Sex ratio and evolution

Huub Segerink

Rijksuniversiteit Groningen

Supervisors: Bernd Riedstra & Cor Dijkstra

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Abstract

Variation in sex ratios is a poorly understood phenomenon that despite having been extensively studied since Darwin, still holds many intriguing questions. In many species sex ratios are unequal, not holding to Fisher's principle of equal sex ratios. Explanations vary a lot, and no single theory is sufficient to explain all that is found. Inherently linked to sex ratios is the system that determines the sex. These systems also show much variation, and their evolution is poorly understood. Currently, many interesting developments involving both sex ratio and the sex determination are underway, most notably in arthropod species. Extreme sex ratio deviations occur, and the concurrent evolution of the sex determination system might provide insight into the dynamics and origins of sex determination systems.

Introduction

Most people would think that whether they will have a son or a daughter is simply a matter of chance, and that that chance for having a boy (or a girl) is 50%. However, this is not the case. The global sex ratio at birth in humans is 105:100, in favor of males (2), meaning that the chance on a son is about 5% higher than that of having a daughter. Interestingly, the mortality rate of males is also reported to be slightly higher, suggesting it may be some form of compensation to keep the reproductively active sex ratios balanced. Much larger deviations from a 50:50 ratio can be found in some populations, e.g. in three countries in the Caucasus, where the ratio varied from 115:100 to as much as 128:100 (68). It is unclear whether this is due to culturally or economically motivated abortions, biased data selection, or a more primal biological reason. However, shortly after WWII, the proportion of sons that were born in the Netherlands increased (1)(fig 1), suggesting the existence of an adaptive sex ratio in humans. It is not clear, however, why such a capacity should exist, and much less how it would work.

There are many different animals in which biased sex ratios have been found, and in some cases the reasons and mechanisms are quite clear. For instance, in some reptiles the ambient temperature of the embryos determines the proportion of males to females born (69), offering them a way to control, or at least influence the sex ratio of their offspring. In many cases though, it remains unclear what the reasons and mechanisms for sex ratio biases are, or if species are at all able to influence their sex ratio.

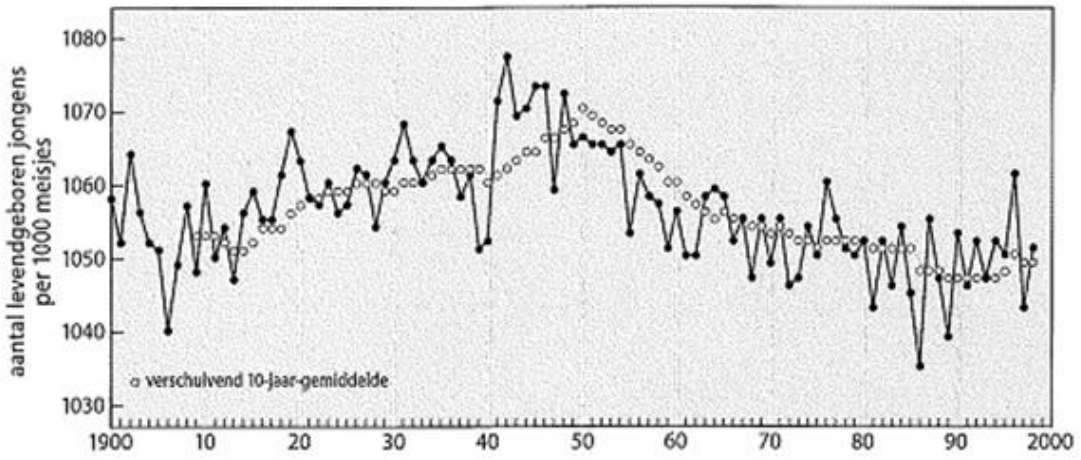

Fig 1 Sex ratio in the Netherlands from 1900 to 2000

Charles Darwin was the first to address this issue in modern times. In the *Descent of Man and Selection in Relation to Sex published in 1871 (7)* he noted that the sex ratio at birth is irrelevant, because competition for a mate occurs at maturity, and it is this that results in progeny. The minority sex would have an advantage there because they would have less competition for a partner. However, in the second edition of the book he concluded that *'…the whole problem is so intricate that it is safer to leave its solution for the future"*.
It becomes clear here that it is not just the sex ratio at birth (called the secondary sex ratio) that might be relevant. Important besides the sex ratio at birth are the sex ratio at fertilization (the primary sex ratio) and the sex ratio of adults (the tertiary sex ratio). This last one is somewhat limited, as not all adults might be sexually active. Hence the operational sex ratio (OSR) is used, which is the ratio of male to female individuals "ready to mate". Factors influencing the OSR beside the ratio of adults are the mating system, the reproductive rates of the sexes, the stability of pair bonds, and other reasons why some individuals would not be ready to mate.

Since Darwins unsuccessful attempt, Düsing, Fisher and Shaw and Mohler expanded on the issue of sex ratios, which is now understood quite well. Currently, much work has been done on different phyla to elucidate the principles underlying sex ratio biases, but so far there has been a wide range of explanations. The questions I will address in this thesis are: How and why do biased sex ratios come about and to what extent can this affect species evolution? Due to the large body of literature on sex ratio and sex determination, I will focus primarily on arthropods and the more extreme cases of sex ratio biases. Arthropods are invertebrate animals with an exoskeleton and a segmented body, are well studied and show extensive variation in sex ratio and sex determination. They form a phylum that includes insects, arachnids (e.g. spiders, mites, ticks) and crustaceans (e.g. crabs, shrimp, krill, lice).

1. Biased sex ratios

Despite the fact that biased sex ratios exist across a wide range of species and are found in many different phyla, the deviations from a 50:50 ratio that are found are usually small (70). But why should most species have a sex ratio of (or close to) 50:50? This is best explained by Düsing (8) and Fisher (6). When the sex ratio of a population is biased, there will be less individuals of one of either sex. Since all offspring have 2 parents (one of each sex), the rarer of the two sexes will make a larger contribution to the total progeny than the more abundant sex. An example of this would be if 40 females and 60 males produced 100 offspring. An average female would then contribute more to the next generation than the average male, since 100/40=2,5 offspring per female and 100/60=1,66 offspring per male. Hence, it is advantageous to produce the scarcer sex since they have more offspring on average, and consequently populations will tend to balance themselves to a 50:50 ratio. It is important to note here that Fisher talks about sex allocation, which means an equal investment in both sexes energetically, and not in raw numbers. If a son were twice as costly to produce, there would be equal investment in the sexes with a 1:2 male to female ratio. It might seem better to produce two daughters and so generate more progeny, but since all females require a male, a single male would (on average) mate with two females and produce twice the offspring that one daughter would.
However, in many species across different phyla deviations from this 50:50 ratio have been found. The big brown bat big *Eptesicus fuscus* produces more females if it gives birth early in the season (77), and mice in laboratory condition bias their sex ratio towards males if they are on a high fat diet, but towards females in a high carbohydrate diet (78). The false clown anemone fish *Amphiprion ocellaris* consists of mostly males, where only the dominant member of the group is a female. Should she die, then the male that is 2nd in line becomes a female (79). The butterfly *H. bolina* can be even more extreme, as on the Samoan islands it actually

had a 99% female sex ratio. I will come back on this example later on (52).
The factors that affect such deviations are numerous, and can be put into three main categories. Genetic sex determination (GSD), environmental sex determination (ESD) and sex ratio distorters (SRDs). In GSD, sex is determined at fertilization, depending on the genetic mechanism of sex determination of the animal. In humans this is determined by whether the female egg carrying an X chromosome receives another X, or the Y chromosome, which carries a dominant factor that effectuates masculinization. In ESD environmental effects determine the sex, and so potentially offer control of the sex after conception. A fine example are reptiles, as in many of them the ambient temperature of the developing embryos determines the sex ratio of each individual, and thus of the clutch (69). The third factor affecting sex ratios are SRDs, they distort the sex ratio to increase their own spread. An example of this is the endosymbiont bacteria called Wolbachia, which resides in the cytoplasm of female eggs and is only transmitted through females. It is able to alter the offspring to a staggering 100% daughters to increase its own spread. I will now review these factors affecting sex ratio in more detail, and later assess their potential impact on species ecology.

2. Sex determining mechanisms

2.1 Genetic sex determination

Genetic sex determination is probably the most common mechanism of sex determination. Though not all species use the same genetic sex system, even those who do often do not use the exact same mechanism to determine the sex. Variation exists in the type of system for genetic sex determination and the details of the mechanisms. The main genetic systems are heterogamety and haplodiploïdy. In the first, two different sex chromosomes determine the sex, where homozygosity (e.g. XX) creates one sex, and heterozygosity (e.g. XY) the other. Male heterogamety (common in mammals) and female heterogamety (common in birds) are both widespread. Having the same system here does not mean the same mechanism however. For example, where in humans a dominant factor on the Y-chromosome determines masculinization through the testes, birds require the W chromosome and estrogens to femininize. And in fruit flies it is the X chromosome to autosome ratio that determines the sex. In the haplo-diploïd system there is only one sex chromosome, and it is the number of those that determine the sex. Usually diploid (XX) here means female and haploid (X) male, although the reverse (thelytoky) is also possible and has been found in some lizards and insects (80). Though even more unusual systems exist, and also species that can change their sex or have both sexes simultaneously, I will not get into these as that would be too comprehensive for this thesis.
Interestingly, great variation exists in these sex determination systems. In some cases, they even vary between different populations of the same species, as is the case in the woodlouse *Armadillidium vulgare* (19), the housefly *Musca domestica* (18, 20, 17) and the shrimp *Gammarus duebeni* (21, 21, 22), while at other times these mechanisms seem to be well preserved. A question that rises is why and how does the evolution of these mechanisms occur? Since offspring sex ratio and sex determination are inherently linked, I will return to this issue later.

2.2 Environmental sex determination

With ESD sex is determined through an interaction of environment and the progeny, for instance temperature-dependent sex determination as seen in reptiles and some other animals. By modifying the place or the depth at which they lay their eggs, reptiles can influence the temperature and so the sex ratio of their offspring. In other animals environmental factors known to have similar effects include pH, salinity, mate availability and nutrition level (3).The theory of local resource competition (LRC) may provide insight into why such a system would occur. This theory explains the advantages of having different sex ratios when environmental changes bestow different fitness benefits upon sons and daughters (4). For instance, when higher temperatures would benefit

males more than females, changing the sex ratio disproportionally with temperature change would be advantageous.

Hamilton (5) was the first to offer such an explanation for ESD. He suggested that when males did not disperse from a group and mated females did, at some point the high competition between males would make it more advantageous to produce daughters. Many similar theories followed, all pointing at different causes as to why differential advantages to the sexes occurred. These causes include different competition for resources between the sexes (4), different fitness returns of parental investment (81), advantaged daughters due to them inheriting their mother's high ranks in a group (9), and many more (5). All these theories have one thing in common: the two sexes have unequal fitness returns from a certain condition of an environmental factor, either abiotic or biotic.

2.3 Sex ratio distorters

The third factor affecting sex ratios is probably the most extreme: selfish elements that promote their own spread by altering the sex ratio or fertility of offspring. These elements can be all sorts of things: endosymbiotic organisms, cellular organelles, cytoplasmic factors, nuclear chromosomal factors or B chromosomes (extra chromosomes with functions not vital for development). These heritable elements can cause all kinds of different sex distortion effects, intended to increase their own spread. Because this offers a disadvantage to the host fitness, especially in populations where the sex ratio is very much distorted, this can have severe implications for population ecology. I will bring this out in Wolbachia, since it is present in many species, is well studied, and uses a range of known strategies of host manipulation.

Wolbachia are endosymbiotic proteobacteria that infect arthropods and nematodes (10), and *Wolbachia pipientis* was first discovered in the mosquito *Culex pipiens (23)*. So far it has been divided into eight supergroups varying in host selection and type of symbiotic bond (24). It is estimated that over 65% (10^6 species) of all insects harbor *Wolbachia (44)*. Host and arthropod *Wolbachia* phylogeny do not concord, due to extensive horizontal transfer and recombination of *Wolbachia,* even among supergroups, making it difficult to resolve their phylogenetic relationships. (28, 29, 30, 31, 32, 33). They are well adapted to manipulatiing host cells, and are known to use the spindle apparatus and dyesin and kinesin motors within cells, allowing efficient transmission during cell division (34, 35, 36). Beside the pandemic distribution among arthropods, another interesting feature of Wolbachia is the diversity of mutualistic and parasitic effects they employ.

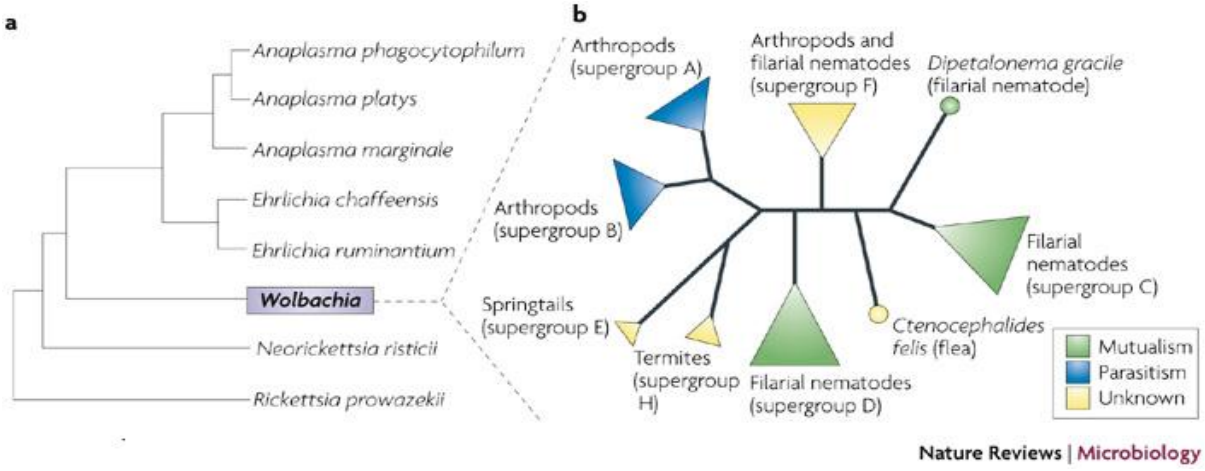

Fig. 1

b | An unrooted phylogenetic tree of the main supergroups of *Wolbachia*. Also shown are the dominant patterns of mutualism and reproductive parasitism across the supergroups. For some supergroups, functional effects of *Wolbachia* have not yet been determined. The G supergroup has been removed because its status is currently unclear (27). The pattern suggests that the main supergroups of *Wolbachia* participate in either mutualism or reproductive parasitism. Rooting of the *Wolbachia* phylogeny, which could help resolve whether mutualism or reproductive parasitism is

ancestral, is problematic owing to long-branch attraction to out-groups. Resolution requires genome-sequence information for additional taxa. Triangle size represents described diversity within each lineage. Circles represent a lineage based on a single *Wolbachia* strain. Part **a** reproduced from (25). Part **b** reproduced from (26).

It is the parasitic effects that are of interest here, since *Wolbachia* has developed a strategy called reproductive parasitism. This means that they affect the reproductive system of their host to promote their own spread. Of the four ways *Wolbachia* has of accomplishing this, the most common form is cytoplasmic incompatibility or CI.

2.3.a Cytoplasmic incompatibility

Cytoplasmic incompatibility consists of two steps. In the first step the bacteria that are present in the cytoplasm of the eggs modify the male spermatogenesis in such a way that it no longer functions. In the second step the bacteria must rescue the sperm by modifying it a second time, so that it can fertilize the eggs again. The molecular mechanism of this is unknown, but an asynchrony of male and female pronucleï at the initial stage of mitosis, likely caused by an altered cdk1 activity (a kinase that drives cell mitosis) is disrupted (38), leading to incomplete condensation of male chromosomes that then fail to be separated properly by the centromere. In diploid animals this leads to aneuploidy (missing chromosomes) and embryonic death, while in haplo-diploid animals this mostly leads to the production of the haploid sex, which is usually male (37). This means that affected male sperm is not compatible with uninfected females, and they cannot be fertilized by these males. This is called unidirectional CI. Bi-directional CI occurs when two different, incompatible types of *Wolbachia* are both present in a population. Females can then only be fertilized by males of which the sperm is modified by the same (or a sufficiently similar) *Wolbachia* strain. This is the only form of reproductive parasitism that does not necessarily alter the sex ratio, as in diploid animals sterility occurs.

2.3.b Male-killing

Another form of reproductive parasitism is male-killing (MK). This is, as the name suggest, the killing of the males present. This usually occurs during the embryonic stage, though there are exceptions, like certain protists who kill mosquitos in a more advanced life stage (39). Male-killing can be found in a variety of sex determining systems; XY, ZW, XO (haplodiploidy) and ESD. The spread of the endosymbiont is promoted by killing the non-transmitting male sex. Females gain further fitness advantages by sibling egg consumption (of the killed males), decreased sibling competition and inbreeding. These advantages are limited, and while often the male-killing is much lower than 100%, it can each high frequencies (40, 43).

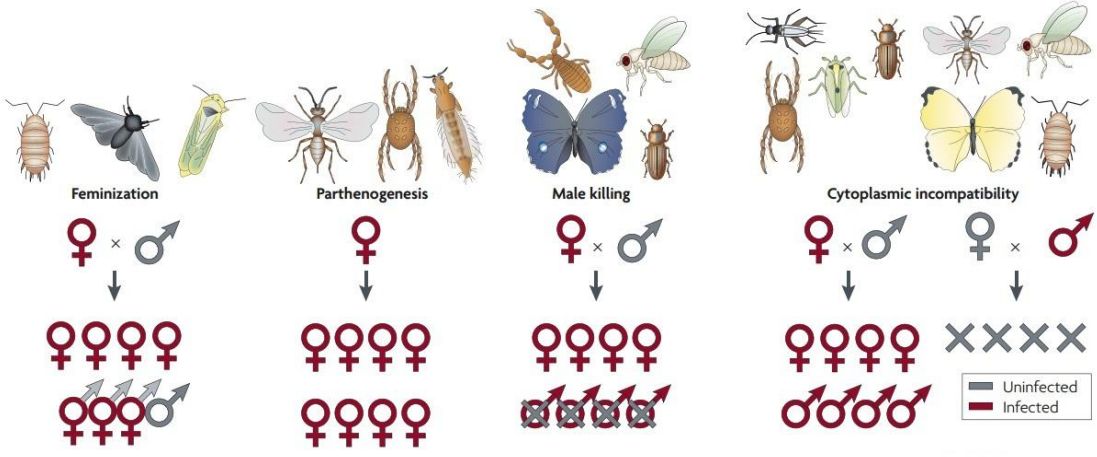

Fig. 3 (44)
The four ways in which *Wolbachia* can distort the sex ratio of its host. Feminization: the conversion of male embryos to females. Parthenogenesis: the conversion of male gametes to female. Male-killing: the killing of males. Cytoplasmic incompatibility: incompatibalization of sperm with uninfected individuals.

The mechanism is unknown, but it seems that the sex determination pathway is involved, and death is induced by lethal feminization (feminization is explained below) (41, 42). Female-killing has not been reported, and for obvious reasons, as male sperm would likely be too poor a habitat to harbor endosymbiont bacteria.

2.3.c Feminization

Feminization is the conversion of a genetic male to a functional female. The key method to accomplish this, and the most common, is found in crustaceans, though this phenomenon is not uncommon in fish either (79). These crustaceans generally have a ZZ ZW sex chromosome system, and a factor on the W chromosome that suppresses male differentiation. *Wolbachia* is able to suppress the adrenal gland, through which male differentiation takes place (40). In effect, this renders the W chromosome obsolete, and *wolbachia* transmission determines sex. In insects however, sex determination is a cellular process, and sex determining pathways are less straightforward. While most of these have heterogametic sex chromosomes, the grasshopper *Z. pullula* has a haplodiploid system, though it is not known whether these converted females are fertile (45, 46, 47, 48). Feminization is usually not complete, producing some completely converted females and some intersex individuals. This might be due to a dosage effect, but the mechanisms are unknown. Though feminization is suspected in many species, it has been shown in only a few species (40).

2.3.d Parthenogenesis induction

The last method described in *Wolbachia* is induction of parthenogenesis. This system has only been found in haplodiploid species, and it works by doubling the haploid genome. Hereby males are converted to females, and spread of the *Wolbachia* is maximized. It seems somewhat similar to feminization, but one main difference is that embryos can develop without fertilization. The mechanism remains unknown, but there seem to be at least three different ways in which parthenogenesis occurs: during the cell cycle in embryonic development it can keep the chromosomes from splitting during cell division, it can fuse successfully split cells with haploid nuclei or it can induce meiotic modification in eggs, depending on the species studied. Sometimes only partial chromosomal repression occurs, and a corresponding degree of intersexuality is found (40).

3. Genetic conflict and sex determination

3.1 Genetic conflict

Such extreme manipulations of *wolbachia* on the sex ratio are not favorable for the host. For in an increasingly female biased population it is not very advantageous to still have a female sex bias. Even worse, it can dramatically lower their fitness, and theoretically lead to extinction of a population when there are no males to mate with. Therefore, selection would favor any genes disrupting the sex ratio distorting effect of *Wolbachia*. Bi-parentally inherited genes (autosomes) would favor an equal sex ratio, conflicting with the *Wolbachia* which benefits by biasing the sex ratio. Selection here takes place in opposite directions, and on different levels. In this case the endosymbionts interests conflict with that of the organism.

When in different genetic elements a gain in fitness for one decreases the fitness of the other, there is a genetic conflict. And the stronger the distortion (and thus the sex ratio bias in the population), the stronger the selection pressure for conflicting autosomal genes. On the other hand, if the sex distortion effect is less strong, species will be less stressed to adapt and evolve genes that repress these effects, granting more time for adaptations to evolve. The evolution of such genes, which are called repressor genes, is one way in which a species can overcome these detrimental effects to their fitness. The other way is to have multiple types of *Wolbachia* or other sex ratio distorters in a population. Nasonia vitripennis harbours at least four SRDs: A cytoplasmic factor causing all-female broods, a male killing bacterium, a

B chromose causing all-male broods and a Wolbachia that produces male-biased sex ratios (12, 13, 14, 15). With some favoring male offspring and others female, equilibrium develops. Though this does not mean repressor genes will not develop, it offers another way for selection to equalize sex ratios. In any case, it is most interesting to see what happens when repressor genes do evolve.

3.2 Adaptations to *Wolbachia*

3.2.a Resistance to feminization

Resistance to feminization has developed in *A. vulgare*, where a polygenic system of resistance genes (R genes) prevents feminization by resisting *Wolbachia* transmission to offspring (16). In this way R genes indirectly impact the sex determination. However, in addition to *Wolbachia* another feminizing agent known as the f element has been found. It is thought that this element is a part of the *Wolbachia* genome that has transferred to the host nuclear genome. It might be a mobile genetic element inciting lateral gene transfer between itself and host genome, something which is frequent in *Wolbachia* (54, 55, 56). The selfish nature of the f element constitutes an intra-genomic conflict and has resulted in the selection for the dominant autosomal masculinizing gene M, which is able to restore the male sex, but is not efficient against the *Wolbachia* feminizing effect (57). This illustrates how parasitic sex factors can catalyze the evolution of sex determining systems.

3.2.b Resistance to male-killing

As it kills all the male embryos, male-killing obviously has a strong negative impact on host fitness, and one that is stronger than feminizing or PI induction. Resistance to male-killing has been documented best in the butterfly *Hypolimnas bolina*. This species is infected with *Wolbachia* which kills the males in Polynesian populations, but it does not do so in Southeast Asia. The MK effect here is suppressed by a single dominant autosomal host gene, and they produce progeny with a balanced sex ratio (51). The spread of the suppressor in *H. bolina* has been monitored on the Samoan islands, where a 99% female sex ratio bias shifted to parity within about 10 generations (52). This is one of the most rapid cases of evolutionary and ecological change observed in the wild. This means that cases may well exist of species that no longer show sex ratio manipulations, but have done so in the recent past.

Surprisingly, the endosymbiont prevalence did not diminish much, as after suppression of MK the surviving males exhibited an already present ability of *Wolbachia* to induce CI (53). The mechanisms used by *Wolbachia* are unknown, but the ability to induce multiple effects manipulating host reproduction may result from the long term presence of a co-evolutionary arms race of endosymbionts and hosts.

3.2.c Resistance to Perthenogenesis

In the haplodiploid wasp *Richogramma kaykai,* PI *Wolbachia* is found to be stable at less than 30% (58). The spread of *Wolbachia* is prevented by the presence of a paternal sex ratio chromosome (PSR). This is a paternally inherited B chromosome that converts diploid fertilized eggs into haploid eggs by destroying the paternal chromosome set, but not itself. It does so by condensing the chromosome into a dense chromatin mass during the first mitotic division after fertilization (58, 59, 60). So diploid females become haploid males, and the paternal chromosome in contact with the PSR is doomed. In this way *Wolbachia* spread is limited and sexual reproduction is maintained (58).

3.3 Repressor genes and the evolution of sex determination systems

In heterogamete sexual systems in arthropods (XX/XY and ZZ/ZW) sex chromosomes usually show no or very little heteromorphy. The different systems are found within closely related species or within species, and simple experimental manipulations can invert sex chromosomes that produce fertile WW or YY animals, indicating very similar if not identical genetic programs for the sexes (61, 62, 63). This suggests that the evolution of sex chromosomes of these species is at an incipient stage of the specialization of a pair of

ancestral autosomes that carry sex determinants (40). As seen in *A. vulgare*, where in the presence of the f element the nuclear M gene became the sex determining factor, a pair of autosomes could become the new sex chromosomes, and at the same time switch from female to male heterogamety. Repeated changes in sex determining genes caused by endosymbionts or other feminizing factors could constantly relocate the sex chromosomes, and explain the incipient level of sex chromosome heteromorphy (60, 61, 62).

The haplodiploid system evolved in insects at least ten times independent of each other (64). It might be that the system evolved from the coevolution of male-killers and their hosts. MK that act by destruction of the male chromosome set in diploid males would favor selection of host genes for haploid rescue of the male.(64, 65, 66, 67) In this way a diplodiploid system could turn into a haplodiploid system of sex determination. But to accommodate the fixation of such a system in a population, high levels of inbreeding (5, 67) or a mutualistic interaction yielding a competitive advantage to the endosymbiont host is required (67), otherwise fully viable haploid males would negate the benefit of resource allocation to females. However, in *H.bolina,* the ability of *Wolbachia* to induce MK as well as cytoplasmic incompatibility might supersede these requirements and be another way for Wolbachia to persist and accomplish fixation in a population. Parthenogenesis inducing endosymbionts can invade haplodiploid species without leading to extinction when fixated, as is the case in some wasp species (49). Also the ability to reproduce sexually has been lost in many populations, making *Wolbachia* an obligate partner for reproduction (71, 72, 73). A hypothesis known as the virginity mutation hypothesis offers an explanation. In a female biased population male production is advantageous, so female genes preventing fertilization would be selected for. In the wasp species *Trichogramma pretiosum*a it is shown that a single nuclear effect can be sufficient to explain the loss of female sexual function (74), suggesting that endosymbionts could play a role in the development of asexuality.

3.4 Sex ratio and species development

So while it often seems most beneficial for species to allocate resources equally to both sexes, many exceptions to this have been found, with varying explanations. A major class of explanations consists of adaptation to varying environment factors, made possible by different fitness returns for the two sexes. In case some factor would skew the fitness returns of one sex more than the other, to adapt the offspring sex ratio to this factor would be beneficial. Many explanations in this regard have been given in various species, and numerous factors to which adaptation occurs have been described (5, 70, 3). Little is known about the evolution of ESD, and for the best studied variant (TSD) only theories on its origin exist. However, an interesting phenomenon in some species exists where *Wolbachia* prevalence and transmission is determined by temperature, creating a strong effect of temperature on sex ratio. (E.g. the mosquito Aedes albopictusis, where higher temperatures resulted in lower vertical transmission of *Wolbachia) (*75, 76). In such a way a first step to a transition from GSD to ESD might be made, in a way similar to *A. vulgare,* where intergenomic conflict between host and *Wolbachia* became an intragenomic conflict for the host and its mechanism of sex determination changed to resolve this conflict.

Beside the ability to bias the sex ratio based on environmental factors, intergenomic conflict caused by endosymbiotic infection of host germ cells can be a major factor in the evolution of sex determination systems. This is seen in *A. vulgare, H. bolina and T. pretiosuma,* where systems of sex determination can change to resolve intergenomic conflicts caused by *Wolbachia.* In many other species (e.g. *N. vitripennis* and *R. kaykai)* diverse and sometimes multiple effects of single *Wolbachia,* or multiple *Wolbachia* with different effects are found. Added to the widespread occurrence of *Wolbachia* in arthropods, the complexity and multitude of interactions it shows, and its ability to exchange genetic material with the host genome, it seems that a coevolutionary endosymbiotic relationship may be present there for a long time, affecting sex determination systems in potentially radical

ways. Phenomena that could be explained by this coevolution include the shift between male and female heterogamety (XX/XY and ZZ/ZW), the evolution of haplodiploidy, obligate asexuality, and undifferentiated sex chromosomes. Also, many other radical interactions of *Wolbachia* and host are present, of which an ultimate explanation, the mechanism and its effects on species are unknown.

To come back to my main question how and why sex ratios are determined, and to what effect they can affect species development, it is clear in the first place that a lot of variation exists in sex ratio determination. All kinds of environmental factors yielding sexually differential benefits, inter-genomic conflicts, and intra-genomic conflicts can cause sex ratio biases, which act against the tendency of the autosomes to produce a balanced sex ratio. Furthermore the specific genetic system of sex determination is important in allowing regulation of sex ratio biases.

Species development is affected depending on the specifics of these sex ratio determining factors in the context of a given species, both of which can be extremely diverse. No singular effect can therefore be ascribed to sex ratio regulation, though this certainly does not mean that there is not an effect worthy of note. Especially the most extreme cases deserve attention since their impact could be tremendous. To develop obligate asexuality or haplodiploidy would strongly influence the extent of genetic variation among a species, so influencing its evolution itself. The specifics and dynamics of sex determination systems also play a major role in evolution.

Furthermore, CI can cause isolation of infected and uninfected individuals of a species, and large sex ratio biases can strongly drive evolution. For these reasons, the effects on species development can be enormous, at least in arthropods.

Though even in insects, where over 65% of species is estimated to be infected by *Wolbachia*, and the studies done have yielded very interesting results, much is still unknown. To accurately estimate the impact of endosymbionts on all insects, not to mention different phyla, it is still too early. However, it is a very interesting field of study where much remains to be discovered, and the effects of endosymbionts and the evolution of sex determination systems certainly is fascinating.

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