# The evolution of sex determination in isopod crustaceans

**Thierry Rigaud, Pierre Juchault and Jean-Pierre Mocquard**

**Summary**

Sex is determined by non-Mendelian genetic elements overriding the sex factors carried by the heterochromosomes in some species of terrestrial isopods. A bacterium *Wolbachia* and a non-bacterial feminizing factor (f) can both force chromosomal males of *Armadillidium vulgare* to become phenotypic functional females. The f factor is believed to be a genetic element derived from the *Wolbachia* genome that becomes inserted into the host nuclear genome. The feminizing factors can be considered to be selfish genetic elements because they bias their host's sex ratio to increase their own transmission. New sex-determining genes are selected (genes resisting the feminizing effects, or the transmission of feminizing elements) as a consequence of the conflict between these elements and the rest of the host's genome. These events drive the sex-determining mechanisms to evolve, and may explain the polymorphism of sex factors and the poor differentiation of the heterochromosomes in isopods.

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## Introduction

Most animals produce the two types of gametes (sperm and oocytes) in separate individuals (males and females). The mechanisms responsible for this separation of the sexes vary considerably among taxa, and also between species within a given taxa[1,2]. The more common system of sex determination is genetic sex determination (GSD), in which the signal initiating the differentiation between sexes is carried on chromosomes. GSD has been found in all animal taxa, but can have several variations. The most common type of GSD is heterogamety, but there are also multi-factor and polyfactor systems[1]. Even the heterogametic system can vary; there are two types (♂XY-♀XX and ♂ZZ-♀WZ), and the initiation of male phenotype in the XY system can be Y-dominant (mammals) or X-recessive (insects and nematodes, where the X chromosome/autosomes ratio determines the sex[1]). Another mechanism for determining sex is environmental sex determination (ESD). This occurs in reptiles[3], fishes[4], crustaceans[5] and worms (reviewed in ref. 1). Here, sex is determined by environmental factors after conception, and the cue for sex determination varies according to the species (temperature, photoperiod or crowding). The third mechanism for determining sex is cytoplasmic sex determination (CSD). CSD has only been found in crustaceans to date. Sex is determined by intracytoplasmic, vertically transmitted micro-organisms. These microbes vary according to the host species[6-8], but they all produce the same phenotypic effect, i.e. changing putative males into functional females. These micro-organisms are parasitic sex factors or feminizing parasites.

This range of sex-determining mechanisms suggests that several evolutionary pathways have been used to produce the same basic phenomenon, separation of the sexes. Several hypotheses or models have been advanced to explain why and how sex-determining mechanisms have evolved. The driving forces for such evolutionary patterns include (1) sex-specific fitness according to environment, for ESD[1,4,5] and (2) selection for 1:1 sex ratios, the relative fitness of adult size and transitions from multi-factor systems to heterogametic sex determination, for GSD[1,9,10]. A few species of crustaceans, dipterans, fishes and reptiles[1] employ more than one method for determining sex, and they provide a unique opportunity for investigating directly the evolution of these systems and their possible interactions and/or interconnections. A good example is the house fly *Musca domestica*. This species has several genetic sex-determining factors, which may be on heterosomes or autosomes. The prevalence of each factor differs among populations[11]. The selection of a given sex factor, leading to changes in heterogamety types, can be driven by the linkage of sex-determining genes with genes conferring resistance to insecticides[12] or by spermatic competition[11].

Some species of crustaceans also have several sex-determining mechanisms. This paper examines how the study of variation in isopod sex-determining mechanisms has helped us to develop a new explanation for the way in

**Table 1.** *The main heterogametic types and heteromorphy in isopods*

| | Heterogamety type | |
|---|---|---|
| Sub-order | ♂ XY / ♀ XX | ♂ ZZ / ♀ WZ |
| Asellota | *Asellus aquaticus*** | *Jaera marina* (5 sub-sp.)*** |
| Flabellifera | | *Dynamene bidentata** |
| Valvifera | | *Idotea balthica* |
| Oniscidea | *Porcellio dilatatus dilatatus** | *Porcellio dilatatus petiti** |
| | | *Porcellio rathkei*** |
| | | *Porcellio laevis*** |
| | *Armadillidium nasatum** | *Armadillidium vulgare** |
| | *Helleria brevicornis* | *Eluma purpurascens* |
| | | *Oniscus asellus* |

Symbols indicate extreme (***), very slight (**) and no (*) heteromorphism of sex chromosomes. No symbol: no data about heteromorphism (heteromorphy is however likely to be absent, because WW or YY individuals are viables and fertiles). The extreme heteromorphisms are due to translocations. From ref. 55.

which sex determination has evolved. The first part is an overview of GSD and CSD systems in these crustaceans. This is followed by a discussion of the implications of parasitic sex factors on the evolution of sex determination. We propose that feminizing elements can drive the evolution of GSD directly by inserting feminizing genes into host chromosomes, or indirectly through the genomic conflicts they induce (by selection of host genes that resist the feminizing effect of parasitic elements).

## Genotypic sex-determining mechanisms in isopods

The earliest studies on sex determination in isopods consisted of diagnosing heterogametic sex by cytological studies, examining the sex linkage of phenotypic markers, and breeding experimentally sex-reversed individuals (see ref. 13 for a review). These studies showed that most species of isopods have heterochromosomal sex determination. But few cases of heteromorphy (morphologically differentiated sex chromosomes) have been described (ref. 14; Table 1). Both heterogametic systems (♂XY/♀XX and ♂ZZ/♀WZ) occur in isopods, sometimes within the same genus and occasionally within the same species, as in *Porcellio dilatatus*. This species has a hierarchy of epistasis, in which Y is epistatic to W and X, which in turn are epistatic to Z (thus a YW individual will be male, an XZ individual will be female)[13].

Several studies have shown that sex determination and sex differentiation are very labile in isopods. Males can readily be changed to females, and females to males, by simple experimental manipulations[13]. This indicates that both sexes possess all the genetic programmes necessary for expression of the opposite sex. More particularly, the sex chromosomes must have large homologous segments, as suggested by the frequent crossing over between sex chromosomes in some species[15], by the near-absence of sex-linked genes[13], and above all by the fact that unusual genetic combinations, such as WW females or YY males, are viable and fertile[13].

The absence of heteromorphy, the presence of mixed heterogametic systems and of large homologous segments shared by sex chromosomes are all generally considered to be signs of a primitive stage of sex chromosome differentiation[1,16]. Incipient sex chromosome differentiation has also been proposed to account for the small morphological difference in the male-specific chromosome in *Asellus aquaticus*[17]. Based on further experimental data, Juchault and Mocquard[18] proposed that males and females of the woodlouse *Armadillidium vulgare* share common genotypes, except that the female chromosome (W chromosome in this species) is a male chromosome (Z) carrying an additional 'female gene' that inhibits the activity of the 'male gene' (Fig. 1A). The sex chromosomes of males and females thus differ only by the presence or absence of a single sex factor, the segregating unit that provides the inherited basis of differences in sex determination[1]. This sex factor need only be a 'trigger' gene, which induces a series of reactions that are controlled by realizer genes present in the genotypes of the both sexes[13]. In fact, the cue for sex determination and differentiation in crustaceans seems to be the 'male gene'. This gene controls the development of the androgenic gland, the organ responsible for producing male hormone. This protein[19] causes male sex differentiation after the third moult of young in isopods[20]. A female phenotype and a female physiology are automatically developed without this hormone[56]. The analysis of several sets of empirical data lead to the proposal that, in female heterogamety, the female gene inhibits the activity of the male gene, allowing the female sex to develop (Fig. 1A)[13].

Some species also have multiple-factor systems of sex determination in addition to heterogametic sex determination. In the marine isopod *Idotea balthica*, heterochromosomic sex determination can be overridden by a polyfactorial system of 'minor' sex factors[21]. These minor sex factors are linked to body-colour genes and have an additive effect. Some of them are masculinizing (they change heterochromosomic WZ females into males), while others are feminizing (they change homochromosomic ZZ males into females). The terrestrial isopod *Porcellio scaber* has a dominant autosomal gene that is responsible for a mottled body appearance and also a strong feminizing influence (see ref. 13). Finally, *Armadillidium vulgare* has an autosomal dominant gene (*M* gene) that possesses a masculinizing influence. Males carrying this gene and captured in the wild are often heterozygous, i.e. ZZ *Mm* males. This gene strongly inhibits the W sex factor, since WZ *Mm* and even WW *Mm* females are changed into functional males[22] (Fig. 1B).

## The incidence of non-Mendelian sex ratio distorters

The isopod crustaceans have long been known to have

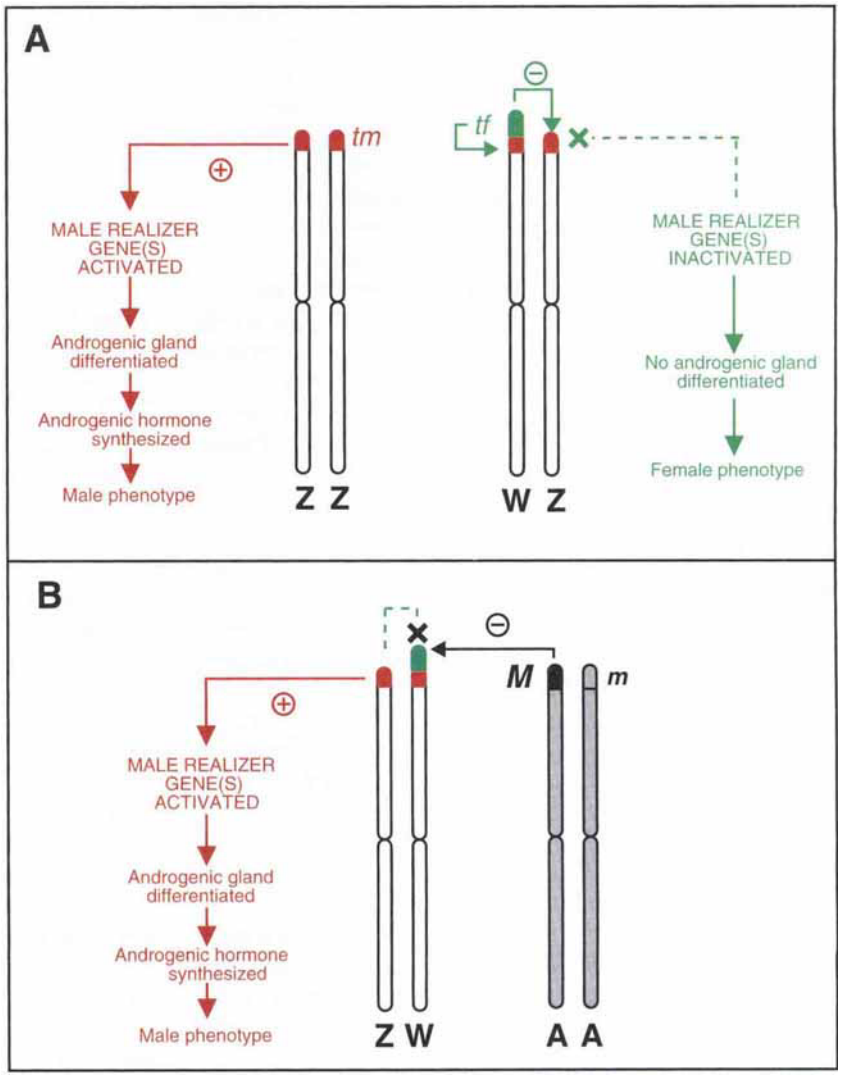


**Fig. 1.** Models for genetic sex determination in the woodlouse *Armadillidium vulgare*. (A) Heterogametic sex determination. The key for male differentiation is the development of the androgenic gland, which is under the control of male sex-determining genes (*tm* for 'trigger male') carried by the Z chromosomes (left). The W and Z chromosomes probably share large homologous segments that can recombine, except in the region of the female sex factor on W (*tf* gene)[18]. This female gene may inhibit the activity of *tm*, preventing differentiation of the androgenic gland. In the absence of the male hormone synthesised by this gland, the undifferentiated gonad spontaneously differentiates into ovaries and the sexual characters evolved toward the female phenotype (right)[13,15,56]. (B) Model for sex determination by a masculinizing autosomal gene (*M* gene)[22,52]. This gene inhibits the feminizing effect of the *tf* gene, which allows the differentiation of a male phenotype in the presence of a female genotype.

abnormal sex ratios[23]. Most of these aberrations have been studied in the woodlouse *Armadillidium vulgare* (Oniscidea). Despite the female heterogamety of this species (Table 1), several genetic studies have shown that some females regularly produce highly female-biased offspring with no differences in the mortality rates, and that this trait is maternally inherited (thelygeny)[23,24]. WZ females produce biased sex ratios after the transplantation of tissue from thelygenic females, suggesting that an infectious agent causes the sex ratio bias[25]. The agent was discovered a few years later[26]. It appears to be a small endocytoplasmic bacterium that lives in the cells of all tissues of such females, but is never found in males. These bacteria belong to the genus *Wolbachia*, a monophyletic group of the $\alpha$ subdivision of purple bacteria[27]. The micro-organisms are concentrated in the oocytes and are maternally inherited. All zygotes inheriting these bacteria, whatever their sexual genotype, will develop a female phenotype. Most notably, the ZZ males are changed to phenotypically functional females, which produce, in turn, female-biased broods. Antibiotics cure the host tissues of these bacteria, and the feminizing effect is suppressed[28].

*Wolbachia* bacteria are thermosensitive. Temperatures up to 30°C destroy many of the symbionts in the host tissues[29]. As a consequence of this destruction, very young *Wolbachia*-infected females of *A. vulgare* reared at 30°C gradually acquire a male phenotype[29]. Older infected females reared at 30°C cannot be changed to males because their sexualised tissues have lost their competence. But when these females are crossed at 30°C, they produce highly male-biased broods[30]. This effect of high temperature is readily understood if it is assumed that all infected females are genetic males (ZZ), and that they possess a female phenotype only because of the symbiont.

All the infected females in populations in which *Wolbachia* occurs are ZZ individuals sexually reversed by the bac-

teria[31,32]. Sex determination of these isopods is therefore under the control of the symbiont in the infected strains: individuals inheriting *Wolbachia* develop into females, while the males are uninfected individuals. *A. vulgare* is therefore a perfect example of cytoplasmic sex determination. As symbiont transmission is about 90% efficient, the sex ratio in broods of infected females is strongly female-biased[31,32]. This sex ratio bias is very stable from generation to generation, indicating a stable transmission rate of the symbiont to offspring. But microbe-induced feminization can nevertheless be incomplete in a few cases, leading to intersexual phenotypes[13]. These phenotypes vary from functional females with tiny male sexual secondary characters to sterile individuals having the sexual characters of both sexes and intersexual non-functional gonads (each gonad consisting of incomplete male and female structures). This variation may be linked to the bacterial density in individuals[28].

This raises the question of how these cytoplasmic sex factors act. *Wolbachia* does not alter the genotypes of infected individuals: males changed to females remain ZZ. The effect is therefore restricted to post-embryonic sexual differentiation. The precise target of feminizing micro-organisms on host sex determination is unknown. This effect can occur at different stages of the differentiation of the androgenic gland, but the androgenic gland never differentiates in reversed males. The symbionts may inhibit the activity of the 'male gene' early in sexual development.

*A. vulgare* is not the only isopod to harbour *Wolbachia*. At least 17 other species belonging to different families are infected by these bacteria[33-36] (Bouchon and Rigaud, unpublished results). These symbionts have feminizing effects in *Armadillidium nasatum*[35] and probably in *A. album*, *Ligia oceanica*[33], *Chaetophiloscia elongata*[36], *Porcellionides pruinosus*[36,37] and the estuarine isopod *Sphaeroma rugicauda*[34].

In contrast, many female-biased strains of *A. vulgare* lack *Wolbachia* symbionts[38]. These lineages possess traits similar to those of *Wolbachia*-infected strains, plus several specific traits. The main similarity is that the females in these lineages are genotypic males reversed by a feminizing factor, as shown by the high-temperature effect, which restores a male-biased sex ratio[39]. The inheritance of the biased sex ratios in these strains is mainly maternal[39], but the sex ratio bias is very unstable. Monitoring individual iso-female lines has revealed wide variations in the sex ratio from generation to generation. In addition, the female proportion often decreases in the successive broods of a single mother (because they can store sperm, female woodlice produce several broods without re-mating). The sex ratio can change from 100% daughters to 100% sons over only three broods[39], as if there is a gradual exhaustion in the transmission or expression of the feminizing factor as the mother ages. These sex ratio variations have never been observed in *Wolbachia*-infected strains. But the main difference from lineages harbouring *Wolbachia* is that these biased sex ratios are occasionally transmitted by males[32,39,40]. Inheritance of the feminizing factor by the male route is very unstable, and has a non-Mendelian pattern[39]. Lastly, the females of these lineages can be experimentally changed to males by the implantation of a male androgenic gland, which is impossible with females harbouring *Wolbachia*. These experimental neo-males are able to transmit the biased sex ratios to their offspring[39]. The feminizing agent responsible for the abnormal sex ratios in these strains was labelled f. Although the precise nature of the f factor remains unknown, its transmission and expression are very similar to those of transposable elements responsible for hybrid dysgenesis[39,41,42] and that of the σ virus in *Drosophila*[43].

The distribution of sex ratio distorters in the wild populations of *Armadillidium vulgare* is shown in Fig. 2[32,44,45]. The most common sex factor is the f factor, and the rarest is *Wolbachia*. Most populations have no symbionts, and *Wolbachia*-infected females, when they are present, are often in a minority. As females infected with *Wolbachia* and/or the f factor produce 70-90% daughters, the population sex ratio depends on the frequency of infected females, but is often female-biased (Fig. 2).

## The evolutionary impact of parasitic sex factors

The most direct effect of *Wolbachia* is the change in the location of female sex determinant from nucleus to cytoplasm. This occurs by a simple selective process. The chromosomal females produce half daughters, while *Wolbachia*-

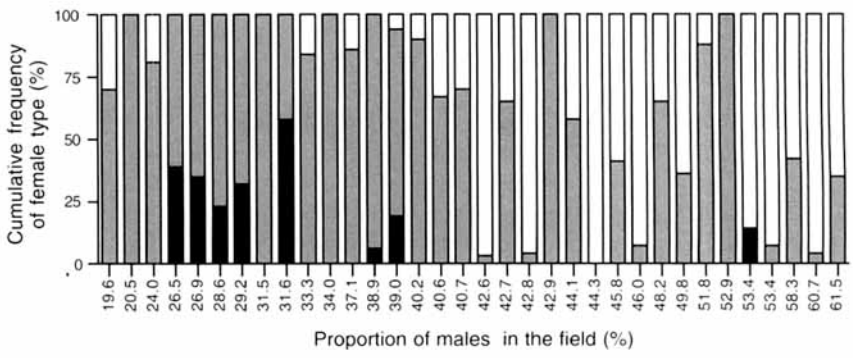


**Fig. 2.** Frequencies of the categories of females as a function of the proportion of males in field populations of *A. vulgare*. One bar represents one population. Black parts of the bars: proportion of ZZ+*Wolbachia* females; grey parts of the bars: proportion of ZZ+f females, white parts of the bars: proportion of WZ females. Data from ref. 32.

infected females produce mainly daughters. As the fecundities of the infected and uninfected females are the same, the infection spreads in the population from generation to generation, and genetic females can be entirely replaced by feminized males in a few generations[1,46]. The W chromosome is therefore rapidly eliminated in species with female heterogamety, and the nuclear female determinant is replaced by the cytoplasmic one (the *Wolbachia*). The dynamics of the f factor in a population is the same, and the competition between WZ females and ZZ females infected with f also leads to the disappearance of WZ females. Field observations on *A. vulgare* are in accordance with this prediction; many field populations are composed entirely of females with a male genotype (females ZZ+f and/or ZZ+*Wolbachia*).

There is a link between the f factor and the *Wolbachia* bacteria in *A. vulgare*[39]. Studies on the offspring of WZ females inoculated with *Wolbachia* and maintained in inbred lines showed that an f lineage appeared spontaneously, following a lack of *Wolbachia* transmission in the descendents of one female. The f factor could therefore be a virus associated with *Wolbachia*, or a part of the bacterial DNA that becomes incorporated into the nuclear genome of the isopod[39]. As no viral particles have been detected in females carrying f, and as no experimental transinfection of the f element is possible by inoculation, f could be a transposon-like factor carrying bacterial feminizing information. But no molecular data are yet available to confirm this. Whatever the underlying mechanism, the association between *Wolbachia* and the isopods seems to have led to the appearance of a new sex-determining mechanism (Fig. 3). This evolution can develop further. The f factor can acquire a Mendelian inheritance in some lineages, which restores a stable balanced sex ratio[18]. Crossing experiments have shown that this stable binding of f occurs to a male chromosome (Z chromosome), and creates a W-like chromosome. This strengthens the idea that there are tiny difference between male and female sexual chromosomes: the only difference between W and Z seems to be the presence or absence of the female determinant, as the rest of the chromosome is identical[18]. This also gives a circular view of the evolution of sex-determining mechanisms in *A. vulgare* (Fig. 3), raising the question of the origin of this evolutionary pattern. It is often assumed that the original sex-determining mechanism is nuclear in crustacean species that are influenced by feminizing factors[13]. However, there are suggestions of cytoplasmic effects on the evolution of sex-determining mechanisms and/or reproductive systems in other taxa, like plants, where nuclear-cytoplasmic interactions might have favoured evolution from hermaphroditism to dioecy (separated sexes)[47]. It is therefore interesting to note that the evolution of sex determination in *A. vulgare* could reflect a more ancient evolution in crustaceans, namely the appearance of separated sexes (and thus the

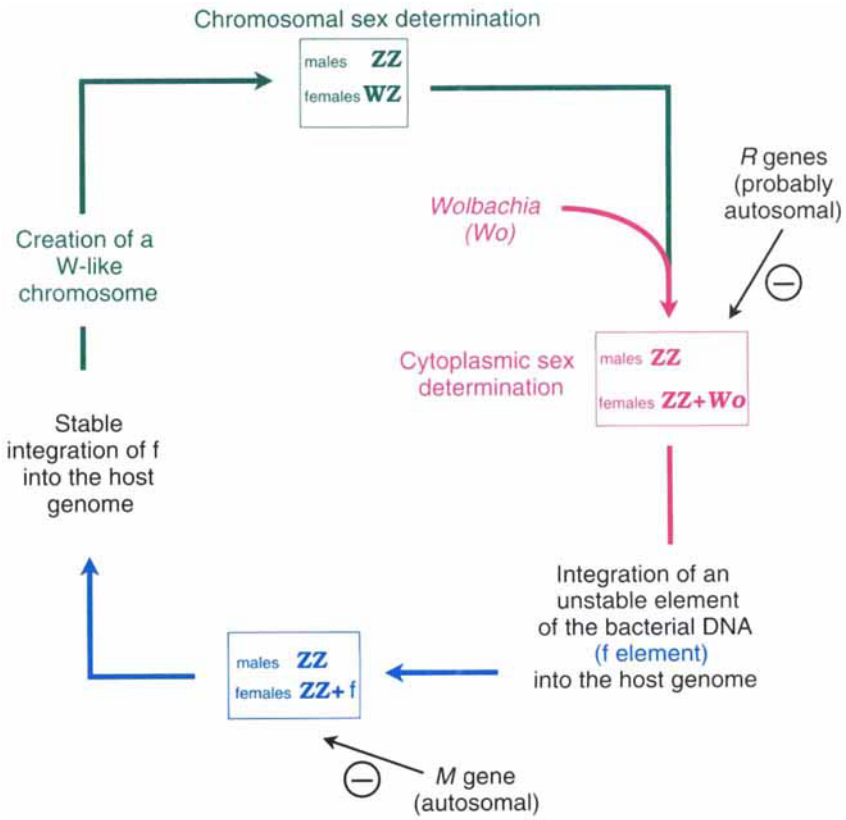


**Fig. 3.** Diagram showing the evolution of sex-determining mechanisms in *Armadillidium vulgare*. The symbiotic association between feminizing *Wolbachia* bacteria may lead to a new non-Mendelian sex factor (f), following a step of cytoplasmic sex determination. The f factor may become inserted stably into a Z chromosome, generating a W-like chromosome. Each step in this evolution is selected for in wild-type populations, but each feminizing factor has its nuclear inhibitor (the *R* and *M* genes of the host), a result of genomic conflicts[51,52]. This evolution may be the origin of the complexity and variability of sex-determining mechanisms found in the wild[32]. Figure modified from ref. 18.

appearance of sexual chromosomes) from a hermaphrodite ancestor *via* cytoplasmic sex determination[18].

The presence of feminizing factors also leads to the selection of other sex factors. As explained by Werren[48] and Hurst[49], cytoplasmic sex ratio distorter genes are in conflict with the autosomal genes of the host because of their different inheritance patterns. Anisogamy (unequal size of the gametes) ensures that vertically transmitted cytoplasmic micro-organisms are maternally inherited (sperm do not transmit cytoplasm to the next generation). The male phenotype is a 'dead end' for these organisms, which explains why the feminization of putative males, and consequently the biased sex ratio, has been selected by *Wolbachia* to increase their transmission to the next generation. The feminizing elements can be considered to be selfish genetic elements[50], but because host nuclear genes are inherited by both parents, they will tend to maintain all mechanisms that lead to a balanced sex ratio. This conflict between cytoplasmic and autosomal genes can be solved by the selection of host autosomal genes that resist the sex ratio distorter (for alternative solutions, see ref. 48). Some evidence for such genes (probably a polygenic system, in fact) was found in *A. vulgare* by selecting an infected strain in which females produced male-biased broods[51]; it was found that their main effect is to limit the transmission of bacteria to offspring, rather than to override the feminizing activity of the symbiont, and they can be referred to as 'resistance' genes (*R* genes). Nevertheless, such genes do intervene indirectly in sex determination by modifying the *Wolbachia* transmission pattern, and may be considered to be new types of sex-determining genes. The presence of the f factor also leads to intragenomic conflict. The autosomal masculinizing gene *M* (described above) can restore a male phenotype in the presence of f[52], as described in the equation (1), where *t* represents the transmission rate of the f factor.

$$♂ZZMm \times ♀ZZmm+f \rightarrow t/2.ZZmm+f(♀) + t/2.ZZMm+f(♂) + (1-t)/2.ZZmm(♂) + (1-t)/2.ZZMm(♂). \quad (1)$$

In accordance with Fisher's predictions, this gene is not selected in populations where sex determination is chromosomal[45], but the frequency of *M* increases with the frequency of the f factor[40], suggesting that it is selected to repress f factor activity. We have previously described this autosomal *M* gene as an example of multi-factorial sex determination, because it also overrides the female sex factor on the W chromosome. Here, in fact, multi-factor sex determination can be seen as a byproduct of the genomic conflict between f and the autosomes. This serial selection of sex-determining genes (i.e. the occurrence of feminizing factors inhibiting male genes, followed by the selection of genes inhibiting the feminizing elements) fits well with the hypothesis for the evolution of the sex-determining pathway proposed by Wilkins[10]. The driving force of this evolution, however, is the genomic conflict induced by factors external to the host genotype.

The multiple sex factors found in *A. vulgare* may have had major consequences for the evolution of sex chromosomes[55]. We have proposed that the evolution of heterogamety in isopods is at an incipient stage, and shown that the chromosomal sex factors are not restricted to a given chromosome pair. The most obvious example is the autosomal masculinizing gene *M*, which became the male sex determinant in the presence of the f factor[52]. Thus, a system similar to male heterogamety can be selected (where $t \approx 1$ in equation 1), but the heterosomal chromosome pair become the autosomal pair carrying the *M* gene. This male heterogamety can be seen as a by-product of the intragenomic conflict in a species with an ancestral female heterogamety, and this suggests that epigenetic sex factors can repeatedly change the location of sex-determining genes on their host chromosomes. The origin of the double heterogamety system in *Porcellio dilatatus* or in the genus *Armadillidium* (Table 1) can perhaps be found in a system similar to the *M* gene[8]. In several taxa, the sex chromosomes are believed to have evolved from the autosomes of hermaphrodite ancestors. The first event of this evolution is the strict linkage of male and female sex factors with a given chromosomal pair, which allows this pair to evolve toward heterogamety. Then, serial phenomena would have enhanced chromosome differentiation, including the failure of recombination between X and Y chromosomes through much of their length, the genetic inertness of a large part of the Y chromosome, and the accumulation of repeated sequences on the Y chromosome[1,16]. In isopods, the repeating of all the changes in the heterogamety system, changes in the sex factor location, changes in the nature of the sex factors (inhibitors, genes avoiding bacterial transmission, etc.) may have prevented the differentiation and evolution of sex chromosomes, because of the instability of the linkage between the sex factors and a given chromosomal pair[8,55].

## Conclusions

The studies on sex determination in isopod crustaceans show complex patterns leading to male and female phenotypes. The evidence for cytoplasmic sex factors (bacteria) has helped us to understand this complexity, and to develop a pattern to describe the evolution of sex determination (see Fig. 3), although several steps in this pathway remain obscure. This evolutionary pattern explains in part the prevalence of the feminizing factor in the wild populations of *A. vulgare*[32]. The recurrent and one-directional evolution from *Wolbachia* to the W chromosome *via* the f factor explains why *Wolbachia* are often found associated with the f factor in populations, why the f factor can increase at the expense of *Wolbachia* in these populations, and why the W chromosome is often found in populations harbouring f (see refs 32,40 for detailed arguments). Other factors are probably involved in the scattered distribution of sex ratio dis-

torters, however, including the selection of resistance genes[51,52], the effect of high temperatures that limit the spread of sex ratio distorters[30] and the loss of infection in small subpopulations[53]. A combination of all these phenomena will lead to the maintenance of polymorphism in the sex-determining systems.

If the f factor is a mobile element of bacterial origin, then f can be seen as a selfish element, driving the host sex determination to promote its own spread. This pattern is similar to that observed in yeast *Saccharomyces cerevisae*, where a mobile element enhances mating frequency in the host and thus promotes its spread in populations more efficiently than in clonal lineages[54]. The f factor in woodlice could be another example of a selfish genetic element becoming integrated into the host sexual cycle.

The variety of sex factors in *Armadillidium vulgare* can be understood by considering that these sex-determining genes have evolved through genomic conflicts, a consequence of the presence of the feminizing sex ratio distorters. This situation illustrates how genetic constraints that induce genomic conflict can be important evolutionary forces[57].

The impact of these feminizing elements on the whole isopod group, their identification in other crustaceans and the precise mode of action of these elements at the molecular level, all remain to be investigated. Several lines of research are possible. The first is to try to define the nature of the feminization. The sequence of the androgenic hormone (see ref. 19; G. Martin, in preparation) opens a new route towards identification of the gene responsible for the synthesis of this protein. The expression and control of this gene must then be investigated to provide more information about its putative inhibitors. A search for mobile elements may also lead to an understanding of the nature of the f element. The discovery of mobile elements does not provide, in itself, clear evidence for their involvement in sex determination, but there may well be a link between such an element and sex ratio distortion. Finally, the involvement of genomic conflicts in the evolution of genetic sex determination should be investigated more thoroughly. As it is probably difficult to obtain more direct experimental evidence to support this hypothesis, theoretical investigations of the conditions required for heterogamety evolution could be useful.

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**Thierry Rigaud, Pierre Juchault** and **Jean-Pierre Mocquard** are at the Université de Poitiers, Laboratoire de Biologie Animale, HUMR CNRS 6556, 40 avenue du Recteur Pineau, F-86022 Poitiers Cedex, France.
E-mail: rigaud@hermes.univ-poitiers.fr