles on X ranging roughly from 0.2 to 1.0, with an average higher than 0.5 (favourable for males) in both sexes (Fig 5.2). The resulting sex ratio equals 0.5. *F* invades the system only if it is linked with the SA locus which possesses SA variation allowing *F* to be linked with genes beneficial for females. Eventually, alleles linked with *F* evolve towards a value of zero. The frequency of *F* increases to 0.5 in females, but since Y possesses alleles detrimental for females it does not fixate and polymorphism for the X and Y chromosomes is maintained (Fig 5.2). The population sex ratio is equal to 0.5. *M* never has a fitness advantage, but can be neutral if *F* is present in the population.

d) Sex specific dominance of SA alleles: In this scenario the allele beneficial for a given sex is expressed. SA alleles on X evolve towards zero and the alleles on Y towards values of one, leading to maximal viability in both sexes and a 1:1 sex ratio. Since Y strongly increases male fitness *M* can never replace it. *F* invades if it is linked with a SA locus, but a full switch to female heterogamety and fixation of Y chromosome is impossible since it has accumulated alleles detrimental to females. As a result a polymorphic system for both X and Y, and *F* and *f* is stable (Fig 5.3).

2. Differentiated sex chromosomes: Under this scenario SA alleles are present only on the X chromosome. Table 5.3 shows a summary of the results.

a) Additivity of SA alleles: As predicted by theory (Rice 1984) not much SA variation accumulates on the X chromosome and the average value of SA alleles in both males and females and the population sex ratio equals 0.5. *M* can invade the system and can replace *Y* (fig 5.1A), but positive selection for *M* seems to be weak. This may be caused by the fact that the presence of *M* decreases variation in male fitness. Invasion is facilitated by linkage of SA genes with *M*. Then *M* replaces *Y* and accumulates SA alleles favourable for males and *m* accumulates SA alleles favourable for females. The sex ratio becomes female biased. If *F* is introduced after *M*, it is favoured if it is linked with the SA locus, but *M* is not. If *F* is introduced first it can spread even without linkage with SA genes. When *F* invades, the SD system switches to female heterogamety, with either *M* or *Y* fixating in the population (depending on which one was already present).

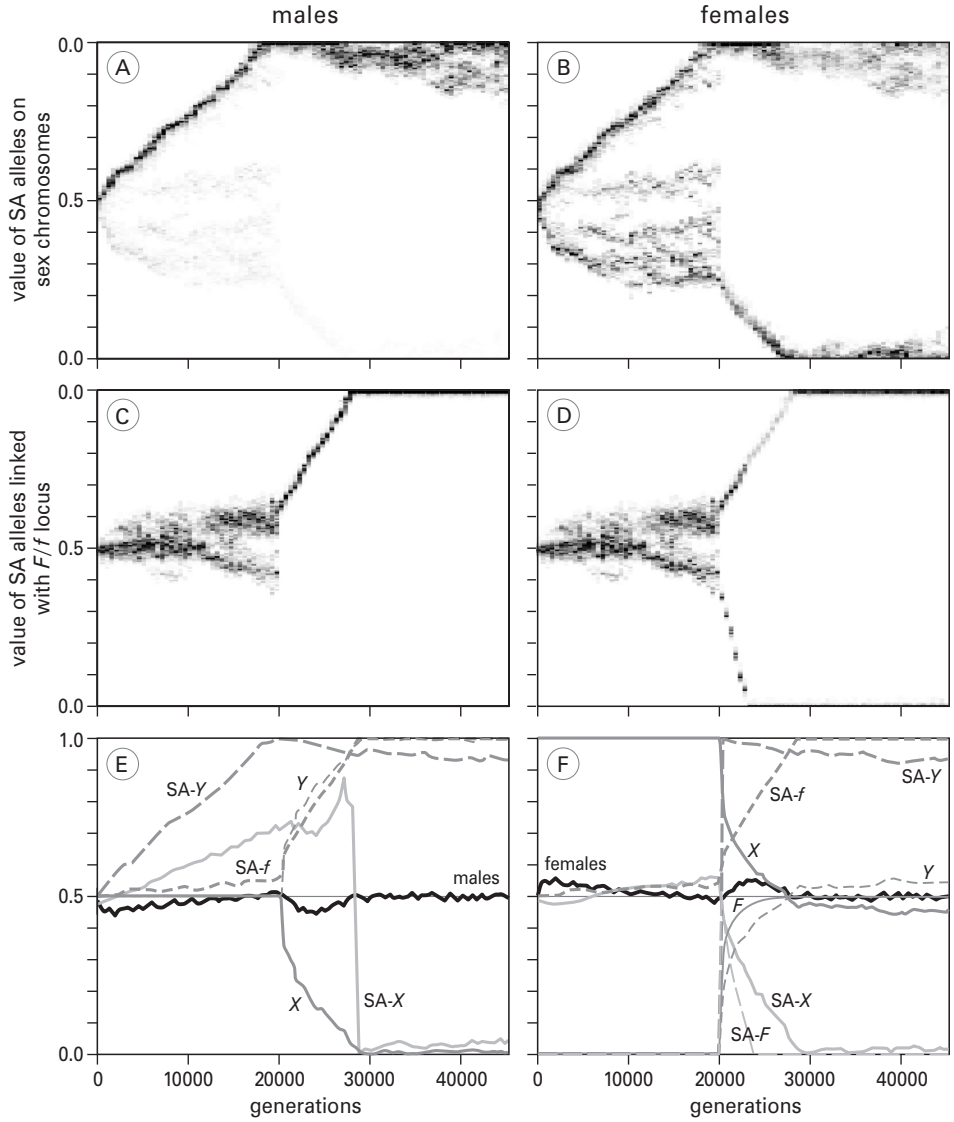

Figure 5.2. Dynamics of the SD system and alleles on SA loci for the scenario with undifferentiated sex chromosomes and female-beneficial alleles dominant. The panels on the left concern males and those on the right females. (A) and (B): distribution of SA alleles on sex chromosomes. The higher the frequency of the allele with a given value the darker the point. In males the SA alleles located on the Y chromosome have the highest value. (C) and (D): distribution of SA alleles on locus linked with *f/F* locus. (E) and (F). Sex ratio, frequency of different SD factors and average value of SA alleles in males and females, respectively. SA-Y denotes SA alleles linked wth Y, etc. Initially a standard XY system is present, *F* is introduced at generation 20000 and spreads leading to a female heterogametic system with polymorphism for X and Y chromosome. Polymorphism on the X linked SA locus decreases and the average allele value is almost zero. The value of the SA alleles linked with *F* decreases to zero and the value of SA alleles linked with *f* increases to 1.

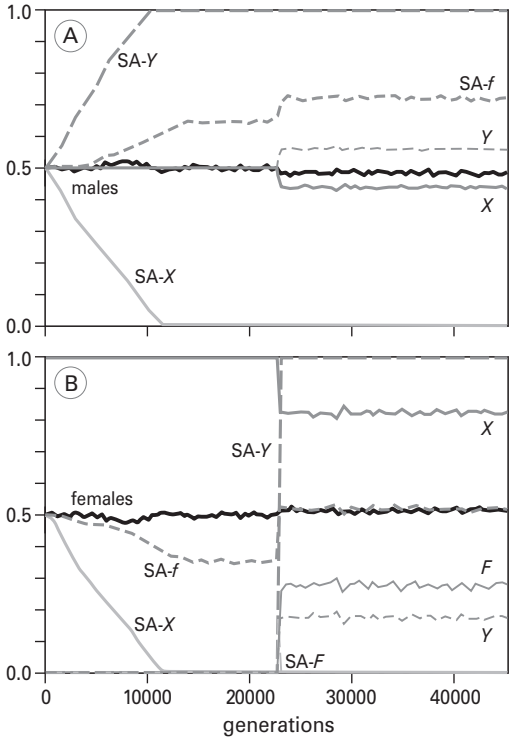

Figure 5.3. Dynamics of the SD system and average value of SA loci for the scenario with undifferentiated sex chromosomes and sex-specific dominance of SA alleles. Shown are sex ratio, frequency of different SD factors and average value of SA alleles in males (**A**) and females (**B**). Initially a standard XY system is present, the SA alleles located on the X chromosome decrease in value to zero and the alleles located on Y increase to one. *F* is introduced at generation 20000 and increases in frequency, but does not spread to fixation. A system polymorphic for two SA loci is maintained: *X* and *Y*, and *F* and *f*.

b) Dominance of male-beneficial alleles: As expected (Rice 1984) there is very little variation of the SA locus on the X chromosome. *M* always invades since homozygosity for X in males increases their fitness, due to the higher chance of the expression of more favourable alleles, especially since in XY system average the value of SA alleles evolves towards a female optimum. For that reason *F* can also invade if it is introduced in the system without *M*, since hemizygous XY females will have higher fitness than XX females in which there is a higher chance that male-beneficial alleles will be expressed.

c) Dominance of female-beneficial alleles: The X chromosome accumulates SA variation (similar to situation in 1c; Fig 5.2A and 5.2B), since both dominant female-beneficial alleles as well as recessive male-beneficial ones can spread (Rice 1984). *M* never invades since homozygosity for X in males is not beneficial due to the increased chance that female-beneficial alleles will be expressed. *F* can invade only if

it can accumulate SA alleles, leading to a switch to female heterogamety and fixation of Y. *F* accumulates female-beneficial and *f* - male-beneficial alleles.

d) Sex specific dominance of SA alleles: As above the X chromosome accumulates SA variation. However, now *M* always invades if it is introduced first, since homozygous XX males (out of two alleles the more beneficial one is expressed) have higher fitness than XY males (also female-beneficial, male-harmful alleles are expressed). *F* is favoured only if it can accumulate female-beneficial alleles. However, it does not fixate if *M* has already accumulated male-beneficial alleles. In that case polymorphism on both the *M/m* and *F/f* locus is maintained (Fig. 5.1D).

Discussion

We showed that sexually antagonistic variation on sex chromosomes may facilitate the spread of new sex determining factors and the switch to a new sex determining system, even if new SD factors are not linked with SA genes. However, this is only the case if sex chromosomes are differentiated and SA genes located only on the X chromosome. In most cases the presence of SA alleles on autosomes in close linkage to a new SD factor facilitates the switch to a new SD system.

Previously, Van Doorn & Kirkpatrick (2007) made an analytical model to investigate how SA variation on sex chromosomes and autosomes influences the invasion of a new autosomal male-determining factor. Their model corresponds to our scenario with undifferentiated sex chromosomes and invasion of *M*, although they considered only one SA allele at each locus. They showed that, all else being equal, SA variation on autosomes facilitates, but SA variation on sex chromosomes hampers the spread of a new SD factor. This is consistent with our results, where sex chromosomes accumulate SA variation, but variation on autosomes is absent or low, and the *M* factor does not invade. However, when sex chromosomes are differentiated and SA genes located only on X, conditions for invasion of new autosomal SD genes are much less restrictive, although still presence of SA variation on autosomes helps new SD factors with establishing in the population. We do not expect much variation of SA genes on autosomes, since alleles having a net advantage averaged over both sexes should spread to fixation (Rice 1984). However, some SA variation can be maintained by mutation (as we saw in our simulations), migration, frequency-dependent selection or be transient during the process of fixation of new alleles (Rice & Chippindale 2001; van Doorn & Kirkpatrick 2007).

It has to be noted that in our model we assumed that the lack of either an X or Y chromosome does not have any negative fitness effects (except for the ones potentially caused by SA alleles). This may be true when sex chromosomes are not yet strongly differentiated. However, differentiation of sex chromosomes may on the one hand lead to degeneration of the Y chromosome and the presence of vital genes only on the X chromosome (Charlesworth 1996), or on the other hand, genes necessary

for male fertility may accumulate on the Y chromosome (Roldan & Gomendio 1999). Differentiation of sex chromosomes may also lead to the evolution of dosage compensation (Charlesworth 1996) and result in the unviability of individuals with novel genotypes (Schütt & Nöthiger 2000). All of these processes may lead to lethality of YY individuals or sterility of XX males and hamper the change of the sex determining system. Therefore, some differentiation of sex chromosomes facilitates changes in SD systems (compare Tables 5.2 and 5.3), but stronger differentiation may in turn prevent them. However, there are species that have morphologically differentiated chromosomes, but the fitness of males and females with unusual genotypes is not lower (Bull 1983; Dübendorfer *et al.* 2002) and SD systems in those species might be especially prone to changes. One could even expect cycles of co-evolution between SA genes and sex chromosomes: the chromosome with the SD factor accumulates SA alleles, which favours reduced recombination and differentiation of sex chromosomes (Rice 1996a; Charlesworth *et al.* 2005). This facilitates the accumulation of SA variation on the X chromosome (Rice 1984), which in turn may lead to the invasion of a new SD factor and the beginning of a new cycle of sex chromosome evolution.

We showed that the mode of expression of SA genes has a profound effect on the fate of new SD factors (Table 5.2 and 5.3). When both the X and Y chromosome possess an SA locus, the condition promoting the highest variation (dominance of female-beneficial alleles) allows the invasion of new SD factors (although only if they are also linked with the SA locus; Table 5.2). The opposite effect is seen in cases where only the X chromosome possesses an SA locus, under dominance of female-beneficial alleles, conditions for the invasion of new SD factors are more restrictive than for other modes of allele dominance (Table 5.3).

Not much is known about the expression of SA genes in nature, but it seems that some of them are at least partly dominant, since their effect can be detected in heterozygous females (Gibson *et al.* 2002). There is no *a priori* reason to assume that all genes show the same pattern of dominance. It is often believed that new deleterious mutations are recessive, but some of them may be also at least partly dominant (Oliver & Parisi 2004). However, SA alleles by definition are deleterious for one sex, and we can imagine that both male- and female- beneficial mutations can be dominant. SA variation seems to be most easily maintained if female-beneficial alleles are dominant and male-beneficial alleles are recessive (Rice 1984). However, polymorphism at SA loci should also be present if alleles have different patterns of dominance, either only transiently during the process of replacement of one SA allele by another (Rice & Chippindale 2001) or when maintained by migration and mutation (van Doorn & Kirkpatrick 2007). Little is known about the patterns of dominance of SA alleles and more empirical research is necessary to estimate what is (if any) the most common pattern for SA genes dominance.

PART II

Empirical data

CHAPTER 6

Evolutionary Ecology Research, submitted

Temperature and the geographical distribution of sex determining mechanisms in the housefly

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Leo W. Beukeboom
Ido Pen

Abstract

Question: Does temperature variation explain the global geographical distribution of sex determining mechanisms (SDM) in the housefly (*Musca domestica*)?

Data studied: SDM frequencies of houseflies collected in various African populations and similar data from the literature.

Results: Housefly populations on the southern hemisphere repeat the pattern earlier found on the northern hemisphere: higher frequencies of non-XY SDM closer to the equator. Statistical analysis suggests that temperature is a better predictor of SDM clines than latitude per se.

Introduction

Sex determining mechanisms vary considerably across taxa and seem to evolve quite rapidly, for reasons that are still poorly understood (Bull 1983, 1985; Marin & Baker 1998; Werren & Beukeboom 1998; Kraak & Pen 2002). However, the vast majority of variation occurs above the species level. Since the housefly (*Musca domestica*) harbors several different sex determining mechanisms it is a particularly interesting model species for studying sex determination. All individual houseflies possess a female determining factor (the *F* factor) which turns on the female developmental pathway, unless a so-called *M* factor is also present and blocks the action of *F*, thus triggering developmental into a male. In "standard" males, the *M* factor is located on the Y chromosome (Dübendorfer *et al.* 1992), but *M* factors can also be located on any of the five autosomes or even on the X chromosome (Table 6.1) (Denholm *et al.* 1983; Dübendorfer *et al.* 2002).

Table 6.1. Relation between genotype and sexual phenotype in the housefly. The female determining factors (F/F^D) are located on chromosome IV; the male determining factors (*M*) can be located on any chromosome. + = wildtype state (no *M*); • = the same phenotype will develop irrespective of the presence or absence of *M*.

Autosomes		Sex chromosomes	
IV	I–V	XX	XY
F/F	+/+	♀	♂
F/F	•/*M*	♂	♂
F/F^D	•/•	♀	♀

In populations where autosomal *M* factors are prevalent, the Y chromosome is often absent and males are either XX or sometimes XO (Denholm *et al.* 1985; Denholm *et al.* 1990; Çakir & Kence 2000). In some populations males may be homozygous for an autosomal *M* factor or possess multiple *M* factors on different autosomes. In such populations, females often possess a special dominant version of the *F* factor, designated F^D, which is not blocked by *M* factors (Tomita & Wada 1989b; Hilfiker-Kleiner *et al.* 1993).

Interestingly, the geographical distribution of different sex determining systems in the housefly appears to be far from random but shows clear latitudinal and altitudinal clines. The first evidence for this was reported by Franco *et al.* (1982), who examined houseflies from 53 localities in Europe from Denmark in the north to Sicily in the south and discovered that frequencies of autosomal *M* factors increased towards the south and decreased with higher altitude. Additional studies from England (Denholm *et al.* 1985), Japan (Tomita & Wada 1989b), Turkey (Çakir & Kence 1996) and the United States (Hamm *et al.* 2005) showed similar patterns with

XY males in the north or at high altitudes and males with autosomal *M* factors dominating in the south or at lower altitudes. It is not entirely clear whether these clines represent stable distributions or whether they are a transient phenomenon. Some authors argued for the latter because before 1948 no study on the housefly revealed any other system than the standard XY system (Franco *et al.* 1982; Denholm *et al.* 1985; Tomita & Wada 1989b; Çakir & Kence 1996).

We have evidence (Kozielska *et al.*, in press) that frequencies of autosomal *M* factors have not changed much for several decades in Europe. This is not entirely unexpected, since recent theoretical models have shown that multiple *M* factors may stably coexistence (Kozielska *et al.* 2006). However, it is still unclear why the clines exist in the first place. Some authors have suggested that autosomal *M* factors "hitchhike" with insecticide resistant genes (Kerr 1970; Franco *et al.* 1982; Tomita & Wada 1989b), but more recent studies did not produce supporting evidence for this (Shono & Scott 1990; Hamm *et al.* 2005). Obviously, any factor which shows pronounced clinal variation could in principle be involved in causing the clinal distribution of sex determining mechanisms. The most obvious factor which varies predictably with both latitude and altitude is temperature, and it has been invoked as a possible explanation by several authors (Franco *et al.* 1982; Çakir & Kence 1996).

No systematic quantitative analysis has yet been performed to investigate to what extent variation in temperature can explain the distribution of sex determining mechanisms in the housefly. In this paper, we present such an analysis, based on previously published data and on newly collected data. All previous studies of geographical distributions of sex determining mechanisms in the housefly have been carried out on populations in the northern hemisphere. If temperature is an important determinant of these distributions, we would expect to find the opposite pattern in the southern hemisphere, i.e. relatively more autosomal *M* factors in the north than in the south. To test this prediction we collected houseflies from several subequatorial populations in Africa and examined them for the presence of autosomal *M* factors and F^D factors. In addition, we collected temperature data for all housefly population studies in the literature that contain suitable data and tested statistically whether temperature or latitude could explain the observed clinal distribution. We discuss several causal hypotheses for an effect of temperature on sex determination in the housefly.

Materials and methods

Sampling and analyses of African housefly populations

We collected houseflies at farms, horse stables and markets at five locations in Tanzania and six locations in South Africa. At every location, approximately 100 adult flies were caught with a sweeping net and stored in boxes supplied with water, milk powder and egg-laying medium (according to the protocol of Hilfiker-Kleiner *et*

al. 1994). For transport from Africa to our laboratory in the Netherlands, of every sampling location 150-200 larvae were stored in 50ml tubes that contained medium. In the laboratory, larvae, flies and eggs were grown under conditions as described by Hilfiker-Kleiner *et al.* (1994) with the following modifications of their protocol: ambient temperature was set at 20ºC, relative humidity at 60% and flies were kept under constant light.

For each sampling location 15 males were crossed with virgin females from a mutant strain recessive for visible traits on each autosome (*ali curly* (*ac*) on linkage group I; *aristapedia* (*ar*) on II; *brown body* (*bwb*) on III; *yellow eyes* (*ye*) on IV; *snip wings* (*snp*) on V). Since mutant females have the standard *F* factor, they only get sons when crossed with males homozygous for an *M* factor, and mixed-sex offspring when crossed with males heterozygous for *M* factors. Thus, by inspecting the F1 sex ratio of each male, we could estimate the frequency of homozygous males. For 10 out of the 15 males for each location, we selected 3 male F1 offspring and crossed each of them with a mutant virgin female to determine on what chromosomes male-determining *M* factors were located (see Franco *et al.*, 1982 for a more detailed description of this technique).

To determine whether females were carrier of a dominant female-determining factor F^D, for each sampling location up to 15 females were crossed with males of a laboratory strain that were homozygous for an autosomal *M* factor. Female offspring of such crosses necessarily carried an F^D factor, since F^D overrides the male determining effects of up to three simultaneously present *M* factors (McDonald *et al.* 1978; Franco *et al*. 1982).

Compilation of published studies

We compiled relative frequencies of males with autosomal *M* factors and females with F^D from four additional published studies (see Table 6.2 and Fig. 6.1). These studies used either cytological techniques to determine the presence/absence of the Y chromosome, or used crosses similar to those described above. In the cytological studies (Denholm *et al*. 1985; Çakir & Kence 1996), autosomal *M* factors were inferred from the absence of Y chromosomes. This procedure can obviously underestimate frequencies of autosomal *M* factors, since males with Y chromosomes can also have autosomal *M* factors. For the studies relying on crosses (Tomita & Wada 1989b; Hamm *et al.* 2005), we also regarded males to be "autosomal" only in the absence of a Y chromosome, in order to make these studies comparable to the cytological studies.

Sources of temperature and latitude data

For each study location, estimates of average daily minimum and maximum temperatures were obtained from WORLDCLIM (www.worldclim.org, see Hijmans *et al.* 2005), which provides global estimates at a spatial resolution of one square kilometer. In the statistical analysis, we used "average temperature" as the mean of

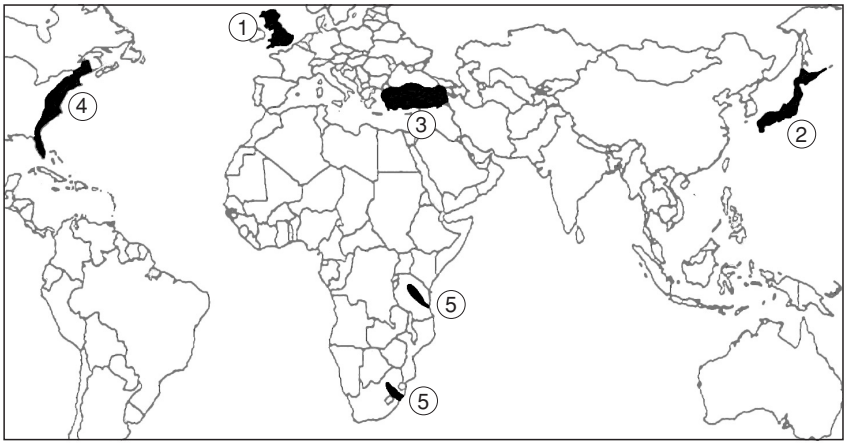

Figure 6.1. Geographical locations and references (see Table 6.2) of housefly studies that were used in the analysis.

Table 6.2. Studies of the geographical distribution of housefly sex determining mechanisms used in our pooled analyses.

Study	# locations	# males	# females
1. Denholm *et al*. 1985; UK	6	430	-
2. Tomita and Wada 1989b; Japan	18	1105	739
3. Çakir and Kence 1996; Turkey	34	1050	-
4. Hamm *et al*. 2005; USA	4	308	-
5. This study; Africa	11	99	126

minimum and maximum temperature. In our study of African houseflies, we used GPS to estimate the latitude of the study locations. For the published studies, latitudes were either explicitly provided in the original study (Turkey: Çakir and Kence 1996; USA: Hamm *et al.* 2005), or we estimated latitude based on the description of the sampling location provided in the original study (Japan: Tomita and Wada 1989b; UK: Denholm *et al.* 1985).

Statistical analysis

Relative frequencies of autosomal males and F^D females were modeled as proportions with mixed model logistic regression in R (R Development Core Team 2006), using the lme4 procedure with the "family=binomial" option (Bates 2005). We used a likelihood-ratio approach to judge significance of model variables, using F tests to correct for overdispersion (Krackow & Tkadlec 2001). Specifically, we calculated the quantity $F = (\Delta\text{dev} / \Delta\text{DF})/(\text{dev/DF})$, where dev denotes the deviance of the final

model (i.e. including the tested variable), DF the residual degrees of freedom of the final model, Δdev the change in deviance due the tested variable, and ΔDF the degrees of freedom in the tested variable. *F* was then compared to an F-distribution with ΔDF and DF degrees of freedom in the numerator and denominator, respectively. For our African dataset we used "country" as a random effect, as we collected the data in two different countries. Similarly, we used "study" as a random effect in the analysis of the pooled data. This is a somewhat conservative approach, since both country and study were correlated with latitude and temperature, whose potential effects might therefore be partly obscured by the random effects. Nevertheless, we felt the inclusion of the random effects would strengthen our confidence in the significance of any additional explanatory power of latitude and temperature. Since the latter two variables were correlated themselves, the order in which they were entered in the models affected their significance. We therefore show results for both orderings. For example, if latitude is significant in a model without temperature, we then proceed to add temperature to the model in order to see if temperature can explain any additional variation. Conversely, if temperature is significant in a model without latitude, we add latitude to judge its ability to explain residual variation.

Results

New African data

In Tanzania, in three out of five sampled populations, all males had autosomal *M* factors and no Y chromosome, while in the remaining two populations 80% of the males had autosomal *M* factors. The autosomal *M* factors were always located on chromosome 2. In South Africa, the overall frequency of males with autosomal *M* factors was about the same as in Tanzania (see Table 6.3), but the *M* factors were found on all chromosomes except chromosome 4. However, males from Tanzania were significantly more often homozygous for *M* factors than males from South Africa (TZ: 62%; SA 26%; logistic regression: $P = 0.02$). Females with F^D were found in all populations. However in South Africa the frequency of F^D was significantly lower (Table 6.3) than in Tanzania, where all females seem to carry the F^D factor in all but one population. After controlling for country in the analysis, latitude had no significant effect on the frequency of autosomal males, or on the frequency of F^D in females. Temperature did not have a significant effect either on the frequencies of autosomal *M* and F^D , although it seemed to explain more variation than latitude (Table 6.4).

Analysis including data from previous studies

The analysis of the combined data is presented in Table 6.5 (see also Fig. 6.2). After controlling for study, both latitude and temperature had a highly significant effect on the frequency of autosomal *M* factors, when both variables were entered separately.

Table 6.3. Frequencies of males with autosomal *M* factors and females with F^D in Tanzanian (TZ) and South African (SA) sampling locations. Temp = average yearly temperature at the sample locations; Chrom *M* = chromosomes on which *M* factors were found; %Auto M = percentage of males carrying the *M* factor explicitly on the autosomes (all other males had *M* on the Y chromosome but also on an autosome); % F^D = percentage of females carrying the F^D factor; n = number of individuals tested.

Location	Latitude	Temp (ºC)	Chrom *M*	% Auto *M* (n)	% F^D (n)
TZ,					
Same	4.07	23.1	II	100 (10)	100 (13)
Moshi	3.33	21.4	II	100 (10)	100 (11)
Makuiuny	3.55	21.2	II,Y	80 (10)	100 (13)
Arusha	3.37	17.4	II	100 (10)	100 (14)
Karatu	3.34	13.3	II,Y	80 (10)	85 (13)
SA,					
Zinkwazi Beach	29.28	20.8	II,III	100 (9)	29 (7)
Umhlali	29.45	20.2	I,II,III,V	100 (10)	79 (14)
Hammarsdale	29.79	18.4	II,III	100 (9)	92 (13)
Ashburton	29.63	16.3	I,II,III	100 (5)	13 (8)
Mooi River	29.18	15.3	II,III	100 (6)	29 (7)
Warden	27.87	14.4	III,Y	70 (10)	15 (13)

Table 6.4. Mixed-model logistic regression analysis of autosomal *M* frequencies (Males) and F^D frequencies (Females) in African houseflies. Lat = latitude, Temp = yearly average temperature.

Model	DF	Δ DF	Deviance	*F*	P
Males					
Null model (intercept)			18.35		
Country (random)	9	1	18.35	0.00	>0.5
Country + Lat	8	1	18.11	0.12	>0.5
Country + Temp	8	1	13.51	3.22	0.11
Females					
Null model (intercept)			80.24		
Country (random)	9	1	44.83	7.11	0.026
Country + Lat	8	1	39.55	1.07	0.33
Country + Temp	8	1	27.62	3.46	0.10

(Regression coefficients for "Males" analysis: Lat = 0.014, Temp = 0.284; "Females" analysis: Lat = -0.154, Temp = 0.524)

Table 6.5. Mixed-model logistic regression analysis of autosomal *M* frequencies (Males) and F^D frequencies (Females) in pooled data. Lat = latitude, Temp = yearly average temperature.

Model	DF	Δ DF	Deviance	*F*	P
Males					
Null model (intercept)	72		1582		
Study (random)	71	1	1405	8.95	0.0038
Study + Lat	70	1	1056	23.13	<0.0001
Study + Temp	70	1	981.5	30.20	<0.0001
Study + Lat + Temp	69	1	934.3	8.99	0.0038
Study + Temp + Lat	69	1	934.3	3.49	0.066
Females					
Null model (intercept)			679.6		
Study (random)	26	1	549.7	6.14	0.020
Study + Lat	25	1	485.1	3.33	0.090
Study + Temp	25	1	476.4	3.85	0.061
Study + Lat + Temp	24	1	472.4	0.65	0.43
Study + Temp + Lat	24	1	472.4	0.20	>0.5

(Regression coefficients for the complete model of "Males" analysis: Lat = -0.262, Temp = 0.005; "Females" analysis: Lat = 0.018, Temp = 0.274)

However, temperature appears to be a better predictor than latitude, because when temperature was added to the model with latitude, temperature again explained a highly significant part of the variation in autosomal *M* frequency, while adding latitude to a model including temperature only marginally improved the fit of the model. After controlling for study, latitude and temperature both had a marginally significant effect on the frequency of F^D in females, although temperature appeared to be a slightly better predictor.

Discussion

This study set out to address two main questions regarding the geographical distribution of sex determining factors in the housefly. The first question was whether the increasing frequency of autosomal *M* factors towards the equator on the northern hemisphere would be matched by a similar pattern on the southern hemisphere. The second question was whether variation in temperature is a better predictor for the observed clines than latitude per se. Our results show that the answers to both questions are affirmative.

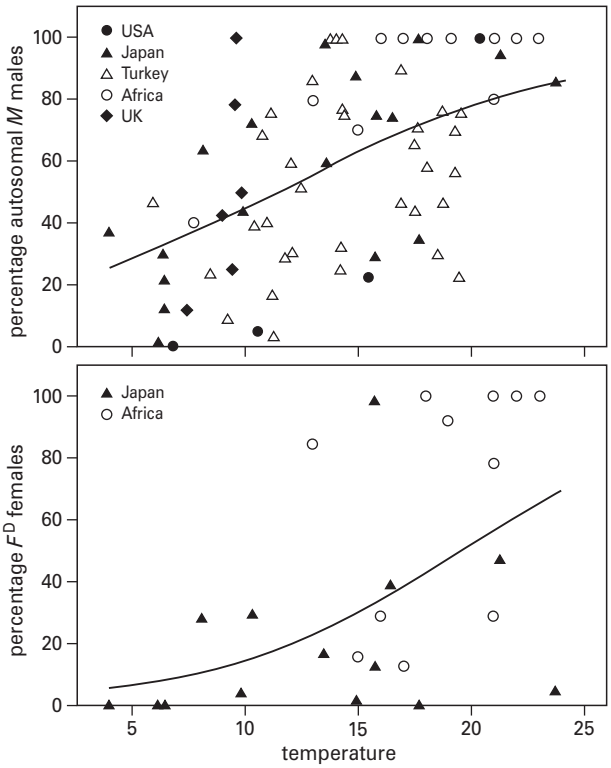

Figure 6.2. Relationship between average temperature and percentage of males with autosomal *M* factors (top) and percentage of females with F^D factors (bottom). The curves were fitted by means of logistic regression with temperature as sole fixed factor.

Unlike the studies of European, North American and Asian housefly populations, where males without autosomal *M* factors are common, we did not find a single male without at least one autosomal *M* factor in the Tanzanian and South-African populations, and the frequency of Y chromosomes was very low (Table 6.3). Nevertheless, the frequency of males homozygous for autosomal *M* factors was considerably higher in Tanzania than South-Africa, indicating that in African populations autosomal *M* factors are more frequent towards the equator, just like they are in populations on the northern hemisphere. Similarly, the frequency of F^D factors in females was much higher in Tanzania than in South-Africa (Table 6.3).

After controlling for random differences between studies, we found temperature to be a consistently better predictor of variation in sex determining factors than latitude per se (Tables 6.4 and 6.5). Indeed, even after controlling for latitude, temperature explains a significant portion of the residual variation in the frequency of males with autosomal *M* factors as well as of females with F^D factors, but the converse is not true.

Although we have obviously not established a causal link between temperature and the variability of sex determining factors in houseflies, we will briefly discuss a few candidate mechanisms for causal effects of temperature on the spread of autosomal *M* factors.

It is conceivable that *M* changes chromosomes via translocation e.g. via transposable elements. This mechanism seems quite plausible since it has been demonstrated in the scuttle fly *Megaselia scalaris*, where the *M* factor resides within a transposable element (Traut & Willhoeft 1990). Theoretical models show that the fixation probability of transposable elements in a population not only depends on the transposition rate but also correlates negatively with generation time (Le Rouzic & Capy 2005). In the case of the housefly this would imply that autosomal *M* is more frequent in warmer regions as more generation cycles are possible per population. Over time one would expect the autosomal *M* factor to spread into colder regions. However Kozielska *et al.* (in press) found that the distribution of autosomal *M* in Europe has not changed over the last 50 years. This suggests that generation time per se cannot be the sole explanation and there has to be an additional mechanism.

One of these mechanisms could be temperature induced segregation distortion by *M* factors. It is well known theoretically that segregation distorters can increase in frequency even at the expense of individual fitness (Haig & Bergstrom 1995; Weissing & van Boven 2001). Jayakar 1987 has shown that sex determining factors linked to segregation distorters may lead to a shift in sex determining mechanisms. In *Drosophila melanogaster*, segregation distorters have been found that are temperature sensitive (Mange 1968; Hartl 1975; Hiraizumi 1993): in some strains a temperature of 25ºC was associated with strongly aberrant segregation ratios, while the degree of distortion was lower at both higher and lower temperatures. There is weak evidence that segregation distortion sometimes occurs in the housefly (Clark 1999), but this has not been linked to temperature.

Another explanation is pleiotropic fitness effects of the *M* factor induced by temperature. Temperature can directly affect developmental mechanisms down to the RNA or protein level leading to a change in fitness (Cowperthwaite *et al.* 2005). In the case of the housefly this could be pleiotropic effects of transcription product of the *M* factor leading to increased viability for example. Alternatively, transient linkage of the *M* factor to genes under selection (hitchhiking) could result in fitness advantage at higher temperatures (Werren & Beukeboom 1998). In the case of *M. domestica* some authors speculated that the *M* factor is linked to insecticide resistance genes (Kerr 1970; Franco *et al.* 1982; Tomita & Wada 1989b). One laboratory study found that the *M* factor was linked to a dominant resistant gene after selection for resistance for several generations (Kerr 1970). A study on a wild population showed that the *M* factor on chromosome 3 was linked to a recessive resistance gene and was negatively – not positively – correlated to male survival (Shono & Scott 1990). A recent study compared insecticide resistance in several North American housefly populations but did not detect any correlation between resistance and autosomal

M frequency (Hamm *et al.* 2005). Thus, linkage between autosomal *M* factors and insecticide resistance genes might explain the spread of the *M* factors in some cases but it is unlikely to provide a general explanation for the clinal patterns. Moreover, no link between insecticide resistance and temperature has been established.

In some organisms it has been shown that temperature can have an effect on the expression of genes with different functions (Maurelli & Sansonetti 1988; Howarth & Ougham 1993; Carroll *et al.* 2003). In the housefly there is evidence that intersexes are more frequent in winter than they are in summer (Milani 1967) suggesting that sex determining factors of the housefly may be temperature sensitive. Moreover, two housefly laboratory strains exist where sex determination is affected by temperature. One strain has a maternal effect mutation, *Arrhenogenic* (*Ag*), that maps to the same position as *M* on chromosome 1 (Vanossi Este & Rovati 1982; Dübendorfer *et al.* 2002). The sex of the offspring depends on the genotype of the mother. If the mother is heterozygous for *Ag* she produces mostly sons and intersexes at lower temperatures and mostly daughters at higher temperatures whereas females without *Ag* only produce daughters (Schmidt *et al.* 1997b). In a second strain the mutation *masculinizer* (*man*) occurs. It maps to the same chromosomal location as *F* but seems to have the properties of a null allele of *F* (Schmidt *et al.* 1997a, b). All individuals homozygous for *man* develop into males whereas all individuals heterozygous for *man* develop into females at low and into males and intersexes at high temperatures. Thus, in these strains temperature directly acts on the sex determining system but, so far, these variants have only been found in the laboratory. Nevertheless, it is conceivable that autosomal *M* factors offer greater protection against development as intersex at high temperatures.

Acknowledgements

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CHAPTER 7

*Genetical Research, in press**

Are autosomal sex determining factors of the housefly (*Musca domestica*) spreading north?

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Abstract

Multiple sex determining factors have been found in natural populations of the housefly, *Musca domestica*. Their distribution seems to follow a geographical cline. The "standard" system, with a male-determining factor, *M*, located on the Y chromosome prevails at higher latitudes and altitudes. At lower latitudes and altitudes M factors have also been found on any of the five autosomes. Such populations often also harbour a dominant autosomal factor, F^D, which induces female development even in the presence of several *M* factors. Autosomal M factors were first observed some 50 years ago. It has been hypothesised that following their initial appearance, they are spreading northwards, replacing the standard XY system, but this has never been systematically investigated. To scrutinize this hypothesis, we here compare the current distribution of autosomal *M* factors in continental Europe, on a transect running from Germany to southern Italy, with the distribution reported 25 years ago. Additionally, we analyzed the frequencies of the F^D factor, which has not been done before for European populations. In contrast to earlier predictions, we do not find a clear change in the distribution of sex determining factors: as 25 years ago, only the standard XY system is present in the north, while autosomal *M* factors and the F^D factor are prevalent in Italy. We discuss possible causes for this apparently stable polymorphism.

Introduction

Sex determination in the housefly, *Musca domestica*, is more variable than in most other species, which usually exhibit just a single sex determining mechanism (Bull, 1983; Dübendorfer *et al.*, 2002). Polymorphism for sex determining factors has been found in many natural populations of the housefly (Franco *et al.*, 1982; Denholm *et al.*, 1985; Tomita & Wada, 1989b; Feldmeyer *et al.*, submitted; Table 7.1). In "standard" strains, sex is determined by a male determining factor, *M*, which is located on the Y chromosome; therefore males are XY and females are XX. During development, the *M* factor blocks the female determining factor *F* located on autosome IV, the activity of which is necessary for female development. In many populations, *M* is located on one of the autosomes or even on the X chromosome (Denholm *et al.*, 1983). In such populations, usually a dominant constitutive mutation of *F* (F^D) is also present, which triggers female development even in the presence of several *M* factors in the same individual (see McDonald *et al.*, 1978; Franco *et al.*, 1982; Dübendorfer *et al.*, 2002; Table 7.1).

Table 7.1. Relation between genotype and gender in the housefly. The female determining factors (F/F^D) are located on autosome IV; the male determining factors (*M*) can be located on any chromosome. A "+" indicates the wild type state (no *M*) and a "•" indicates that an *M* or + allele on this locus will not influence the sex.

Autosomes		Sex chromosomes	
IV	I–V	XX	XY
F/F	+/+	♀	♂
F/F	•/*M*	♂	♂
F/F^D	•/•	♀	♀

The XY system is probably ancestral in the housefly, since it also most common in closely related species (Boyes *et al.*, 1964) and the first reports on autosomal sex determining (SD) factors appeared only around 1960 (reviewed by Franco *et al.*, 1982). Since then, the geographical distribution of different SD factors has been studied on most of the continents and appears to follow geographical clines. In general, the Y chromosome is more common at higher latitudes and altitudes and its frequency gradually decreases with decreasing latitude and altitude leading to populations with only autosomal sex determining factors (autosomal *M* and F^D) closer to the equator and at low altitudes (Franco *et al.*, 1982; Tomita & Wada, 1989b; Çakir & Kence, 1996; Hamm *et al.*, 2005; Feldmeyer *et al.*, submitted). It is not clear what forces are responsible for the distribution of different SD factors, but temperature seems to be an important factor (Feldmeyer *et al.*, submitted).

There is some evidence that autosomal sex-determining factors have spread in some populations replacing the standard XY system (Franco *et al.*, 1982; Tomita & Wada, 1989a, b). It has been hypothesized (Franco *et al.*, 1982; Denholm *et al.*, 1985; Tomita & Wada, 1989a, b; Çakir & Kence, 1996) that the observed distributions are a transient state. In particular, Franco and colleagues (1982) suggested that autosomal *M* factors are spreading north in Europe, but their hypothesis was based only on the change in frequency of the Y chromosome in a few populations before 1980. No systematic or recent studies have been done on the dynamics of different SD factors in natural populations of the housefly. The last study in continental Europe dates from 25 years ago (Franco *et al.*, 1982) in which cytological data were used to show a clear latitudinal cline with the standard XY system exclusively present in the north of Europe (Iceland, Denmark, the Netherlands, Germany and Switzerland) and entirely autosomal populations (lacking the Y chromosome) in southern Italy, at altitudes below 100 m. In northern Italy mixed populations have been found with the frequency of the Y chromosome increasing with higher altitudes and latitudes.

The aim of this study was to investigate whether the distribution of SD factors in the housefly has changed in Europe over the last 25 years. Therefore, we sampled a number of European populations on a north-south transect from Germany to southern Italy, and compared the frequency of males that carry the Y chromosome and autosomal *M* factors with the data published by Franco and colleagues (1982). Additionally, we analyzed the frequencies of the F^D factor and we publish the frequencies of *M* factors located on different chromosomes, which has not been done before for European housefly populations.

Material and methods

Collection and rearing of the flies

We sampled populations along a north-south transect from north Germany to south Italy in July 2006 (see Fig. 7.1 and Table 7.2 for details on the sampling locations). Most of the sampling sites were chosen to be close to the ones studied by Franco *et al.* (1982), as far as we could judge from the limited information. For Germany and Switzerland, they only gave the name of a state (Baden-Württemberg) or a canton (Mittelland) and our sampling sites lie within these areas. For Italy, Franco and colleagues published a map indicating sampling sites together with information on altitudes, but precise geographical coordinates were lacking. We judged their locations visually and used altitudes within 110 m, but usually within a 50 m range. The exception is population IT5 where the altitude given by Franco *et al.* (1982) does not match the area indicated by them, so to match the altitude we sampled 50 km west of their indicated location. Ultimately, our sampling sites were distributed approximately homogeneously along a north-south transect, with some areas having sampling sites at different altitudes.

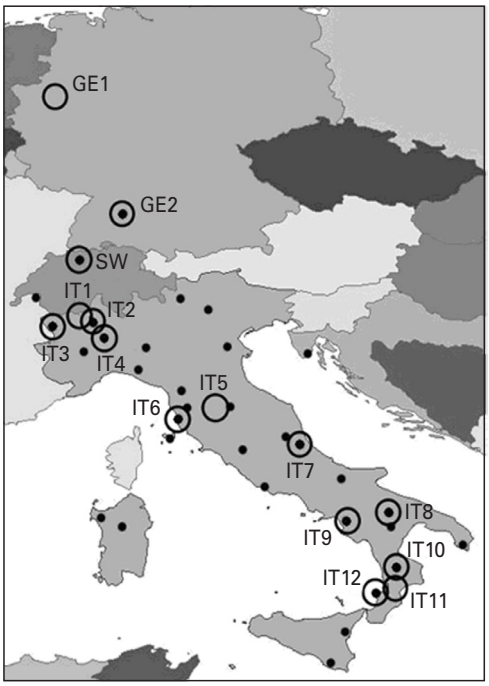

Figure 7.1. Sampling locations in the study of Franco and colleagues (1982; dots) and in the present study (circles). Locations from the present study are labelled with population codes as in Table 7.2.

Table 7.2. Geographical coordinates, altitudes (in meters above sea level) and average yearly temperatures of the sampling sites.

Population code	Latitude N	Longitude E	Altitude (m)	Temperature (°C)
GE1	51° 19,4’	7° 10,9’	220	9.1
GE2	48° 29,5’	9° 2,0’	347	9.0
SW	47° 17,8’	7° 51,8’	410	9.4
IT1	45° 46,6’	8° 2,5’	794	8.8
IT2	45° 42,3’	8° 14,1’	470	10.1
IT3	45° 35,4’	7° 8,0’	1700	4.2
IT4	45° 17,8’	8° 33,1’	121	12.3
IT5	43° 29,2’	11° 33,1’	313	13.2
IT6	43° 11,0’	10° 31,7’	18	15.4
IT7	42° 32,6’	13° 49,3’	367	13.3
IT8	40° 45,7’	16° 14,3’	562	13.3
IT9	40° 32,5’	15° 6,4’	63	16.1
IT10	39° 21,4’	16° 26,5’	1194	10.4
IT11	38° 48,0’	16° 20,3’	690	13.9
IT12	38° 40,6’	15° 54,6’	49	17.7

Sampling sites are ordered according to their latitude.
Letters in the code indicate the country of origin: GE – Germany, SW – Switzerland, IT – Italy.

For each location, we obtained data on average monthly minimum and maximum temperatures from WORLDCLIM (www.worldclim.org, see Hijmans *et al.*, 2005) which provides global estimates at a resolution of one square kilometre. We estimated average yearly temperatures as the mid-point between minimum and maximum temperatures (Table 7.2). Since all these measures of temperature are highly correlated ($p<0.0001$, Pearson's product-moment correlation test), we used only the average yearly temperature in our statistical analysis (see below).

Flies were sampled at farms and horse stables. At each location we caught approximately 50 adult males and females (except for IT3, where only 10 females were found). The flies were caught with sweeping nets, placed in plastic containers and provided with water and milk powder as food. They were also provided with egg laying medium (according to Hilfiker-Kleiner *et al.*, 1994) on which females laid eggs within a few days. Larvae were transferred to bigger containers after a few days and fed *ad libitum* on the same medium. Flies from all the locations (or their offspring) were successfully transported to the laboratory and populations were established and maintained in cages at population size of approximately 500 individuals.

Analysis of the sex determining factors

M factors: The presence of different *M* factors in males was determined by two generations of single-pair crosses with standard XX (without an F^D factor) virgin females, from a marker strain that carries visible recessive mutations in homozygous state on each of the five autosomes (Tomita & Wada, 1989b). The sex ratio of F1 offspring shows whether the father was homozygous for at least one *M* factor (only sons are produced) or heterozygous for all *M* factors (daughters are also present among the offspring). Sex-linked inheritance of visible markers in the second generation of backcrosses to marker-strain females shows on which chromosomes *M* factors are located. This is a standard procedure in our laboratory and it gives a good estimation of the frequency of *M* factors located on different autosomes (for details see Denholm *et al.*, 1983). However, if a focal wild type male was homozygous for *M* (producing all-male offspring) and all of his sons appeared to have two (or more) *M* factors (e.g. *M* on autosome II and V), we could not unambiguously determine if the father was homozygous for *M* on only one or on both chromosomes, especially if the number of sons was small. For example, M^{II}/M^{II}; M^{V}/M^{V}, $M^{II}/+$; M^{V}/M^{V} and M^{II}/M^{II}; $M^{V}/+$ males all produce $M^{II}/+;M^{V}/+$ sons when mated with standard females. This happened a few times (13 males in total, with a maximum of 4 males per population). For each chromosome involved in a population, we calculated both the minimal frequency of *M* (assuming that all ambiguous males were heterozygous for *M*), and the maximal frequency of the *M* factor (assuming that all ambiguous males were homozygous for *M*) on the given chromosome. We then used the mid-point value between the two extremes as a population estimate.

We used 20 males from each population for the first series of crosses and 3 sons from each of them for the F1 backcrosses (although we did not obtain offspring from

all males). Males used for analysis were either the ones caught in the field (IT3), or from the first generation in the lab (offspring of the wild caught flies; IT6, IT7, IT8, IT10, IT11, IT12), the third generation in the lab (GE1, GE2, SW, IT1, IT2, IT4, IT5) or the fourth generation (IT9). Because of the lack of visible markers on the X and the Y chromosome, in cases in which we assigned M to a sex chromosome, we cannot be sure whether it was located on the Y or the X (as has been found in Britain: Denholm *et al.*, 1983, 1985). If *M* was located on a sex chromosome we will call this chromosome Y, but we will discuss this issue in more detail later.

F*^{D} *factor: *F* and F^{D} factors have been sequenced at the University of Zürich (M. Hediger and D. Bopp, personal communication). F^{D} has two deletions compared to *F* in all populations analyzed (of European, Asian and African origin). We used primers designed for one of these deletions to distinguish between *F* (one band present) and F^{D} (two bands) females. We used approximately 20 females from each population, either females caught in the field (populations: GE2, SW, IT5 and all 10 females from IT3) or from the first generation in the lab (all the other populations). Additionally, we took 2-3 females from each population and crossed them individually with a male homozygous for *M* located on autosome III. Females without F^{D} produce only sons, but the ones with F^{D} also produce daughters, because F^{D} is dominant over *M*. After determining the sex of the offspring, we also analyzed the mothers molecularly and found without exception that the results of the molecular analysis were consistent with those obtained from the crosses. This shows that the deletion in the F^{D} factor is also present in the populations we collected and justifies the use of the molecular technique for analyzing frequencies of F^{D} in our populations.

Statistical analysis

We performed a logistic regression analysis using the glm function with quasi-binomial errors in R (R Development Core Team, 2006) to investigate the influence of latitude, altitude and temperature on the frequency of autosomal *M* males (with at least one autosomal *M* factor) and on the frequency of females with the F^{D} factor. We started with a full model (including all two-way interactions between explanatory variables) and used backward selection to find the minimal adequate model. The significance of the difference between models was assessed with the likelihood-ratio approach, using F-tests to correct for under- and overdispersion (Krackow & Tkadlec, 2001).

A statistical comparison between the frequencies of different SD factors in the past and present is only possible to a limited extent, since Franco *et al.* (1982) only performed cytological observations. They used the frequency of XX males as a measure for the frequency of autosomal males. They checked the linkage of autosomal *M* factors with crosses similar to ours, but they do not provide the exact frequencies of different factors. They also do not provide data on frequencies of the F^{D} factor. Moreover, due to the lack of data on the number of males tested by Franco and colleagues (1982), in each autosomal and standard population separately (except for GE2), we could only include eight populations (GE2, IT2, IT3, IT4, IT5, IT7, IT8

and IT10) in a statistical analysis to compare frequencies of autosomal males (without Y chromosome) between ours and their study. For this analysis, we performed a mixed-model logistic regression analysis in R using the lmer function with binomial errors from the lme4 package. The full model included population as a random effect and "study" (Franco *et al.*, 1982 or this study) as a fixed effect. Significance of the effect of "study" was judged using the likelihood-ratio approach, using an F-test to correct for overdispersion (Krackow & Tkadlec, 2001). For each of the eight populations we also performed a binomial test, to see if there is a significant change in the frequency of XX males between the past and the present.

Results

Distribution of sex determining factors in 2006

We found *M* factors on the sex chromosomes and on each of the autosomes (Table 7.3, Fig. 7.2A). *M* located on autosome III was the most frequent among autosomal *M* factors and the frequencies of *M* on autosome IV and V were very low. We did not

Table 7.3. Estimated frequencies of females with F^D factor and frequencies of *M* factors in males in samples from different housefly populations.

Pop. code	# females	frequency of females with F^D	# males	frequency of *M* on					
				sex chromosome	autosome				
					I	II	III	IV	V
GE1	20	0.00	18	0.50	0	0	0	0	0
GE2	19	0.00	20	0.50	0	0	0	0	0
SW	21	0.05	20	0.50	0	0	0	0	0
IT1	20	0.44	20	0.52	0	0	0.12	0	0
IT2	21	0.43	16	0.44	0	0.25	0.09	0	0
IT3	10	0.10	11	0.50	0	0	0	0	0
IT4	20	1.00	19	0.42	0.12	0.09	0.45	0	0
IT5	22	1.00	20	0.62	0.02	0.17	0.50	0	0.09
IT6	20	0.95	19	0.68	0.03	0.13	0.32	0	0
IT7	23	0.78	18	0.17	0	0.03	0.53	0	0
IT8	22	1.00	19	0.16	0.09	0.03	0.86	0.03	0.03
IT9	22	0.86	18	0.06	0.08	0.17	0.46	0	0
IT10	19	0.95	19	0	0.03	0	0.55	0.03	0
IT11	23	0.96	17	0.03	0	0	0.76	0	0
IT12	19	0.47	18	0.08	0	0	0.56	0	0

Frequencies of *M* are given separately for each chromosome (a value of 1.0 would indicate complete homozygosity for *M* on this chromosome). The sum of *M* frequencies over all chromosomes may exceed 1.0 when males carry multiple *M* factors. Population codes as in Table 7.2 and Fig. 7.1.

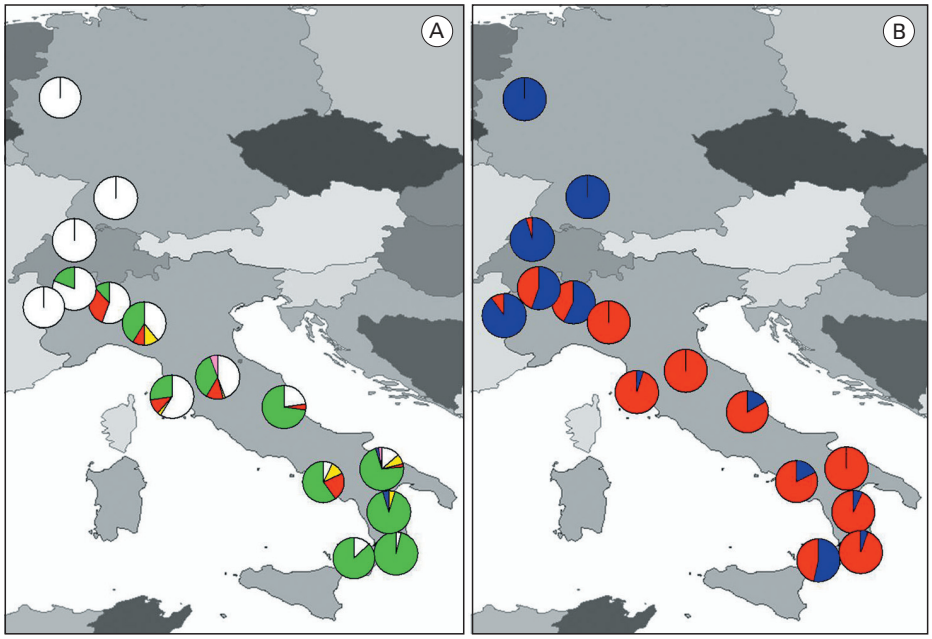

Figure 7.2. Distribution of sex determining factors in the housefly in 2006. (A) Relative frequencies of *M* factors located on different chromosomes: white – sex chromosome, yellow – autosome I, red – autosome II, green – autosome III, blue – autosome IV, pink – autosome V. (B) Frequencies of females with (red) and without (blue) the F^D factor.

detect any autosomal *M* in the German and Swiss populations and in one northern Italian population from the highest altitude (IT3). In populations with autosomal SD factors, often single males with multiple *M* factors, located on up to four different chromosomes, were observed (data not shown). Statistical analysis showed that altitude, latitude, temperature and interaction of temperature and latitude (and to a lesser extent interaction between temperature and altitude) influence the frequencies of autosomal *M* males (Table 7.4).

We did not find F^D in populations from Germany and only at low frequencies in Switzerland and at the highest location from northern Italy (IT3; Table 7.3, Fig. 7.2B). In most of the Italian populations frequencies of F^D females were above 0.75 and in three populations F^D appeared to be at fixation. Statistical analysis showed that the frequency of females with F^D is influenced by latitude, temperature and the interaction of the two (Table 7.4).

Comparison with the past

A comparison between our results and the results of Franco and colleagues (1982) shows that there is no clear evidence for the spread of autosomal *M* factors northwards during the last 25 years (Fig. 7.3). In the two northernmost populations and

Table 7.4. Logistic regression analysis of (A) frequencies of autosomal *M* males and (B) frequencies of females with F^D. Parameter estimates (logit scale) and their standard errors (SE) are shown for the final models, after the removal of non-significant variables.

Source of variation	Parameter	SE	Δdev	*F*	*P*
(A) Males					
Intercept	277.4	33.2			
Altitude (A)	–0.014	0.002	19.12	70.6	<0.0001
Latitude (L)	–5.521	0.652	28.39	104.9	<0.0001
Temperature (T)	–12.07	1.493	24.24	89.6	<0.0001
A*T	0.0004	0.0002	1.53	5.6	0.042
L*T	0.222	0.029	22.28	82.3	<0.0001
(B) Females					
Intercept	124.470	27.495			
Latitude	–2.884	0.606	108.25	40.95	<0.0001
Temperature	–8.684	1.822	82.04	31.04	<0.0005
L*T	0.204	0.042	85.32	32.28	<0.0005

Temperature refers to the average yearly temperature. Δdev indicates the change in deviance resulting from removing the given variable from the final model. The F-tests for significance of removed variables have 1 and residual degrees of freedom of the final model (DF) for numerator and denominator, respectively.
Final models: (A) deviance=3.05, residual DF = 9; (B) deviance =27.28, residual DF = 11.

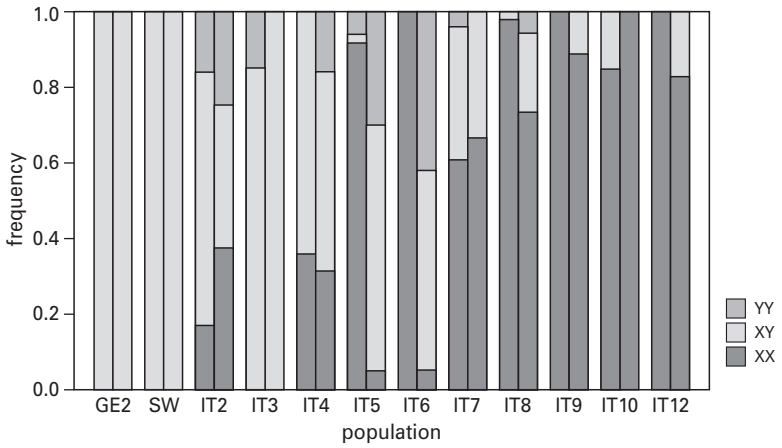

Figure 7.3. Comparison of karyotype frequencies in males in the past and the present (2006). For each population the left bar corresponds to the data from Franco *et al.* (1982) and the right bar to the data from this study. We inferred karyotypes from our crosses assuming that Y is the sex chromosome bearing the *M* factor (see Material and Methods). Three populations analyzed by us are not included in the figure since they were not studied by Franco and colleagues. Populations are ordered according to the decreasing latitude of the sampling sites (see Table 7.2).

Table 7.5. Logistic mixed-model analysis of the frequencies of XX males in the study of Franco et al. (1982) and this study. The full model includes population as a random effect and study (data from Franco *et al.*, 1982 or from our study) as a fixed effect under analysis.

Model	DF	Deviance	*F*	*P*
Population (random) + study	13	107.7		
Population (random)	14	117.5	1.19	0.7

No significant difference was found between the studies.

in IT3, which lacked XX males in the past, we also did not find any autosomal *M* factor. Furthermore, all populations described by Franco and colleagues (1982) as mixed or autosomal were found to have autosomal *M* factors in 2006. However, in the populations which were described by Franco and colleagues as autosomal in 1982 (IT6, IT9 and IT12) we also found *M* on a sex chromosome. Statistical analysis based on the eight populations for which comparable data were available shows no significant systematic change in the frequencies of autosomal males in the last decades (Table 7.5). Statistical analysis for each population separately, shows a significant decrease in the frequency of XX males for two populations: IT5 and IT8 ($p<0.002$, which is also significant after Bonferroni correction for multiple tests).

The distribution of F^D also seems to be relatively stable in time. F^D frequencies were not analyzed by Franco *et al.* (1982), but the presence of F^D can be deduced from the occurrence of at least one homozygous *M* male in all autosomal populations and the occurrence of XY females and YY males in mixed populations (Franco *et al.*, 1982), implying that 25 years ago F^D (or a similar genetic element) was present across the entire range of Italy, as it is now. However, we did find F^D in Switzerland, where it was not detected before 1982 suggesting that the F^D factor has spread slightly northwards.

Discussion

Our results show that autosomal *M* factors have not spread northwards in Europe over the last 25 years, in contrast to what was predicted by Franco *et al.* (1982). One may argue that we have overlooked low frequencies of autosomal *M* factors in Switzerland and Germany due to insufficient sample size. Although this may be true, very low frequencies of autosomal factors still support the hypothesis that the standard XY system is not being replaced by autosomal factors in northern populations. In line with our results, we suggest that after their initial spread in southern localities (see Franco *et al.*, 1982), autosomal *M* factors reached a stable distribution.

Our results indicate that some factors prevent the spread of autosomal *M* in

populations north of Italy. In the transect we studied, the Alps may be considered as a barrier, although the biology of the housefly and its ease of spread with human transportation seem to preclude this physical barrier as being important for the potential long-term spread of autosomal *M* factors. In fact, the presence of the F^D factor north of the Alps and the *M* factor on autosome II in flies collected in eastern France in 2004 (results not shown) suggests that geographical barriers do not prevent the northward spread of autosomal *M* factors. More likely, some climatic factors are responsible for the stability of the distribution of *M*. The most obvious climatic factor related with latitude is temperature, which has been shown to be a strong predictor of the frequencies of different sex determining factors in the housefly worldwide (Feldmeyer *et al.*, submitted). However, it is not obvious how temperature might influence the evolution and distribution of SD mechanisms (discussed in detail in Feldmeyer *et al.*, submitted).

Our statistical analysis reveals an effect of temperature, but also a significant interaction between temperature and latitude on the frequency of autosomal SD factors (Table 7.4). The interaction stems from the fact that at higher latitudes temperature has a positive effect on the frequencies of autosomal SD factors, whereas the opposite pattern is present at lower latitudes (not shown). This may suggest that autosomal SD factors reach the highest frequencies at intermediate temperatures. However, autosomal SD factors have been found at high frequencies in places where average temperatures are higher than at our sampling sites (Feldmeyer *et al.*, submitted). A more likely explanation is that temperature interacts with other climatic factors (like humidity) that could be correlated with latitude (and altitude) in our study area. This could also explain why an *M* factor on autosome III and F^D have been found at locations in England where the yearly range of temperatures is similar to Germany and Switzerland (Denholm *et al.*, 1985; data on temperatures from WORLDCLIM, not shown). Additionally, *M* factors located on different autosomes may be differently affected by temperature.

It has also been proposed that autosomal *M* factors have spread due to their linkage with insecticide resistance genes (Kerr, 1970; Franco *et al.*, 1982), since the isolation of autosomal *M* factors coincided with the appearance of insecticide resistance in natural populations of the housefly (Tomita & Wada, 1989b). Also, in a number of resistant populations autosomal *M* males have been found (Tsukamoto, 1983) and one laboratory experiment showed replacement of standard XY males by autosomal *M* males after several generations of selection for DDT resistance (Kerr, 1970). However, even though linkage with insecticide resistance genes could facilitate spread of autosomal *M* factors, it is not clear how it could contribute to the clinal distribution of SD factors in the housefly. One could argue that in warmer climates more generations of flies are produced and more applications of insecticides are used, allowing faster spread of *M* factors linked with insecticide resistant genes. However, since pesticides have been used in whole Europe for decades and resistance genes are widespread also in northern populations (Keiding, 1977, 1999), one

would expect that, although slower, *M* factors would be increasing in frequency also in the north. As we showed in this study, this is not the case. Another argument is that there is no correlation between the frequency of autosomal *M* males and insecticide resistance in housefly populations from eastern United States (Hamm *et al.*, 2005). Therefore, linkage with insecticide resistance genes might explain spread of autosomal *M* factors is some cases, but it seems unlikely to provide a general explanation for the clinal distribution of SD factors in the housefly.

Interestingly, autosomal *M* factors are not fixed in most populations and multiple factors on several or even all chromosomes can be maintained in a single population. This polymorphism was one of the reasons underlying the opinion of earlier researchers that the sex determining mechanism in the housefly is in a transient state (e.g. Franco *et al.*, 1982; Denholm *et al.*, 1985; Tomita & Wada, 1989b). However, theoretical models reveal that such a polymorphism can be stable not only for specific fitness values of different genotypes (Bull & Charnov, 1977; Jayakar, 1987), but also when different genotypes have the same viability and fertility (Kozielska *et al.*, 2006). Therefore, the conditions for a stable polymorphism may be much less restrictive than previously thought, and it may well be that the multifactorial SD system of the housefly is stable.

Unfortunately, we do not have data on the frequencies of different autosomal *M* factors in the past to see whether these frequencies have changed. Franco and colleagues (1982) did not find any *M* factors located on autosomes I, IV or V, but they do not provide the number of males investigated. If these factors were present in the past at low frequencies as they are now (Table 7.3), Franco *et al.* (1982) might not have detected them in small sample sizes. They reported that *M* was more common on autosome III than on autosome II. The same pattern is seen in this study and in several other studies (Tomita & Wada, 1989b; Denholm *et al.*, 1990; Hamm *et al.*, 2005; except for Tanzanian populations, Feldmeyer *et al.*, submitted.). This suggests that *M* on autosome III confers the largest fitness gain to its bearer, but this may only be a conditional effect (e.g. frequency- or temperature-dependent) since the *M* on autosome III did not replace other *M* factors during the last decades in the Italian populations.

Another explanation for the high polymorphism in genomic location of *M* factors is that the *M* factor is part of a transposable element, as is known for the *M* factor in *Megaselia scalaris* (Traut & Willhoeft, 1990). In this species transposition rate differs depending on which chromosome *M* is located (Green, 1980). This might not only explain why *M* factors are more common on some autosomes than others, but also the clinal distribution of *M* factors, since transposition rate is known to be dependent on temperature and often increases with increasing temperature (Lampe *et al.*, 1998; Ohtsubo *et al.*, 2005; but see Hashida *et al.*, 2003). Molecular studies are necessary to establish whether the *M* factor is always the same gene located on a transposable element or whether *M* factors on different chromosomes are different genes blocking the female determining factor *F* (see Dübendorfer *et al.*, 2002).

Our crosses suggest that the frequency of the Y chromosome has increased over the last decades in some Italian populations. We found an *M* factor on the sex chromosomes in some populations that were described as purely autosomal by Franco and colleagues (1982; Fig. 7.3). It is difficult to assess what the cause of these changes in particular populations is; some local factors may be involved. For population IT5, the difference between past and present frequencies of XX males might reflect the fact that we could not locate accurately the sampling site of Franco and colleagues (1982; see Material and Methods). Moreover, it should be noted that due to the absence of visible markers on the sex chromosomes of the housefly, our crosses did not allow us to determine whether the *M* factor was present on the Y or on the X chromosome (as found in England: Denholm *et al.*, 1983, 1985). Without additional information, the data obtained from the crosses could easily lead to the incorrect classification of XX^M males as XY males. Therefore, we performed additional cytological investigations, using orcein staining, a standard technique used in cytological studies of the housefly (Hiroyoshi, 1964; Franco *et al.*, 1982; Denholm *et al.*, 1983, 1985). Our preliminary results (not shown) confirm that males from the northernmost populations (GE1, GE2, SW and IT3) are of karyotype XY. Unfortunately, we could not unambiguously distinguish between XX, XY and YY karyotypes in the other populations, because the length polymorphism of the housefly sex chromosomes (also know from other strains: Boyes *et al.*, 1964; Boyes, 1967; Milani, 1971; Franco *et al.*, 1982; Denholm *et al.*, 1983, 1985; Hediger *et al.*, 1998) did not allow a reliable distinction between X and Y chromosomes. Therefore, we cannot exclude the possibility that the X chromosome (rather than the Y chromosome) bears the *M* factor in the southern populations.

In conclusion, even if the distribution of the Y chromosome in European populations is difficult to assess, our main conclusion that autosomal *M* factors have not spread northwards in the last 25 years still holds. This suggests that the polymorphism of the SD factors in natural housefly populations is not transient but stable. Additional studies, both at the ecological and the molecular level, are required to unravel the factors responsible for the stable coexistence of various SD factors. Undoubtedly, better understanding of the housefly SD system will also provide general insights into the evolution of sex determination, which is still poorly understood in other taxa as well.

Acknowledgements

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CHAPTER 8

Temperature and fitness of houseflies with different sex determining factors.

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Abstract

Multiple sex determining mechanisms persist in natural populations of the housefly, *Musca domestica*. Their geographical distribution follows geographical clines, with the standard XY system present mainly at higher latitudes and altitudes and autosomal sex determining factors prevalent at low latitudes and altitudes. Previous studies showed a positive correlation between temperature and frequency of autosomal factors in natural populations, suggesting that they have a fitness advantage over the XY system at higher temperatures. In this study, we experimentally investigated the relative fitness of flies with autosomal sex determining factors versus standard flies under different temperature conditions. We determined whether autosomal *M* factors could invade the standard XY populations. We obtained different results for different *M* factors: the *M* factor on autosome II replaced the Y, but *M* on autosome III did not increase in frequency. However, we did not find an effect of temperature on the outcome. We also compared fitness of females with and without F^D. We found great variation between populations, but no effect of temperature on the fitness of *F* and F^D females. We discuss our results in the context of natural variation in housefly sex determining factors. We conclude that the role of temperature on the spread and distribution of different sex determining mechanism in the housefly still remains unclear. Future experiments should also include interaction of different sex determining factors under different temperatures.

Introduction

Multiple sex determining factors co-exist in many populations of the housefly, *Musca domestica* (Dübendorfer *et al.* 2002; Table 1). The distribution of these factors follows geographical clines. The "standard" system, with a male-determining factor, M, located on the Y chromosome prevails at higher latitudes and altitudes. At lower latitudes and latitudes *M* factors have also been found on any of the five autosomes. Such populations often also harbour a dominant autosomal factor, F^D, which induces female development even in the presence of several *M* factors (Çakir & Kence 1996; Franco *et al.* 1982; Hamm *et al.* 2005; Tomita & Wada 1989b; Kozielska *et al.*, in press; Feldmeyer *et al.*, submitted). It has been proposed that this distribution is governed to a great extent by temperature (for details see Kozielska *et al.*, in press; Feldmeyer *et al.*, submitted). Support for this hypothesis comes from the correlation between the frequencies of autosomal sex determining (SD) factors and the ambient temperature in natural populations of houseflies (Kozielska *et al.*, in press; Feldmeyer *et al.*, submitted). The prevalence of autosomal SD factors in warmer localities and their lack in colder ones suggests that autosomal SD factors have a fitness advantage over the XY system at higher temperatures and a disadvantage at lower temperatures. However, it has never been shown experimentally that this is indeed the case.

Numerous studies have been performed to measure different fitness components at different temperatures of houseflies collected in various localities (e.g. Bryant 1980; Chapman & Goulson 2000; Elvin & Krafsur 1984; Fletcher *et al.* 1990; Lysyk 1991; West 1951), often with contrasting results (see Lysyk 1991; West 1951), but virtually none of them took the sex determining mechanism of the investigated flies into account. To our knowledge, only one study intended to compare the competitive abilities of houseflies from autosomal and standard populations (Çakir & Kence 1999). Çakir and Kence found that the frequency of XX males increased in most of the treatments, but they did not know the exact frequencies of different SD factors, neither *M* nor F^D. They also did not control for the genetic background of different factors, which makes the interpretation of their results difficult.

The objective of the present study was to more directly compare the fitness of flies with different SD factors under different temperatures. For *M* we measured the invasion success of two different autosomal *M* factors into a standard XY population at two different temperatures. This approach reflects presumed ancestral conditions when autosomal *M* factors emerged in XY populations (Franco *et al.* 1982). A similar approach was impossible for comparing the fitness of standard *F* females with F^D females (see below). Therefore we decided to measure lifetime reproductive success of females with and without F^D from different populations at two different temperatures.

Because we used two different approaches, we will present our experiments in two separate sections. Part I contains the methods, results and a short discussion of the experiment on invasion of autosomal *M* factors. Part II includes methods, results

and discussion of the experiment measuring fitness of *F* and F^D females. At the end of the chapter, we present a general discussion on the effect of temperature on different SD factors in the housefly.

PART I: INVASION OF AUTOSOMAL *M* FACTORS

Material and methods

Housefly strains

We used several strains with different with *M* located on different chromosomes.

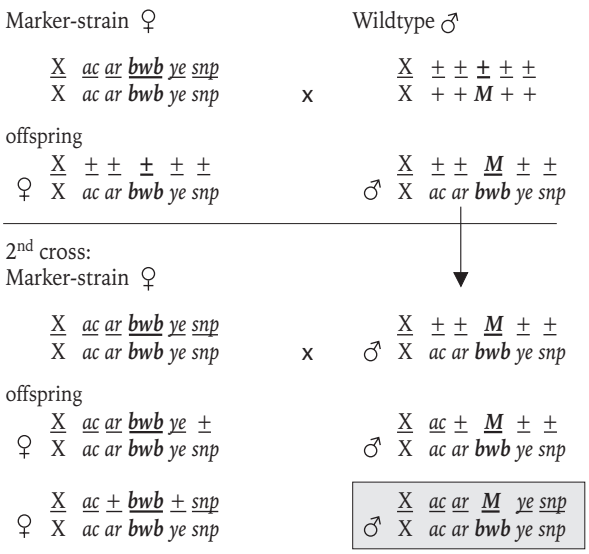
1) Marker XY strain – a lab marker strain homozygous for five recessive visible mutations: *ac* (*ali curve* – tips of the wings are curved upwards), *ar* (*aristopedia* – aristae of antennae are substituted by tarsal segments), *bwb* (*brown body*), *ye* (*yellow eyes*) and *snp* (*snip wings* – part of the wing is missing) on autosome I, II, III, IV and V, respectively. This strain has the standard XY sex determining system.

2) SFE-M^{II} autosomal strain – a lab strain created by a number of generations of backcrosses of one wild type XX male with an *M* factor located on autosome II with the marker-strain females (described in Table 8.1). A wild type male used for generation of this strain came from the strain collected in Santa Fe, Spain, in 2004. All females in this strain are homozygous for all five autosomal markers similar to females from the marker strain; males are homozygous for the mutations on all the autosomes except II. They are heterozygous for autosome II: one autosome comes from the marker strain and the other one is the wild type autosome II with *M*. Since in male houseflies there is almost no recombination the *M* factor is always linked to the wild type *ar*+ allele and males always develop normal antennae.

3) CAM - M^{III} autosomal strain - a lab strain created by a number of generations of backcrosses of one wild type XX male with an *M* factor located on autosome III with the marker-strain females (Table 8.1). A wild type male used for generation of this strain came from the strain CAM collected in Camargue, France, in 2004. Similar to the SFE-M^{II} strain, all females are homozygous for all markers. Males are homozygous for the mutations on all the autosomes except III. They are heterozygous for autosome III: one autosome comes from the marker strain (with *bwb* allele) and the other one is the wild type autosome III with *M*. Males and therefore black, since the *M* factor is linked with the wild type bwb^+ allele.

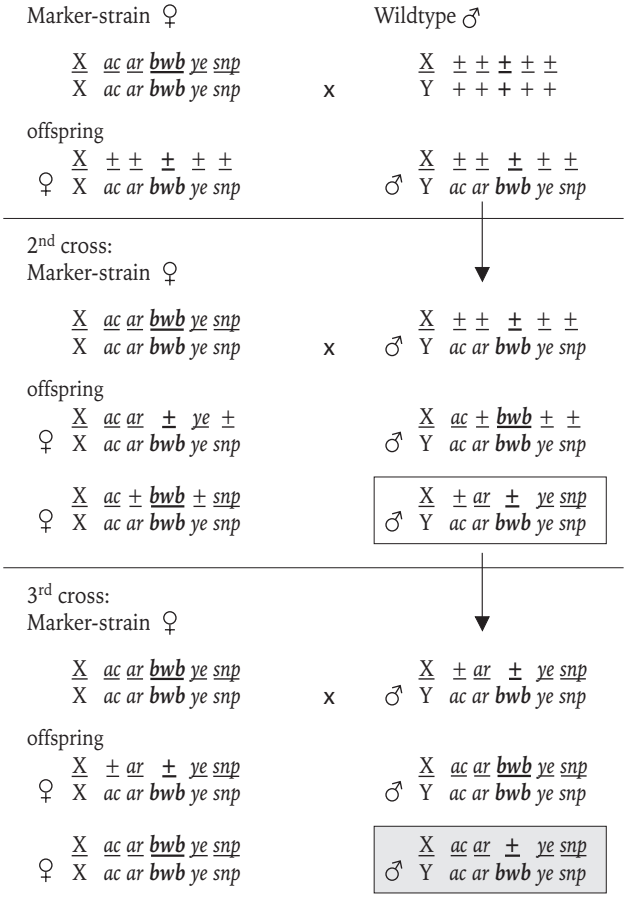
Since there are no visible mutations on the X or Y chromosome it is possible that both an X chromosome from the XY marker strain and an X chromosome from the original wild type males is present in both autosomal strains. However, since there have been no structural genes described so far on the X or Y chromosome (see Dübendorfer *et al*. 2002), we do not expect much effect of sex chromosomes from different strains. Both autosomal strains were created approximately one year (approximately 12 generations) before the start of the experiment in July 2005.

Table 8.1. Schematic representation of the crosses performed to create the CAM-M^{III} strain. *ac*, *ar, bwb, ye* and *snp* represent recessive visible mutations on each of the autosomes (autosome III in bold); + represents a wild type allele of any of the mutations and *M* is always linked with the wild type allele of *bwb*, since there is no recombination in males. In the first generation, wildtype males are crossed with marker-strain females, resulting in heterozygous progeny with a wildtype phenotype. Male offspring is then crossed again with marker-strain females yielding a variety of phenotypes among the F2 (four examples represented here). All females are homozygous for *bwb* and show the brown body phenotype, all males are heterozygous and show the wildtype phenotype (black body). Males homozygous for all visible mutations, except *bwb*, (framed) were again crossed with marker-strain females to establish the CAM-M^{III} strain. The SFE-M^{II} strain was obtained in a similar way, but there the *M* was linked with the *ar*$^+$ allele.



Usage of the strains described above allows us to compare the performance of males with a Y chromosome (strain 1) or autosomal *M* factor (strains 2 and 3) in the same genetic background (except for the genes located on the autosome with the *M* factor). Additionally, the presence of visible markers linked with autosomal *M* factors allows us to precisely score the frequencies of different *M* factors each generation. This is particularly important since there are no molecular markers to distinguish between *M* factors on different autosomes. So far *M* location can be checked only after a tedious procedure involving two generations of backcrosses to marker strains (see Kozielska *et al.*, in press), making analysis of frequencies of different *M* factors from a large number of males difficult. However, a potential drawback is that in our autosomal strains *M* is linked with the wild type phenotype, which may confer an increase in fitness, compared to XY marker-strain males which are homozygous for all mutant alleles. Therefore, we created control males to assess the effect of the wild type marker by separating it from the effect of the *M* factor.

Table 8.2. Schematic representation of the crosses performed to create C-III control males. The procedure is similar to the one present in Table 8.1, but now the *M* is located on the Y chromosome and all visible mutations segregate randomly in both sexes (some examples of offspring genotypes are shown). Male offspring from the 2nd cross heterozygous for *bwb* (open frame) were crossed with marker-strain females, and F3 males homozygous for all visible mutations except *bwb* (grey frame) were used as C-III males in the control experiment.



4) C-III - control males were created by single pair backcrosses of XY males from the same wild type CAM strain from which CAM-M^{III} males were derived, to virgin marker-strain females (Table 8.2). Males whose F2 offspring did not show a sex limited inheritance of visible markers possessed the Y chromosome and no autosomal *M* factor (see e.g. Denholm *et al.* 1983). These male offspring were used in one more generation of backcrosses to marker-strain females from which male offspring with all visible mutations except for brown body were used as a control to CAM-M^{III} males, since they were also homozygous for the four mutant alleles, but heterozygous for wild type autosome III, but without the *M* factor. They possessed a

Y chromosome (to assure maleness), in contrast to autosomal *M* males, which were XX (Table 8.1 and 8.2). This should not influence the results considerably, since both the X and the Y chromosome seem to be equivalent with respect to viability and fertility (Dübendorfer *et al.* 2002; Franco *et al.* 1982). Construction of the control males for the SFE-MII strain was impossible, since in the original wild type strain all males were homozygous for M^{II}, therefore there was no autosome II without an *M* factor present in that population.

Experiment setup

We set up population cages to measure the fitness of males with different *M* factors. Each experimental cage started (generation 0) with 150 females and 120 males from the marker strain and 30 males from one of the autosomal *M* strains or C-III control males. We kept populations at a temperature of either 20°C or 25°C and replicated each treatment five times (five cages per strain per temperature). We used this narrow range of temperatures, since under laboratory conditions there is a very high mortality of larvae below 20°C and a high mortality of adults above 25°C (personal observation).

We kept the adult flies in population cages (13x13x22cm) and provided them with constant access to water, sugar water and milk powder (as food). When flies were about 5 (in 25°C) or 7 (in 20°C) days old, females reached full maturity and were most prone to lay eggs (personal observation) and film boxes with standard egg laying medium (see Hilfiker-Kleiner *et al.* 1994) were placed in the cages. After one day at 25°C or two days at 20°C they were replaced by a second set of boxes of egg laying medium and eggs were transferred to bigger boxes where larvae could develop. The second egg laying medium was collected again after two or one day(s) (in 20/25°C) and eggs were transferred to new larval boxes, leading to two larval boxes per population. This protocol for egg collection yields many eggs and at the same time prevents large age differences between offspring. Larvae were fed *at libidum* with the same medium which was used for egg collection. When larvae from a box pupated, 150 random pupae per box were collected and placed in a new population cage while 150 other random pupae were collected in separate boxes and later used to calculate the frequencies of different *M* factors (see below). Since the pupal emergence rate is almost 100% (personal observation), each population cage contained approximately 300 flies each generation. These rearing conditions reflect the standard fly-keeping procedure used in our lab, except that temperature is usually 20°C and adult population density is approximately 500 flies. The experiment lasted for 8 generations under the same protocol and rearing conditions.

In experiments with the invasion of M^{II} and M^{III} males, every generation we calculated the frequency of males with a Y chromosome (all five mutations present) and males with an autosomal *M* factor (only four mutations; see Table 8.1). For the control we scored the number of black and brown males and females. *bwb* is a recessive mutation and we estimated the frequencies of the wild type *bwb+* allele

assuming a Hardy-Weinberg equilibrium. In the first generation we tried to score the phenotypes of adult flies after the new generation had been started and adults had been killed by freezing in -20°C. However, antennae get damaged very easily after death and scoring the *ar* mutation after freezing was impossible. Therefore, from the 2nd generation onwards, we phenotyped adults from a different, but representative batch of pupae, that was not used for further culturing (see above).

Statistical analysis

For the statistical analysis we used the proportion of M^{II} males, the proportion of M^{III} males or the proportion of the wild type *bwb+* allele in the last generation of the experiment. We analyzed each of these proportions separately with a generalized linear model with binomial errors in R (R Development Core Team 2006). We used a likelihood-ratio approach to judge the significance of the effect of temperature, using an F test to correct for overdispersion. We compared the final frequencies of M^{II} and M^{III} males and the frequency of the *bwb+* allele with their initial frequencies using a binomial test.

Results and discussion

The average frequencies of the *M* factor located on autosome II increased significantly during the course of the experiment at both temperatures (Table 8.4, Fig 8.1A and B). The average proportion of autosomal *M* males after eight generations was one or close to one in most populations and did not differ between temperatures (Table 8.3), although at higher temperature M^{II} seems to reach fixation faster (Fig 8.1). This suggests that males with the autosomal *M* factor on the second chromosome have a selective advantage over males with *M* located on the Y chromosome in the temperature range we used.

Unfortunately, we cannot exclude the possibility that other genes linked with an autosomal *M* factor, in particular a wild type *ar+* allele, have an effect on the fitness of autosomal *M* males. As described above, we were not able to set up a control experiment to test whether a wildtype autosome II without an *M* factor would invade as well. We have some evidence that flies which are homozygous for the *ar* mutation do not have decreased egg to adult viability comparing to heterozygous *ar/ar+* males (not shown), but mal-developed antennae might have a detrimental effect in the adult stage.

The frequency of the *M* factor located on autosome III was not affected by the temperature and it did not significantly increase during the experiment ($P>0.5$ in binomial test for both temperatures pulled together; Table 8.3; Fig 8.1C and D). Although on average the frequencies of autosomal *M* males did slightly increase when compared to the initial frequencies, they were relatively stable between generations. Therefore, males with an M^{III} factor do not seem to have a noticeable fitness

Table 7.3. Absence of a temperature effect on the frequency of autosomal males and *bwb*+ allele. Results from a generalized linear model analysis of the frequencies of males with the *M* factor located on autosome II (A), autosome III (B) and frequencies of the *bwb*+ allele (C) in the last generation of the invasion experiment. There is no effect of temperature on the frequency of any of the genetic factors studied.

Model	DF	Deviance	*F*	*P*
A. M^{II}				
Temperature	7	391.38		
Null model*	8	392.44	0.016	>0.5
B. M^{III}				
Temperature	8	622.97		
Null model*	9	631.20	0.132	>0.5
C. Control				
Temperature	8	392.36		
Null model*	9	449.10	1.370	>0.2

* - includes only intercept

Table 7.3. Changes in the frequencies of males with M^{II} and M^{III}, and the frequency of *bwb*+ allele in the control. The initial and final frequencies (in generation 8) are given, together with the P value from the binomial test comparing them. For each experiment the results from the two temperatures were pooled together, since there is no difference between them. The frequency of males with M^{II} and *bwb*+ allele in control increased significantly during the experiment.

Experiment	Initial frequency	Final frequency	P
Males with M^{II}	0.20	0.85	<0.001
Males with M^{III}	0.20	0.38	>0.05
Control – *bwb*+ allele	0.05	0.11	<0.01

advantage over XY males. This result is puzzling, since the M^{III} factor is the most common among autosomal *M* factors in most of the studied populations worldwide (Denholm *et al.* 1990; Franco *et al.* 1982; Hamm *et al.* 2005; Tomita & Wada 1989b; Kozielska *et al.*, in press).

Moreover, in contrast to the M^{III} factor, the average frequency of the *bwb*+ allele in the control experiment increased significantly during the experiment ($P<0.01$ for both temperatures pooled together; Fig 8.1), suggesting that the *M* on autosome III actually confers a fitness disadvantage to its bearer (Sokal & Sullivan 1963; Sullivan & Sokal 1965). However, we cannot exclude the alternative explanation for this pattern, that some genes on the wild type autosome III are incompatible with the marker-strain background. When linked with the *M* factor, they could not be

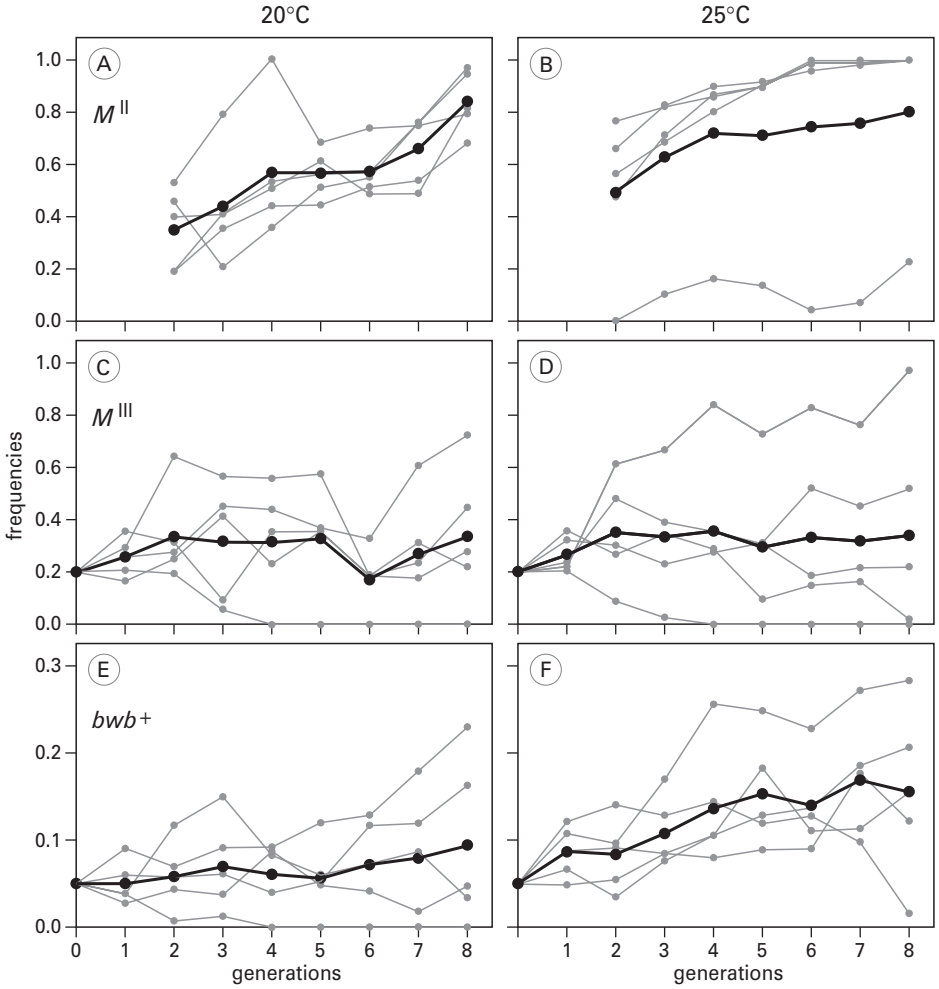


Figure 8.1. Frequencies of males with the M^{II} factor (A and B), males with the M^{III} factor (C and D) and the frequency of *bwb*+ allele in control (E and F) at two different temperatures (20ºC and 25ºC) during the invasion experiment. Grey lines represent five different replicates and the black line their average. Data for generation 1 for the invasion of M^{II} is lacking (see Material and Methods).

removed from the population by recombination, since they were present only in males and crossing-over does rarely occur in males (see Franco *et al.* 1982). In contrast, in the control experiment the wild type autosome could also be present in females in which recombination could have removed initial linkage of incompatible wildtype alleles with the *bwb*+ allele. Future experiments measuring the invasion success of autosomal *M* factors under variable genetic backgrounds may be able to minimize the effect of genetic incompatibility on the spread of autosomal *M* factors.

Although the invasion experiments allow a more realistic assessment of competitive abilities associated with different SD factors than individual fitness essays, they still may not be able to include all fitness aspects. For example, if the presence of an autosomal factor confers a fitness advantage mainly in the later lifetime period, our experiment would not have measured it, since for logistic reasons we only allowed females to lay eggs for a relatively short period. Therefore, only early life time fitness was taken into account in our experiment. Also, all males and females emerged within a relatively short time period, which may increase competition between males above levels seen in nature. Alternatively, if males with M^{III} have a slightly longer developmental time than marker-strain males (Sokal & Sullivan 1963), they may miss most of the mating possibilities, since female houseflies usually mate only once before laying eggs (Andres & Arnqvist 2001; Hicks *et al.* 2004; Riemann *et al.* 1967).

PART II: RELATIVE FITNESS OF FEMALES WITH THE F^D FACTOR

Material and Methods

Housefly strains

Introduction of F^D factor into different genetic background is very slow and labour-intensive, since usually multiple *M* factors segregate in different lines and both types of females (with and without F^D) are produced. Therefore, instead of performing an invasion experiment, we decided to assess life time fitness (and some of its components) of individual F^D and *F* females. We used females from three different wild type strains:

1) CAM – a wild type strain where the frequency of F^D females is around one quarter. This strain possesses *M* factors located on the Y chromosome and autosome III. This strain was established from flies collected in Camargue, France, in 2004. It is the same wild type strain from which the CAM-M^{III} strain used in the *M* factor invasion experiment was established. It was maintained at a population size of approximately 500 flies prior to the experiment (as were all the other strains).

2) FVG – a wild type strain in which the frequency of F^D is around 0.5. The *M* factor has been found on autosome II, but since fewer than 5 males were checked, it can be present also on other chromosomes. This strain was established from flies caught in Faverges, France, in 2004

3) UML – a wild type strain in which the frequency of F^D females is around 0.5, *M* factors are located on autosomes I, II, III and V. It was established from flies caught in South Africa in 2005 (Feldmeyer *et al.*, submitted).

Experimental procedure

F and F^D females cannot be distinguished phenotypically. Therefore we measured several fitness components of 50 randomly chosen females from each population.

After death the genotype of those females was determined using the molecular technique described in Kozielska *et al.*, in press. The experiment started in February 2006.

Since the temperature sensitive period of development starts already during oogenesis (Schmidt *et al.* 1997a), we placed mothers of focal females at the experimental temperatures just after emergence. 50 females and 50 males were placed in population cages at two different temperatures: 20°C and 27°C. We used a slightly wider temperature range than for the *M* invasion experiments to increase the chance of detecting an effect of temperature. This increase of temperature was possible because higher temperatures do not seem to affect adult flies as negatively when they are kept in singe pairs, compared to larger numbers of flies in population cages. A further increase in temperature would largely exceed conditions found in nature (see below). A lower temperature than 20°C would have yielded very low offspring numbers, especially from single-female egg batches (personal observation). The rearing conditions were the same as in the invasion experiment unless mentioned otherwise.

When the females reached maturity they were allowed to lay eggs which later developed at the same temperature as experienced by the mothers. After pupation around 1000 pupae from each population and temperature were collected and when the flies started to emerge in large numbers, 50 females from each temperature treatment of each population were collected within 24 hours after emergence and weighed individually on an electronic laboratory scale. All 50 females used in the experiment emerged within one day or sometimes two days. Each female was placed individually with two males from the same population and of the same age in 180 ml transparent containers and provided with sugar water and milk powder. At the same time we collected around 50 additional males and placed them together with an equal number of females. These males were used to replace dead males in containers with experimental females. We used two males per female to reduce the chance that a female would not produce eggs should her mate be infertile. After 7/5 days (in 20/27°C) females were provided with egg-laying medium. Every 5/3 days the egg-laying medium together with eggs or larvae was transferred to bigger boxes where the larvae developed at the same temperature as the mothers and new egg-laying medium was provided to females. Every day we checked for dead females, which were frozen for later molecular analysis. We let all the offspring develop till the adult stage and we counted all emerging flies.

Statistical analysis

Many females did not have any offspring, leading to a strongly skewed distribution of offspring number with an excess of zeros. Therefore, the lifetime offspring production was modelled with a hurdle model in R, using the hurdle function from the pscl package (Zeileis *et al.* 2007). It is a two-component model: a truncated count component is employed for positive counts and a hurdle component models zero vs. larger counts. For the latter a binomial distribution was used. Females' lifespan was

modelled with Generalized Linear Models with gamma errors in R (Crawley 2007). In both models, for lifespan and offspring production, weight was used as a continuous explanatory variable and temperature, population and SD factor (F^D vs. F) as discrete variables. We started with a full model (including all interactions between discrete variables) and used backward selection to find a minimum adequate model. Significance of the models was assessed with a likelihood-ratio approach.

Results and discussion

Average weight, lifespan and lifetime offspring production of females with F and F^D from different populations and under different temperature conditions are presented in Fig 8.2. Neither the SD factor (F/F^D) nor the interaction of the SD factor with temperature had a significant effect on female fitness. Female fitness is differentially affected by temperature in different populations, as shown by a significant effect of interaction between temperature and population on the females' lifespan and on the lifetime offspring production (Table 8.5). Offspring production seems not to be governed only by differences in lifespan, since at higher temperature lifespan was always shorter (as expected: Fletcher *et al.* 1990; Lysyk 1991), but higher temperatures had a positive effect on lifetime reproductive success in two populations (CAM and UML) and a negative effect in one (FVG).

The different effects of temperature on the lifetime fitness of females from different populations could stem from adaptation to the local conditions of the original population. In the field the FVG population probably only rarely experienced temperatures above 20°C, whereas the average temperatures experienced by CAM are about 5°C higher, and average maximum daily temperatures exceed 22°C

Table 7.5. Factors affecting female fitness. Results of statistical analysis of the lifespan and lifetime offspring production of females. Only statistically significant effects are listed. Δ DF represents the difference in degrees of freedom between the final model and the model without the listed variable.

Model	Δ DF	$\chi 2$	P
Lifespan[1]			
PopulationxTemperature	2	5.529	0.006
Lifetime offspring production[2]			
Weight	2	10.925	0.004
PopulationxTemperature	4	26.901	<0.001

[1] – Final model (Population + Temperature + PopulationxTemperature) has residual DF = 278 and deviance 85.115 [2] – Final model (Weight + Population + Temperature + PopulationxTemperature) has DF = 15 and log-likelihood = -958.45

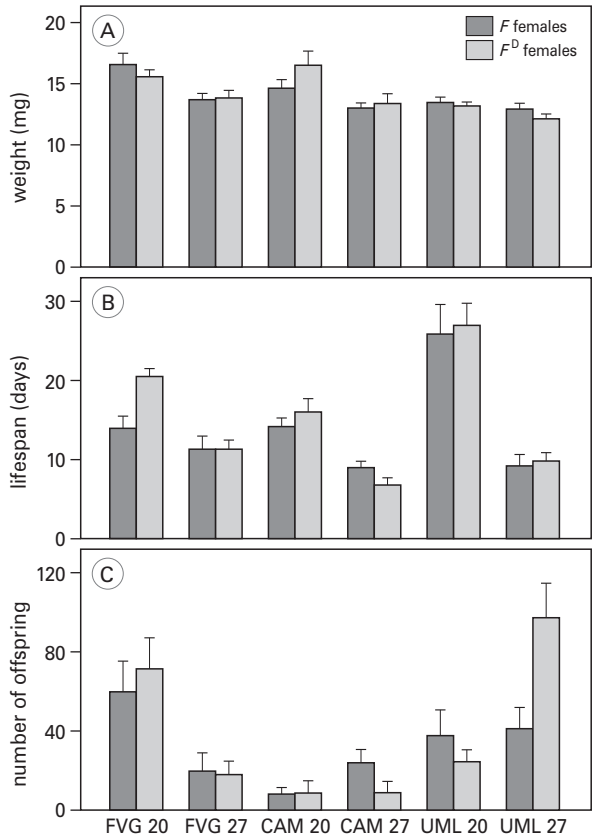


Figure 8.2. Average weight (A), lifespan (B) and lifetime offspring production (C) of females with *F* and F^D from different populations at 20 and 27°C (as listed after the strain name). Error bars represent standard errors.

throughout the year in the location from which the UML population originated (temperature data from http://www.worldclim.org; Hijmans *et al.* 2005; not shown). The low fitness of CAM females can be an indicator of their general low genetic quality and may also explain the low fitness of males from this population (see invasion experiment above).

We did not find any evidence that F^D females have higher fitness at higher temperatures and *F* females under lower temperatures, or any other effect of SD factor (*F* vs. F^D) on female fitness. Theoretically, it is possible that under the temperatures we studied *F* and F^D are neutral and only higher temperatures are favourable for the F^D factor, but temperature data from natural populations would contradict this hypothesis (see General Discussion). A more plausible explanation is that the fitness differences between F^D and *F* females are visible only under more competitive conditions than experienced by the females and their offspring in this experiment.

General discussion

We did not find a clear general effect of temperature neither on the fitness of autosomal *M* males nor on the fitness of females with or without an F^D factor. One might argue that the temperature range we used was too narrow and not representative of the temperatures experienced by the houseflies in nature. Although there may be some truth to this explanation, it does not fully explain our results.

Since M^{II} spread quickly in both temperatures, it may be that the temperatures we used were too high to detect a fitness advantage of XY males that presumably exists under low temperatures in nature (see Feldmeyer *et al.*, submitted). Indeed, average yearly ambient temperatures of 25°C or even 20°C are rare (at least in Europe; temperature data from http://www.worldclim.org; Hijmans *et al.* 2005) and high frequencies of autosomal *M* factors already occur at lower temperatures (Kozielska *et al.*, in press; Feldmeyer *et al.*, submitted). In contrast to M^{II}, M^{III} did not increase in frequency during the experiment, suggesting one obvious explanation that the range of temperature studied was too low for the M^{III} factor to show its fitness advantage. However, as discussed above, this is rather improbable. Similarly, female fitness at different temperatures was not affected by the presence or absence of the F^D factor, suggesting that the *F* and F^D factors are neutral at the used temperatures. As before, this explanation is improbable, since high frequencies of F^D females were also found in populations in which even in summer months the maximum daily temperature is below 20°C (e. g. in most of Italy, Kozielska *et al.*, in press; temperature data not shown).

Under natural conditions ambient temperatures are much more variable than in our experiments and although average yearly temperatures correlate with the frequencies of autosomal SD factors (Feldmeyer *et al.*, submitted), the effect of temperature may be a complex phenomenon (see Feldmeyer *et al.*, submitted). Seasonal or daily temperature extremes or temperature fluctuations may be more important for the long term fitness of different SD factors than average temperatures *per se*. In the wild, flies can also actively seek temperatures that are optimal for them, which may be different at different developmental stages (West 1951). Another possibility is that other climatic factors, e.g. humidity, interact with temperature, creating the geographical distribution of SD factors seen today (see Kozielska *et al.*, in press).

Different SD factors may need to be studied together, since they can affect each other dynamics. For example, the F^D factor may not by itself be affected by temperature, but if at higher temperatures autosomal *M* factors confer higher fitness to both sexes, then F^D females would indirectly gain fitness since they, in contrast to standard *F* females, can possess autosomal *M* factors. The fact that only females from UML seem to follow the expected pattern of higher fitness of F^D females under higher temperatures could support this hypothesis, since in this population all males, and presumably all F^D females, possess at least one autosomal *M* factor

(Feldmeyer *et al.*, submitted). In the CAM population, on the other hand, the frequency of XY males was around 65% (4 months prior to the experiment, results not shown). Therefore, the frequency of autosomal *M* factors in F^D females is probably relatively low. We do not know the exact frequencies of different *M* factors in the FVG population. Indirect fitness gain of F^D females through possessing autosomal *M* could explain why F^D has been found mainly in populations in which autosomal *M* factors were present (Denholm *et al.* 1990; Franco *et al.* 1982; Tomita & Wada 1989b; Kozielska *et al.*, in press; Feldmeyer *et al.*, submitted). Also, even if autosomal *M* factors were beneficial only to males, that would lead to male biased sex ratios and consequently could facilitate spread of F^D to assure even sex ratios. Future experiments controlling for the presence of *M* factors in F^D females are necessary to determine any fitness effect of the presence of *M* factors in females.

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CHAPTER 9

Do *M* factors show segregation distortion in European populations of the housefly?

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Abstract

Multiple sex determining factors coexist in natural populations of the housefly, *Musca domestica*. It has been shown theoretically that linkage to segregation distorters may facilitate the spread of autosomal male-determining *M* factors. Association between autosomal *M* factors and sex ratio distortion has been found to be common in North American populations. Here, we assess the prevalence of *M*-linked segregation distortion in European housefly populations. In this study, we sampled eight populations in Western Europe and introgressed one or two *M* factors from each population into a genetic background of a standard laboratory strain in order to eliminate any possible suppressors of distortion. During each generation of introgression, we analyzed the offspring sex ratio from mass crosses between males with *M* factors and females from the laboratory strain. We found that males with a Y chromosome produced unbiased or even female-biased sex ratios, suggesting that Y chromosomes do not posses segregation distorters. Only one autosomal *M* factor was associated with a consistent, strong male-bias sex ratio. This could have been caused by an *M*-linked distorter, but sex-specific mortality could not be excluded. Offspring sex ratios of other autosomal *M* males were often male-biased, but the sex ratios varied a lot between generations. Therefore, we conclude that *M*-linked segregation distortion is not common in European housefly populations. This suggests that association with sex ratio distorters does not play a major role in maintaining the variability in autosomal sex determining factors in the housefly.

Introduction

Sex chromosome segregation distortion (one of the chromosomes segregates to the majority of gametes of heterogametic individuals) may lead to transitions between sex determining systems (see Chapter 4). In particular, autosomal *M* segregation distortion has been proposed as a force leading to the spread of autosomal sex determining factors in the housefly, *Musca domestica* (Clark 1999; see Chapter 1 for a description of the sex determining mechanism in the housefly).

An almost complete lack of recombination in male houseflies (see Franco *et al.* 1982; Hiroyoshi *et al.* 1982; Inoue & Hiroyoshi 1982; personal observations) may indeed favour the evolution of distorter alleles linked with male determining factors. The most widely accepted model of segregation distortion assumes that two loci are involved: a "distorter" locus and a "responder" locus (Lyttle 1991; Jaenike 2001; Burt & Trivers 2006). A distorter allele shows segregation distortion against the chromosome with a sensitive responder allele. To be selectively favoured, the distorter allele has to be linked with an insensitive responder allele. Therefore, segregation distortion is expected to evolve more easily on sex chromosomes, where recombination is usually restricted (Lyttle 1991; Jaenike 2001). In the male housefly there is little or no recombination along the whole genome, both in XY and autosomal *M* males (see Franco *et al.* 1982; Hiroyoshi *et al.* 1982; Inoue & Hiroyoshi 1982; personal observations). One could therefore expect that a distorter allele will easily spread if it is linked with male determining factors, since the linkage between distorter alleles and insensitive responder will not be broken by recombination. Conversely, male determining factors showing biased segregation due to linkage to distorter alleles are also expected to spread (Chapter 4).

An autosomal *M* factor located on autosome III shows strong segregation distortion (75-90%) in most investigated natural populations of the housefly in the USA (Clark 1999). However, no studies have been done to investigate the presence of *M*-linked distorters in natural populations of houseflies outside the USA.

Determining the sex ratio among offspring of females caught in the field or taken directly from laboratory populations is not very informative, since usually multiple sex determining factors are present in the population (Tomita & Wada 1989b; Kozielska *et al.*, in press), and family sex ratios can be strongly male or female biased even without sex ratio distorters (Kozielska *et al.* 2006). Additionally, in populations with segregation distorters, suppressors are expected to evolve in order to restore equal sex ratios. (Hurst *et al.* 1996; Jaenike 2001; Burt & Trivers 2006). Suppressors of distortion have been found not only on the Y chromosome, but also on autosomes in many species with sex-ratio distorters (Cazemajor *et al.* 1997; Capillon & Atlan 1999; Jaenike 1999; Montchamp-Moreau *et al.* 2001; Atlan *et al.* 2003). Therefore, segregation distortion is usually more effective and easier to detect in an alien background, after a number of generations of backcrosses with individuals from different populations which harbour no suppressors of segregation distortion, or population-

specific suppressors (see e.g. Atlan *et al.* 2003; Burt & Trivers 2006; Tao *et al.* 2007).

Here we present the results of a pilot study to asses the prevalence of *M*-linked segregation distortion in European populations of the housefly. We sampled a number of populations from Western Europe and from each of them two males were backcrossed to marker strain females for a number of generations. We score the sex ratios among the progeny, which is expected to be male-biased if *M* is linked to distorter allele.

Material and methods

The experiment presented here was the first experiment conducted in our laboratory with housefly cultures. It was meant as a pilot study and was performed on a small scale. We collected adult flies from a number of locations in Europe (see below) between May and August 2004. After transportation to the laboratory, strains from different locations were kept under standard conditions (see Chapter 8) at a population size of approximately 500 flies.

In the experiment flies from the following strains were used:

1) CAP – from Campineira, southern Spain; *M* factor on autosome II.
2) HOS –Salobrena, southern Spain; *M* factor on autosome II.
3) SFE –Santa Fe, southern Spain; *M* factor on autosome II.
4) FVG – Faverges, central-eastern France; *M* factor on autosome II.
5) MON –Monachil, southern Spain; *M* factors on autosomes II and III.
6) CAM – Camargue region, southern France; *M* factor on Y chromosome and autosome III.
7) SDF –Seedorf, central Switzerland; standard XY sex determining system.
8) MID – Midlaren, the northern Netherlands; XY system.

The experiment started in the summer of 2004 with two generation of single pair crosses of two males from each strain (although we did not obtain offspring from all of them) with marker-strain females in order to establish on which chromosome(s) *M* factors are located. Flies from the marker strain are homozygous for five visible recessive mutations: *ac, ar, bwb, ye* and *snp* on autosomes I, II, III, IV and V, respectively. This strain possesses a standard XY sex determining system. After the first generation of backcrosses, five male offspring from each parental male were each crossed again individually with a virgin marker-strain female. Sex linked segregation of visible markers in the offspring of this cross indicates on which chromosome an *M* factor is located (for details see e.g. Kozielska *et al.*, in press).

Since backcrosses with marker-strain females were necessary to establish the location of *M* factors in each population, we continued the backcrossing to this strain in order to replace the genetic background of the test strain except for the chromosome with the *M* factor. Thus potential suppressors of segregation distortion were likely to be removed from the experimental line, allowing easier detection of sex

ratio distortion (see Introduction). From each male from the wild-caught strain, an experimental line was established. Therefore, in each line only one chromosome with the *M* factor was segregating.

After the two generations of single-pair backcrosses described above, the following protocol was used for propagation of the lines and sex ratio estimation: 25 freshly emerged male offspring were placed with 25 virgin marker-strain females in population cages and provided with water, sugar water and milk powder *at libitum*. After 5-7 days when females are most prone to lay eggs, egg laying medium (according to Hilfiker-Kleiner *et al.* 1994) was placed in each cage for two days. The eggs laid during that period were then transferred to bigger boxes where the larvae developed and were fed *at libitum* on the same medium until pupation. If too many larvae were present, some were discarded at an early stage, to keep the total number between 100 and 300 and prevent crowding. The flies were kept at 25ºC during the entire developmental period. Adults that emerged from the pupae were counted and sexed. The sex ratio (proportion males) was calculated. The whole life cycle lasted approximately 21 days.

25 male offspring were used for the next generation of backcrosses. For the crosses we selected the males with the largest number of visible mutations, in order to faster replace the original background of each *M* factor with the genetic background of the marker strain. This was achieved from the 4^{th} or 5^{th} generation onwards, when all males were homozygous for either all five visible markers (XY males) or for four visible markers, excluding the one linked with the *M* factor, in the case of autosomal *M* males. Since recombination in males is very rare (Franco *et al.* 1982; Hiroyoshi *et al.* 1982; Inoue & Hiroyoshi 1982) it was impossible to replace the genetic background of the chromosome on which the *M* factor was located, implying that autosomal *M* males were heterozygous for a marker-strain and a wild type chromosome. We did the backcrosses for a few more generations to increase the chance that the X chromosome from the original strain was also replaced with the X chromosome from the marker strain (no visible mutation present). However, we do not expect much influence of the X chromosome on the sex ratio since it seems to possess very few genes (Dübendorfer *et al.* 2002) and the last few generations of backcrosses should reveal whether a sex ratio bias, if present, is stable.

Due to logistic reasons not all the experimental lines started at the same time, but they were divided into three blocks. Lines resulting from the strains MON, CAM and MID were started first. About one generation later the lines from CAP, HOS, FVG and SDF were established and the two lines from the SFE strain were the last. For all the lines the experiment stopped at the same time resulting in five to seven generations of backcrosses. Due to chance events sex ratio data were not obtained in a few cases (SFE lines in generation 4; MON lines in generation 3 and CAM line with Y chromosome in generation 3), but the experiment was continued normally.

The sex ratio at emergence does not necessarily result from biased segregation during spermatogenesis. Therefore, we tried to assess the viability from egg to adult

in order to check whether differential mortality between males and females might cause sex ratio bias. To this end, we randomly selected 100 individual eggs from each cage, let them develop until adulthood and scored all emerging offspring. Unfortunately, even though special care was taken not to dehydrate or damage individual eggs during counting, survival was very low, often below 20% and in many cases even zero. Therefore, most of the mortality probably stemmed from handling and we were not able to assess the potential role of differential mortality.

Each generation, binomial tests were used to assess whether offspring sex ratios from mass crosses deviated significantly from 50:50. Additionally, we checked whether sex ratios produced by lines with a Y chromosome differed from sex ratios produced by lines with autosomal *M* factors. We performed a mixed effect logistic regression for longitudinal data, using the lmer function (from the lme4 package) with quasibinomial errors in R (R Development Core Team 2006). We treated the *M* location (Y vs. autosomal) as a fixed effect, line nested within the *M* location, and generations as repeated measurements. We compared the full model with the one without *M* location effect using a likelihood-ratio approach, using an F-test to correct for overdispersion (Krackow & Tkadlec 2001; Feldmeyer *et al.*, submitted). It should be noted that since we do not know the exact number of fathers siring offspring in our mass crosses the statistical analysis should be interpreted with caution.

Results

Fig 9.1 shows the sex ratios in generations 3 to 7 among the offspring of males from various lines. There is considerable variation between different strains, but also between generations. Only one of the lines shows a consistently biased sex ratio – CAMa with *M* on autosomal III, with the frequency of males between 0.6 and 0.7.

Sex ratios in the lines with autosomal *M* factors are often male biased, but only in a few cases significantly so (Fig 9.1). The lines with a Y chromosome show more equal sex ratios or even a slight bias towards females. However, there is no statistically significant difference in sex ratios between lines with an *M* factor on a Y chromosome and lines with an *M* factor on an autosome ($p=0.65$; $F=0.904$).

Discussion

We did not find clear evidence for *M*-linked segregation distortion in the studied European housefly populations. In a number of cases, the sex ratio in autosomal *M* lines was much higher than 0.5, but this usually happened during only one or two generations per population. There was strong variation in sex ratios between generations, but it is difficult to determine its cause.

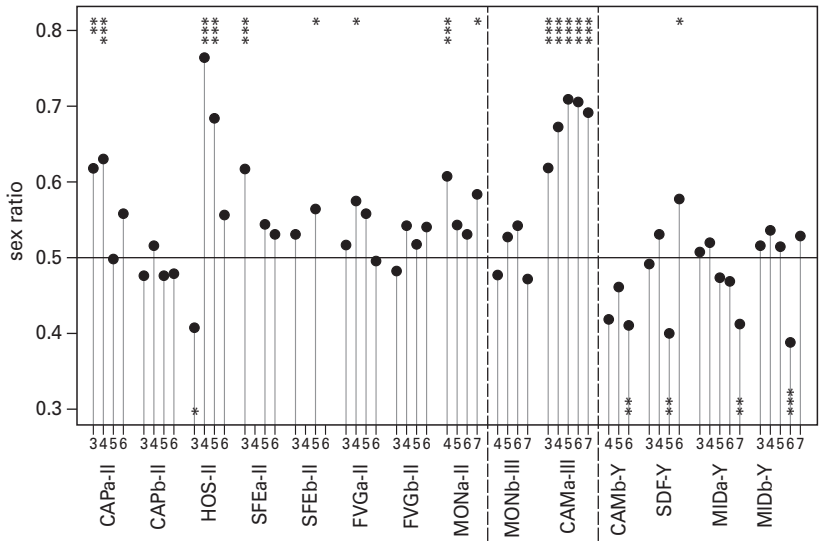

Figure 9.1. Sex ratios (proportion males) in experimental lines with different *M* factors. Different lines are encoded by a three-letter code (CAP, etc.). When two different males from the original populations were used to start the experimental lines, these lines are indicated by "a" and "b". The location of the *M* factor in a given line is indicated by II or III, for autosome II and III, respectively, or by Y for the Y chromosome. For each line, the sex ratio from a number of generations of backcrosses is indicated. Sex ratios significantly higher than 0.5 are indicated by stars in the top part of the figure and the ones lower than 0.5 on the bottom.
* - $p<0.5$; ** - $p<0.01$; *** - $p<0.001$.

It has to be noted that the sex ratio on emergence does not necessarily reflect a biased segregation during spermatogenesis. Differential mortality between males and females may be another cause of sex ratio bias. We tried to assess egg to adult mortality in our lines, but without success. Therefore, we can not exclude that any sex ratio bias observed during the experiment was merely caused by sex-specific mortality. Some uncontrolled environmental conditions (e.g. humidity) affecting sex-specific mortality might have been the reason for the large variation in sex ratio between generations.

Only one *M* factor, on autosome III, in the population from France (CAMa line) showed a strong, consistent male-biased sex ratio. As mentioned above, we cannot exclude the possibility that this sex ratio bias was caused by a higher mortality of females. In our experiment, females were homozygous for a visible mutation on autosome III (brown body). In contrast, males were heterozygous, showing a wild type phenotype (black body). As shown in Chapter 8, the wild type phenotype is probably fitter than the mutant one, which might have caused a relatively lower mortality of males and a biased sex ratio (for more discussion see Chapter 8). However, this is probably only a weak effect, since *M* located on autosome III from

the other line (MONb) is not associated with any sex ratio bias. Alternatively, since the marker strain is inbred, hybridisation with an unrelated strain may lead to increased fitness in hybrid males (the so-called heterosis effect; Lippman & Zamir 2007). This effect may be present in the CAMa line, but not in the MONb line. A decreased fitness of homozygous mutant females or the heterosis effect could also contribute to the on average higher frequency of males in most of the lines with autosomal *M* factors. Since heterosis is known to be affected by environmental conditions (Lippman & Zamir 2007), it could also contribute to the high variation in sex ratio between generations. Clearly, studies measuring egg to adult mortality are necessary to be able to unambiguously show segregation distortion.

The problems of the fitness effect of the marker mentioned above should not have affected the lines with a Y chromosome. In these lines both males and females posses all autosomes from the marker strain. Since the sex chromosomes of the housefly do not posses any known functional genes and seem to be equivalent with respect to fertility and fecundity (Dübendorfer *et al.* 2002), we would not expect a difference in mortality between males and females. Therefore, the equal (or even slightly female biased) sex ratios observed in lines with a Y chromosome are strongly indicative of a lack of Y chromosome segregation distortion.

Interestingly, all distorter *M* factors found so far in the housefly are located on autosome III (possibly line CAMa in this study; Clark 1999). It is possible that this chromosome possessed a segregation distorter before the *M* factor was present, while there were no distorters on autosome II. It would be interesting to investigate if there are any non-*M*-linked distorter alleles in the housefly genome, similar to the Segregation Distorter complex on chromosome II in *Drosophila melanogaster* (Lyttle 1991).

Our sample was too small to assess the frequency of M^{III}–linked (on autosome III) distorters, and the presence of M^{II}–linked distorters in other European populations can also not be excluded. However, our results suggest that the linkage of *M* factors with segregation distortion is not a common phenomenon in European housefly populations. This may not be a surprise, taking into account that sex ratio distorters in other species are usually also present at low frequencies in natural populations (Lyttle 1991; Jaenike 1996; Burt & Trivers 2006; Wilkinson *et al.* 2006). These low frequencies are usually attributed to the low fitness of individuals carrying distorter alleles, both males and females (Wallace 1948; Curtsinger & Feldman 1980; Beckenbach 1983; Jaenike 1996; Atlan *et al.* 2004; Wilkinson *et al.* 2006).

Interestingly, in the North America, seven out of the nine investigated *M* factors from different natural populations showed a considerable sex ratio bias (75-90% males) while in Europe the total prevalence of *M* factors showing segregation distortion seems to be much lower. Additionally, the frequency of F^{D} is very low in the USA (McDonald *et al.* 1975; Hamm *et al.* 2005), while it is frequent in Europe (present in all autosomal populations, see Kozielska *et al.*, in press; Franco *et al.*

1982). The low frequency of recombination in male houseflies (Franco *et al.* 1982; Hiroyoshi *et al.* 1982; Inoue & Hiroyoshi 1982) should facilitate the spread of *M* factors linked with distorters (see Introduction), but only if F^D is absent, as it is in North America. The presence of F^D causes *M* to also segregate in females. This leads to easier breakage of the association between the *M* factor and distorter allele, which may explain the lower frequencies of *M* factors with segregation distortion in European populations. However, the low frequency of F^D in North America is surprising, since the presence of *M*–linked distorters should favour the spread of F^D (Chapter 4).

It has never been investigated systematically whether segregation distorters on X chromosomes exist in the housefly. They exist in many other Dipteran species (for review see e.g. Jaenike 2001; Burt & Trivers 2006) and X chromosome distortion could facilitate the spread of autosomal *M* factors (Chapter 4). Therefore, it would be interesting to investigate whether X chromosome segregation distortion is also present in the housefly. However, the current lack of X chromosomal distortion in populations, does not exclude that X chromosome segregation distortion was an important factor in the past, facilitating changes in sex determining mechanism (Chapter 4).

Our small scale pilot study suggests that autosomal segregation distortion does not play a major role in the maintenance of the variety of sex determining factors in the housefly, since most of the autosomal *M* factors do not seem to show segregation distortion. However, we cannot exclude that the initial spread of autosomal *M* factors was facilitated by linkage with segregation distortion, which later was broken by the segregation through females due to the F^D factor. Presently different sex determining factors may just coexist in stable neutral polymorphism (Kozielska *et al.* 2006; Kozielska *et al.*, in press). Large scale studies, with larger sample sizes per population and including viability measures are necessary to more accurately assess the importance of segregation distortion in the evolution of the housefly sex determination system.

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CHAPTER 10

Epilogue

The evolution of sex determination is an interesting problem. Even though one would intuitively expect that such a fundamental developmental process should be evolutionarily conserved, this is not the case. Comparative studies have shown that sex determining (SD) mechanisms can evolve rapidly and that shifts between different mechanisms are common in various lineages (e.g. Bull 1983; Kraak & Pen 2002; Janzen & Phillips 2006). Comparative studies at the molecular level support the hypothesis that SD cascades evolved from the bottom up (Wilkins 1995). Comparative studies could also potentially be used to test the predictions made by theoretical models. However, comparisons between different species should always be taken with caution. Species differ in many characteristics and it is impossible to correct for all the differences.

An excellent alternative to comparative studies would be to study the evolution of sex determination in species in which multiple SD mechanisms coexist. Studying the variety of SD mechanisms in natural populations of these species allows the verification of existing theories and development of new ones. Moreover, species with multiple SD mechanisms could also be used in controlled laboratory experiments to test theoretical models. However, there are only very few species in which the SD mechanism is not fixed (Bull 1983). The housefly, *Musca domestica*, is one of these exceptions.

This project was inspired by the multiple SD factors which are present in natural and laboratory populations of the housefly (Dübendorfer *et al.* 2002). This natural polymorphism poses an interesting question by itself. However, more importantly the housefly seemed like a good species to test theoretical models in controlled laboratory experiments. Mechanistic models also needed to be developed in order to understand the dynamics of this specific system as well as to gain insight into the evolution of SD mechanisms in general.

A number of aspects of the results of this project have already been discussed in the previous chapters. Here, I would like to bring the results together, discuss them in a somewhat broader context and further speculate on their implications. First, I will concentrate on the evolution of sex determination in the housefly in the light of the empirical and theoretical results of this project. Second, I will present my conclusions concerning the theoretical approach to the evolution of SD mechanisms in general.

Insights into the evolution of sex determination in the housefly

One of the goals of this thesis was to better understand the evolution of the multiple SD mechanisms in the housefly. In "standard" strains of this species, sex is determined by a male determining factor, *M*, which is located on the Y chromosome; therefore males are XY and females are XX. During development, the *M* factor blocks the female determining factor *F* located on autosome IV, the activity of which

is necessary for female development. In many populations, *M* is located on one of the autosomes or even on the X chromosome. In such populations a dominant constitutive mutation of *F* (F^D) is usually also present, which triggers female development even in the presence of several *M* factors in the same individual (for details see Dübendorfer *et al.* 2002; Chapter 1). The distribution of SD factors in the housefly follows geographical clines, with the standard XY system present at high latitudes and altitudes and the frequency of autosomal factors (*M* and F^D) increasing with decreasing latitude and altitude (Franco *et al.* 1982; Tomita & Wada 1989b; Çakir & Kence 1996; Hamm *et al.* 2005).

The variety of SD factors in the housefly poses a number of questions. Why did autosomal SD factors invade the standard system? Why are they only present at lower latitudes or, in other words, what forces are responsible for the distribution of SD mechanisms? Is the coexistence of SD mechanisms in different populations stable, and if so, how is polymorphism maintained? The empirical data and the results of the theoretical models obtained in this project help to, at least partly, answer these questions.

The results presented in this thesis suggest that the worldwide distribution of SD factors in the housefly is governed mainly by differences in temperature: higher frequencies of autosomal factors are associated with higher ambient temperatures (Chapter 6). However, additional environmental factors can be also involved (see Chapters 7 and 8). Contrary to the expectations of early researchers (Franco *et al.* 1982; Tomita & Wada 1989b) this distribution seems to be stable and autosomal SD factors are not replacing standard XY system at higher latitudes (at least in Europe; Chapter 7). These results suggest that conditions in colder regions favour the standard XY system, but in warmer regions autosomal SD factors seem to have an advantage. However, since multiple SD factors coexist in many populations (Denholm *et al.* 1985; Tomita & Wada 1989b; Denholm *et al.* 1990; Chapter 7), a multi-factorial SD system may be sometimes favoured by selection.

There is a number of hypotheses trying to account for the evolution and distribution of different SD factors in the housefly, most of which I have already discussed (Chapters 2, 6-9). Here, I will present the different hypotheses in a more systematic manner (see Chapter 1) and will discuss in more detail how the theoretical models presented in this thesis can help to understand the evolution of SD mechanisms in the housefly. I will concentrate on three aspects: the initial spread of autosomal SD factors, their clinal distribution and the maintenance of polymorphism of different factors in single populations (Table 10.1).

Indirect selection

It is conceivable that different SD factors are not themselves targets of selection, but that they hitchhike with other genes under positive selection. Since the first isolation of autosomal *M* factors coincided with the appearance of DDT resistance in natural housefly populations, it has been proposed that the linkage with insecticide resistance

Table 10.1. Summary of different selective forces studied with theoretical models in this thesis and their ability to explain the evolution of sex determination in the housefly. Three aspects are considered: invasion of autosomal SD factors, maintenance of multi-factorial system (polymorphism) and the clinal distribution of different SD factors in natural housefly populations. A plus sign means that the given selective force could be involved and a minus that it is unlikely to be, according to the models presented in this thesis and available empirical data. See main text for details.

Selective force	invasion	polymorphism	clinal distribution	Chapter #
Selection against intersexes	+			3
Sex ratio selection	+	+	–	2,3
Maternal-offspring conflict	+			3
Segregation distortion	+	+	–	4
SA variation	+			5

genes led to the spread of autosomal *M* factors (Kerr 1970; Franco *et al.* 1982; Tomita & Wada 1989b). Although this mechanism could indeed have played some role in the spread of autosomal *M* factors, it is unlikely to explain the clinal distribution of SD factors, because insecticide use is not limited to warmer climates and furthermore there is no correlation between frequency of autosomal *M* factors and insecticide resistance in the eastern United States (for details see Chapter 7).

Alternatively, autosomal SD factors might be linked with genes beneficial at higher temperatures, but detrimental at lower temperatures. Such a linkage could explain the initial spread, clinal distribution and polymorphism of different factors at intermediate temperatures. However, there is not enough data to support or exclude this hypothesis. Isozymes of the lactate dehydrogenase show a clinal distribution in Japan (Agatsuma & Takeuchi 1978), but their linkage with autosomal *M* factors is unknown. To my knowledge, nothing is known about the clinal variation of other genes or their linkage with autosomal *M* factors.

Selection against intersexes

As has been shown in Chapter 3, selection against intersexes can be a powerful force in the evolution of SD mechanisms. It is possible that it also plays a role in the evolution of the housefly SD system. SD factors of the housefly seem to be at least partly temperature sensitive, leading to the production of intersexes under some conditions. In natural populations more intersexes have been observed in winter than in summer (Milani 1967). A clear temperature effect has also been observed in some laboratory strains, in which under either low or high (depending on strain) temperatures many intersexes are produced (Vanossi Este & Rovati 1982; Schmidt *et al.* 1997a; Schmidt *et al.* 1997b; see also Box 2 in Chapter 1). We did not explicitly

model the evolution of the housefly system under temperature dependent viability and fertility. However, it is conceivable that if production of intersexes under the standard XY system increases with increasing temperatures, autosomal SD factors could invade, if they lead to a more reliable developmental cue. This could explain the current distribution and polymorphism of SD factors in natural populations. However, this has yet to be verified theoretically and empirically. It would therefore be interesting to study the expression pattern of different SD factors under different temperatures. However, before this is possible, a molecular characterisation of *M* factors would be required.

Sex ratio selection

Theoretical models show that sex ratio selection could maintain polymorphism for multiple SD factors (Chapter 2 and 3). The frequency of different factors depends on the direction (whether male- or female-biased sex ratios are favoured) and the strength of sex ratio selection (Figure 2.3 in Chapter 2). When selection favours male-biased sex ratios, at equilibrium only the *M* factor which had the highest initial frequency persists in the population. No F^D is present and the male heterogametic system is stable. If selection favours female biased sex ratios, polymorphism at the *F* and *M* locus is maintained and neither F^D nor *M* reaches fixation. However, again only one *M* factor is present at equilibrium, the one with higher initial frequency. Can these results explain the geographical clines in the distribution of SD factors in the housefly?

According to the results of the model, the stability of the ancestral XY system at lower temperatures would suggest selection for male-biased sex ratios. Polymorphism for multiple factors in populations from warmer localities (Franco *et al.* 1982; Tomita & Wada 1989b; Chapters 6 and 7) would suggest selection for female-biased sex ratios. Little is known about presence of sex ratio selection in natural housefly populations (see Chapter 2). One of the factors involved could be differences in size between males and females. Female houseflies are bigger than males (Goulson *et al.* 1999; Chapman & Goulson 2000) which could potentially lead to selection for male-biased sex ratios. However, this effect could explain only the stability of the XY system in colder localities and it is unlikely that it could be reversed by higher temperatures. Selection for female-biased sex ratios is expected under inbreeding (Hamilton 1967; Werren & Hatcher 2000) and there is some evidence that local housefly populations may sometimes be small enough to experience some inbreeding (Krafsur 1985; Black & Krafsur 1986a). However, long-lasting reduction of population size is rather expected in colder climates, where during the winter housefly breeding is restricted to farms with very limited dispersal between local populations. When ambient temperatures are high enough houseflies can also breed outdoors and there is no differentiation between local populations (Black & Krafsur 1986a, 1986b). Therefore, selection for female-biased sex ratios under inbreeding should be strongest in colder climates, leading to the spread of F^D. This is clearly not the case.

Moreover, our model predicts that under selection for female-biased sex ratios only one *M* factors can be present in the population at equilibrium. In contrast, in natural populations multiple *M* factors coexist in most populations with autosomal factors (Tomita & Wada 1989b; Çakir & Kence 2000; Chapters 6 and 7) and this polymorphism seems to be stable (Chapter 7).

Taking all the above into account, sex ratio selection may play some role in the spread of autosomal SD factors and maintenance of polymorphism in some populations of the housefly. However, it seems unlikely that is the sole cause for their distribution in natural populations.

Genetic conflict

Maternal-offspring conflict

We showed that maternal-offspring conflict over the sex ratio may lead to changes in SD mechanisms (Chapter 3). Since expression of both maternal and offspring genes is necessary for sex determination in the housefly (Dübendorfer & Hediger 1998), maternal-offspring conflict could potentially shape the evolution of SD mechanisms in this species. However, our model was not tailored to the housefly SD system and we cannot draw any firm conclusions yet. Moreover, as discussed above, little is known about the possibility for sex ratio selection, and therefore maternal-offspring conflict, in natural housefly populations.

Segregation distortion

It has been proposed that the segregation distortion of autosomal *M* factors could be responsible for the spread of autosomal sex determining factors in the housefly (Clark 1999). Indeed, autosomal *M* factors seem to show segregation distortion in most of the studied American populations (Clark 1999), but it is probably not common in European populations (Chapter 9). However, it is worth considering whether segregation distortion could have played a role in the evolution of SD factors of the housefly.

My theoretical model (Chapter 4) predicts that when M shows even a slight segregation bias it will invade the population and will persist, even if it does not reach fixation. The presence of a driving M can also, under some circumstances, facilitate the spread of F^D. Therefore, segregation distorters could potentially lead to the spread of autosomal SD factors and multi-factorial SD system. But could it explain clinal patterns in the distribution?

It is known from segregation distorters in *Drosophila* that the strength of drive can be temperature dependent. Both an increase and a decrease of distortion level with temperature have been reported for different distorters in different species (Darlington & Dobzhansky 1942; Mange 1968). Nothing is known about the temperature sensitivity of segregation distortion in the housefly. However, if the strength of segregation distortion were to increase with temperature it could lead to a higher frequency of autosomal *M*, and to some extend F^D, at higher temperatures.

But increase in the frequency of autosomal SD factors with the strength of distortion is expected only if individuals homozygous for driving *M* have low fitness (Chapter 4).

Homozygosity for autosomal *M* factors is common in natural populations (personal observation; Chapter 7), but laboratory strains in which homozygous individuals are lethal are also known (D. Bopp and *M*. Hediger, personal communication). Although the link between fitness and segregation distortion is not known in the housefly, the lethality and sterility assumption may not be valid for this species. Moreover, our model often predicts the fixation of the Y chromosome. This is not the case in natural populations, where populations with autosomal *M* are often (nearly) fixed for the X chromosome (Tomita & Wada 1989b; Hamm *et al.* 2005; Chapter 6). However, it cannot be excluded that lower viability of YY males, postulated by some researchers (Franco *et al.* 1982) could prevent fixation of Y, allowing for stable polymorphism of multiple SD factors.

Importantly, not all autosomal *M* factors show segregation distortion (Clark 1999, Chapter 9). Although non-driving autosomal *M* factors could have spread as a result of biased sex ratios caused by other driving chromosomes: X, Y or possibly other autosomes with driving *M*, it is unlikely that this would result in the clinal distribution of the SD factors (Chapter 4).

Therefore, my studies (Chapter 4 and 9) suggest that the segregation distortion might have contributed to the spread of autosomal SD factors the housefly and possible present of multi-factorial system in some populations. However, it seems unlikely that it plays a role in the maintenance of its current distribution.

Sexually antagonistic alleles

We showed that sexually antagonistic (SA) variation on sex chromosomes and autosomes may lead to changes in SD systems (Chapter 5). However, this factor is probably of little importance in the evolution and maintenance of SD mechanisms of the housefly. Sex chromosomes in the housefly are probably undifferentiated with respect to gene content, since individuals with a single sex chromosome, either X or Y seem to have the same viability and fertility (Milani 1967; Dübendorfer *et al.* 2002). Lack of differentiation of sex chromosomes according to our model usually prevents the spread of new SD factors. One the other hand, there is probably little scope for the accumulation of SA variation on the sex chromosomes, since no functional genes have been described on them, even though a large variety of mutations is known for autosomes (Hiroyoshi 1977; Dübendorfer *et al.* 2002). The lack of genetic variation on sex chromosomes should facilitate the spread of new SD factors if they are linked with SA alleles (Van Doorn & Kirkpatrick 2007; own results, not shown). There is no reason to assume that SA variation is absent on the autosomes, since it has been found in the genomes of other organisms, although data are often indirect (Forsman 1995; Vieira *et al.* 2000; Chippindale *et al.* 2001; Rice & Chippindale 2001; Fedorka & Mousseau 2004; Kozielska *et al.* 2004). Therefore, SA

variation on the autosomes could theoretically facilitate the spread of new SD factors in the housefly. However, it is difficult to see how it could lead to the geographical clines in the distribution of different SD factors (Chapter 5).

Therefore, according to the theoretical results and empirical data available so far, multiple factors could have been involved in the spread of autosomal SD factors (Table 10.1). Some of them could also lead to stable coexistence of multiple SD factors and multi-factorial SD system. However, none of them can be shown yet to be responsible for the clinal distribution of the SD factors in the housefly and some of them are even unlikely to do so (Table 10.1). Therefore, available data do not allow any conclusions on how the current distribution of SD factors in natural housefly populations in maintained. Potential candidates are a strong linkage of autosomal SD factors with genes under positive selection in warmer climates or selection for reliable developmental cues. It is also possible that there is not one major selective force involved or that another yet unknown factor plays a role, e.g. cytoplasmic sex ratio distorters. To better understand the evolution of sex determination in the housefly a better understanding of its ecology is necessary. For example data on population sizes, migration patterns and the relation between brood sex ratio and the fitness of the mother and offspring, would allow estimation of the scope for sex ratio selection and maternal-offspring conflict.

The housefly and testing of evolutionary hypotheses

This project was started with the idea that the housefly could be used to test various hypotheses concerning the evolution of SD mechanisms. Multiple SD factors and possibility to achieve male or female heterogametic systems, as well as monogeny and some dose of temperature dependent sex determination (Dübendorfer *et al.* 2002; Chapter 1) make the SD mechanism of the housefly more flexible than any other species (Bull 1983). The housefly is also more suitable for evolutionary studies in comparison to mammal or fish species with multiple SD factors (Fredga *et al.* 1976; Orzack *et al.* 1980) due to its much shorter generation time. However, during this project I realised that some issues should be resolved before the housefly can be used more profitably in experimental studies.

Different environmental factors, especially temperature, but maybe also humidity or other factors, can probably differently influence fitness of the flies depending on the SD factors present (Chapter 7 and 8). However, these environmental effects are not yet clear. Understanding of the influence of environmental conditions on the fitness of flies with different SD factors is necessary, to avoid any confounding effects in experiments.

Currently there are no good markers for different *M* factors and large scale studies are not possible. To assess the frequency of different factors, two generations of backcrosses to a multi-marked strain are necessary, which is very time and labour consuming. Alternatively strains with visible markers could be used, but these

markers are probably not neutral in respect to fitness (see Chapter 8). Therefore, molecular markers for *M* factors located on different chromosomes are needed in order to be able to screen large numbers of flies. However, the development of universal molecular markers to distinguish between *M* factors located on different chromosomes may be very difficult if closely linked markers (e.g. microsatellites) are population-specific. This may not be of much concern if the *M* factors located on different chromosomes are different genes and primers specific for each of them could be developed. However, if *M* factors are one and the same gene located on a transposable element (see Chapter 1), the sequence of the *M* factors located on different chromosomes will be the same.

A fine scale linkage map and the whole genome sequencing would allow the study of the linkage of different SD factors with other genes under selection. The molecular identification of *M* factors would allow the study of their expression level and, therefore, of hypotheses concerning selection for reliable developmental clues and maternal-offspring conflict (Chapter 3). Sequencing the genome could be achieved relatively easily with the current developments in whole-genome sequencing techniques, especially given that the housefly genome is only approximately one and a half times as large as the genome of *D. melanogaster* (Gao & Scott 2006).

In conclusion, currently there are too many unknowns in our understanding of the housefly SD mechanisms and not enough molecular tools to use this species to experimentally test theoretical models. I think the first step would be to develop molecular tools. This would allow larger scale studies in the field in order to understand the population structure, migration pattern and other ecological parameters of the housefly populations. This, coupled with laboratory experiments, could help understand the evolution of SD mechanism in this species. Only a better understanding of natural variation will allow use of the housefly to study general hypotheses on the evolution of sex determination.

General insights from the models

The models presented in this thesis help to understand the evolution of SD mechanisms in the housefly, but they also have more general implications. Many of them have been already discussed in other chapters (Chapters 2-5). Here, I will present my more general conclusions, especially concerning the approach to theoretical studies of the evolution of sex determination.

Mechanistic approach

Many models for the evolution of SD mechanisms are either very abstract and have little connection to the real systems (Caubet *et al.* 2000; Werren & Hatcher 2000; Werren *et al.* 2002) or are tailored to one specific species (McVean & Hurst 1996;

Charlesworth & Dempsey 2001; Pomiankowski *et al.* 2004). The first approach is usually very elegant and manageable analytically, but since it ignores the mechanisms by which sex is determined it may be too simplistic to explain real-world SD systems. The second approach includes many species-specific details and, although informative for the species under study, is usually brings little general insights. Therefore, a mechanistic approach in which sex determination is based on our knowledge of real-world mechanisms and measurable parameters, but at the same time general enough to be applicable to different organisms should be in many cases most informative.

I took such a mechanistic approach in this thesis. The use of a three-locus SD system in three of my models (Chapters 2, 4 and 5) was inspired by the housefly SD mechanism. However, these models are relatively general, and one can easily imagine how they might apply to many other organisms. In many species sex is determined by a dominant male-determining factor, which can be located on different chromosomes in closely related species or even within one species (Martin *et al.* 1980; Traut & Willhoeft 1990). Female-determining genes are known to block male determining genes from the SD cascade of *Caenorhabditis elegans* (Cline & Meyer 1996). In this species feminizing mutations which can override masculinizing genes are also known (Hodgkin 2002). Such genes and mutations probably also appeared during the evolution of other SD cascades (Shearman 2002). Moreover, the model for the evolution of regulatory SD genes with quantitative effects on the amount of feminizing and masculinizing products (Chapter 3), was based on recent insights from molecular work on the sex determination of a variety of species (Schmidt *et al.* 1997a; Schmidt *et al.* 1997b; Nagamine *et al.* 1999; Tarone *et al.* 2005; Otake *et al.* 2006; for more details see Chapter 3).

Taking mechanistic details into account is especially important in studies of sex ratio selection. Many analytical studies have studied evolutionarily stable sex ratios under many different circumstances (Karlin & Lessard 1986; Hardy 2002). However, taking into account constraints of real-world SD mechanisms shows that expected sex ratios may not be achieved (Chapters 2 and 3). Models allowing for any arbitrary sex ratio show that under maternal-offspring conflict the stable population sex ratio lies between the maternal and offspring ESS (Eshel & Sansone 1991). However, my mechanistic model shows that there is no truly stable sex ratio and maternal-offspring conflict can lead to rapid changes in sex ratio, from no bias to maternal ESS (Chapter 3).

As noted by Hodgkin (2002), the evolution of SD mechanisms has "been extensively studied at a theoretical level with some success, but it seems likely that adequate understanding will depend also on knowledge of the basic molecular machinery involved in sex determination." Going one step further, understanding the molecular details allows the incorporation of this knowledge into theoretical models leading to even better understanding of evolutionary processes shaping sex determination. However, many (or even most) genes involved in sex determination differ

between different species. These genes can also exert their function in different ways. Most genes in the *Drosophila* SD cascade are splicing factors (Schütt & Nöthiger 2000) whereas in *C. elegans* a membrane receptor and a protease are also involved in sex determination. Clearly, including too much detail will make the models very species-specific (e.g. Pomiankowski *et al.* 2004).

An increased knowledge of the molecular mechanisms of sex determination in different species allows the recognition of some general patterns. These patterns can in turn be incorporated in the models for the evolution of SD mechanisms. I took this approach in chapter 3, which is based on the recent finding that SD genes exert quantitative effects and that individuals with reduced fecundity are produced when the developmental cue is ambiguous (Schmidt *et al.* 1997a; Schmidt *et al.* 1997b; Nagamine *et al.* 1999; Tarone *et al.* 2005; Otake *et al.* 2006). The results show that selection for reliable developmental cues has a profound effect on the evolution of SD mechanisms and leads to the evolution of dominant SD factors.

I think similar models, taking into account properties of genetic cascades involved in sex determination should be used to study the evolution of SD mechanisms under different selection pressures. I concentrated on genetic sex determination, but these types of models could be especially useful in studying transition between environmental and genetic SD mechanisms, by allowing the level of expression of SD genes to be temperature sensitive (Quinn *et al.* 2007).

Pluralistic approach

The results of my project strongly suggest that models including different selective forces are necessary to better understand the evolution of SD mechanisms. First of all, the current geographical variation in SD factors of the housefly is difficult to explain as a consequence of only one selective force (see above). Second, models including multiple selective forces may lead to different outcomes than those with only one selective force. For example, a lower fitness of some genotypes may prevent the changes in SD mechanisms which one would expect under sex ratio selection or when segregation distorters are present (Chapters 2 and 4). Also, the outcome of the maternal-offspring conflict differs depending on whether selection for reliable developmental cues is included in the model or not (Chapter 3).

The influence of the joint action of different selective forces on the outcome of evolution has been already shown before (e.g. Bengtsson 1977; Charlesworth & Dempsey 2001; Hoekstra & Hoekstra 2001), but very few studies have addressed this problem so far. One of the reasons may be that including multiple selective forces in a model leads to an increase in complexity, especially given that the number of factors which can play a role is quite big (see Chapter 1). Nowadays due to modern computational techniques, relatively complicated simulations can be performed relatively quickly. However, with a larger number of parameters it may become difficult to understand the interaction of evolutionary forces.

Another complication is that information on whether a new SD factor will invade

or not is not enough to predict the equilibrium state of the system. A new SD factor may either reach fixation leading to a switch to a new SD system or it does not reach fixation and multiple SD factors segregate within a population. This may depend on whether biased or equal sex ratios are favoured or whether additional selective forces (e.g. fitness effects) prevent the fixation (Chapters 2 and 4). Alternatively, some selective factors may be transient. For example, a segregation distorter may lead to changes in SD mechanism, but eventually be lost from the population (Chapter 4). Therefore, invasion analysis or equilibrium analysis alone may not be informative. Studying the full dynamics of the SD system seems necessary.

The question is how to include multiple forces in a dynamic model, but at the same time avoid too much complexity. One of the solutions would be to identify those selective forces which lead to similar changes in SD mechanisms. For example, both a driving Y chromosome and accumulation of SA variation with dominance of male-beneficial alleles lead to a switch from a male to a female heterogametic system (Chapters 4 and 5). Combining these two forces in one model would probably not bring any new insights. In contrary, including two opposing selective forces could be more interesting. For example, selection for male biased sex ratios when the cost of sons and daughter differs prevents the spread of a female-determining factor (Chapter 2), but a driving Y chromosome promotes it. The outcome of the interaction of two opposing forces is not intuitive and therefore worth modelling.

In the context of housefly sex determination, it may be necessary to incorporate multiple selective forces to understand the geographical distribution of SD factors in the species. For example, one could imagine that along the whole distribution range selection favours autosomal *M* factors because they provide a more reliable developmental clue than an *M* factor located on a heterochromatic Y chromosome (Hediger *et al.* 1998). However, if selection for a male-biased sex ratio is stronger in colder regions (e.g. due to stronger population subdivision and male-specific dispersal; Black & Krafsur 1986a; Pen 2006) it may prevent changes in SD mechanism (Chapter 2). Although this hypothesis cannot yet be verified empirically it might be worth studying theoretically. Of course, a combination of other selective forces leading to the clinal distribution is also possible.

Selection can act not only at the population level leading to changes in the frequencies of SD factors, but also at the molecular level in order to increase the reliability of the developmental cue (Chapter 3). I think incorporating selection on this level is important in mechanistic models of the evolution of sex determination, especially when they are explicitly based on genetic sex determining cascades. Selection for reliable developmental clues is probably the most basic and universal force (Belote & Baker 1982; Schmidt *et al.* 1997a; Wallace *et al.* 1999) acting in the evolution of sex determination. Incorporating it in models with other selection pressures is important, since it has a profound effect on the outcome of evolution (Chapter 3).

Final remarks

As is probably often the case with scientific research, my project raises more questions that it answers. However, I believe that this thesis is one more step towards understanding the evolution of SD mechanisms. Even though it is still not clear what (selective) forces are responsible for the variety of SD factors in the housefly, I showed that this polymorphism and its geographical distribution is probably stable. This is supported by my theoretical models and field studies. I hope I also showed the use of mechanistic models in theoretical studies of the evolution of sex determination.

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Summary

Many organisms reproduce sexually and possess two separate sexes: males and females. Males and females differ in their reproductive organs and many morphological and behavioural traits. In these species sex determination is a fundamental developmental process. One might, therefore, expect sex determining (SD) mechanisms to be highly conserved. However, SD mechanisms vary greatly between different taxonomic groups and even between closely related species. For example, in humans the presence of a Y chromosome (or more specifically a male-determining gene located on this chromosome) leads to male development. Males are, therefore, a heterogametic sex and posses two different sex chromosomes: X and Y. Females are the homogametic sex with an XX genotype. In birds the opposite is true: females are the heterogametic sex. In wasps and bees males develop from unfertilized and females from fertilized eggs. In many reptiles the sex of an individual depends on the temperature experienced by it during development (so-called environmental sex determination). Many other mechanisms are also known.

This variety of SD mechanisms poses obvious questions of how it evolved. Multiple selective forces have been proposed to lead to changes in SD mechanisms (**Chapter 1**). They include indirect selection, where the target of selection is a gene not involved in sex determination, but a new SD gene hitchhikes with it, leading to changes in the SD mechanism. Selection can also directly act on genes involved in sex determination in order to increase their accuracy in initiating development of males or females and therefore to increase the individual's viability and fertility. Since SD genes influence the sex of the offspring, selection for and against biased sex ratios is likely an important force in the evolution of SD mechanisms. Sex ratio selection may act differentially on maternal and offspring genes leading to conflict over sex determination. Genetic conflict can occur also within one individual, between genes on different loci or even between alleles at the same locus. Genetic conflict on these different levels of organisation can lead to changes in SD mechanisms.

Although a number of theoretical models have been developed to study the influence of different selective forces on the evolution of sex determination, we are still far from a thorough understanding of this process. Additionally, the empirical studies on the evolution of sex determination are rather limited, since SD mechanisms are usually fixed within a species and this prevents a direct observation of evolutionary changes.

The aim of this project was to combine both theoretical and empirical approaches to gain more insights in the evolution of sex determination. We built mechanistic models based on the knowledge of SD mechanisms found in nature. Some of these models were inspired by the SD system of the housefly, which is one of few species in which multiple SD mechanisms coexist. To better understand the evolutionary forces shaping sex determination of the housefly, we collected field data and we performed controlled experiments in the laboratory.

Theoretical models

Part I of the thesis presents the results of theoretical investigations in which an influence of a number of selective forces on the evolution of SD mechanisms were studied. In **Chapter 2** we investigate theoretically the effect of sex ratio selection on the dynamics of a multi-factorial sex determining system. The system considered resembles the naturally occurring three-locus system of the housefly which allows for male heterogamety, female heterogamety and a variety of other mechanisms. Sex ratio selection is modeled by assuming cost differences in the production of sons and daughters, a scenario leading to a strong sex ratio bias in the absence of constraints imposed by the mechanism of sex determination. We show that, despite the presumed flexibility of the sex determining system considered, equilibrium sex ratios never deviate strongly from 1:1. Even if daughters are very costly, a male-biased sex ratio can never evolve. If sons are more costly, the sex ratio can be slightly female biased but even in case of large cost differences the bias is very small (<10% from 1:1). Therefore, genetic constraints of SD mechanisms may prevent achievement of sex ratios favourable by selection. Sex ratio selection can lead to a shift in the sex determining mechanism, but cannot be the sole cause of complete switches from one sex determining system to another. In fact, more than one locus remains polymorphic at equilibrium.

In **Chapter 3** we present a mechanistic model for the evolution of SD mechanisms based on recent insights from molecular studies, showing quantitative effects of SD genes and production of individuals with reduced fecundity when the SD signal is ambiguous. Specifically, we use individual-based simulations to model the gradual evolution of regulatory genes with a quantitative effect on the amount of a feminizing product. The amount of product must surpass a noisy threshold level to trigger female development, otherwise males are produced, or sterile intersexes if the amount of product is too close to the threshold. We impose sex ratio selection by assuming cost differences in the production of sons and daughters. By letting both maternal genes and offspring genes affect the level of feminizing product in the developing offspring, maternal-offspring sex ratio conflict drives the evolution of the regulatory genes. Selection against intersexes is an important force in the evolution of SD mechanisms. It leads to evolution of two alleles of either offspring genes or maternal genes, but not both. When a dimorphism evolves in offspring genes, either a female-heterogametic or a male-heterogametic sex determining system is the outcome, and the sex ratio stabilizes at equality. In contrast, when maternal genes evolve to a dimorphic state, monogeny evolves; that is, all females produce single-sex families, and the population sex ratio evolves to the maternal optimum. Which system evolves is to some extent random but can be partially predicted by initial conditions and the direction and strength of sex ratio selection. To simulate the growth of sex determining pathways, we perturbed the evolved equilibrium by introducing a new masculinizing gene in the population. The result is a series of rapid switches between sex determining systems, interspersed by long periods of apparent

stability, where population sex ratio and frequency of different SD factors do not change. We conclude that our simple mechanistic model is able to capture much of the observed dynamics and variability of extant sex determining mechanisms.

Genetic conflict can be also present within a genome of an individual, an example being segregation distortion. Segregation distorters, alleles able to bias their own segregation and be eventually present in more than 50% of the functional gametes of heterozygous individuals, have been found in many species. Sex chromosomal distorters lead to biased sex ratios, which may select for changes in sex determining systems. In **Chapter 4** we present a model in which we analyze the conditions for the spread of new sex determining factors in a system with a driving sex chromosome. We consider three scenarios: a driving X chromosome, a driving Y chromosome, and a driving autosome with a male determining factor. We investigate how the invasion prospects of a new sex determining factor are affected by the strength of distortion and the fitness effect of the distorter allele. We show that in many cases segregation distortion may induce changes in the sex determining mechanism. When the drive leads to female biased sex ratios, a new masculinizing gene can invade leading to male heterogamety at a new locus. When the drive leads to male biased sex ratios, a feminizing factor can invade, leading to a switch to female heterogamety. Although the presence of driving alleles induces the spread of new sex determining factors, the change in the sex determining system may eventually lead to loss of the driving alleles from the population, if they reduce individual fitness. Therefore, distorter alleles may be present in a population only in a transient state between turnovers of sex determining mechanisms. This shows that it may be impossible to infer the past forces responsible for changes in sex determining systems and the role of meiotic drive in this process may be underestimated.

Genetic conflict leading to changes in SD mechanisms can also occur within one locus. The conflict stems from the fact that a gene can be subject to selection in the opposite direction when expressed in males compared to when expressed in females. Sexually antagonistic (SA) alleles, beneficial to one sex but detrimental to the other, seem to be present in genomes of many species. Theory, supported by experimental data, predicts that SA variation is especially prone to accumulate on sex chromosomes. Accumulation of SA alleles close to SD genes may in turn facilitate reduced recombination and eventually differentiation between sex chromosomes. Although sex determining systems strongly influence the pattern of SA variation little theoretical work has been done on how SA variation can influence the evolution of sex determination. In **Chapter 5** we present a model to investigate the conditions under which new SD factors can spread in response to accumulation of SA variation on the original sex chromosomes. We start with a XY system and let the sex chromosomes accumulate SA variation, and then introduce new male- or female-determining genes to see if they can spread in the population. We investigate the effect of sex chromosome differentiation, dominance effect of different SA alleles and linkage of new SD factors with SA loci on the outcome of the evolutionary dynamics. Our results show that for the

system with undifferentiated sex chromosomes (both X and Y chromosome posses homologous SA locus) a new male-determining factor never has a fitness advantage. A new female-determining factor can spread only if it can accumulate SA variation and female-beneficial alleles are dominant or SA alleles show sex-specific dominance. If sex chromosomes are differentiated and only X possesses a SA locus, the conditions under which new SD factors can spread are much less restrictive and new SD factors can spread even if they are not linked with SA alleles, although linkage facilitates their spread. After their initial spread new SD alleles can reach fixation leading to a switch to a new male or female heterogametic SD system. In some cases a new SD factor does not spread to fixation, but a SD system polymorphic on multiple loci is maintained.

Empirical data

Empirical tests of theoretical models of the evolution of sex determination are usually constrained to comparative studies, since SD mechanisms are usually fixed within a species. In very few species multiple SD mechanisms coexist, the housefly, *Musca domestica*, being one of them. Polymorphism for sex determining factors has been found in many natural populations of the housefly. In "standard" strains, sex is determined by a male determining factor, *M*, which is located on the Y chromosome; therefore males are XY and females are XX. During development, the *M* factor blocks the female determining factor *F* located on autosome IV, the activity of which is necessary for female development. In many populations, *M* is located on one of the autosomes or even on the X chromosome. In such populations, usually a dominant constitutive mutation of *F* (F^D) is also present, which triggers female development even in the presence of several *M* factors in the same individual. Due to the variety of SD mechanisms, the housefly could potentially be used to experimentally test hypotheses on the evolution of SD mechanisms. However, first a better understanding of the selective forces behind evolution and the variety of SD factors in natural housefly populations are necessary. For this reason we collected field data and performed laboratory experiments.

The distribution of SD factors in the housefly follows geographical clines in the northern hemisphere: autosomal *M* factors and F^D factors are more common in southern populations and at lower altitudes, while at higher latitudes and altitudes the standard XY system is prevalent. These clines led several authors to speculate that higher temperatures might favour autosomal *M* factors and/or F^D factors, but this idea has never been investigated systematically. In **Chapter 6** we present data from several populations in the southern hemisphere that corroborate this suggestion: populations closer to the equator have higher frequencies of autosomal *M* factors and F^D factors. A joint analysis of these results and earlier studies, combined with temperature data from the various study locations, demonstrates that the geographical clines can be explained better by variation in temperature than by latitude per se. However, it is not yet clear what (if any) the causal effect of temperature is on the spread of autosomal SD factors.

Autosomal *M* factors were first observed some 50 years ago. It has been hypothesised that following their initial appearance, they are spreading northwards, replacing the standard XY system, but this has never been systematically investigated. To scrutinize this hypothesis, in **Chapter 7** we compare the current distribution of autosomal *M* factors in continental Europe, on a transect running from Germany to southern Italy, with the distribution reported 25 years ago. Additionally, we analyze the frequencies of the F^D factor, which has not been done before for European populations. In contrast to earlier predictions, we do not find a clear change in the distribution of sex determining factors: as 25 years ago, only the standard XY system is present in the north, while autosomal *M* factors and the F^D factor are prevalent in Italy. Therefore, it seems that the polymorphism for multiple SD factors in natural housefly populations is not transient, but stable.

The positive correlation between temperature and frequency of autosomal factors in natural populations suggests that they have a fitness advantage over the XY system at higher temperatures but a disadvantage at lower temperatures. In **Chapter 8** we experimentally investigated the relative fitness of flies with autosomal sex determining factors versus standard flies under different temperature conditions. We determined whether autosomal *M* factors could invade the standard XY populations. We obtained different results for different *M* factors: the *M* factor on autosome II replaced the Y, but *M* on autosome III did not increase in frequency. However, we did not find a significant effect of temperature on the outcome. We also compared fitness of females with and without F^D. We found great variation between populations, but no effect of temperature on the fitness of *F* and F^D females. Therefore, the role of temperature on the spread and distribution of different SD factors in the housefly still remains unclear.

We showed theoretically that linkage to segregation distorters may facilitate the spread of autosomal male-determining *M* factors. Association between autosomal *M* factors and sex ratio distortion has been found to be common in North American populations. In **Chapter 9** we assess the prevalence of *M*-linked segregation distortion in European housefly populations. We sampled eight populations in Western Europe and introgressed one or two *M* factors from each population into a genetic background of a standard laboratory strain in order to eliminate any possible suppressors of distortion. During each generation of introgression, we analyzed the offspring sex ratio from mass crosses between males with *M* factors and females from the laboratory strain. We found that males with a Y chromosome produced unbiased or even female-biased sex ratios, suggesting that Y chromosomes do not posses segregation distorters. Only one autosomal *M* factor was associated with a consistent, strong male-bias sex ratio. This could have been caused by an *M*-linked distorter, but sex-specific mortality could not be excluded. Offspring sex ratios of other autosomal *M* males were often male-biased, but the sex ratios varied a lot between generations. Therefore, it seems that *M*-linked segregation distortion is not common in European housefly populations. This suggests that association with sex

ratio distorters does not play a major role in maintaining the variability in autosomal sex determining factors in the housefly.

Conclusions

Theoretical models and empirical data presented in this thesis show that multiple selective forces could have played a role in the evolution of SD mechanisms in the housefly. However, none of these forces seems to be the sole factor maintaining the current distribution of SD factors in this species (discussed in detail in **Chapter 10**). Models incorporating multiple selective forces seem necessary to bring new insights into the evolution of SD mechanisms, not only in the housefly. Models based on our knowledge of the real-world SD mechanisms and molecular details of SD cascades allow studying genetic constraints in evolution of sex determination. Such mechanistic models would also allow making predictions testable with empirical data.

Samenvatting

Vele organismen planten zich geslachtelijk voort en hebben twee aparte geslachten: mannetjes en vrouwtjes. Zij onderscheiden zich middels hun geslachtsorganen en vele aspecten van hun morfologie en gedrag. In deze soorten is geslachtsbepaling ofwel sexdeterminatie (SD) een fundamenteel ontwikkelingsproces. Men zou daarom verwachten dat SD mechanismen in sterke mate geconserveerd zijn gebleven tijdens de evolutie. SD mechanismen verschillen echter sterk tussen verschillende taxonomische groepen en zelfs tussen nauw verwante soorten. In mensen bijvoorbeeld leidt de aanwezigheid van een Y-chromosoom (of, preciezer, een gen voor mannelijke ontwikkeling dat op dit chromosoom ligt) tot een mannelijke ontwikkeling. Mannetjes zijn dus het heterogametische geslacht en in het bezit van twee verschillende geslachtschromosomen: X en Y. Vrouwtjes zijn het homogametische geslacht met een XX genotype. In vogels is echter het omgekeerde het geval en zijn vrouwtjes het heterogametische geslacht. In wespen en bijen ontwikkelen mannetjes zich uit onbevruchte en vrouwtjes uit bevruchte eitjes en zijn er geen verschillende geslachtschromosomen. In vele reptielen hangt het geslacht van een individu af van de temperatuur tijdens de ontwikkeling (zogenaamde omgevings-SD). Hiernaast zijn nog vele andere mechanismen bekend.

Deze diversiteit in SD mechanismen leidt uiteraard tot de vraag hoe die mechanismen geëvolueerd zijn. Meerdere selectieve krachten zijn gesuggereerd als mogelijke oorzaak van verandering in SD mechanismen (**Hoofdstuk 1**). Een oorzaak is indirecte selectie, waarbij een gen dat niet betrokken is bij SD het doelwit van selectie is, en een nieuw SD gen meelift op deze selectie via genetische koppeling, leidend tot een verandering van SD mechanisme. Selectie kan ook direct op de bij SD betrokken genen werken om hun precisie van de ontwikkeling van mannetjes of vrouwtjes te vergroten, en zo de levensvatbaarheid en vruchtbaarheid van het individu te verhogen. Aangezien SD genen het geslacht van de nakomelingen beïnvloeden is selectie voor of tegen ongelijke sexratios (proportie vrouwtjes en mannetjes) waarschijnlijk een belangrijke kracht in de evolutie van SD mechanismen. Het is mogelijk dat sexratioselectie differentieel op moederlijke- en nageslachtsgenen werkt, wat leidt tot een generatieconflict over SD. Een genetisch conflict kan ook binnen één individu plaatsvinden, tussen genen op verschillende loci of zelfs tussen allelen op hetzelfde locus. Genetisch conflict op al deze verschillende niveaus kan leiden tot veranderingen in SD mechanisme.

Hoewel er een aantal theoretische modellen ontwikkeld is om de invloed van verschillende selectieve krachten op de evolutie van SD te onderzoeken zijn wij nog verre van een grondig begrip van dit proces. Hiernaast zijn de empirische onderzoeken naar de evolutie van SD nogal beperkt, aangezien SD mechanismen meestal onveranderlijk zijn binnen een soort, wat het onmogelijk maakt evolutionaire veranderingen direct waar te nemen en de voor- en nadelen van verschillende mechanismen te toetsen in eenzelfde organisme

Het doel van dit project was om theoretische en empirische benaderingen te combineren om meer inzicht in de evolutie van SD te verkrijgen. We hebben mecha-

nistische modellen geconstrueerd die gebaseerd zijn op de beschikbare kennis over SD mechanismen zoals deze in de natuur worden aangetroffen. Sommige van deze modellen zijn geënt op het SD systeem van de huisvlieg, wat een van de weinige bekende soorten is waarin meerdere SD mechanismen naast elkaar voorkomen. Om de devolutie van SD van de huisvlieg beter te begrijpen hebben wij data verzameld in het veld en experimenten in het laboratorium gedaan.

Theoretische modellen

Deel I van dit proefschrift bespreekt de resultaten van theoretisch onderzoek, waarin de invloed van een aantal selectieve krachten op de evolutie van SD mechanismen wordt bestudeerd. In **Hoofdstuk 2** onderzoeken we theoretisch het effect van sexratio selectie op de dynamiek van een 'multi-factoriaal' SD systeem. Het beschouwde systeem is vergelijkbaar met het in de natuur voorkomende drie-locus systeem van de huisvlieg. Dit systeem biedt ruimte voor zowel mannelijke als vrouwelijke heterogamie en een scala aan andere mechanismen. Sexratio selectie wordt gemodelleerd door aan te nemen dat de kosten voor de productie van zonen dan wel dochters verschillen. Dit scenario leidt tot een zeer scheve sexratio wanneer er geen beperkingen worden uitgeoefend door het SD mechanisme. Wij laten zien dat ondanks de verwachte flexibiliteit van het SD systeem onder beschouwing de sexratio nooit sterk afwijkt van 1:1. Zelfs als dochters veel meer kosten leidt dit niet tot een sexratio met meer mannetjes. Als zonen meer kosten kan de sexratio licht doorslaan naar meer vrouwtjes maar zelfs als de verschillen in kosten zeer groot zijn blijft de afwijking zeer klein (<10% van 1:1). Wij concluderen dat de genetische beperkingen van SD mechanismen wellicht voorkomen dat de sexratios bereikt worden die ongelimiteerde selectie zouden bewerkstelligen. Sexratio selectie kan een verandering van SD mechanisme bevorderen maar kan niet de enige oorzaak zijn van totale omschakelingen van het ene SD systeem naar het andere. Sterker nog, meer dan één locus blijft polymorf in het equilibrium.

In **Hoofdstuk 3** presenteren wij een mechanistisch model voor de evolutie van SD systemen dat gebaseerd is op recente bevindingen uit moleculair onderzoek die aantoonden dat SD genen een kwantitatief effect kunnen hebben en dat individuen met een verlaagde fitness worden geproduceerd als het SD signaal ambivalent is. We gebruiken individugebaseerde simulaties om de geleidelijke evolutie van regulator genen met een kwantitatief effect op de productie van een ‘feminizing’ gen te modelleren. De transcriptie van het gen moet een drempelwaarde overschrijden om vrouwelijke ontwikkeling in gang te zetten, zo niet, dan worden er mannetjes geproduceerd, of steriele ‘intersexes’ als de transcriptie te dicht bij de drempelwaarde ligt. We passen sexratioselectie toe door verschillen in de kosten voor de productie van zonen en dochters te veronderstellen. Door zowel moederlijke als nakomelinggenen invloed uit te laten oefenen op de hoeveelheid ‘feminizing’ product in de zich ontwikkelende nakomelingen wordt de evolutie van de regulatorgenen aangedreven door het moeder-nakomelingsconflict. Selectie tegen ‘intersexes’ blijkt een belang-

rijke kracht te zijn in de evolutie van SD mechanismen. Het leidt tot dimorfisme van nakomelings- of moedergenen, maar niet van beide tegelijk. Als een dimorfisme in de nakomelingsgenen evolueert leidt dit tot een SD systeem van ofwel vrouwelijke ofwel mannelijke heterogamie en een sexratio van uiteindelijk 1:1. Als de moedergenen echter naar een dimorphisme evolueren is het eindresultaat monogenie, wat wil zeggen dat alle vrouwtjes nageslacht produceren van één geslacht. Het resultaat is dat de sexratio in de populatie evolueert naar het moederoptimum. Welk van deze twee evolutierichtingen wordt gekozen is deels willekeurig, maar het is mogelijk voorspellingen te doen op basis van de beginsituatie van het systeem en de richting en kracht van de sexratioselectie. Om de groei van SD gencascades te simuleren verstoorden wij de geëvolueerde sexratio door een nieuw 'masculinizing' gen te introduceren in de populatie. Het resultaat is een serie snelle wisselingen tussen SD systemen, afgewisseld met lange perioden van ogenschijnlijke stabiliteit waarin de sexratio en de frequenties van de diverse SD factoren niet veranderen. We concluderen dat ons eenvoudige mechanistische model in staat is om veel van de dynamiek en diversiteit van bestaande SD mechanismen na te bootsen.

Genetisch conflict kan ook aanwezig zijn binnen het genoom van een individu, bijvoorbeeld het segregatie distortie complex. Segregatiedistortie refereert naar allelen die in staat zijn hun eigen overerving te bevoordelen om zo in meer dan 50% van de functionele gameten van heterozygote individuen aanwezig te zijn. Ze zijn in vele organismen aangetroffen. Sex chromosoom verstoorders (ook wel aangeduid met meiotic drive sex chromosomen) leiden tot ongelijke sexratios, wat weer kan leiden tot selectie ten voordele van een verandering in SD systeem. In **Hoofdstuk 4** presenteren wij een algemeen model waarin wij de voorwaarden voor het verspreiden van nieuwe SD factoren analyseren in een systeem met een 'driving' sex chromosoom. Wij beschouwen drie scenario's: een 'driving' X-chromosoom, een 'driving' Y-chromosoom en een 'driving' autosoom met een 'male determining' factor. We onderzoeken hoe de invasiekansen van een nieuwe SD factor beïnvloed worden door de kracht van de distortie en het fitness effect van het distortie allel. We tonen aan dat in veel gevallen 'meiotic drive' tot een verandering van SD mechanisme kan leiden. Als de 'drive' tot een vrouwelijke sexratio leidt kan het gevolg een succesvolle invasie van een 'masculinizing' gen zijn wat op zijn beurt leidt tot een omslag naar vrouwelijke heterogametie. Hoewel de verspreiding van nieuwe SD factoren het resultaat is van de aanwezigheid van 'driving' allelen is het mogelijk dat de verandering van SD systeem uiteindelijk leidt tot het verdwijnen van deze 'driving' allelen uit de populatie, namelijk als zij nadelig zijn voor de individuele fitness. Hierdoor is het mogelijk dat distortie allelen zich alleen in de populatie bevinden tijdens overgangen van SD mechanismen. Dit leidt tot de conclusie dat het wellicht onmogelijk is om de krachten die ooit tot een verandering in SD mechanisme hebben geleid te bepalen, en dat de rol van 'meiotic drive' in dit proces wellicht onderschat wordt.

Genetisch conflict dat tot veranderingen in SD mechanismen leidt kan ook optreden binnen één locus. Het conflict ontstaat doordat een gen onderhevig kan zijn

aan selectie in tegengestelde richting in mannetjes dan wanneer het in vrouwtjes tot uitdrukking komt. Seksueel antagonistische (SA) allelen die voordelig zijn voor het ene geslacht maar nadelig voor het andere lijken in het genoom van veel organismen aanwezig te zijn. Zoals theoretisch voorspeld is wordt SA variatie vooral op de geslachtschromosomen aangetroffen. De accumulatie van SA allelen dichtbij SD genen zou de vermindering van recombinatie en uiteindelijke differentiatie tussen de geslachtschromosomen kunnen vergemakkelijken. Hoewel SD systemen in sterke mate het patroon van SA variatie bepaalt, is er tot nu toe weinig onderzoek gedaan naar hoe SA variatie de evolutie van sex determinatie kan beïnvloeden. In **Hoofdstuk 5** gebruiken we een model om de omstandigheden te onderzoeken waaronder nieuwe SD factoren zich kunnen verspreiden in respons op accumulatie van SA variatie op de oorspronkelijke geslachtschromosomen. We beginnen met een XY-systeem en laten de geslachtschromosomen SA variatie verzamelen, waarna we nieuwe genen voor mannelijkheid of vrouwelijkheid in de populatie introduceren om te onderzoeken of zij zich verspreiden in de populatie. We onderzoeken het effect van geslachtschromosoomdifferentiatie, dominantie effecten van verschillende SA allelen en koppeling van nieuwe SD factoren met SA loci op het verloop van de evolutionaire dynamiek. Onze resultaten tonen aan dat voor het systeem met ongedifferentieerde geslachtschromosomen (zowel het X- als het Y-chromosoom hebben een homoloog SA locus) een nieuwe 'masculinizing' factor nooit een fitnessvoordeel heeft. Een nieuwe 'feminizing' factor kan zich alleen verspreiden door de populatie als deze SA variatie weet te accumuleren en allelen die voordelig zijn voor vrouwtjes de overhand hebben, of wanneer SA allelen een geslachtsspecifieke dominantie ten toon spreiden. Als geslachtschromosomen gedifferentieerd zijn en slechts het X chromosoom een SA locus bezit zijn de voorwaarden waaronder nieuwe SD factoren zich kunnen verspreiden veel minder beperkt. In dat geval kunnen nieuwe SD factoren zich zelfs als zij niet met SA allelen gekoppeld zijn verspreiden, hoewel een koppeling dit proces vergemakkelijkt. Na hun initiële verspreiding kunnen nieuwe SD allelen gefixeerd raken, wat leidt tot een omslag naar een nieuw mannelijk of vrouwelijk heterogametisch SD systeem. In sommige gevallen verspreidt een nieuwe SD factor zich niet tot fixatie, maar ontstaat er een stabiel nieuw SD systeem dat polymorf is op meerdere loci.

Empirische data

Empirische toetsen van theoretische modellen beperken zich meestal tot vergelijkend onderzoek, aangezien SD mechanismen meestal binnen een soort onveranderlijk zijn. In slechts zeer weinig soorten komen meerdere SD mechanismen voor, waarvan de huisvlieg, *Musca domestica*, er één is. Polymorfisme van SD factoren is in vele natuurlijke populaties van de huisvlieg aangetroffen. In standaard lijnen wordt het geslacht bepaald door een factor voor mannelijkheid, *M*, welke op het Y chromosoom ligt; dit betekent dat mannetjes XY zijn en vrouwtjes XX. Tijdens de ontwikkeling blokkeert de *M* factor de factor voor vrouwelijkheid, *F*, die op autosoom IV gelegen is en die actief moet zijn voor de ontwikkeling van vrouwtjes. In vele populaties ligt *M* op een

van de autosomen of zelfs op het X chromosoom. In dergelijke populaties is meestal ook een dominante 'altijd aan (constitutive)' mutatie van *F* (F^D) aanwezig die zelfs in de aanwezigheid van meerdere *M* factoren vrouwelijke ontwikkeling bewerkstelligt. Vanwege de variatie in SD mechanismen zou de huisvlieg mogelijkerwijs gebruikt kunnen worden om hypothesen over de evolutie van SD mechanismen experimenteel te toetsen. Er is echter eerst een beter begrip van de selectieve krachten achter de evolutie en variatie van SD factoren in natuurlijke huisvliegpopulaties nodig. Daartoe hebben wij data uit het veld verzameld en laboratoriumexperimenten verricht.

De verspreiding van SD factoren in de huisvlieg toont een geografisch verloop ('clines') op het noordelijk halfrond: autosomale *M* factoren en F^D factoren komen meer voor in zuidelijke populaties en op lagere hoogten terwijl op hogere breedtegraden en hoger boven zeeniveau het standaard XY systeem de overhand heeft. Deze 'clines' hebben enkele auteurs ertoe geleid te speculeren dat hogere temperaturen autosomale *M* en/of F^D factoren positief beïnvloeden, maar dit idee is nog niet systematisch onderzocht. In **Hoofdstuk 6** presenteren wij gegevens uit verschillende populaties van het zuidelijk halfrond die deze suggestie ondersteunen: populaties in Afrika hebben dichter bij de evenaar hogere frequenties van autosomale *M* en F^D factoren. Een gecombineerde analyse van deze resultaten en eerder onderzoek toont samen met temperatuurgegevens van de diverse onderzoekslocaties aan dat geografische 'clines' beter verklaard kunnen worden door temperatuursvariatie dan door breedtegraad. Het is echter nog niet duidelijk wat het oorzakelijk verband is tussen temperatuur en de verspreiding van autosomale SD factoren.

Autosome *M* factoren zijn 50 jaar geleden voor het eerst waargenomen. Het is geopperd dat zij zich richting het noorden aan het verspreiden zijn. Om deze hypothese te onderzoeken vergelijken we in **Hoofdstuk 7** de huidige verspreiding van autosomale *M* factoren in continentaal Europa, op een transect van Duitsland naar Zuid-Italië , met de verspreiding zoals die 25 jaar geleden beschreven is. Hiernaast analyseren wij de frequenties van de F^D factor, wat nog niet eerder is gedaan voor Europese populaties. In tegenstelling tot de voorspelling vinden wij geen duidelijke verandering in de verspreiding van SD factoren: evenals als 25 jaar geleden komt in het noorden alleen het standaard XY systeem voor, terwijl autosomale *M* factors en F^D in Italië de overhand hebben. Het lijkt er dus op dat het polymorfisme voor meerdere SD factoren in natuurlijke populaties van de huisvlieg geen overgangsfase is, maar een stabiel patroon.

De positieve correlatie tussen temperatuur en frequentie van autosomale factoren in natuurlijke populaties suggereert dat er een fitnessvoordeel voor het XY-systeem is bij hogere temperaturen, maar een fitnessnadeel bij lagere temperaturen. In **Hoofdstuk 8** vergelijken wij experimenteel de fitness van huisvliegen met autosomale SD factoren ten opzichte van de fitness van vliegen met het standaard XY-systeem onder verschillende temperaturen. We onderzoeken of het mogelijk is voor autosomale *M* factoren om zich in een populatie met het standaard XY-systeem te verspreiden. We verkrijgen verschillende resultaten voor verschillende *M* factoren: de

M factor op autosoom II verdringt de Y, maar de *M* op autosoom III neemt niet in frequentie toe. We vinden echter geen significant effect van de temperatuur op de uitkomst van de experimenten. We vergelijken ook de fitness van vrouwtjes met en zonder F^D. We vinden grote variatie tussen populaties, maar geen effect van temperatuur op de fitness van *F* en F^D dragende vrouwtjes. De invloed van de temperatuur op de verspreiding en frequentie van verschillende SD mechanismen in de huisvlieg blijft dus nog steeds onduidelijk.

Het is theoretisch aangetoond dat een koppeling met uitsplitsingsverstoorders ('segregation distorters') de verspreiding van autosomale *M* factoren kan vergemakkelijken. In Noord Amerika treft men vaak een associatie aan tussen autosomale *M* factoren en sexratioverstoorders. In **Hoofdstuk 9** toetsen wij in hoeverre *M*-gekoppelde uitsplitsingverstoring in Europese huisvliegpopulaties voorkomt. We nemen steekproeven uit acht populaties in West Europa en kruizen één of twee *M* factoren uit iedere populatie in, in de genetische achtergrond van een standaard laboratoriumlijn om alle mogelijke onderdrukkers van uitsplitsingverstoring te elimineren. Tijdens iedere introgressie-generatie analyseren we de sexratio van het nageslacht in massakruisingen tussen mannetjes met *M* factoren en vrouwtjes van de laboratoriumlijn. Het resultaat is dat mannetjes met een Y chromosoom gelijke of zelfs overwegend vrouwelijke sexratios produceren, wat suggereert dat Y chromosomen geen sexratioverstoorders bezitten. Slechts één autosomale *M* factor was geassocieerd met een consistente, sterk mannelijke sexratio. Dit zou het resultaat kunnen zijn van een *M*-gekoppelde verstoorder maar geslachtsspecifieke sterfte kan niet worden uitgesloten. De sexratios van het nageslacht van andere autosomale *M* mannetjes zijn vaak richting meer mannetjes verschoven, maar variëren sterk tussen generaties. Het lijkt er dus op dat *M*-gekoppelde uitsplitsingsverstoring niet veel voorkomt in Europese huisvliegpopulaties. Dit suggereert dat een associatie met sexratioverstoorders geen grote rol speelt in het handhaven van de diversiteit in autosomale SD factoren in de huisvlieg.

Conclusies

De theoretische modellen en empirische gegevens die in dit proefschrift voorgelegd worden, laten zien dat meerdere selectieve krachten een rol gespeeld kunnen hebben in de evolutie van SD mechanismen in de huisvlieg. Geen van deze krachten lijkt op zichzelf de huidige verspreiding van SD factoren in stand te houden (in detail besproken in **Hoofdstuk 10**). Om nieuwe inzichten in de evolutie van SD mechanismen (ook in andere organismen dan de huisvlieg) te verkrijgen zijn modellen nodig waarin meerdere selectieve krachten tegelijk verwerkt zijn. Modellen die gebaseerd zijn op onze kennis van natuurlijk voorkomende variatie in SD mechanismen en de moleculaire details van SD cascaden maken het mogelijk om de genetische beperkingen in de evolutie van SD mechanismen te bestuderen. Dergelijke mechanistische modellen maken het ook mogelijk om voorspellingen te doen die empirisch te toetsen zijn.

Streszczenie

Wiele zwierząt rozmnaża się płciowo i posiada osobniki dwóch oddzielnych płci: samce i samice. Każda z płci posiada specyficzne organy płciowe, ale także wiele cech morfologicznych i zachowań. Determinacja płci jest więc fundamentalnym procesem rozwoju osobniczego. Można by się zatem spodziewać, że mechanizmy determinacji płci są takie same u większości zwierząt. Jednak różnią się one bardzo nawet pomiędzy blisko spokrewnionymi gatunkami. Przykładowo, u ludzi obecność chromosomu Y (a ściślej genu determinującego płeć męską położonego na tym chromosomie) prowadzi do rozwoju mężczyzn. U ludzi więc mężczyźni są heterogametyczną płcią i mają dwa różne chromosomy płciowe: X i Y, a kobiety są homogametyczną płcią i mają dwa jednakowe chromosomy X. U ptaków sytuacja jest odwrotna, samice są heterogametyczną płcią. U os i pszczół samce rozwijają się z niezapłodnionych, a samice z zapłodnionych jaj. U większości gadów płeć zależy od temperatury, w jakiej inkubowane są jaja. Jest jeszcze wiele innych mechanizmów determinacji płci.

To zróżnicowanie mechanizmów determinacji płci nasuwa oczywiste pytanie o to, dlaczego i jak ono wyewoluowało. Różnych czynniki doboru naturalnego mogą prowadzić do zmian w determinacji płci (przegląd w Rozdziale 1). Na przykład dobór może działać na gen niezwiązany z determinacją płci, ale nowy gen determinacji płci, jeśli jest położony blisko takiego genu, może rozprzestrzenić się i spowodować zmiany w mechanizmie determinacji płci. Dobór może działać także bezpośrednio na geny odpowiedzialne za determinację płci w celu zwiększenia niezawodności sygnału potrzebnego do prawidłowego rozwoju płciowego, a tym samym zwiększenia przeżywalności i płodności. Ponieważ geny determinacji płci wpływają na proporcje płci w potomstwie, dobór za i przeciw odchyleniom w proporcji płci jest ważnym czynnikiem w ewolucji mechanizmów determinacji płci. Taki dobór na proporcje płci może działać inaczej na geny matczyne i te ulegające ekspresji w potomstwie i dlatego prowadzić do konfliktu genetycznego o determinację płci. Konflikt może także wystąpić pomiędzy różnymi genami obecnymi w jednym osobniku, a nawet pomiędzy różnymi allelami tego samego genu. Konflikt genetyczny na różnych poziomach organizacji może prowadzić do zmian w mechanizmie determinacji płci.

Pomimo tego, że wiele teoretycznych modeli zostało stworzonych w celu studiowania wpływu różnych czynników doboru naturalnego na ewolucję determinacji płci, zrozumienie tego procesu jest ciągle niewielkie. Dodatkowo, badania empiryczne ewolucji determinacji płci są raczej ograniczone, ponieważ mechanizm determinacji płci jest najczęściej niezmienny dla danego gatunku.

Ten projekt miał na celu połączenie modeli teoretycznych i danych empirycznych w celu lepszego zrozumienia ewolucji determinacji płci. W trakcie jego trwania, na podstawie znajomości mechanizmów determinacji płci obecnych w naturze, zostały stworzone teoretyczne modele. Część z tych modeli była zainspirowana systemem determinacji płci muchy domowej, jednego z niewielu

gatunków, u których są obecne wielorakie mechanizmy determinacji płci. W celu lepszego zrozumienia ewolucji determinacji płci u muchy domowej, zostały przeprowadzone badania terenowe i eksperymenty laboratoryjne.

Teoria

Pierwsza część niniejszej pracy obejmuje wyniki stworzonych w czasie trwania projektu modeli teoretycznych, w których badano wpływ różnych czynników doboru na ewolucję mechanizmów determinacji płci. W rozdziale 2 opisano badania wpływu doboru na proporcję płci na dynamikę trój-czynnikowego systemu determinacji płci. System ten przypomina naturalnie występujący mechanizm determinacji płci muchy domowej. Pozwala on na heterogametyczność samców i samic oraz mechanizmy pośrednie. W modelu zostało założone, że dobór na proporcję płci działa przez różnice w kosztach produkcji synów i córek. Przykładowo, samice są większe i potrzebują większego nakładu zasobów. Przy takich założeniach i braku ograniczeń narzuconych przez mechanizm determinacji płci, oczekiwany stosunek liczbowy płci jest odchylony (nawet znacznie) na korzyść „tańszej" płci. Wyniki stworzonego modelu wskazują, że pomimo pozornej elastyczności modelowanego systemu determinacji płci, w stanie równowagi proporcje płci nie odbiegają znacznie od 1:1. Odchylenie na korzyść samców nigdy nie występuje, nawet jeśli córki są bardzo kosztowne. Jeśli synowie są bardziej kosztowni, proporcja płci może być nieznacznie odchylona na korzyść samic, ale nawet w przypadku znacznych różnic w kosztach, odchylenie jest bardzo małe (poniżej 10% od 1:1). Z tego wynika, ze genetyczne ograniczenia mogą uniemożliwić osiągniecie proporcji płci faworyzowanej przez dobór. Dobór na proporcje płci może prowadzić do zmian w mechanizmie determinacji płci, jednak nie powoduje on całkowitej zmiany z jednego systemu na inny. W stanie równowagi więcej niż jeden locus pozostaje polimorficzny.

W rozdziale 3 przedstawiono mechanistyczny model ewolucji mechanizmów determinacji płci na podstawie najnowszej wiedzy z badań molekularnych. Badania te pokazują, że geny determinacji płci dają ilościowe efekty i że osobniki o obniżonej płodności są produkowane, gdy sygnał determinujący płeć jest niejasny. W celu modelowania stopniowej ewolucji genów regulatorowych, które mają ilościowy wpływ na poziom feminizującego produktu, użyto symulacji komputerowych. Ilość feminizującego produktu musi być większa niż pewien progowy poziom, aby doprowadzić do rozwoju płci żeńskiej, w przeciwnym wypadku osobnik rozwinie się jako samiec, albo nawet jako niepłodny osobnik interseksualny, jeśli ilość feminizującego produktu jest zbyt bliska poziomu progowego. Dodatkowo, dobór na proporcje płci został narzucony przez założenie, że koszty produkcji synów i córek są różne. Ponieważ w modelu ilość feminizującego produktu jest zależna i od genów ulegających ekspresji u matki, i genów ulegających ekspresji w potomstwie, konflikt pomiędzy matką a potomstwem o proporcje płci przyczynia się do ewolucji mechanizmów determinacji płci. Wyniki przeprowadzonych symulacji wskazują, że dobór przeciw interseksualnym osobnikom jest ważną siłą w ewolucji determinacji

płci. Prowadzi on do powstania dwóch alleli w genach matczynych albo w genach u potomstwa, ale nigdy w obu. Jeśli dwa allele wyewoluują w genach ulegających ekspresji u potomstwa, rezultatem jest heterogametyczność samców albo samic, a proporcja płci wynosi 1:1. Jeśli dwa allele wyewoluują w genach matczynych, rezultatem jest system, w którym część samic produkuje tylko córki, a reszta tylko synów. W tym wypadku proporcja płci w populacji równa się matczynemu optimum. Który system wyewoluuje, zależy częściowo od przypadku, ale także od warunków początkowych oraz kierunku i siły doboru na proporcje płci. W celu symulacji powstawania kaskad genów determinacji płci obecnych w naturze, równowaga systemu została zakłócona przez dodanie nowego maskulinizującego genu. W rezultacie nastąpiła seria gwałtownych zmian z jednego systemu determinacji płci w drugi. Pomiędzy zmianami występowały okresy pozornej stabilności, w czasie których stosunek płci w populacji i frekwencja różnych czynników determinacji płci pozostawała niezmienna. Zatem zastosowany stosunkowo prosty mechanistyczny model jest w stanie odtworzyć wiele z dynamiki i różnorodności mechanizmów determinacji płci.

Konflikt genetyczny może być także obecny wewnątrz genomu pojedynczego osobnika. Przykładem takiego konfliktu jest zaburzenie segregacji. Allele zaburzające segregację potrafią wyeliminować homologiczny allel z funkcjonalnych gamet i dzięki temu są obecne w ponad połowie potomstwa heterozygotycznych osobników. Takie allele zaburzające segregację są obecne w genomach wielu gatunków. Jeśli znajdują się one na chromosomach płci, prowadzą do zaburzenia proporcji płci i mogą prowadzić do zmian w mechanizmie determinacji płci. W rozdziale 4 zaprezentowano model, przy pomocy którego analizowano warunki potrzebne do rozprzestrzenienia się nowych genów determinacji płci w obecności czynników zaburzających segregację. Wzięto pod uwagę trzy scenariusze: zaburzenie segregacji chromosomu X, zaburzenie segregacji chromosomu Y i zaburzenie segregacji genu determinującego płeć męską położonego na autosomie. Badano, jak szansa na to, że nowy gen determinacji płci rozprzestrzeni się, zależy od tego, jak silne jest zaburzenie segregacji i od tego, czy allel zaburzający segregację ma negatywny wpływ na dostosowanie (przeżywalność lub płodność) osobników. Z rezultatów wynika, że w wielu przypadkach zaburzenie segregacji może prowadzić do zmian mechanizmu determinacji płci. Jeśli zaburzenie segregacji powoduje odchylenie stosunku płci na korzyść samic, nowy maskulinizujący gen rozprzestrzeni się, prowadząc do ewolucji systemu, gdzie samce są heterogametyczną płcią na nowym loci determinującym płeć. Jeśli zaburzenie segregacji powoduje odchylenie stosunku płci na korzyść samców, nowy feminizujący gen rozprzestrzeni się, prowadząc do ewolucji systemu, gdzie samice są heterogametyczną płcią. Chociaż obecność alleli zaburzających segregację powoduje rozprzestrzenienie się nowego genu determinującego płeć, zmiana mechanizmu determinacji płci może prowadzić do usunięcia allelu zaburzającego segregację z populacji, jeśli powoduje on obniżenie dostosowania. Z tego wynika, że allel zaburzający segregację może być obecny w populacji tylko w

stanie przejściowym pomiędzy zmianami w mechanizmie determinacji płci. To pokazuje, że wnioskowanie o czynnikach doboru odpowiedzialnych w przeszłości za zmiany w mechanizmie determinacji płci może być trudne, a rola czynników zaburzających segregację może być niedoceniona w tym procesie.

Konflikt genetyczny prowadzący do zmian w mechanizmie determinacji płci może także wystąpić w ramach jednego locus. Taki konflikt jest powodowany tym, że dobór naturalny może działać na dany gen w przeciwnych kierunkach w zależności od tego, czy jest on obecny u samicy czy u samca. Przykładem może być gen na masę ciała u gatunków, w których samce i samice różnią się znacznie optymalną wielkością. Takie antagonistyczne płciowo (ang. sexually antagonistic) allele, korzystne dla jednej płci, ale niekorzystne dla drugiej, są obecne w genomach wielu organizmów. Teoria, poparta badaniami eksperymentalnymi, przewiduje, że płciowo antagonistyczna zmienność ma skłonność do akumulacji na chromosomach płci. Nagromadzenie antagonistycznych alleli blisko genów determinujących płeć może z kolei prowadzić do zmniejszenia częstości rekombinacji i ostatecznie do zróżnicowania między chromosomami płci. Chociaż system determinacji płci ma silny wpływ na nagromadzenie się antagonistycznych alleli, niewiele teoretycznych prac badało wpływ antagonizmu płciowego na ewolucję determinacji płci. W rozdziale 5 przedstawiono model, za pomocą którego badano warunki rozprzestrzenienia się nowego genu determinującego płeć w odpowiedzi na nagromadzenie antagonistycznych alleli. Zaczęto od systemu, w którym samce są heterogametyczną płcią (XY) i antagonizm płciowy może się swobodnie akumulować. Następnie wprowadzono nowy gen determinujący płeć męską albo nowy gen determinujący płeć żeńską, aby zobaczyć, czy może on rozprzestrzenić się w populacji. Badano, jaki jest wpływ zróżnicowania między chromosomami płci, typu dominacji antagonistycznych alleli i sprzężenia nowych genów determinacji płci z antagonistycznymi płciowo allelami na ewolucję determinacji płci. Wyniki pokazały, że jeśli chromosomy płciowe nie są zróżnicowane (i chromosom X i Y posiadają antagonistyczne geny), nowy gen determinujący płeć męską nigdy nie ma przewagi. Nowy gen determinujący płeć żeńską może się rozprzestrzenić tylko jeśli sam jest sprzężony z płciowo antagonistycznymi allelami, które są dominujące w sposób korzystny dla samic, albo dla tej płci, w której są obecne. Jeśli chromosomy płciowe są zróżnicowane i tylko X zawiera antagonistyczne geny, warunki w których nowe geny determinacji płci mogą się rozprzestrzenić są szersze i nowy gen determinacji płci może się rozprzestrzenić nawet jeśli nie jest sprzężony z antagonistycznymi płciowo allelami, chociaż takie sprzężenie ułatwia ich rozprzestrzenienie się. Nowe geny determinacji płci mogą się utrwalić w populacji i doprowadzić do całkowitej zmiany mechanizmu determinacji płci. W niektórych przypadkach nowy gen determinacji płci nie zostaje utrwalony, ale w stanie równowagi utrzymuje się polimorfizm na rożnych loci determinacji płci.

Dane empiryczne

Empiryczne testy modeli teoretycznych są najczęściej ograniczone do badań porównawczych, ponieważ większość organizmów posiada jeden mechanizm determinacji płci. Tylko u niewielkiej liczby gatunków obecny jest więcej niż jeden mechanizm determinacji płci. Mucha domowa (*Musca domestica*) jest jednym z takich wyjątków. Zróżnicowanie pod względem mechanizmu determinacji płci jest obecne w naturalnych populacjach i liniach laboratoryjnych. Standardowo płeć męska jest determinowana przez czynnik *M* położony na chromosomie Y, a wiec samce są XY a samice XX (system XY). W czasie rozwoju zarodkowego czynnik *M* blokuje feminizujący czynnik *F*, położony na czwartym autosomie, którego aktywność jest niezbędna do rozwoju samic. W wielu populacjach *M* jest położony na autosomie (autosomalne czynniki *M*) albo nawet na chromosomie X. W takich populacjach najczęściej jest również obecna dominująca mutacja czynnika *F* (F^D), która jest niewrażliwa na *M* i dlatego umożliwia rozwój samic, nawet jeśli osobnik posiada parę czynników *M* (patrz Fig. 1.3 i Tabela 6.1). Dzięki takiemu zróżnicowaniu czynników determinacji płci, mucha domowa mogłaby potencjalnie służyć do testowania hipotez dotyczących ewolucji determinacji płci. Jednakże najpierw potrzebne jest lepsze zrozumienie sił ewolucyjnych odpowiedzialnych za zróżnicowanie determinacji płci w naturalnych populacjach muchy domowej. W tym celu zostały przeprowadzone badania terenowe i eksperymenty laboratoryjne.

Czynniki determinacji płci w naturalnych populacjach muchy domowej są rozmieszczone według specyficznego gradientu geograficznego: na półkuli północnej autosomalne czynniki *M* i czynnik F^D są częstsze na niższych szerokościach geograficznych, a na wyższych szerokościach geograficznych powszechny jest standardowy system XY. Takie rozmieszczenie sprowokowało wielu badaczy do spekulacji, że wysokie temperatury faworyzują autosomalne czynniki *M* i czynnik F^D. Jednak ta teza nie była nigdy systematycznie badana. W rozdziale 6 przedstawiono dane zebrane z kilku populacji z półkuli południowej, które potwierdzają tą hipotezę: w populacjach położonych bliżej równika frekwencja autosomalnych czynników *M* i czynnika F^D jest większa. Analiza statystyczna przeprowadzona na tych nowych danych i danych z wcześniejszych badań z różnych miejsc na świecie pokazuje, że frekwencje różnych czynników determinacji płci są lepiej wyjaśnione przez różnice w temperaturze niż przez samą szerokość geograficzną. Jednak ciągle niejasne jest przyczynowe powiązanie pomiędzy temperaturą i rozprzestrzenieniem się autosomalnych czynników determinacji płci u muchy domowej.

Autosomalne czynniki *M* zostały po raz pierwszy zaobserwowane w latach 50. poprzedniego stulecia. Pierwsi naukowcy postulowali, że po początkowym pojawieniu się, autosomalne czynniki rozprzestrzeniają się w kierunku północnym, wypierając system XY. Jednak ta hipoteza nigdy nie była systematycznie studiowana. W celu jej przetestowania, w rozdziale 7 porównano obecne rozmieszczenie różnych czynników determinacji płci w Europie z rozmieszczeniem sprzed 25 lat, na odcinku

pomiędzy południowymi Włochami a Niemcami. Dodatkowo, po raz pierwszy dla europejskich populacji, przeanalizowano frekwencję czynnika F^D. W przeciwieństwie do wcześniejszych przewidywań, nie zaobserwowano wyraźnej zmiany w rozmieszczeniu różnych czynników determinacji płci: tak jak 25 temu, tylko standardowy system XY jest obecny na północy, a autosomalne czynniki *M* i czynnik F^D są powszechne we Włoszech. Z tego wynika, ze polimorfizm czynników determinacji płci w naturalnych populacjach muchy domowej jest stabilny, a nie przejściowy.

Korelacja pomiędzy temperaturą a frekwencją autosomalnych czynników determinacji płci (autosomalme czynniki *M* i czynnik F^D) sugeruje, że mają one przewagę nad systemem XY w wyższych temperaturach, a są niekorzystne w niskich temperaturach. W rozdziale 8 zostały opisane wyniki eksperymentów, w których badano dostosowanie much z autosomalmymi czynnikami determinacji płci względem much ze standardowym systemem XY w dwóch różnych temperaturach. Dla czynników *M* przeprowadzono eksperyment, w którym badano, czy autosomalme czynniki *M* mogą się rozprzestrzenić w populacji z systemem XY. Otrzymano różne wyniki dla różnych czynników *M*: czynnik *M* położony na drugim autosomie całkowicie zastąpił chromosom Y, ale nie wzrosła frekwencja *M* na trzecim autosomie. Nie znaleziono jednak znaczącej różnicy pomiędzy dwoma temperaturami. W innym eksperymencie porównano dostosowanie (długość życia i liczbę potomstwa) pomiędzy samicami z czynnikiem *F* i samicami z czynnikiem F^D. Znaleziono silne zróżnicowanie pomiędzy populacjami, ale żadnego wpływu temperatury na dostosowanie samic z różnymi czynnikami determinacji płci. Zatem wpływ temperatury na rozprzestrzenienie się i rozmieszczenie różnych czynników determinacji płci u muchy domowej ciągle pozostaje niejasny.

W jednym z modeli w rozdziale 4 zostało pokazane, że sprzężenie autosomalnego czynnika *M* z czynnikiem zaburzającym segregację może przyczynić się do jego rozprzestrzenienia. Takie sprzężenie znaleziono wcześniej w populacjach muchy domowej w Ameryce Północnej. W rozdziale 9 starano się oszacować powszechność sprzężonego z *M* zaburzenia segregacji w zachodnio-europejskich populacjach muchy domowej. Z ośmiu różnych populacji jeden albo dwa samce były wstecznie krzyżowane przez parę pokoleń z samicami z linii laboratoryjnej w celu wyeliminowania z genomu potencjalnych supresorów zaburzenia segregacji. W każdym pokoleniu introgresji została sprawdzona proporcja płci potomstwa. Samce z chromosomem Y produkowały potomstwo o proporcji płci 1:1 albo nieznacznie odchylonym na korzyść samic, co sugeruje, że na chromosomie Y nie ma czynników zaburzających segregację. Tylko jeden czynnik *M* był konsekwentnie związany z silnym odchyleniem proporcji płci na korzyść samców. Mogło to być spowodowane sprzężeniem z czynnikiem zaburzającym segregację, ale wyższa śmiertelność samic nie może być wykluczona. Proporcja płci potomstwa samców z innymi czynnikami *M* była często odchylona na korzyść samców, ale odchylenie było bardzo zmienne pomiędzy pokoleniami i czasem nawet występowało

odchylenie na korzyść samic. Zatem wydaje się, że zaburzenie segregacji sprzężone z *M* nie jest częste w europejskich populacjach. To sugeruje, że sprzężenie z czynnikami zaburzającym segregację nie gra ważnej roli w utrzymaniu różnorodności czynników determinacji płci u muchy domowej.

Podsumowanie

Modele teoretyczne i dane empiryczne przedstawione w tej pracy pokazują, że różnorakie czynniki doboru mogą być odpowiedzialne za ewolucję determinacji płci u muchy domowej. Jednakże żaden z tych czynników doboru nie wydaje się być wystarczający do utrzymania obecnego rozmieszczenia różnych czynników determinacji płci (szczegółowa dyskusja w rozdziale 10). Modele biorące pod uwagę wielorakie siły selekcyjne wydają się niezbędne do lepszego zrozumienia ewolucji determinacji płci, nie tylko u muchy domowej. Modele takie powinny bazować na wiedzy o naturalnie występujących mechanizmach determinacji płci i danych molekularnych dotyczących kaskad genów regulujących determinację płci. Takie mechanistyczne modele pozwalałyby także na tworzenie hipotez testowalnych empirycznie.

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Magdalena

Curriculum vitae

Magdalena Kozielska was born on 19th March 1979 in Bielsko-Biała, Poland. She finished her primary and secondary education in her home town. In 1998 she moved to Kraków (Poland) to study at the Jagiellonian University. She followed the Individual Interdisciplinary Mathematics and Natural Sciences Studies. During her studies she obtained a Socrates/Erasmus Scholarship to do a five-month theoretical research project on the stability of food webs at the Linköping University in Sweden. After that, she did her master project "Polyandry and the offspring fitness in the bulb mite, *Rhizogryphus robini*" at the Jagiellonian University where she also obtained her Master's degree in Biology in 2003. The same year she started her PhD project in the Evolutionary Genetics and Theoretical Biology groups at the University of Groningen in the Netherlands. The project combined theoretical and empirical approaches to study the evolution of sex determination and its results are presented in this book. Currently she is working on mechanism-based pharmacokinetic-pharmacodynamic modelling as a post-doc in the Pharmacokinetics and Drug Delivery group at the University of Groningen.

Drawing by Adam Kozielski