sulfated polysaccharides is involved in host cell infection. *J. Biol. Chem.* 274, 1267–1276
53 Muller, H.M. *et al.* (1993) Thrombospondin related anonymous protein (TRAP) of *Plasmodium falciparum* binds specifically to sulfated glycoconjugates and to HepG2 hepatoma cells suggesting a role for this molecule in sporozoite invasion of hepatocytes. *EMBO J.* 12, 2881–2889
54 Sim, B.K. *et al.* (1992) Localization of the 175-kilodalton erythrocyte binding antigen in micronemes of *Plasmodium falciparum* merozoites. *Mol. Biochem. Parasitol.* 51, 157–159
55 Jakobsen, P.H. *et al.* (1998) Identification of an erythrocyte binding peptide from the erythrocyte binding antigen, EBA-175, which blocks parasite multiplication and induces peptide-blocking antibodies. *Infect. Immun.* 66, 4203–4207
56 Hettmann, C. *et al.* (2000) A dibasic motif in the tail of an apicomplexan myosin is an essential determinant of plasma membrane localization. *Mol. Biol. Cell* 11, 1385–1400
57 Dobrowolski, J.M. *et al.* (1997) Participation of myosin in gliding motility and host cell invasion by *Toxoplasma gondii*. *Mol. Microbiol.* 26, 163–173
58 Sultan, A.A. *et al.* (1997) TRAP is necessary for gliding motility and infectivity of plasmodium sporozoites. *Cell* 90, 511–522
59 Wengelnik, K. (1999) The A-domain and the thrombospondin-related motif of *Plasmodium falciparum* TRAP are implicated in the invasion process of mosquito salivary glands. *EMBO J.* 18, 5195–5204
60 Dessens, J.T. (1999) CTRP is essential for mosquito infection by malaria ookinetes. *EMBO J.* 18, 6221–6227
61 Kappe, S. (1999) Conservation of a gliding motility and cell invasion machinery in apicomplexan parasites. *J. Cell Biol.* 147, 937–944

Inherited microorganisms, sex-specific virulence and reproductive parasitism

Claudio Bandi, Alison M. Dunn, Gregory D.D. Hurst and Thierry Rigaud

Parasites show an amazing repertoire of adaptations, highlighted by complex life cycles that allow both survival in the host and transmission among hosts. However, there is one heterogeneous group of microorganisms whose adaptations are perhaps even more surprising: parthenogenesis induction, feminization of genetic males, killing of male hosts and sperm-mediated sterilization of uninfected eggs. The common feature of these microorganisms is their mode of transmission: inheritance from mother to offspring. Here, we present an introduction to hereditary symbiosis, focusing on microsporidia and bacteria that manipulate host reproduction in arthropods (reproductive parasites). We also discuss the implications of one of these microorganisms, *Wolbachia*, for the control of arthropod pests and vectors and for the therapy of filarial diseases. Finally, we discuss whether some parasites of vertebrates might show sex-specific virulence.

It is still held that natural selection will reduce the virulence of parasites to a state of peaceful coexistence. The ecological and pathogenic conditions leading to virulence reduction are not always easy to assess. The mode of transmission of parasites is thought to be a key determinant in the evolution of virulence[1]. For example, it is accepted that increased virulence is favoured where parasite transmission is horizontal, where there is a high chance that a host is infected by several parasite strains, and where parasite virulence is associated with parasite fecundity and with the likelihood of transmission. However, where parasites are vertically transmitted (from parent to offspring), the reproductive success of host and parasite are more tightly interlinked, and selection should favour more benign parasite strains. Indeed, selection might also promote a positive contribution of the inherited agent to the host. Thus, vertical transmission is also thought to promote the development of mutualistic interactions. Vertically transmitted parasites and symbionts are, in most cases, microorganisms, either eukaryotic or prokaryotic, referred to here as inherited microorganisms.

Evolutionary biology of inherited microorganisms

The interaction between host and inherited microorganisms outlined above is a simplification of the way in which selection acts upon these agents. These microorganisms, being inherited, have interests in common with their host – more precisely, they are maternally inherited, and thus have interests in common with female hosts. The selective pressures on maternally inherited elements differ from those on the genome of the host[2]. First, selection on inherited microorganisms should select directly for beneficial effects towards the host sex responsible for their transmission (i.e. in general, females)[3]. Beneficial effects on males might follow indirectly, but are not a consequence of direct selection. In fact, selection might favour microorganism strains that cause a reduction of the fitness of individuals not involved in their transmission (males or uninfected females), if these effects are associated with an increase in the fitness of females bearing the infection. Thus, in addition to becoming benign or even beneficial to the hosts responsible for their transmission (infected females), selection on inherited microorganisms can favour the spread of phenotypes that are detrimental to those hosts not involved in their transmission (males or uninfected females)[3–7]. Indeed, maternal inheritance underlies

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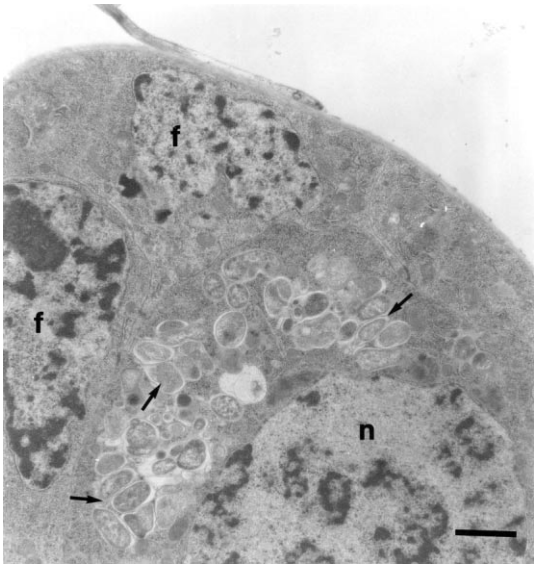

Fig. 1. Electron micrograph showing an egg of the mosquito *Culex pipiens* harbouring numerous *Wolbachia* bacteria (arrows). In mosquitoes and in other arthropods, various strains of *Wolbachia* determine cytoplasmic incompatibility (Fig. 3). Other reproductive manipulations determined by *Wolbachia* are parthenogenesis, feminization of genetic males and death of male embryos. Abbreviations: f, nuclei of follicular cells of the ovariole; n, oocyte nucleus. Scale bar = 0.9 μm. Photograph by Luciano Sacchi and Claudio Bandi.

the different forms of microorganism-induced reproductive manipulations observed in arthropods. These manipulations frequently involve determining a female-biased sex ratio: for maternally inherited microorganisms, males are dead ends. In some cases, all-female broods are produced by converting the female host to a particular form of asexuality (thelytokous parthenogenesis)[8,9]; in other cases, sexual reproduction is continued, but progeny that would ordinarily develop as males instead develop as females (they are feminized)[10]. Apart from these manipulations, certain inherited microorganisms are relatively benign in females, but kill male larvae or embryos, if this either facilitates their horizontal transmission, or if it enhances the survival of sisters of the dead male[11–14]. Finally, some inherited microorganisms cause cytoplasmic incompatibility: through the sperm of infected males they sterilize those females that do not harbour them[5–7].

These different microbial manipulators of host reproduction are known as reproductive parasites[3]. Reproductive parasites occur in various arthropod hosts (insects, mites, crustaceans) and have been assigned to different prokaryotic lineages, as well as to the microsporidia (eukaryotic protists)[3,4]. One special assemblage of reproductive parasites is represented by the bacteria of the genus *Wolbachia* (Fig. 1), which determine different kinds of reproductive manipulations (from parthenogenesis induction to feminization to the killing of male embryos), and which are also thought to be beneficial in filarial nematodes[5–7]. Maternal inheritance underlies reproductive parasitism, but reproductive parasites can also be transmitted horizontally. Reproductive parasites can thus be contagious, as in the case of the male-killing microsporidia in mosquitoes or of parthenogenesis-inducing *Wolbachia* in certain parasitoid wasps[9].

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Parthenogenesis induction

The development of unfertilized eggs into females (thelytokous parthenogenesis) would increase the fitness of a maternally transmitted microorganism. In the early 1990s, it was first reported that thelytokous parthenogenesis in certain species of wasps could be cured by the administration of antibiotics. This indicated the possible involvement of some microorganism in parthenogenesis induction. Parthenogenesis in these and a range of other Hymenoptera was subsequently shown to be associated with infection with certain strains of *Wolbachia*[8]. More recently, *Wolbachia* has also been shown to be associated with parthenogenesis in insects phylogenetically distant from the Hymenoptera (Arakaki *et al.*; Vandekerchove *et al.*, Abstracts)*. In Hymenoptera, male progeny arise from (haploid) unfertilized eggs and females from (diploid) zygotes that are the product of fertilization. Sex determination is based on ploidy. *Wolbachia* induces the production of all-female broods through doubling the chromosome constitution of unfertilized eggs, restoring diploidy, and thus producing female rather than male development[15]. Another possible case of parthenogenesis induction is seen in the mite *Leptotrombidium tsutsugamushi*, where infection with the bacterium *Orientia tsutsugamushi* is associated with female-biased sex ratios[16].

Parthenogenesis-inducing *Wolbachia* are frequently fixed within the population, converting the sexual host species to an asexual one. In some of these species, the ability to reproduce sexually has been lost over time. Although antibiotics give rise to the production of male progeny, these males are not functional. In other species, the infection remains at a polymorphic equilibrium, with both uninfected and infected individuals remaining. Polymorphism is thought to be promoted by host factors that resist the action or transmission of the bacterium[15].

Feminizing microorganisms, sex determination and genetic conflicts

Parasitic feminization is induced by both bacterial and protozoan microorganisms and has been reported in several crustaceans and in one insect[10,17,18]. These microorganisms distort sex determination, converting genotypic males into functional, phenotypic females[17,19]. By feminizing a male host, a maternally transmitted microorganism ensures its transmission to new hosts (i.e. the offspring) and the relative frequency of infected females in the population is increased[17].

At least four ultrastructurally distinct species of feminizing microsporidia have been reported infecting the crustacean *Gammarus duebeni*[20–22]. The best studied, *Nosema granulosis*, is characterized by low pathogenicity and is present in up to 46% of

* Results presented at the First International *Wolbachia* Conference, Kolymbari, Crete, 7–12 June 2000.

females in natural populations[23]. In terrestrial isopods (woodlice), bacteria of the genus *Wolbachia* are responsible for sex reversal[19]. Around half of the woodlice species belonging to different families are infected, each species carrying a single *Wolbachia* lineage[24]. In the most intensively studied species, *Armadillidium vulgare*, the prevalence of *Wolbachia* in females ranges from 0% to 64%, but most populations are uninfected[19,25]. In other species, however, the *Wolbachia* can be at fixation: that is, all females are infected[26].

Feminization has implications for host ecology and for the evolution of host sex ratios and mechanisms of sex determination. As a feminizing microorganism spreads through the host population, the sex ratio will become more female biased. This sex ratio bias might lead to conflict between inherited microorganisms and hosts over sex ratio. Selection on the microorganisms will favour a female-biased sex ratio, because only females transmit the infection. However, selection acting on the host genes will generally favour a 1:1 primary sex ratio[27]. As a result, host autosomal genes that determine a greater investment in males (the rarer sex) might be favoured, leading to compensatory sex ratio evolution[28,29]. Co-evolutionary feedback between host sex ratio and prevalence can drive microorganism prevalence upwards and can lead to the emergence of novel sex determining mechanisms in the host[28–30]. This feedback between microorganism prevalence and host sex ratio is predicted to lead to fixation of the microorganism in females (all females are infected) and monogeny in the host (all hosts are genetically male)[28,29]. The conflict between autosomes and inherited microorganisms might also select for host resistance to microorganism transmission or feminization[30].

Resistance to *Wolbachia* has been found in *A. vulgare* through either suppression of the feminizing effect (albeit incompletely) or suppression of microorganism transmission[31]. There is also evidence for resistance to microorganism transmission and feminization in *G. duebeni* infected by feminizing microsporidia[20,21,23]. In some *A. vulgare* populations, sex ratio evolution in the presence of a feminizing *Wolbachia* has gone to the limit, as predicted theoretically. In infected lineages, all individuals are genetically male and infected individuals are feminized by the bacteria, which has become the female sex-determining factor in this host[19,32]. In this context, by controlling microorganism functions and/or transmission, resistance genes can be seen as new sex-determining factors. Although sex ratio evolution has been reported for populations infected with *N. granulosis*, there is no evidence for parasite fixation. Feminization is not 100% efficient for these microorganisms[23] and so the drive to monogeny and fixation will be less strong[28].

Feminizing microorganisms might also affect host population stability and extinction[33,34]. Even if a feminizer does not become fixed in the host population, theoretical models predict that the host population might be driven to extinction if males become too rare to sustain the population through reproduction[33,34]. However, microsporidian sex ratio distorters appear to be relatively stable in natural *G. duebeni* populations[17], and recent models show that metapopulation structure might enable parasite–host coexistence, despite the transience of local populations through turnover of uninfected, infected and extinct patches[34].

The mechanism of sex differentiation in Crustacea might provide an opportunity for feminization by inherited microorganisms of this arthropod group[10]. In crustaceans, sex differentiation is controlled by the male hormone produced by the androgen gland[35]. If the androgen gland differentiates, the circulating hormone induces male development; in the absence of androgen gland differentiation, female development takes place. It has been suggested that these microorganisms suppress the development of the gland to induce feminization of the host[32], a task that is relatively easy to achieve because only one tissue has to be targeted by the microorganism. In woodlice, some *Wolbachia* strains cause feminization through action on the androgen gland and androgen hormone reception, whereas other strains disrupt only the gland development, resulting in imperfect feminization efficiency, with some males harbouring *Wolbachia*[26,36].

Several microsporidian species feminize *G. duebeni*, and it has been suggested that the mechanism of environmental sex determination (ESD) might increase vulnerability of the host to feminization[17]. ESD is an adaptive sexual strategy under which the sex of an individual is matched to its size-related future fitness according to environmental cues. The level of ESD varies between *G. duebeni* populations[37], and a study of microsporidian prevalence across a series of populations found that feminizer prevalence was higher in populations with a high level of ESD. This supports the idea that the delay in sex determination under ESD makes *G. duebeni* vulnerable to parasitic manipulations of sex, and hence to the invasion of sex ratio disorters[17].

Killing of male embryos

Inherited bacteria that selectively kill male embryos are diverse and are found in a wide variety of insect hosts[11,12,14,38]. Host species typically lay eggs in batches (although exceptions such as the butterfly *Danaus chrysippus* exist). In addition, male-killer prevalence is highest in groups of species where unhatched eggs are consumed by siblings shortly after hatching, or where there is competition between the hatched siblings for a limiting resource[12]. In these cases, the death of male hosts enhances the survival of sibling female hosts, which bear the same bacteria by descent.

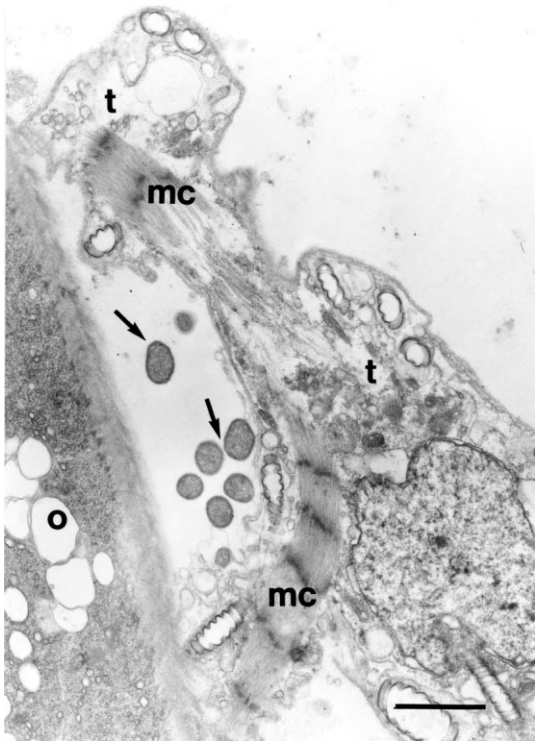

Fig. 2. Electron micrograph showing male-killing bacteria (arrows) from the ladybird beetle *Adonia variegata*. The bacteria are located between the ovariole surface and the envelope formed by tracheocytes (t). mc, myo-connective fibres; o, oocyte. Scale bar = 1.7 μm. Photograph by Luciano Sacchi and Greg Hurst.

Male-killing bacteria have been recorded within the genus *Spiroplasma* (insect-associated mollicutes), the flavobacteria (Fig. 2), the gamma proteobacteria (*Escherichia coli* relatives) and the alpha proteobacteria[38]. In this last group, it is notable that members of the genus *Rickettsia* have been recorded as showing the male-killing trait, in addition to *Wolbachia* male killers[38,39]. A particular host species might be infected by more than one male-killing bacterium[40]. Thus, it is not possible to score the prevalence of the associated bacteria solely from their phenotype.

The prevalence of male-killing bacteria varies between host–bacterium associations. Most commonly, prevalence is in the range of 5–50% of female hosts. However, extremely low- and high-prevalence infections do occur. In *Drosophila willistoni*, fewer than 1% of females are infected[41]. By contrast, in the butterfly *Acraea encedon*, a male-killing *Wolbachia* is found in 86% of female hosts in certain populations, and results in sex role reversal in its host, with females competing for access to a small pool of males[42]. The prevalence achieved by these bacteria is determined by the effect of male host death on the survival of their sister hosts, the direct effects of infection on female host physiology, and the efficiency with which the bacteria are transmitted from a female to her progeny. This last parameter might be affected by both host and microorganism factors.

Killing of male larvae

Late male killing appears to have evolved only in microsporidia that infect mosquito hosts. Male-killing microsporidia display sex-differential development and virulence in their hosts. These parasites cause a relatively benign infection in females, which go on to transmit the parasite to the next host generation. However, in males, a different developmental sequence occurs. Massive parasite replication in the fat body results in death during the fourth larval instar stage and subsequent release of spores into the aquatic environment for horizontal transmission. Varying degrees of male killing have been reported[13,14]. True male killers (type 1 infections)[13] are typified by *Thelohania californica*, which kills transovarially infected males but forms a benign infection in females. By contrast, most hosts that inherit *Amblyospora campbelli* die and there is little sex-specific mortality, although surviving females transmit the parasite to their progeny. Sweeney *et al.*[43] and Hurst[14] suggested that the different parasite–host interactions reflect the ecology of the host and the relative efficiency of horizontal versus vertical transmission. There is no selective advantage of a benign infection in males because male hosts do not transmit the infection vertically, whereas male-specific virulence will be selected if host death leads to horizontal transmission. Virulence in females should be selected if the opportunities for horizontal transmission are high. For example, larval death in mosquitoes infected with *A. campbelli* releases spores that infect a copepod intermediate host[44]. By contrast, a benign infection in females infected with *T. californica* is adaptive because the host mosquito is univoltine and vertical transmission is important for parasite maintenance outside of the host breeding season.

Cytoplasmic incompatibility

In cytoplasmic incompatibility (CI), embryonic death is seen after mating between males infected by certain strains of *Wolbachia* and females that are either uninfected or infected with an incompatible *Wolbachia* strain. *Wolbachia* that induce CI are found in a wide variety of arthropods, particularly in insects, but also in mites and isopod crustaceans[5–7]. Different strains of *Wolbachia* can produce CI, and these might be mutually incompatible (bidirectional CI); that is, a male infected with strain w1 is incompatible with a female infected with strain w2, and vice versa.

CI is thought to be determined by some as yet undetermined *Wolbachia*-induced modifications of sperm from infected males (e.g. through a toxin: *Wolbachia* is not transmitted through sperm). In diploids, modified spermatozoans render inviable those eggs they enter that do not harbour *Wolbachia* (or harbour a different compatibility type of *Wolbachia*). In infected eggs, the presence of *Wolbachia* leads to the rescue of the egg (e.g. through an antitoxin) and successful development can take place. In other words, *Wolbachia* kills those eggs that

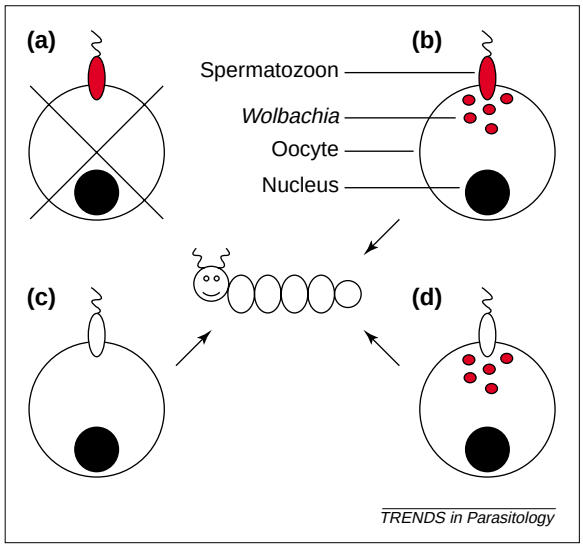

Fig. 3. Diagram illustrating unidirectional cytoplasmic incompatibility. Spermatozoa coming from infected males (modified spermatozoa) and *Wolbachia* bacteria are red. The meeting between modified spermatozoa and uninfected oocytes leads to early embryonic death (a). In infected females, *Wolbachia* is thought to produce a rescue factor that allows the normal development of oocytes fertilized by modified spermatozoa (b). Spermatozoa from uninfected males can successfully fertilize both uninfected (c) and infected (d) oocytes. Drawing by Maurizio Casiraghi.

do not carry *Wolbachia* (Fig. 3). It is also notable that a *Wolbachia* type exists that rescues the host egg from incompatibility, while being unable to modify the sperm to induce it (mod– res+ *Wolbachia*)[45].

CI-determined death occurs during early development, and appears to be associated with alterations of the proper condensation/decondensation of the paternal chromatin[5–7]. This association with paternal chromatin is witnessed most spectacularly in haplodiploid species, where improper condensation produces haploidy and male development. The behaviour of CI can be seen as one where the bacteria is interfering with the production of uninfected offspring, and thus indirectly inducing the spread of the infection. Field experimental evidence from populations of *Drosophila simulans* confirms the rapidity with which *Wolbachia*-induced CI spreads[46].

CI is probably the most widespread of all the manipulations produced by *Wolbachia*. It is also the most benign. This is because incompatibility is produced only in crosses between infected males and uninfected females. A strain that produces a strong incompatibility spreads to high prevalence; so that strong incompatibility becomes associated with the presence of few uninfected females, and thus a rare occurrence of the incompatibility phenotype within the population.

Wolbachia is also known to have effects on sperm production and sperm precedence[5–7]. By increasing the relative fertility of infected males, CI *Wolbachia* might increase the frequency of CI induction in uninfected females. In *D. simulans*, by contrast, *Wolbachia* infection is associated with reduced sperm number[47].

Reproductive parasitism and control of insect pests and vectors

Because of their theoretical rapid spread in the host population and the effect they might have on reproduction, transovarially transmitted microorganisms have the potential to provide powerful help in controlling agricultural pests or insect vectors[48]. Their use could be direct or indirect. One direct way to use reproductive parasites is to enhance the reproductive potentialities of parasites or predators of particular pest species. Numerous Hymenoptera are already used as agents for biological control. The occurrence of *Wolbachia* inducing parthenogenesis in numerous parasitoid wasps could be helpful because, as only females parasitize their hosts, the symbionts provide a unique opportunity to double the 'parasitically active' population size of the parasitoid.

The direct use of microorganisms inducing CI has been shown to be a useful tool to suppress local pest populations[49]. However, increasing knowledge on the mechanisms and dynamics of CI in wild populations suggest that this direct use could be very complex in large-scale practice. An indirect strategy would be to use these microorganisms as gene vectors in their host[48]. If a gene suppressing the transmission of a given microbial disease in its insect vector could be introduced into *Wolbachia*, the potential spreading of this modified *Wolbachia* through the vector population could limit disease transmission. To achieve this goal, several steps forward need to be taken: *Wolbachia* have to be transformed in cell culture and re-introduced into the insect vector, and the spread of this organism in pest populations must be ensured. Trials of control await the further development of these procedures. Another potential way to control arthropod populations would be to exploit the mechanism used by reproductive parasites to manipulate host reproduction. Does *Wolbachia* induce CI through some toxin carried by spermatozoa? Do some male-killing bacteria produce a male-specific toxin? Studying the biochemistry and genetics of reproductive parasites, as well as the responses of the arthropod hosts to their presence, will facilitate understanding of the molecular mechanisms involved in reproductive manipulations, and reveal new molecular strategies for the control of arthropod pests and vectors. In addition, understanding how reproduction is manipulated might provide new insight into the biology of reproduction.

Wolbachia and the control of filarial diseases

The potential implications of the presence of *Wolbachia* in filarial nematodes have recently attracted a great deal of attention[50–52]. As in arthropods, *Wolbachia* in filarial nematodes is maternally transmitted. As discussed above, uniparental transmission is thought to have promoted the evolution of mechanisms to manipulate host reproduction in arthropod *Wolbachia*: should nematode *Wolbachia* behave differently from the arthropod one? In filarial nematodes, the phylogeny of *Wolbachia* matches the host phylogeny[53]. In addition, in filarial species positive for *Wolbachia*, all examined specimens have been shown to harbour

these bacteria[50,51]. This distribution pattern is also seen at higher taxonomic levels: the association with *Wolbachia* is typical of whole branches in the filarial phylogenetic tree[53]. This differs from that seen in arthropods, where *Wolbachia* is patchily distributed and where host and symbiont phylogenies are not congruent[5–7]. Thus the evolutionary dynamics of *Wolbachia* in arthropods and nematodes is different, indicating losses of the infection and horizontal transmission events in arthropods, stable infection, co-speciation and a long co-evolutionary history in nematodes.

Long co-evolution of host and symbiont is expected to result in co-adaptation and reciprocal dependence. Indeed, bacteriostatic drugs, such as tetracycline, which are known to be effective against *Wolbachia* in arthropods, have been shown to have detrimental effects on filarial nematodes that harbour *Wolbachia*. In addition, tetracycline has been recorded to be ineffective against *Achantocheilonema viteae*, a filaria that does not harbour *Wolbachia*[51,52]. These results support the hypothesis that those filarial nematodes that harbour *Wolbachia* require the presence of this bacterium, at least during some stages of their life cycle. *Wolbachia* could thus represent a useful target for the control of filariases. Indeed, it has recently been shown that doxycycline inhibits microfilaria production in *Onchocerca volvulus*[52]. In addition, *Wolbachia* seems to play an important role in the pathogenesis of filarial diseases[52]. Treatments aimed at reducing *Wolbachia* density in filarial worms could thus become useful; for example, in reducing the side effects of microfilaricidal treatments.

In view of the wide range of phenotypic effects caused by *Wolbachia* in arthropods, it would not be surprising if different *Wolbachia* endosymbionts behave in different ways in different filarial nematodes, being beneficial in some hosts and determining reproductive manipulations in others. The presence of *Wolbachia* might also result both in reproductive alterations and physiological benefits in the same host. Investigation of this subject awaits further developments.

Reproductive parasites in vertebrates: why not?

Are reproductive parasites restricted to invertebrate animals? A prerequisite for reproductive parasitism is inheritance through a single sex, a modality of transmission that occurs among vertebrate parasites, even though it is usually coupled with some form of horizontal transmission. Should we expect to find reproductive parasites among protozoans such as *Toxoplasma* and *Neospora*, or among the various other microorganisms and viruses transmitted through the placenta? Should we expect to find some degree of male-specific virulence in these parasites? Should we expect to find sex ratio biases among aborted fetuses, where abortion is caused by transplacentally transmitted parasites? The key factor is probably the relative importance of vertical and horizontal transmission in the life cycle of a given parasite. Even a low level of horizontal transmission could allow the perpetuation of the parasite, thus reducing selective pressures for becoming less virulent towards females. However, as in the case of male-killing microsporidia, low levels of sex-specific virulence can still exist in the presence of efficient horizontal transmission. An aborted foetus or weak offspring harbouring coccidian microorganisms could ensure horizontal transmission of these parasites if consumed by carnivorous mammals (where the sexual phase of the cycle takes place). But why renounce the possibility of also being vertically transmitted through females? This would obviously require that congenitally infected females transmit the microorganism, even inefficiently, to the progeny during subsequent pregnancies. We predict that future research on some transplacentally transmitted parasites will lead to the discovery of some level of male-specific virulence.

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References

1 Ebert, D. and Herre, E.A. (1996) The evolution of parasitic diseases. *Parasitol. Today* 12, 96–101
2 Cosmides, L. and Toby, J. (1981) Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* 89, 83–129
3 Werren, J.H. and O'Neill, S.L. (1997) The evolution of heritable symbionts. In *Influential Passengers, Inherited Microorganisms and Arthropod Reproduction* (O'Neill, S.L. *et al.*, eds), pp. 1–41, Oxford University Press
4 O'Neill, S.L. *et al.*, eds (1997) *Influential Passengers, Inherited Microorganisms and Arthropod Reproduction*, Oxford University Press
5 Werren, J.H. (1997) Biology of *Wolbachia*. *Annu. Rev. Entomol.* 42, 587–609
6 Bourtzis, K. and Braig, H.R. (1999) The many faces of *Wolbachia*. In Rickettsia *and Rickettsial Diseases at the Turn of the Third Millennium* (Raoult, D. and Hackstadt, T., eds), pp. 199–219, Elsevier
7 Stouthamer, R. *et al.* (1999) *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53, 71–102
8 Stouthamer, R. *et al.* (1993) Molecular identification of organisms associated with parthenogenesis. *Nature* 361, 66–68
9 Huigens, M.E. *et al.* (2000) Infectious parthenogenesis. *Nature* 405, 178–179
10 Rigaud, T. (1997) Inherited microorganisms and sex determination of arthropod hosts. In *Influential Passengers, Inherited Microorganisms and Arthropod Reproduction* (O'Neill, S.L. *et al.*, eds), pp. 81–101, Oxford University Press
11 Skinner, S.W. (1985) Son-killer: a third extrachromosomal factor affecting the sex ratio in the parasitoid wasp, *Nasonia* (= *Mormoniella*) *vitripennis*. *Genetics* 109, 745–759
12 Hurst, G.D.D. *et al.* (1997) Cytoplasmic sex-ratio distorters. In *Influential Passengers, Inherited Microorganisms and Arthropod Reproduction* (O'Neill, S.L. *et al.*, eds), pp. 125–154, Oxford University Press
13 Kellen,W.R. *et al.* (1965) Host–parasite relationship of some *Thelohania* from mosquitoes (Nosematidae: Microsporidia). *J. Invertebr. Pathol.* 7, 161–166
14 Hurst, L.D. (1991) The incidences and evolution of cytoplasmic male killers. *Proc. R. Soc. London B Biol. Sci.* 244, 91–99
15 Stouthamer, R. (1997) *Wolbachia*-induced parthenogenesis. In *Influential Passengers, Inherited Microorganisms and Arthropod Reproduction* (O'Neill, S.L. *et al.*, eds), pp. 102–124, Oxford University Press
16 Takahashi, M. *et al.* (1997) Occurrence of high ratio of males after introduction of minocycline in a colony of *Leptotrombidium fletcheri* infected with *Orientia tsutsugamushi*. *Eur. J. Epidemiol.* 13, 79–86
17 Dunn, A.M. *et al.* (1995) Evolutionary ecology of vertically transmitted parasites: strategies of transovarial transmission of a microsporidian sex ratio distorter in *Gammarus duebeni*. *Parasitology* 111, S91–S110
18 Kageyama, D. *et al.* (1998) Female-biased sex ratio in the Asian corn borer, *Ostrinia furnacalis*: evidence for the occurrence of feminizing bacteria in an insect. *Heredity* 81, 311–316

19 Juchault, P. *et al.* (1993) Evolution of sex determination and sex ratio variability in wild populations of *Armadillidium vulgare* (Latr) (Crustacea, Isopoda): a case study in conflict resolution. *Acta Oecologia* 14, 547–562
20 Bulnheim, H.P. (1978) Interaction between genetic, external and parasitic factors in sex determination of the crustacean amphipod *Gammarus duebeni*. *Helgolander wiss Meeresunters* 31, 1–33
21 Dunn, A.M. *et al.* (1993) Transovarial transmission and sex ratio distortion by a microsporidian parasite in a shrimp. *J. Invertebr. Pathol.* 61, 248–252
22 Terry, R.S. *et al.* (1999) Ultrastructural characterisation and molecular taxonomic identification of *Nosema granulosis* n. sp., a transovarially transmitted feminising (TTF) microsporidium. *J. Euk. Microbiol.* 46, 492–499
23 Terry, R.S. *et al.* (1998) Impact of a novel feminizing microsporidian parasite on its crustacean host. *J. Eukaryot. Microbiol.* 45, 497–501
24 Bouchon, D. *et al.* (1998) Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. *Proc. R. Soc. London B Biol. Sci.* 265, 1081–1090
25 Rigaud, T. *et al.* (1999) Mitochondrial polymorphism, sex ratio distorters and population genetics in the isopod *Armadillidium vulgare*. *Genetics* 152, 1669–1677
26 Marcadé, I. *et al.* (1999) Mitochondrial DNA and *Wolbachia* infection in two sibling woodlice species. *Heredity* 83, 71–78
27 Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*, Clarendon Press
28 Hatcher, M.J. and Dunn, A.M. (1995) Evolutionary consequences of cytoplasmically inherited feminizing factors. *Philos. Trans. R. Soc. London Ser. B* 348, 445–456
29 Werren, J.H. (1987) The coevolution of autosomal and cytoplasmic sex ratio factors. *J. Theor. Biol.* 124, 313–334
30 Taylor, D.R. (1990) Evolutionary consequences of cytoplasmic sex ratio distorters. *Evol. Ecol.* 4, 235–248
31 Rigaud, T. and Juchault, P. (1993) Conflict between feminizing sex ratio distorters and an autosomal masculinizing gene in the terrestrial isopod *Armadillidium vulgare* Latr. *Genetics* 133, 247–252
32 Rigaud, T. *et al.* (1997) The evolution of sex determination in isopod crustaceans. *BioEssays* 19, 409–416
33 Hatcher, M.J. *et al.* (1999) Population dynamics under parasitic sex ratio distortion. *Theor. Popul. Biol.* 56, 11–22
34 Hatcher, M.J. *et al.* (2000) The co-existence of hosts and sex ratio distorters in structured populations. *Evol. Ecol. Res.* 2, 185–205
35 Charniaux-Cotton, H. and Payen, G. (1985) Sexual differentiation. In *The Biology of Crustacea* (Vol. 9) (Bliss, D.E and Mantel, L.H., eds), pp. 217–299, Academic Press
36 Rigaud, T. *et al.* (1999) *Wolbachia* infection in the terrestrial isopod *Oniscus asellus*: sex ratio distortion and effect on fecundity. *Heredity* 83, 469–475
37 Watt, P.J. and Adams, J. (1993) Adaptive sex determination and population dynamics in a brackish water amphipod. *Estuarine Coastal and Shelf Sci.* 37, 237–250
38 Hurst, G.D.D. and Jiggins, F.M. (2000) Male-killing bacteria in insects: mechanisms, incidence and importance. *Emerg. Infect. Dis.* 6, 329–336
39 Hurst, G.D.D. *et al.* (1999) Male-killing *Wolbachia* in two species of insect. *Proc. R. Soc. London Ser. B Biol. Sci.* 266, 735–740
40 Hurst, G.D.D. *et al.* (1999) Invasion of one insect species, *Adalia bipunctata*, by two different male-killing bacteria. *Insect Mol. Biol.* 8, 133–139
41 Williamson, D.L. and Poulson, D.F. (1979) Sex ratio organisms (spiroplasmas) of *Drosophila*. In *The Mycoplasmas* (Whitcomb, R.F. and Tully, J.G., eds), pp. 175–208, Academic Press
42 Jiggins, F.M. *et al.* (1999) Sex ratio distorting *Wolbachia* causes sex role reversal in its butterfly host. *Proc. R. Soc. London Ser. B Biol. Sci.* 267, 69–73
43 Sweeney, A.W. *et al.* (1990) Life cycle of *Amblyospora indicola* (Microspora: Amblyosporidae) a parasite of the mosquito *Culex sitiens* and of *Apocyclops* sp. copepods. *J. Invertebr. Pathol.* 55, 428–434
44 Dickson, L. and Barr, A.R. (1990) Development of *Amblyospora campbelli* (Microsporidia: Amblyosporidae) in the mosquito *Culiseta incidens* (Thomson). *J. Protozool.* 37, 71–78
45 Bourtzis K. *et al.* (1998) Rescuing *Wolbachia* have been overlooked. *Nature* 391, 852–853
46 Turelli, M. and Hoffmann, A.A. (1991) Rapid spread of an inherited incompatibility factor in California *Drosophila*. *Nature* 353, 440–442
47 Snook, R.R. *et al.* (2000) Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*. Analyses of fecundity, fertility and accessory gland proteins. *Genetics* 155, 167–178
48 Curtis, C.F. and Sinkins, S.P. (1998) *Wolbachia* as a possible means of driving genes into populations. *Parasitology* 116, S111–S115
49 Laven, H. (1967) Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature* 207, 383–384
50 Bandi, C. *et al.* (1998) Phylogeny of *Wolbachia* in filarial nematodes. *Proc. R. Soc. London Ser. B Biol. Sci.* 265, 2407–2413
51 Taylor, M.J. and Hoerauf, H. (1999) *Wolbachia* bacteria of filarial nematodes. *Parasitol. Today* 15, 437–442
52 Taylor, M.J. *et al.* (2000) *Wolbachia* bacteria of filarial nematodes: a target for control? *Parasitol. Today* 16, 179–180
53 Casiraghi, M. *et al.* (2001) A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbiont. *Parasitology* 122, 93–103

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