



HAL
open science

Manipulation of arthropod sex determination by endosymbionts: diversity and molecular mechanisms

Wen-Juan Ma, Fabrice Vavre, Leo W. Beukeboom

► To cite this version:

Wen-Juan Ma, Fabrice Vavre, Leo W. Beukeboom. Manipulation of arthropod sex determination by endosymbionts: diversity and molecular mechanisms. *Sexual Development*, 2014, pp.59-73. hal-01092616

HAL Id: hal-01092616

<https://inria.hal.science/hal-01092616>

Submitted on 3 Jul 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Manipulation of arthropod sex determination by endosymbionts: diversity and**
2 **molecular mechanisms**

3

4 Wen-Juan Ma^{1*}, Fabrice Vavre², Leo W. Beukeboom¹

5 1. Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of
6 Groningen, Groningen, The Netherlands

7 2. Université de Lyon, Lyon; Université Lyon 1; Laboratoire de Biométrie et Biologie Evolutive,
8 Villeurbanne, France

9

10

11

12 ***Correspondence:**

13 Wen-Juan Ma, Evolutionary Genetics, Centre for Ecological and Evolutionary Studies,
14 University of Groningen, PO Box 11103, 9700 CC Groningen, Groningen, The Netherlands.

15 Email: wenjuanma84@gmail.com

16 Phone: +31 50 363 2336

17

18

19

20

21

22

23 **Running title:** Endosymbiont Manipulation of Arthropod Sex Determination

24

25

26 **Abstract**

27 Arthropods exhibit a large variety of sex-determination systems both at the chromosomal and
28 molecular level. Male heterogamety, female heterogamety, and haplodiploidy occur
29 frequently, but partially different genes are involved. Endosymbionts, such as *Wolbachia*,
30 *Cardinium*, *Rickettsia* and *Spiroplasma*, can manipulate host reproduction and sex
31 determination. Four major reproductive manipulation types are distinguished: cytoplasmic
32 incompatibility, thelytokous parthenogenesis, male killing and feminization. In this review, we
33 summarize the effects of these manipulation types, and how they interfere with arthropod sex
34 determination in terms of host developmental timing, alteration of sex determination and
35 modification of sexual differentiation pathways. Transitions between different manipulation
36 types occur frequently, which suggests that they are based on similar molecular processes. We
37 discuss how mechanisms of reproductive manipulation and host sex determination can be
38 informative on each other, with a special focus on haplodiploidy. We end with future
39 directions on how study of endosymbiont manipulation of host reproduction can be key to
40 further study of arthropod sex determination.

41

42

43

44

45

46 **Key Words:** endosymbiont, arthropods, molecular mechanism, sex determination, sexual
47 differentiation, hormonal signaling, epigenetics

48

49

50

51

52

53

54 **Introduction**

55 Arthropods cover over 1,2 million described species that account for about 80% of all
56 known living animal species. They have colonized virtually all habitats on Earth. In line with
57 this broad adaptation to many conditions they exhibit an enormous variety of life histories and
58 reproductive modes. They also show surprisingly large variation and turnover in sex-
59 determination systems. It is therefore a prime group of organisms to study how changes in
60 sex-determination mechanism come about, a current topic in evolutionary biology that is not
61 well understood. A particular aspect of arthropod biology is their frequent infection with
62 microorganisms that can be mutualistic, parasitic or commensal. A specific group are
63 endosymbionts, such as *Wolbachia*, *Cardinium*, *Rickettsia*, *Spiroplasma* and *Arsenophonus*
64 bacteria, microsporidia and viruses, that manipulate their host's reproduction in a variety of
65 ways [reviewed in Hurst et al., 1996; Werren et al., 2008; Kageyama et al., 2012]. These
66 intracellular parasites are maternally transmitted through the egg cytoplasm. As males are an
67 evolutionary dead end for them, any symbiont having the capability to increase female
68 production is at an advantage and can invade host populations [Partridge and Hurst, 1998;
69 Duron et al., 2008; Werren et al., 2008; Cordaux et al., 2011]. This can be realized through
70 causing thelytokous parthenogenesis, male killing or feminization. As they enhance their own
71 transmission at the expense of their host's fitness, their presence generates genetic conflicts
72 between the two sexes, and possibly an ensuing coevolutionary arms race over offspring sex

73 [Hurst and Werren, 2001; Werren, 2011]. It has been suggested that such conflict can drive the
74 evolution of changes in host reproduction and sex-determination mechanisms [Werren and
75 Beukeboom, 1998; Stouthamer et al., 2010; Cordaux et al., 2011; Beukeboom, 2012]. Hence,
76 these endosymbionts may be important evolutionary drivers of turnovers in arthropod sex
77 determination.

78 Here, we review and discuss the current knowledge about manipulative actions of
79 endosymbionts on arthropods. We first briefly summarize the current knowledge about
80 arthropod sex determination and the four major endosymbiont manipulation types of host
81 reproduction. We then move to a specific focus on how symbionts might interfere with host
82 sex determination based on the current knowledge about the molecular bases of host
83 manipulation. We end by proposing future directions on how these reproductive phenotypes
84 may be key to further study of arthropod sex determination. As epigenetic effects are
85 becoming more apparent in insect development, we pay special attention to the possibility of
86 epigenetic regulation.

87

88

89 **Arthropod sex determination**

90 Sex determination in arthropods is generally genetically determined by factors on sex
91 chromosomes, with some exceptions in crustaceans in which it is under either temperature or
92 photoperiod control [Bouchon et al., 1998; Cordaux et al., 2011; Kageyama et al., 2012]. Most
93 knowledge comes from insects, where sex determination occurs through a cascade of genes
94 with a highly conserved master switch gene (*doublesex*) at the bottom, but more divergence in
95 the upstream genes (e.g. *transformer*) and the primary signals at the top of the cascade
96 [Wilkins, 1995; Beye et al., 2003; Verhulst et al., 2010; Beukeboom, 2012]. The chromosomal
97 constitutions serve as primary signals and vary between orders. In most insect orders (22 out
98 of 29), the chromosomal constitutions are either XO or XY male heterogamety [Blackman,
99 1995; Beukeboom and Perrin, 2014]. For instance, most Diptera (flies) and Coleoptera (beetles)
100 have male heterogamety with presence of a Y chromosome (XX/XY), and most Orthoptera

101 (grasshoppers), Odonata (dragonflies), and Mantodea (mantids) have male heterogamety
102 without a Y (XX/XO). All Lepidoptera (butterflies, moths) and Trichoptera (caddisflies) have
103 female heterogamety (either ZW/ZZ or ZO/ZZ). Hymenoptera (sawflies, wasps, bees and ants)
104 and Thysanoptera (thrips) do not have specific sex chromosomes and reproduce by
105 haplodiploidy (haploid males, diploid females). In addition to these common types of sex
106 determination, more rare variations occur, such as multiple sex chromosomes and X-
107 chromosome or paternal genome loss [Bull, 1985; Sánchez, 2008].

108 The chromosomal constitutions are translated into different downstream signals that are
109 also diverse among insect orders. In diploids they include X (or Z) chromosome counting
110 elements, dominant masculinizing factors, and dominant feminizing factors. In haplodiploids,
111 allelic complementarity, at one or more sex-determination loci, and maternal effect genetic
112 imprinting have been documented. In most species these signals converge downstream to
113 regulate a key sex-determination gene *transformer*, which directly regulates the sex master
114 switch gene *doublesex (dsx)* [Bull, 1985; Nöthiger and Steinmann-Zwicky, 1985; Wilkins, 1995;
115 Marín and Baker, 1998; Raymond *et al.*, 1998; Schütt and Nöthiger, 2000; Graham *et al.*, 2002;
116 Saccone *et al.*, 2002; Sánchez, 2008; Verhulst *et al.*, 2010; Gempe and Beye, 2011]. Exceptions
117 seem to occur in Lepidoptera where *transformer* has not been found [Suzuki *et al.*, 2001, 2008;
118 see also Geuverink and Beukeboom in this issue]. *Doublesex* in turn regulates genes for sex
119 specific development [Wilkins, 1995; Raymond *et al.*, 1998; Schütt and Nöthiger, 2000], and
120 together with the *fruitless* gene regulates sexual differentiation including sexual behaviour
121 [Waterbury *et al.*, 1999; Rideout *et al.*, 2010].

122 Much less is known about arthropod sex determination outside of the insects, in particular
123 at the level of genes. In crustaceans, heterogametic sex determination appears to be most
124 common [Legrand *et al.*, 1987]. The *transformer* gene has been only identified in the water
125 flea *Daphnia magna*, but does not show sex differences in expression or splicing patterns,
126 rendering it unlikely to be involved in sex determination [Kato *et al.*, 2010]. An important
127 difference from insects is that sex determination in crustaceans is an endocrine process
128 mediated by the androgenic hormone synthesized by the androgenic gland [Ventura *et al.*,
129 2011]. Basically, individuals have all the genetic information to develop as male or female, but

130 their fate is determined by a feminizing gene that inhibits the development of the androgenic
131 gland and the synthesis of the androgenic hormone. In absence of the androgenic hormone,
132 female differentiation is induced. In Acari (mites, ticks), both diploidy and haplodiploidy occur,
133 but virtually nothing is known about the genetic regulation of sex determination [Norton et al.
134 1993; Arakaki et al., 2001]. The same holds for myriapods (millipedes, centipedes) that have
135 male heterogametic sex determination [Fontanetti et al., 2002]. No sex-determination genes
136 have been identified in any of these arthropod groups yet.

137

138

139 **Endosymbiont diversity and manipulation types**

140 Over 40% of all arthropods are infected with endosymbionts that live in the cytoplasm of
141 their cells and are vertically transmitted through eggs of females [Werren, 1997; Werren and
142 O'Neill, 1997; Zchori-Fein et al., 2001; Zchori-Fein and Perlman, 2004; Zug and Hammerstein,
143 2012]. Some of these are obligate mutualists such as *Buchnera* in aphids [Douglas, 1998; Koga
144 et al., 2003], but many others are reproductive parasites. The most prevalent of these host
145 manipulators are the alpha-proteobacteria *Wolbachia pipientis* and *Rickettsia sp.*, the
146 bacteroidetes *Cardinium hertigii*, the gamma-proteobacterium *Arsenophonus* and the
147 mollicutes *Spiroplasma poulsonii* and *S. ixodetis*, which belong to very distantly related
148 bacterial clades [Duron et al., 2008]. Four broad categories of host reproduction manipulation
149 are distinguished: induction of cytoplasmic incompatibility between egg and sperm,
150 thelytokous parthenogenetic reproduction, killing of male offspring and feminization of
151 genotypic males [Hurst et al., 2002; Werren et al., 2008; Kraaijeveld et al., 2011]. The
152 molecular genetic details of the mechanisms by which these endosymbionts exert the effects
153 on their hosts are not yet well known. Given the diversity of effects and the variety of
154 microorganisms involved, different questions arise: is this true convergence or are horizontal
155 gene transfers between symbionts involved? If this is convergence among symbionts, is it only
156 at the phenotypic level or also at the mechanistic level? How can we explain the seemingly
157 easy evolution of these manipulations? Do different types of manipulation share common

158 mechanisms? Answering these questions requires a better understanding of the molecular
159 mechanisms at play, which in turn will pave the way to better understand the basic processes
160 of sex determination and their evolution. Before getting into these questions we briefly
161 present the different types of reproductive manipulations. The common theme is that host sex
162 determination is somehow manipulated by the endosymbionts to increase their own
163 transmission, which is by vertical transmission through females. Recent evidence suggests that
164 some of these manipulative actions may be attained by directly interfering with host sex-
165 determination genes [Sugimoto and Ishikawa, 2012; Beukeboom, 2012].

166

167 *Cytoplasmic incompatibility*

168 Cytoplasmic incompatibility (CI) is considered as the most widespread endosymbiont
169 manipulation among arthropods [Werren et al., 2008; Kageyama et al., 2012]. It has been
170 found in Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, Isopoda,
171 Trombidiformes, Mesostigmata [Tram and Sullivan, 2002; Werren et al., 2008; Kageyama et al.,
172 2012] (table 1). Despite this broad phylogenetic distribution, CI induction has thus far only
173 been attributed to *Wolbachia* and *Cardinium*. CI is a form of post-zygotic reproductive isolation
174 occurring in crosses between infected males and uninfected females, or when mates harbor
175 different strains of the symbiont [O'Neill et al., 1992; Turelli and Hoffmann, 1995; Werren,
176 1997]. In diploid species, incompatible crosses produce severe cell cycle defects in the male
177 derived pronucleus, resulting in abnormal chromosome condensation at metaphase and
178 aberrant segregation during anaphase of the first mitotic division, which leads to early
179 embryonic mortality [Serbus et al. 2008]. In haplodiploids, CI crosses lead to male-biased
180 offspring sex ratios because elimination of the paternal chromosome set restores haploidy and
181 results in male development [Breeuwer and Werren, 1990, 1993; Breeuwer, 1997;
182 Raychoudhury and Werren, 2012]. However, in some species haploid embryos may also die in
183 an early stage depending on the host species, genotype or the symbiont complement [Vavre et
184 al., 2000, 2001; Perrot-Minnot et al., 2002; Hunter et al., 2003; Mouton et al., 2005], due to
185 the incomplete elimination of paternal chromosomes resulting in aneuploidy, and thus
186 unviable embryos [Tram et al., 2006]. The exact mode of action is not fully understood, but the

187 current model is based on a chromosome marking effect during male gametogenesis that is
188 rescued in the egg if endosymbionts (inherited from the mother via the egg cytoplasm) of
189 similar type are present [Werren et al., 2008]. CI thus results from a delayed paternal effect as
190 *Wolbachia* or *Cardinium* are not present in the sperm. The sequencing of a CI-inducing
191 *Cardinium* genome was expected to provide insights into the mechanisms of CI, but the recent
192 publication of this genome did not bring more information. Interestingly though, it suggests
193 that CI has an evolutionary independent origin in *Wolbachia* and *Cardinium*, since no recent
194 horizontal gene transfer between these two symbionts has been detected [Penz et al., 2012].
195 CI-*Wolbachia* can readily spread in populations, because infected females have an advantage
196 over uninfected females in that they are compatible with uninfected and infected males
197 (Werren 1997).

198

199 *Thelytokous parthenogenesis*

200 Several types of endosymbionts have been found to induce thelytokous parthenogenesis
201 including *Wolbachia*, *Cardinium* and *Rickettsia* [Werren, 1997, 2008; Giorgini et al., 2010]
202 (table 1). Parthenogenesis induction (PI) by microbes entails making the host reproduction
203 independent of fertilization. This results in progeny that consist entirely of females if the
204 parthenogenesis-induction is 100% effective. Parthenogenetic development of eggs requires
205 special adaptations to the mode of oogenesis, i.e. the diploid complement needs to be
206 restored after meiosis. There are many ways in which this could be accomplished
207 [Suomalainen et al., 1987; Stenberg and Saura, 2009], including several modifications of
208 meiosis, but the mechanisms used by endosymbionts appear rather limited (see below).
209 Moreover, the taxonomic distribution of endosymbiont-induced-thelytokous-parthenogenesis
210 in arthropods is quite restricted. It has thus far only been documented for haplodiploids, like
211 hymenopterans, thrips and mites (table 1). In these groups the endosymbionts cause doubling
212 of the chromosomes in the egg without subsequent cell division. Because of haplodiploid sex
213 determination, the haploid eggs that would normally develop into males are converted into
214 diploid eggs that develop into females [Werren et al., 2008]. In other words the sex reversal is
215 opposite to that of CI: genetic males are converted into genetic females by changing the

216 chromosome complement of the zygote from haploidy to diploidy. Curing of hosts from their
217 endosymbionts with antibiotics typically results in the production of haploid eggs that develop
218 into males.

219 Cytological studies on a number of hymenopterans have revealed several different post-
220 meiotic mechanisms of diploidy restoration. In *Trichogramma pretiosum*, *T. deion*, and *T. nr.*
221 *deion*, diploidization is due to a segregation failure of the two sets of chromosomes in the first
222 mitotic anaphase [Stouthamer and Kazmer, 1994]. A similar mechanism occurs in *Leptopilina*
223 *clavipes* [Pannebakker et al., 2004]. In *Muscidifurax uniraptor*, however, the normal first
224 mitotic anaphase is followed by fusion of the adjacent first mitotic nuclei [Gottlieb et al., 2002],
225 a process known as gamete duplication. The result is two identical sets of chromosomes and
226 completely homozygous progeny. In the haploid mite *Bryobia praetiosa*, reproduction is
227 functionally apomictic with all progeny identical in genotype to their mother and
228 heterozygosity being maintained [Weeks and Breeuwer, 2001]. The similar functionally
229 apomictic cloning mechanism was also found in the heterozygous offspring of *Rickettsia*-
230 infected parasitoid wasp *Neochrysocharis formosa* [Adachi-Hagimori et al., 2008].

231 PI is the ultimate strategy for a maternally-transmitted symbiont: as fertilization is super-
232 fluous, fixation of the symbiont within populations or entire species is possible. Curing of hosts
233 from their endosymbionts with antibiotics typically results in male production [e.g. Zchori-Fein
234 et al., 2001; Kremer et al., 2009]. However, restoration of sexual lines has yet proved impossi-
235 ble in species in which the endosymbiont is fixed. Sexual traits have decayed either both in
236 males and females, or males partially retain functionality. Two alternative explanations have
237 been proposed. The neutral mutation hypothesis states that if traits involved in sexual repro-
238 duction are neutral under asexuality, relaxed selection might take place and allow mutations
239 to accumulate, for instance, in male sexual traits such as courtship behavior and fertility. The
240 selective hypothesis considers that sexual traits decay can be selected for in females. First, if
241 sexual traits are costly and no longer provide fitness benefits, they are expected to be strongly
242 negatively selected. This applies stronger to female than male sexual traits, like pheromone
243 production, spermatheca functionality, and egg fertilization, because the males are absent
244 under asexuality [Fong et al. 1995; Schwander et al. 2013]. Second, when *Wolbachia* infection

245 remains polymorphic through inefficient transmission of the symbiont, nucleo-cytoplasmic
246 conflict over sex-ratio may select nuclear alleles for higher male production, referred to as
247 “virginity mutants”, which can be achieved through losing the ability to use sperm, or losing
248 the ability to mate [Stouthamer et al., 2010]. Whatever the mechanism at play, PI-symbionts
249 are associated with loss of traits involved in the normal process of sexual reproduction, and
250 this process can be either neutral or actively selected for, which opens up the possibility that
251 endosymbionts take over the role of genes involved in sex determination and sexual differen-
252 tiation.

253

254 *Male killing*

255 Male killing (MK) is induced by a large diversity of endosymbiont taxa and found in a
256 variety of arthropod host orders (table 1). *Wolbachia*, *Spiroplasma*, *Rickettsia*, *Arsenophonus*,
257 *Flavobacteria*, as well as microsporidia have all been reported to cause male killing [reviewed
258 in Hurst and Jiggins, 2000; Kageyama et al., 2012]. Male-killing occurs if sons of infected
259 mothers are killed by the endosymbiont during development [Bonte et al., 2008; Werren et al.,
260 2008]. Endosymbiont-induced male lethality has been reported from six different arthropod
261 orders, i.e. Coleoptera, Diptera, Pseudoscorpiones, Hemiptera, Lepidoptera and Hymenoptera
262 [Werren et al., 2008; Kageyama et al., 2012] (table 1). The MK phenotype is variable and can
263 be divided into two broad categories according to the timing of action: early male killing at
264 embryonic stages and late male killing at late larval or early pupal stages [Hurst, 1991;
265 Kageyama et al., 2007]. Of interest, male-killing is found in species with either male or female
266 heterogamety, as well as haplodiploidy, which suggests, together with developmental timing
267 variation, that male-killing is the outcome of different molecular mechanisms (table 1; fig. 1).
268 Early male-killing is typically encountered in species where intra-brood competition is high;
269 killing brothers allows sisters to have more resources for survival. Late male-killing is
270 associated with parasites having both vertical and horizontal transmission. The
271 microorganisms gain the maximal benefit from it, because male hosts which do not contribute
272 to vertical transmission are killed at the late larval stage when the number of infected cells is
273 maximal allowing for the maximal horizontal transmission [Hurst, 1991; Kageyama et al., 2007;

274 Nakanishi et al., 2008]. Importantly, the presence of male-killing selfish elements leads to
275 selection for host resistance. This is notably what occurred in the butterfly *Hypolimnas bolina*
276 where Asian populations harbor a dominant resistant allele to the male-killing phenotype,
277 although the mechanistic details are not yet known [Hornett et al., 2008]. Interestingly, the
278 rapid spread of resistance has been monitored in natural populations of the South Pacific,
279 highlighting both the dynamic nature of these interactions and the intensity of the selective
280 pressures generated by reproductive manipulators [Charlat et al., 2007].

281

282 *Feminization*

283 Conversion of genotypic males into phenotypic and functional females is known as
284 feminization (FM) [Bouchon et al., 1998; Kageyama et al., 1998]. Endosymbiont-induced
285 feminization has been reported from seven arthropod orders: Lepidoptera, Hemiptera,
286 Hymenoptera, Thrombidiformes, Isopoda, Ephemeroptera and Amphipoda [reviewed in
287 Werren et al., 2008; Kageyama et al., 2012]. Feminization is associated with different sex-
288 determination mechanisms in these groups, such as male or female heterogamety,
289 haplodiploidy, and some unknown mechanisms for crustacean species (table 1). Feminization
290 seems to be more frequent in crustaceans than in insects, which could be due to the easiness
291 to manipulate sexual phenotypes in the former. Indeed, simple manipulation of hormonal
292 levels in crustaceans leads to sex reversion. In the well-studied woodlouse *Armadillidium*
293 *vulgare* (Isopoda), *Wolbachia* feminizes ZZ males by interfering with the
294 production/perception of the androgenic hormone from the male developmental gland during
295 sexual differentiation [Bouchon et al., 2008; Cordaux et al., 2011]. This resembles the shrimp
296 *Gammarus duebeni*, in which microsporidian parasites, such as *Octosporea effeminans* and
297 *Nosema granulosis*, change males into functional females [Bulnheim and Vávra, 1968; Terry et
298 al., 1998; Rodgers-Gray et al., 2004]. Feminization has also been found in insects where
299 different mechanisms could be at play, such as disrupting methylation patterns and genetic
300 imprinting in the male-heterogametic leafhopper *Zyginidia pullula* [Negri et al., 2006, 2009], or
301 altering splicing of *doublesex* in the female-heterogametic butterfly *Eurema mandarina* [Narita
302 et al., 2007]. Feminization also occurs in haplodiploid species. Giorgini et al. [2009] found that

303 in *Encarsia hispida*, curing from *Cardinium* does not lead to haploid but diploid males,
304 suggesting that the endosymbionts are not responsible for genome duplication
305 (parthenogenesis) but rather cause feminization of diploid males. Moreover, in the *Cardinium*
306 infected mite *Brevipalpis phoenicis*, consisting exclusively of haploid females, Weeks et al.
307 [2001] reported that curing of the bacterium changes haploid daughters into haploid sons.

308 Under endosymbiont-induced feminization, scarcity of males within host populations
309 generates a strong nucleo-cytoplasmic conflict. Resistance forms have been detected in some
310 cases, notably in *A. vulgare*. In this species, together with masculinizing genes, other
311 feminizing factors have been evidenced, but encoded by the nuclear genome [Juchault and
312 Mocquard, 1993]. There is some evidence that this nuclear feminizing factor originates from a
313 horizontal gene transfer from *Wolbachia*. The *A. vulgare* system is a good illustration of the
314 dynamic nature of sex determination where female and male heterogamety are evolving in
315 response to feminizing *Wolbachia* [Cordaux et al., 2011]. The high diversity and dynamics of
316 sex determination systems and the absence of sex chromosome differentiation in crustaceans
317 makes it likely that this pattern occurs more widespread in crustaceans [Rigaud et al., 1997].

318

319

320 **Mechanisms of reproductive and sex determination manipulations**

321 With respect to genetic mechanisms we delineate a typology of reproductive manipulations.
322 CI, PI, MK and FM differ in their actions in relation to the timing at which they interfere with
323 the host sex determination and differentiation processes. Taking the master sex switch gene
324 *doublesex* as the central point (“bottleneck in an hourglass”), manipulations can target earlier
325 events constituting the primary signals, *doublesex* itself, or downstream processes including
326 sexual differentiation (fig. 1). This typology integrates phylogenetic and empirical information,
327 and allows us to consider different reproductive-manipulation mechanisms in a phylogenetic
328 context. It indicates that endosymbionts have the potential to undergo rapid evolutionary
329 shifts in phenotypes [Werren, 1997; Jaenike, 2007; Kraaijeveld et al., 2011]. Below we discuss

330 how mechanisms of reproductive manipulation may be informative for the molecular bases of
331 host sex determination.

332

333 *Interference with primary signals*

334 Interference with primary sex determination signals concerns notably manipulation of
335 chromosomal behavior. This is clearly established for CI where paternal effects lead to ploidy
336 changes in the early fertilized egg. CI-endosymbionts in diploid arthropod species obviously do
337 not interfere with host sex determination because they cause lethality through haploidization
338 of eggs [Serbus et al., 2008]. However, in haplodiploids, conversion of diploid female eggs into
339 haploid male eggs occurs by changing the zygotic chromosomal constitution that act as
340 primary signal for sex determination. This is very similar to PI-endosymbionts that also act
341 early during sex determination as they alter the number of chromosomal complements at the
342 end of the first or beginning of the second mitotic division. As transcription is probably limited
343 at that time, PI is certainly a parental effect, but contrary to CI, it is limited to a maternal effect.
344 It is still unknown how endosymbionts precisely alter the molecular regulation of mitosis to
345 induce diploidization of the host eggs. Why parthenogenesis inducing microbes have not been
346 found in diploid species remains another mystery. One explanation is that PI evolves more
347 easily in haplodiploids because of the pre-existing cellular machinery of full development from
348 unfertilized haploid eggs. The interaction between mechanisms of sex determination and PI
349 endosymbionts are particularly complex and further elaborated in Box 1. The early MK type
350 can also act on the zygotic chromosome constitution that serves as the primary signal in the
351 host sex-determination pathway. In the wasp *Nasonia vitripennis*, *Arsenophonus nasoniae* kills
352 male offspring by blocking maternal centrosome formation during oogenesis [Ferree et al.,
353 2008]. In *Drosophila bifasciata*, infected male embryos show severe defects of chromatin
354 remodeling and spindle organization, a phenotype strikingly similar to the phenotype
355 observed in CI [Riparbelli et al., 2012].

356 Early acting endosymbionts that alter the chromosomal constitution, a feature of PI, CI and
357 early MK, suggests similar target host genes that have a relatively broad function. This would
358 explain why the manipulations occur in such a diversity of host taxa regardless of their sex-

359 determination system. There are many molecules that endosymbionts could target to change
360 the chromosome constitution of the egg. Of particular interest, CI is associated with impaired
361 histone deposition in the male pronucleus, which could lead to activation of cell cycle
362 checkpoints [Landmann et al., 2009]. Other examples include the inhibition of the proper
363 digestion of cohesions that would result in failure of chromosome separation during meiosis
364 or mitosis [Ferree et al., 2008; Schurko et al., 2009]. A similar effect might be achieved by
365 interfering with the signals that regulate the M checkpoint in the cell cycle. An interesting class
366 of potential target genes are meiosis related genes which code for Argonaute proteins or
367 mitotic division related genes coding for cell cycle proteins [Schurko et al., 2009; Kraaijeveld
368 and Bast, 2012]. Informatively, *Wolbachia*-induced CI can transit to MK (fig. 2), as was found in
369 two *Drosophila* species and two moth species. MK occurred when uninfected males of
370 *Drosophila subquinaria* mated with hybrid females from the cross between *Drosophila recens*
371 females with the CI phenotype and endosymbiont uninfected *D. subquinaria* males [Sasaki et
372 al., 2002, 2005; Jaenike, 2007]. Interestingly, the same transition but in opposite direction,
373 from MK to CI, occurred in the butterfly *Hypolimnas bolina* [Hornett et al., 2008]. The
374 suppression of the MK phenotype in infected individuals resulted in male production, which
375 upon mating with uninfected females induced CI (fig 2). These studies suggest that it is
376 relatively easy to shift between male killing and cytoplasmic incompatibility, and point towards
377 similar mechanisms. Transitions can also occur from PI to CI. In *Asobara japonica*, male
378 offspring produced by PI-*Wolbachia*-infected females induced (moderate) CI against
379 uninfected females [Kraaijeveld et al., 2011] (fig. 2).

380

381 *Direct interference with doublesex*

382 Late acting endosymbionts are associated with sexual differentiation, and must recognize
383 maleness resulting from male specifically expressed genes during development. It is now
384 evident that endosymbionts can directly interfere with the expression of sex determination
385 genes. For example, male killing in the moth *Ostrinia scapularis*, is accomplished by altering
386 the splicing of *doublesex* [Sugimoto and Ishikawa, 2012]. Altered splicing is also found in the
387 butterfly *Eurema mandarina*, in which *Wolbachia*-infected genetic males (ZZ) are

388 morphologically and behaviorally fully female and completely fertile. The splicing pattern of
389 the sex-determining gene *dsx* changes according to the *Wolbachia* infection status. Intersex
390 individuals express both female and male *dsx* splice variants. The lethal effects normally occur
391 during late embryonic or early larval developmental stages, and might be due to disruption of
392 dosage compensation [Kageyama and Traut, 2004; Narita et al., 2007; Sakamoto et al., 2007;
393 Sugimoto et al., 2010; Sugimoto and Ishikawa, 2012]. It is still unknown whether *Wolbachia*
394 directly acts on *dsx* splicing, or (more probably) on an upstream splicing regulator of *dsx* in this
395 female heterogametic system [Beukeboom, 2012]. In the *Spiroplasma* infected ladybird beetle
396 *Anisosticta novemdecimpunctata*, males are killed in the early embryonic stage [Tinsley and
397 Majerus, 2006], but the genetic mechanism is still unknown, as is true for all MK types in
398 ladybirds [Balayeva et al., 1995; Hurst et al., 1996]. These examples of early MK show that the
399 microbes have evolved different ways of killing males. The MK in *Ostrinia* is the first well
400 documented case of direct seizure of endosymbionts upon host sex-determination genes. Due
401 to being the central gear of the key sex-determination gene, *transformer* is expected to be a
402 particularly likely target for such manipulation in holometabolous insect sex determination
403 [Beukeboom, 2012; Negri and Pellicchia, 2012].

404

405 *Interference during sexual differentiation*

406 Male killing can also occur in the sexual differentiation phase of embryonic or larval
407 development. A functional dosage-compensation complex, a major component of sexual
408 differentiation in *Drosophila melanogaster*, is required for male-killing by *Spiroplasma*.
409 *Spiroplasma* failed to kill males lacking any of the five protein required for proper dosage-
410 compensation [Veneti et al., 2005]. Dosage compensation is tightly connected with sex
411 determination in *Drosophila*, as the gene *sex lethal* which has both a function in dosage
412 compensation and in sex determination as a splice-regulator of *transformer* [Cline, 1984].
413 Although yet speculative, it may be that the MK *Spiroplasma* targets the *sex lethal* gene [Starr
414 and Cline, 2002]. In the mosquito *Aedes stimulans*, *Amblyospora* microsporidia kill males in
415 the fourth larval stage [Andreadis, 1985], which is another example of late male killing.
416 Furthermore, an unknown RNA virus was found responsible for late male killing in the oriental

417 tea tortrix, *Homona magnanima*, in which male death occurs in the larval or pupal stage
418 [Nakanishi et al. 2008].

419 Hormonal signaling pathways are frequently involved in the regulation of symbiotic
420 interactions. In parasitic interactions such as host-parasitoid relationships, they play a central
421 role in synchronizing host and parasite cycles, and manipulation of hormonal signaling by each
422 party has been found [Sagi and Khalaila, 2001; Negri, 2011; Jahnke et al., 2013]. Hormonal
423 signaling as part of sexual differentiation can also be manipulated by endosymbionts. This is
424 apparent in crustaceans where the establishment of the sexes is a hormonal process. Notably,
425 injection of *Wolbachia* in young males of *A. vulgare* induces the hypertrophy of the androgenic
426 gland and the feminization of tissues [Rigaud and Juchault, 1995]. This result indicates that
427 *Wolbachia* may interfere with the androgenic hormone receptors and either antagonize the
428 fixation of the androgenic hormone on these receptors, or decrease their production. The
429 androgenic hormone is related to insulin and/or insulin-like growth factors, which is
430 interesting for two reasons. First, *Wolbachia* has been shown to interact with the insulin
431 pathway in *Drosophila* [Ikeya et al., 2009]. Even though this pathway is not directly involved in
432 sex determination, insulin-like peptides regulate ecdysteroid synthesis, and recent results
433 indicate that 20hydroxyecdysone could play the role of a sex hormone in insects (Negri et al.,
434 2010; Negri and Pellecchia, 2012). Hormonal manipulation seems mostly associated with
435 feminization, but male-killing may also make use of hormonal signals that are different
436 between the sexes. It should however be noted that sex determination in insects is generally
437 considered as a cellular genetic process, and that the importance of hormonal signaling is still
438 under debate [Steinmann-Zwicky et al., 1989; Schütt and Nöthiger, 2000; Negri and Pellecchia,
439 2012]. This is informative for the transition between MK and FM. The phenotype transition
440 from MK to FM is observed in the moth *Ostrinia scapularis*. Antibiotic treatment induced
441 intersex individuals suggesting that MK-inducing *Wolbachia* were also responsible for
442 feminization [Kageyama and Traut, 2004; Sakamoto et al., 2008; Sugimoto and Ishikawa, 2012].
443 In addition, transition from PI to FM occurred in the parasitoid wasp *Trichogramma kaykai*. In *T.*
444 *kaykai* with PI phenotype, diploid intersex individuals were produced under high temperature,
445 suggesting that PI *Wolbachia* are also responsible for feminization that is *Wolbachia* density

446 dependent [Tulgetske and Stouthamer, 2012] (fig. 2). A small proportion of diploid males is
447 also regularly detected in the parasitoid wasp *Asobara japonica*, which suggests that PI-
448 *Wolbachia* are required for feminization and that this effect is dependent on *Wolbachia*
449 density [W.J. Ma, unpublished data].

450

451 **Box 1 Interaction between PI and FM endosymbionts, and haplodiploid host sex**
452 **determination**

453 The interaction between endosymbionts and haplodiploid host sex determination is
454 complex, because the mechanisms by which diploidization of the egg takes place also affects
455 the outcome. In some cases it dictates whether particular endosymbionts can establish (fig. 3).
456 Several hymenopteran groups have complementary sex determination (CSD) in which sex is
457 determined by the allelic composition of the sex locus: heterozygotes develop into females,
458 hemizygotes and homozygotes into males [Whiting, 1933; Cook, 1993a; Beye et al., 2003]. CSD
459 and PI-inducing endosymbionts that cause gamete duplication are incompatible [Cook, 1993b;
460 van Wilgenburg et al., 2006], because this form of diploidization results in complete
461 homozygosity in most documented species so far [Stouthamer and Kazmer, 1994;
462 Pannebakker et al., 2004; Gottlieb et al., 2002]. The reason is that under CSD diploid
463 homozygotes develop into males whereas female development is required for PI
464 endosymbionts to invade a host. There is indeed a phylogenetic association between absence
465 of CSD and presence of PI endosymbionts [Heimpel and de Boer, 2008]. Interestingly, some
466 CSD species do reproduce parthenogenetically, such as *Venturia canescens*, but in such species
467 the diploidization mechanism is different (e.g. central or terminal fusion) and apparently
468 retains sex locus heterozygosity [Suomalainen et al., 1987; Beukeboom and Pijnacker, 2000;
469 Mateo-Leach et al., 2009]. Functionally apomictic cloning mechanism is also the case for the
470 haploid mite *Bryobia praetiosa* and the parasitoid wasp *Neochrysocharis formosa* [Weeks and
471 Breeuwer, 2001; Adachi-Hagimori et al., 2008].

472 The other known genetic mechanism of sex determination in Hymenoptera is maternal
473 effect genomic imprinting sex determination (MEGISD). Under MEGISD female development

474 requires a paternal genome for activation of the *transformer* gene in the zygote, which is
475 silenced on the maternal complement [Verhulst et al., 2010, see also Verhulst and van de
476 Zande in this issue]. It has thus far only been documented for *Nasonia vitripennis*
477 (Chalcidoidea). The broader phylogenetic distribution of the MEGISD model has been
478 challenged, because it is difficult to reconcile with parthenogenetic female reproduction in
479 which a non-imprinted male genome is missing in the egg. One solution would be that the
480 maternally provided imprint is not copied onto the duplicated genome copy during the
481 diploidization process, providing an active *transformer* copy to the zygote without fertilization.
482 Under this assumption, PI endosymbionts would be able to infect species with MEGISD. On the
483 other hand, if the maternal imprint would be passed on, zygotic diploidy would result in
484 males, and PI endosymbionts cannot establish (fig. 3). For other forms of diploidization to
485 establish, such as central and terminal fusion, it is necessary to assume that the
486 endosymbionts can remove the maternal imprint, because fusion of two meiotic nuclei, each
487 with a maternal imprint, would lead to diploid males. Only gamete duplication without imprint
488 copying alleviates the requirement of endosymbiont interference with MEGISD (fig. 3). Further
489 information is needed on the phylogenetic distribution of the MEGISD system before these
490 issues can be solved.

491 In the chalcidoid *Encarsia hispida*, diploid males are produced when females are cured from
492 *Cardinium*. The type of endosymbiont action, following the above rationale, is thus informative
493 for the sex determination mechanism of this species: it may have MEGISD without the
494 maternal imprint copy (fig. 3, see the green lines originating from MEGISD). Taking the
495 opposite argumentation, having MEGISD may have prevented it from being infected by PI
496 endosymbionts (fig. 3, see the red lines originating from MEGISD). How egg diploidization and
497 feminization occurs in this system is not yet known. Removal of the bacteria yields diploid
498 males indicates that egg diploidy is controlled by the host genotype. Assuming MEGISD, one
499 possibility is that *Cardinium* prevents transmission of the maternal imprint to the duplicated
500 genome copy, turning diploid male eggs into diploid female eggs.

501

502

503 **Conclusion and future directions**

504 Although there have been several reviews on endosymbiont manipulation of arthropod
505 host reproduction, we have taken a specific focus on the mechanisms by which endosymbionts
506 may interfere with host sex determination. From considering the four major endosymbiont
507 manipulation types, it is clear that the diverse endosymbionts can target the host at different
508 developmental stages, ranging from spermatogenesis stage or the first mitotic division to the
509 late pupal stage. The evolution of similar manipulation types in distantly related endosymbiont
510 taxa shows that convergent evolution has probably occurred repeatedly. Many of the
511 intricacies of endosymbiont-host interactions remain to be discovered because in most
512 instances it is still unknown what developmental pathways are exploited by the
513 endosymbionts to exert their effects on host reproduction. We have proposed that the
514 transitions between endosymbiont phenotypes suggest partly similar mechanisms for
515 apparent divergent phenotypes. We have also argued that the mechanism of endosymbiont
516 manipulation must be considered in the context of the host sex determination mechanism and
517 that both of these processes may be mutually informative on each other.

518 With the development of Next-Generation DNA sequencing techniques, it is getting easier
519 to acquire genomic information on non-model organisms, which makes the unraveling of the
520 genetic basis of endosymbiont manipulation very promising and exciting. A first question to
521 answer is whether the diversity of effects and the variation of microorganisms involved,
522 reflects true convergence or merely horizontal gene transfer between symbionts. Future
523 studies should compare different endosymbiont genomes for gene composition, as well as
524 compare gene products that might affect developmental pathways of their host [e.g. Moreno
525 et al. 2011]. For instance, the comparison of genomes between *Wolbachia* and *Cardinium*
526 suggests that CI has an evolutionary independent origin in these two symbionts and reveals no
527 evidence for recent horizontal gene transfer [Penz et al., 2012]. Comparison of transcriptomes
528 and proteomes of infected and uninfected hosts may also be rewarding [e.g. McNulty et al.
529 2012]. Moreover, the integration of knowledge about evolutionary dynamics and genomic

530 data should make it possible to identify genomic signatures that can lead to the identification
531 of genes involved in host reproduction and sex determination manipulation. Attention should
532 be paid to host sex determination genes such as *transformer* and *doublesex*, candidate targets
533 for disruption by endosymbionts, whose regulation may be altered in several ways, including
534 their sex-specific splicing or imprinting. In addition, cell cycle genes involved in meiosis and
535 mitosis, particularly those related to histone regulation or genes coding for Argonaute proteins
536 are good candidates (Kraaijeveld and Bast, 2012).

537 There is growing evidence for epigenetic control of developmental processes in insects [Lyko
538 & Maleszka 2011], as well as in host-parasite interactions [Gómez-Díaz et al., 2012]. Given the
539 multiple evolution of reproductive manipulations, it is tempting to propose that these
540 phenotypes may actually be mechanistically very close to other physiological mechanisms
541 involved in host-parasite interactions that could represent pre-adaptations to reproductive
542 manipulations [Vavre et al., 2003]. As reproductive manipulations often involve parental
543 effects, epigenetic manipulation by endosymbionts clearly requires attention. An obvious
544 candidate is chromatin remodeling which can lead to alteration of chromosomal behavior, as
545 well as to variation in gene expression or splicing processes. It is thus possible that many of
546 the symbiont phenotypes rely on epigenetic mechanisms, particularly those related to histone
547 regulation. Moreover, paternal effects of CI, maternal effects of PI, and mechanisms of early
548 MK, may all involve some form of genomic imprinting [Werren 2011; Negri and Pellecchia,
549 2012; Rabeling and Kronauer, 2013]. The currently strongest evidence for a role of epigenetics
550 was found by Negri et al. [2009], who showed that *Wolbachia* interferes with host sexual
551 differentiation in the leafhopper *Zyginidia pullula* by disrupting methylation patterns and
552 genetic imprinting. In *Drosophila* species, *Wolbachia* prophage DNA adenine
553 methyltransferase genes might be involved in the modification or rescue process of CI
554 [Saridaki et al., 2011]. These studies are first indications for a role epigenetics in host
555 manipulation, but we are only at the beginning of elucidating the precise molecular and
556 biochemical pathways involved. Technological developments now allow for easier
557 characterization of epigenetic marks, and transcriptome and proteome comparison of infected
558 and uninfected individuals in various systems may be a promising way forward. Without doubt,

559 more mechanistic studies of host reproduction manipulation are going to reveal novel and
560 intriguing insights into the co-evolution between host and endosymbiont reproduction.

561

562

563 **Acknowledgements**

564 We thank Ken Kraaijeveld for input on molecular mechanisms of endosymbiont manipula-
565 tion, Eveline Verhulst, Louis van de Zande and Bart Pannebakker for discussion on sex deter-
566 mination, and an anonymous reviewer for constructive criticism. The research was supported
567 by TOP grant no. ALW 854.10.001 of the Netherlands Organization for Scientific Research and
568 the Agence National de la Recherche (ANR-2010-BLAN-170101).

569 **References**

570 Adachi-Hagimori T, Miura K, Stouthamer R: A new cytogenetic mechanism for bacterial
571 endosymbiont-induced parthenogenesis in Hymenoptera. Proc R Soc B 275:2667–2673
572 (2008).

573 Andreadis TG: Experimental transmission of a microsporidian pathogen from mosquitoes to an
574 alternate copepod host. Proc Natl Acad Sci USA 82:5574–5577 (1985).

575 Arakaki N, Miyoshi T, Noda H: *Wolbachia*-mediated parthenogenesis in the predatory thrips
576 *Franklinothrips vespiformis* (Thysanoptera: Insecta). Proc R Soc B 268:1011–1016 (2001).

577 Balayeva NM, Eremeeva ME, Zakharov IA: Genotype characterization of the bacterium
578 expressing the male-killing trait in the ladybird beetle *Adalia bipunctata* with specific
579 *rickettsial* molecular tools. Appl Environ Microbiol 61:1431-1437 (1995).

580 Beukeboom LW: Microbial manipulation of host sex determination. Endosymbiotic bacteria
581 can directly manipulate their host's sex determination towards the production of female
582 offspring. BioEssays 34:484–488 (2012).

- 583 Beukeboom LW, Perrin N: Evolution of Sex Determination (Oxford University Press, Oxford
584 2014, in press).
- 585 Beukeboom LW, Pijnacker LP: Automictic parthenogenesis in the parasitoid *Venturia canescens*
586 (Hymenoptera: Ichneumonidae) revisited. *Genome* 43:939–944 (2000).
- 587 Beye M, Hasselmann M, Fondrk MK, Page RE, Omholt SW: The gene *csd* is the primary signal
588 for sexual development in the honeybee and encodes an SR-Type protein. *Cell* 114:419–
589 429 (2003).
- 590 Blackman RL: Sex determination in insects, in Hardie J, Leather SR (eds): *Insect Reproduction*,
591 pp 57–94 (CRC Press, Boca Raton 1995).
- 592 Bonte D, Hovestadt T, Poethke H-J: Male-killing endosymbionts: influence of environmental
593 conditions on persistence of host metapopulation. *BMC Evol Biol* 8:243 (2008).
- 594 Bouchon D, Cordaux R, Greve P: Feminizing *Wolbachia* and the evolution of sex determination
595 in isopods, in Bourtzis K, Miller T (eds): *Insect Symbiosis*, pp 273–294. (Taylor and Francis
596 Group LLC, Boca Raton 2008).
- 597 Bouchon D, Rigaud T, Juchault P: Evidence for widespread *Wolbachia* infection in isopod
598 crustaceans: molecular identification and host feminization. *Proc R Soc B* 265:1081–1090
599 (1998).
- 600 Breeuwer JAJ: *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus*
601 *urticae* and *T. turkestanii*. *Heredity* 79:41–47 (1997).
- 602 Breeuwer JAJ, Werren JH: Microorganisms associated with chromosome destruction and
603 reproductive isolation between two insect species. *Nature* 346:558–560 (1990).
- 604 Breeuwer JAJ, Werren JH: Cytoplasmic incompatibility and bacterial density in *Nasonia*
605 *vitripennis*. *Genetics* 135:565–574 (1993).

- 606 Bull JJ: Sex determining mechanisms: an evolutionary perspective. *Experientia* 41: 1285-
607 1295 (1985).
- 608 Bulnheim HP, Vávra J: Infection by the microsporidian *Octosporea effeminans* in the amphipod
609 *Gammarus duebeni*. *J Parasitol* 54:241–248 (1968).
- 610 Charlat S, Davies N, Roderick GK, Hurst GDD: Disrupting the timing of *Wolbachia*-induced
611 male-killing. *Biol Lett* 3:154–156 (2007).
- 612 Cline TW: Autoregulatory functioning of a *Drosophila* gene product that establishes and
613 maintains the sexually determined state. *Genetics* 107:231–277 (1984).
- 614 Cook JM: Sex determination in the Hymenoptera, a review of models and evidence. *Heredity*
615 71:421–435 (1993a).
- 616 Cook JM: Experimental tests of sex determination in *Goniozus nephantidis* (Hymenoptera,
617 Bethyridae). *Heredity* 71:130–137 (1993b).
- 618 Cordaux R, Bouchon D, Grève P: The impact of endosymbionts on the evolution of host sex-
619 determination mechanisms. *Trends Genet* 27:332–341 (2011).
- 620 Douglas AE: Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic
621 bacteria *Buchnera*. *Annu Rev Entomol* 43: 17-37 (1998).
- 622 Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstädter J, et al.: The diversity of
623 reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol* 6:27
624 (2008).
- 625 Ferree PM, Avery A, Azpurua J, Wilkes T, Werren JH: A bacterium targets maternally inherited
626 centrosomes to kill males in *Nasonia*. *Curr Biol* 18:1409–1414 (2008).

- 627 Fong DW, Kane TC, Culver DC: Vestigialization and loss of nonfunctional characters. *Annu Rev*
628 *Ecol Syst* 26: 249–268 (1995).
- 629 Fontanetti CS, Campos KA, Prado RA, da Silva Souza T: Cytogenetic studies in Diplopoda.
630 *Cytologia* 67:253-260 (2002).
- 631 Gempe T, Beye M: Function and evolution of sex determination mechanisms, genes and
632 pathways in insects. *BioEssays* 33:52–60 (2011).
- 633 Giorgini M, Bernardo U, Monti MM, Nappo AG, Gebiola M: *Rickettsia* symbionts cause
634 parthenogenetic reproduction in the parasitoid wasp *Pnigalio soemius* (Hymenoptera:
635 Eulophidae). *Appl Environ Microbiol* 76:2589–2599 (2010).
- 636 Giorgini M, Monti MM, Caprio E, Stouthamer R, Hunter MS: Feminization and the collapse of
637 haplodiploidy in an asexual parasitoid wasp harboring the bacterial symbiont *Cardinium*.
638 *Heredity* 102:365–371 (2009).
- 639 Gómez-Díaz E, Jordà M, Peinado MA, Rivero A: Epigenetics of host-pathogen interactions: the
640 road ahead and the road behind. *PLoS Pathog* 8:e1003007 (2012).
- 641 Gottlieb Y, Zchori-Fein E, Werren JH, Karr TL: Diploidy restoration in *Wolbachia*-infected
642 *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae). *J Invert Pathol* 81:166–174 (2002).
- 643 Graham P, Penn JK, Schedl P: Masters change, slaves remain. *Bioessays* 25: 1-4 (2002).
- 644 Heimpel GE, de Boer JG: Sex determination in the hymenoptera. *Annu Rev Entomol* 53:209–
645 230 (2008).
- 646 Hornett EA, Duploux AMR, Davies N, Roderick GK, Wedell N, Hurst GDD, et al.: You can't keep a
647 good parasite down: evolution of a male-killer suppressor uncovers cytoplasmic
648 incompatibility. *Evolution* 62:1258–1263 (2008).

649 Hunter MS, Perlman SJ, Kelly SE: A bacterial symbiont in the *Bacteroidetes* induces cytoplasmic
650 incompatibility in the parasitoid wasp *Encarsia pergandiella*. Proc R Soc B 270:2185–2190
651 (2003).

652 Hurst GD, Hammarton TC, Obrycki JJ, Majerus TMO, Walker LE, Bertrand D, et al.: Male-killing
653 bacterium in a fifth ladybird beetle, *Coleomegilla maculata* (Coleoptera:Coccinellidae).
654 Heredity 77 :177–185 (1996).

655 Hurst GD, Jiggins FM: Male-killing bacteria in insects: mechanisms, incidence, and implications.
656 Emerg Infect Diseases 6:329–336 (2000).

657 Hurst GD, Jiggins FM, Pomiankowski A: Which way to manipulate host reproduction?
658 *Wolbachia* that cause cytoplasmic incompatibility are easily invaded by sex ratio-distorting
659 mutants. Amer Nat 160:360–373 (2002).

660 Hurst GD, Werren JH: The role of selfish genetic elements in eukaryotic evolution. Nature Rev
661 Genet 2:597–606 (2001).

662 Hurst LD: The incidences and evolution of cytoplasmic male killers. Proc R Soc B 244:91–99
663 (1991).

664 Ikeya T, Broughton S, Alic N, Grandison R, Partridge L: The endosymbiont *Wolbachia* increases
665 insulin/IGF-like signalling in *Drosophila*. Proc R Soc B 276:3799–3807 (2009).

666 Jaenike J: Spontaneous emergence of a new *Wolbachia* phenotype. Evolution 61:2244–2252
667 (2007).

668 Jahnke M, Smith JE, Dubuffet A, Dunn AM: Effects of feminizing microsporidia on the
669 masculinizing function of the androgenic gland in *Gammarus duebeni*. J Invertebr Pathol
670 112:146–151 (2013).

671 Juchault P, Mocquard JP: Transfer of a parasitic sex factor to the nuclear genome of the host : a
672 hypothesis on the evolution of sex-determining mechanisms in the terrestrial Isopod
673 *Armadillidium vulgare* Latr. J Evol Biol 6:511–528 (1993).

674 Kageyama D, Anbutsu H, Shimada M, Fukatsu T: *Spiroplasma* infection causes either early or
675 late male killing in *Drosophila*, depending on maternal host age. Naturwissenschaften
676 94:333–337 (2007).

677 Kageyama D, Hoshizaki S, Ishikawa Y: Female-biased sex ratio in the Asian corn borer *Ostrinia*
678 *furnacalis*: evidence for the occurrence of feminizing bacteria in an insect. Heredity
679 81:311–316 (1998).

680 Kageyama D, Narita S, Watanabe M: Insect sex determination manipulated by their
681 endosymbionts: incidences, mechanisms and implications. Insects 3:161–199 (2012).

682 Kageyama D, Traut W: Opposite sex-specific effects of *Wolbachia* and interference with the sex
683 determination of its host *Ostrinia scapularis*. Proc R Soc B 271:251–258 (2004).

684 Kato Y, Kobayashi K, Oda S, Tatarazako N, Watanabe H, Iguchi T: Sequence divergence and
685 expression of a *transformer* gene in the branchiopod crustacean, *Daphnia magna*.
686 Genomics 95:160–165 (2010).

687 Koga R, Tsuchida T, Fukatsu T: Changing partners in an obligate symbiosis: a facultative
688 endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an
689 aphid. Proc R Soc B 270:2543–2550 (2003).

690 Kraaijeveld K, Bast J: Transposable element proliferation as a possible side effect of
691 endosymbiont manipulations. Mobile Genet Elem :253–256 (2012).

692 Kraaijeveld K, Reumer BM, Mouton L, Kremer N, Vavre F, van Alphen JJM: Does a
693 parthenogenesis-inducing *Wolbachia* induce vestigial cytoplasmic incompatibility?
694 Naturwissenschaften 98:175–180 (2011).

- 695 Kremer N, Charif D, Henri H, Bataille M, Prévost G, Kraaijeveld K, et al.: A new case of
696 *Wolbachia* dependence in the genus *Asobara*: evidence for parthenogenesis induction in
697 *Asobara japonica*. *Heredity* 103:248–256 (2009).
- 698 Landmann F, Orsi GA, Loppin B, Sullivan W: *Wolbachia*-mediated cytoplasmic incompatibility is
699 associated with impaired histone deposition in the male pronucleus. *PLoS Pathog*
700 5:e1000343 (2009).
- 701 Legrand JJ, Legrand-Hamelin E, Juchault P: Sex determination in crustacea. *Biol Rev* 62:439–
702 470 (1987).
- 703 Ma W-J, Kuijper B, De Boer JG, Van de Zande L, Beukeboom LW, Wertheim B, et al.: Absence of
704 complementary sex determination in the parasitoid wasp genus *Asobara* (Hymenoptera:
705 Braconidae). *PLoS ONE* 8:e60459 (2013).
- 706 Marín I, Baker BS: The evolutionary dynamics of sex determination. *Science* 281: 1990-1994
707 (1998).
- 708 Mateo-Leach I, Pannebakker BA, Schneider MV, Driessen G, Van de Zande L, Beukeboom LW:
709 Thelytoky in Hymenoptera with *Venturia canescens* and *Leptopilina clavipes* as case studies,
710 in Schön I, Martens K, Dijk P (eds). *Lost Sex*, pp 347–375 (Springer Netherlands, Dordrecht
711 2009).
- 712 McNulty SN, Abubucker S, Simon GM, Mitreva M, McNulty NP, Fischer K, et al.: Transcriptomic
713 and proteomic analyses of a *Wolbachia*-Free filarial parasite provide evidence of trans-
714 kingdom horizontal gene transfer. *PLoS ONE* 7: e45777 (2012).
- 715 Moreno Y, Gros P-P, Tam M, Segura M, Valanparambil R, Geary TG, et al.: Proteomic analysis of
716 excretory-secretory products of *Heligmosomoides polygyrus* assessed with next-generation
717 sequencing transcriptomic information. *PLoS Negl Trop Dis* 5: e1370 (2011).

- 718 Mouton L, Henri H, Bouletreau M, Vavre F: Multiple infections and diversity of cytoplasmic
719 incompatibility in a haplodiploid species. *Heredity* 94:187–192 (2005).
- 720 Nakanishi K, Hoshino M, Nakai M, Kunimi Y: Novel RNA sequences associated with late male
721 killing in *Homona magnanima*. *Proc R Soc B* 275:1249–1254 (2008).
- 722 Narita S, Kageyama D, Nomura M, Fukatsu T: Unexpected mechanism of symbiont-induced
723 reversal of insect sex: feminizing *Wolbachia* continuously acts on the butterfly *Eurema*
724 *hecabe* during larval development. *Appl Environ Microbiol* 73:4332–4341 (2007).
- 725 Negri I: *Wolbachia* as an “infectious” extrinsic factor manipulating host signaling pathways.
726 *Front Endocrinol* 2:115 (2011).
- 727 Negri I, Franchini A, Gonella E, Daffonchio D, Mazzoglio PJ, Mandrioli M, et al.: Unravelling the
728 *Wolbachia* evolutionary role: the reprogramming of the host genomic imprinting. *Proc R*
729 *Soc B* 276: 2485-2491 (2009).
- 730 Negri I, Pellecchia M: Sex steroids in insects and the role of the endosymbiont *Wolbachia*: A
731 New Perspective, in Dubey RK (eds): *Sex Hormones*, pp 353-374 (InTech, Winchester 2012).
- 732 Negri I, Pellecchia M, Grève P, Daffonchio D, Bandi C, Alma A: Sex and stripping: The key to the
733 intimate relationship between *Wolbachia* and host? *Commun Integr Biol* 3: 110-115 (2010).
- 734 Negri I, Pellecchia M, Mazzoglio PJ, Patetta A, Alma A: Feminizing *Wolbachia* in *Zyginidia*
735 *pullula* (Insecta, Hemiptera), a leafhopper with an XX/X0 sex-determination system. *Proc R*
736 *Soc B* 273:2409–2416 (2006).
- 737 Norton RA, Kethley JB, Johnston DE, O’ Connor BM: Phylogenetic perspectives on genetic
738 systems of reproductive modes of mites, in Wrensch DL, Ebbert DL (eds): *Evolution and*
739 *Diversity of Sex Ratio in Mites and Insects*, pp 8-99 (Chapman & Hall, New York 1993).

- 740 Nöthiger R and Steinmann-Zwicky M: A single principle for sex determination in insects. Cold
741 Spring Harb Symp Quant Biol 50:615–621 (1985).
- 742 O’Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM: 16S rRNA phylogenetic analysis of
743 the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc
744 Natl Acad Sci USA 89:2699–2702 (1992).
- 745 Pannebakker BA, Pijnacker LP, Zwaan BJ, Beukeboom LW: Cytology of *Wolbachia*-induced
746 parthenogenesis in *Leptopilina clavipes* (Hymenoptera: Figitidae). Genome 303:299–303
747 (2004).
- 748 Partridge L, Hurst LD: Sex and conflict. Science 281:2003–2008 (1998).
- 749 Penz T, Schmitz-Esser S, Kelly SE, Cass BN, Müller A, Woyke T, et al.: Comparative genomics
750 suggests an independent origin of cytoplasmic incompatibility in *Cardinium hertigii*. PLoS
751 Genet 8:e1003012 (2012).
- 752 Perrot-Minnot M-J, Migeon A, Navajas M: Intergenomic interactions affect female
753 reproduction: evidence from introgression and inbreeding depression in a haplodiploid
754 mite. Heredity 93:551–558 (2002).
- 755 Rabeling C, Kronauer DJC: Thelytokous parthenogenesis in ensocial Hymenoptera. Annu Rev
756 Entomol 58:273-292 (2013).
- 757 Raychoudhury R, Werren JH: Host genotype changes bidirectional to unidirectional
758 cytoplasmic incompatibility in *Nasonia longicornis*. Heredity 108:105–114 (2012).
- 759 Raymond CS, Shamu CE, Shen MM, Seifert KJ, Hirsch B, Hodgkin J, et al.: Evidence for
760 evolutionary conservation of sex-determining genes. Nature 391: 691-695 (1998).

- 761 Rideout EJ, Dornan AJ, Neville MC, Eadie S, Goodwin SF: Control of sexual differentiation and
762 behavior by the doublesex gene in *Drosophila melanogaster*. *Nature Neurosci* 13:458–466
763 (2010).
- 764 Rigaud T: Inherited microorganisms and sex determination of the hosts, in O'Neill SL, Hoff-
765 mann AA, Werren JH (eds): *Influential Passengers: Inherited Microorganisms and Arthro-*
766 *pod Reproduction*, pp 81-101 (Oxford University Press, Oxford 1997).
- 767 Rigaud T, Juchault P: Success and failure of horizontal transfers of feminizing *Wolbachia*
768 endosymbionts in woodlice. *J Evol Biol* 8:249–255 (1995).
- 769 Riparbelli MG, Giordano R, Ueyama M, Callaini G: *Wolbachia*-mediated male killing is
770 associated with defective chromatin remodeling. *PLoS ONE* 7:e30045 (2012).
- 771 Rodgers-Gray TP, Smith JE, Ashcroft AE, Isaac RE, Dunn AM: Mechanisms of parasite-induced
772 sex reversal in *Gammarus duebeni*. *Int J Parasitol* 34:747–753 (2004).
- 773 Saccone G, Pane A, Polito LC: Sex determination in flies, fruitflies and butterflies. *Genetica*
774 116: 15-23 (2002).
- 775 Sagi A, Khalaila I: The crustacean androgen: a hormone in an isopod and androgenic activity in
776 decapods. *Amer Zool* 41:477–484 (2001).
- 777 Sakamoto H, Kageyama D, Hoshizaki S, Ishikawa Y: Sex-specific death in the Asian corn borer
778 moth (*Ostrinia furnacalis*) infected with *Wolbachia* occurs across larval development.
779 *Genome* 50:645–652 (2007).
- 780 Sakamoto H, Kageyama D, Hoshizaki S, Ishikawa Y: Heat treatment of the Adzuki bean borer,
781 *Ostrinia scapularis* infected with *Wolbachia* gives rise to sexually mosaic offspring. *J Insect*
782 *Sci* 8:1–5 (2008).
- 783 Sánchez L: Sex-determining mechanisms in insects. *Int J Dev Biol* 52:837–856 (2008).

- 784 Saridaki A, Sapountzis P, Harris HL, Batista PD, Biliske JA, Pavlikaki H, et al.: *Wolbachia*
785 prophage DNA adenine methyltransferase genes in different *Drosophila-Wolbachia*
786 associations. PloS ONE 6:e19708 (2011).
- 787 Sasaki T, Massaki N, Kubo T: *Wolbachia* variant that induces two distinct reproductive
788 phenotypes in different hosts. Heredity 95:389–393 (2005).
- 789 Sasaki T, Kubo T, Ishikawa H: Interspecific transfer of *Wolbachia* between two lepidopteran
790 insects expressing cytoplasmic incompatibility: a *Wolbachia* variant naturally infecting
791 *Cadra cautella* causes male killing in *Ephesia kuehniella*. Genetics 162:1313–1319 (2002).
- 792 Schilthuisen M, Honda J, Stouthamer R: Parthenogenesis inducing *Wolbachia* in *Trichogramma*
793 *kaykai*. Ann Entomol Soc Am 91:410–414 (1998).
- 794 Schurko AM, Logsdon JM, Eads BD: Meiosis genes in *Daphnia pulex* and the role of
795 parthenogenesis in genome evolution. BMC Evol Biol 9:78 (2009).
- 796 Schütt C, Nöthiger R: Structure, function and evolution of sex-determining systems in Dipteran
797 insects. Development 127:667–677 (2000).
- 798 Serbus LR, Casper-Lindley C, Landmann F, Sullivan W: The genetics and cell biology of
799 *Wolbachia*-host interactions. Ann Rev Genet 42:683–707 (2008).
- 800 Starr DJ, Cline TW: A host – parasite interaction rescues *Drosophila* oogenesis defects. Nature
801 418:76–79 (2002).
- 802 Steinmann-Zwicky M, Schmid H, Nöthiger R: Cell-autonomous and inductive signals can
803 determine the sex of the germ line of *Drosophila* by regulating the gene *Sxl*. Cell 57:157–
804 166 (1989).
- 805 Stenberg P, Saura A: Cytology of asexual animals, in Schon I, Martens K, Peter D (eds): Lost Sex,
806 pp 63–74 (Springer Netherlands, Dordrecht 2009).

807 Stouthamer R, Kazmer DJ: Cytogenetics of microbe-associated parthenogenesis and its
808 consequences for gene flow in *Trichogramma* wasps. *Heredity* 73:317–327 (1994).

809 Stouthamer R, Russell JE, Vavre F, Nunney L: Intragenomic conflict in populations infected by
810 parthenogenesis inducing *Wolbachia* ends with irreversible loss of sexual reproduction.
811 *BMC Evol Biol* 10:229 (2010).

812 Sugimoto TN, Fujii T, Kayukawa T, Sakamoto H, Ishikawa Y: Expression of a *doublesex*
813 homologue is altered in sexual mosaics of *Ostrinia scapularis* infected with *Wolbachia*.
814 *Insect Biochem Mol Biol* 40:847–854 (2010).

815 Sugimoto TN, Ishikawa Y: A male-killing *Wolbachia* carries a feminizing factor and is associated
816 with degradation of the sex-determining system of its host. *Biol Lett* 8: 412-415 (2012).

817 Suomalainen E, Saura A, Lokki J: *Cytology and Evolution in Parthenogenesis*, pp 23-52 (CRC
818 Press, Inc, Boca Raton 1987).

819 Suzuki MG, Imanishi S, Dohmae N, Nishimura T, Shimada T, Matsumoto S: Establishment of a
820 novel in vivo sex-specific splicing assay system to identify a trans-acting factor that
821 negatively regulates splicing of *Bombyx mori dsx* female exons. *Mol Cell Biol* 28:333–343
822 (2008).

823 Suzuki MG, Ohbayashi F, Mita K, Shimada T: The mechanism of sex-specific splicing at the
824 *doublesex* gene is different between *Drosophila melanogaster* and *Bombyx mori*. *Insect*
825 *Biochem Mol Biol* 31:1201–1211 (2001).

826 Schwander T, Crespi BJ, Gries R, Gries G: Neutral and selection-driven decay of sexual traits in
827 asexual stick insects. *Proc R Soc B* 280: 20130823. [http://dx.doi.org/10.1098/rspb.2013.](http://dx.doi.org/10.1098/rspb.2013.0823)
828 0823.

829 Terry RS, Smith JE, Dun AM: Impact of a novel feminising microsporidium on its crustacean
830 host. *J Euk Microbiol* 45:497–501 (1998).

- 831 Tinsley MC, Majerus MEN: A new male-killing parasitism: *Spiroplasma* bacteria infect the
832 ladybird beetle *Anisosticta novemdecimpunctata* (Coleoptera: Coccinellidae). *Parasitology*
833 132:757–765 (2006).
- 834 Tram U, Fredrick K, Werren JH, Sullivan W: Paternal chromosome segregation during the first
835 mitotic division determines *Wolbachia*-induced cytoplasmic incompatibility phenotype. *J*
836 *Cell Sci* 119:3655–3663 (2006).
- 837 Tram U, Sullivan W: Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-
838 induced cytoplasmic incompatibility. *Science* 296:1124–1126 (2002).
- 839 Tulgetske GM: Investigation into the mechanisms of *Wolbachia* induced parthenogenesis and
840 sex determination in the parasitoid wasp *Trichogramma*. PhD thesis (University of
841 California, Riverside 2010).
- 842 Tulgetske GM, Stouthamer R: Characterization of intersex production in *Trichogramma kaykai*
843 infected with parthenogenesis-inducing *Wolbachia*. *Naturwissenschaften* 99:143–152
844 (2012).
- 845 Turelli M, Hoffmann AA: Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and
846 parameter estimates from natural populations. *Genetics* 140: 1319-1338 (1995).
- 847 Van Wilgenburg E Van, Driessen G, Beukeboom LW: *Frontiers in Zoology*. Single locus
848 complementary sex determination in Hymenoptera: an "unintelligent" design. *Front Zool*
849 15:1–15 (2006).
- 850 Vavre F, Dedeine F, Quillon M, Fouillet P, Fleury F, Bouletreau M, et al.: Within-species diversity
851 of *Wolbachia*-induced cytoplasmic incompatibility in haplodiploid insects. *Evolution*
852 55:1710–1714 (2001).

- 853 Vavre F, Fleury F, Varaldi J, Fouillet P, Boulétreau M: Evidence for female mortality in
854 *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic
855 and evolutionary consequences. *Evolution* 54:191–200 (2000).
- 856 Vavre F, Fouillet P, Fleury F: Between- and within-host species selection on cytoplasmic
857 incompatibility-inducing *Wolbachia* in haplodiploids. *Evolution* 57:421–427 (2003).
- 858 Veneti Z, Bentley JK, Koana T, Braig HR, Hurst GDD: A functional dosage compensation complex
859 required for male killing in *Drosophila*. *Science* 307:1461–1463 (2005).
- 860 Ventura T, Rosen O, Sagi A: From the discovery of the crustacean androgenic gland to the
861 insulin-like hormone in six decades. *Gen Comp Endocr* 173:381–388 (2011).
- 862 Verhulst EC, Beukeboom LW, van de Zande L: Maternal control of haplodiploid sex
863 determination in the wasp *Nasonia*. *Science* 328:620–623 (2010).
- 864 Waterbury JA, Jackson LL, Schedl P: Analysis of the doublesex female protein in *Drosophila*
865 *melanogaster*: role in sexual differentiation and behavior and dependence on intersex.
866 *Genetics* 152:1653–1667 (1999).
- 867 Weeks AR, Breeuwer JAJ: *Wolbachia* – induced parthenogenesis in a genus of phytophagous
868 mites. *Proc R Soc B* 268: 2245–2251 (2001).
- 869 Weeks AR, Marec F, Breeuwer JA: A mite species that consists entirely of haploid females.
870 *Science* 292:2479–2482 (2001).
- 871 Werren JH: Biology of *Wolbachia*. *Annu Rev Entomol* 42: 587- 609 (1997).
- 872 Werren JH: Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proc Natl*
873 *Acad Sci USA* 108:10863–10870 (2011).

874 Werren J, Baldo L, Clark M: *Wolbachia*: master manipulators of invertebrate biology. Nat Rev
875 Microbiol 6:741–751 (2008).

876 Werren JH, Beukeboom LW: Sex determination, sex ratios, and genetic conflict. Annu Rev Ecol
877 Syst 29:233–261 (1998).

878 Werren JH, O'Neill SL: The evolution of heritable symbionts, in O'Neill SL, Hoffmann AA,
879 Werren JH (eds): Influential Passengers Inherited Microorganisms and Arthropod
880 Reproduction, pp 1-41 (Oxford University Press, Oxford 1997).

881 Whiting PW: Selective fertilization and sex-determination in Hymenoptera. Science 78:537–
882 538 (1933).

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

885 Zchori-Fein E, Gottlieb Y, Kelly SE, Brown JK, Wilson JM, Karr TL, et al.: A newly discovered
886 bacterium associated with parthenogenesis and a change in host selection behavior in
887 parasitoid wasps. Proc Natl Acad Sci USA 98:12555–12560 (2001).

888 Zchori-Fein E, Perlman SJ: Distribution of the bacterial symbiont *Cardinium* in arthropods. Mol
889 Ecol 13:2009–2016 (2004).

890 Zug R, Hammerstein P: Still a host of hosts for *Wolbachia*: analysis of recent data suggests that
891 40% of terrestrial arthropod species are infected. PLoS ONE 7:e38544 (2012).

892

893

894

895

896 **Titles and legends to figures**

897 **Fig. 1.** The four manipulation phenotypes of endosymbionts that affect different
898 developmental stages of arthropods (using a butterfly life cycle as an example). Red arrows:
899 thelytokous parthenogenesis induction (PI), purple arrows: cytoplasmic incompatibility (CI),
900 blue arrows: feminization (FM), green arrows: male-killing (MK), light green: early male killing
901 (EMK) and dark green: late male killing (LMK) in terms of the developmental stage at which
902 MK occurs. Each arrow indicates the corresponding host developmental stage at which
903 endosymbiont manipulation takes place. The sex-determination-differentiation pathway is
904 enlarged to depict the position in the gene cascade and timing during development at which
905 endosymbionts interfere. *Transformer (tra)* is the central gear to transmit the primary signals
906 to the conserved master switch gene *doublesex (dsx)*, which regulates the downstream sexual
907 differentiation. The question mark next to *tra* refers to insects in which *transformer* appears to
908 be absent, such as Lepidoptera.

909

910 **Fig. 2.** Transitions between the four different manipulative phenotypes of *Wolbachia*. FM:
911 feminization, EMK: early male killing, LMK: late male killing, CI: cytoplasmic incompatibility
912 and PI: thelytokous parthenogenesis induction. The reported species (and orders) are
913 indicated at each arrow, as well as their mode of sex determination.

914

915 **Fig. 3.** PI-inducing endosymbionts and haplodiploid host sex determination. CSD:
916 complementary sex determination, MEGISD: maternal effect genomic imprinting sex
917 determination. Red arrows: incompatible combinations. Green arrows: compatible
918 combinations. CSD is only compatible with PI if diploidization is other than by gamete
919 duplication (e.g. premeiotic doubling, central fusion or terminal fusion). MEGISD species can
920 only have PI if the maternal imprint, that prevents female development, is not copied during
921 gamete duplication, or the endosymbionts remove it before or after diploidization. The mode
922 of sex determination and diploidization are mutually informative on each other: in *Lepidoptera*
923 *clavipes* and *Trichogramma kaykai*, PI occurs by gamete duplication and CSD is excluded as sex

924 determination [Schilthuizen et al., 1998; Pannebakker et al., 2004; Tulgetske, 2010]; in
925 *Asobara japonica* CSD is absent and diploidization could be by gamete duplication [Kremer et
926 al., 2009; Ma et al., 2013]; and in *Encarsia hispida* feminization of diploid males can occur
927 under MEGISD if *Cardinium* removes the imprint [Giorgini et al. 2009].

928

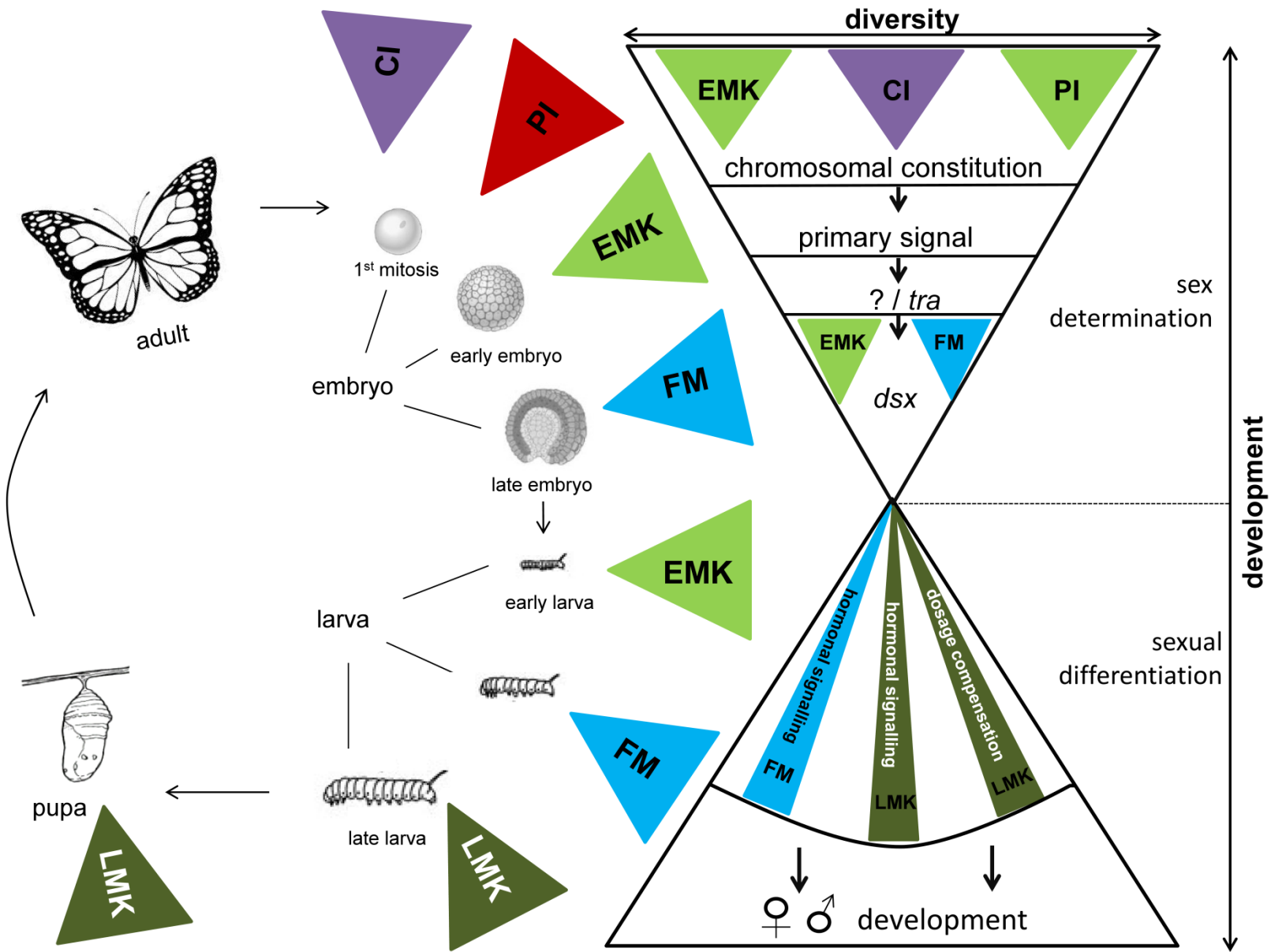
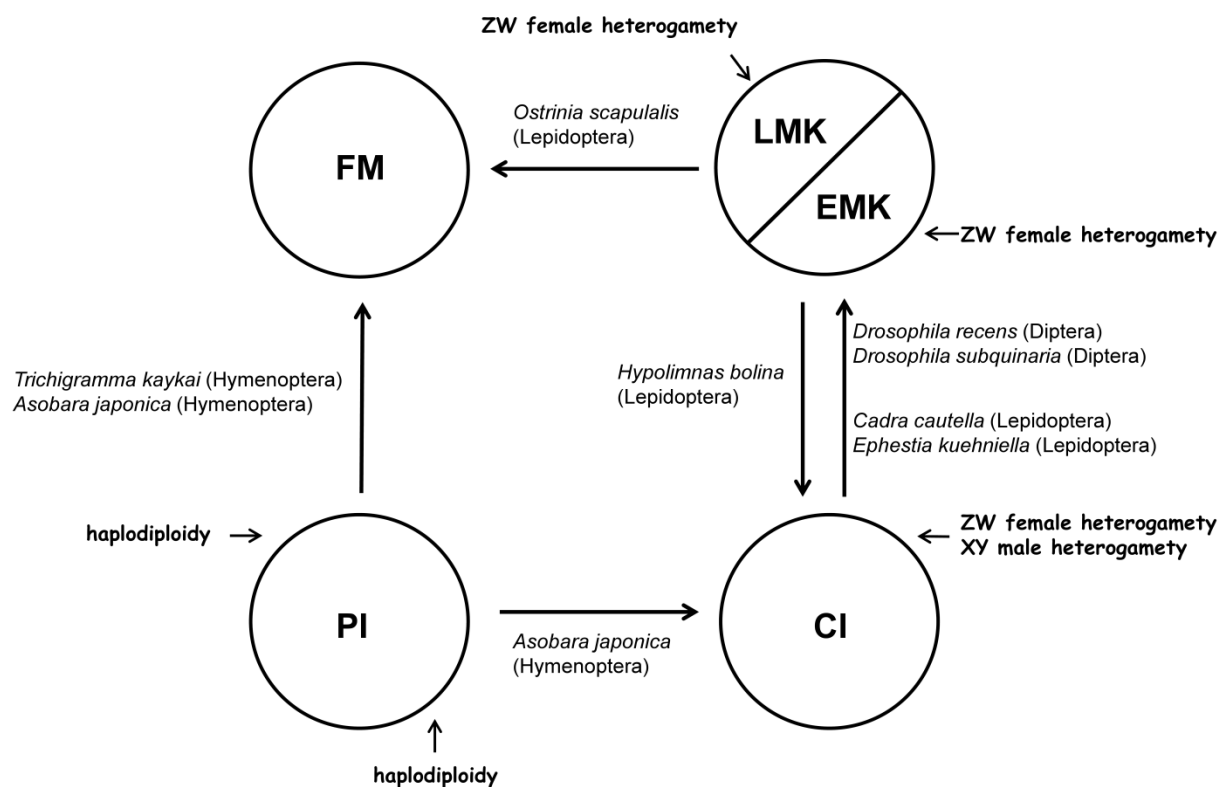
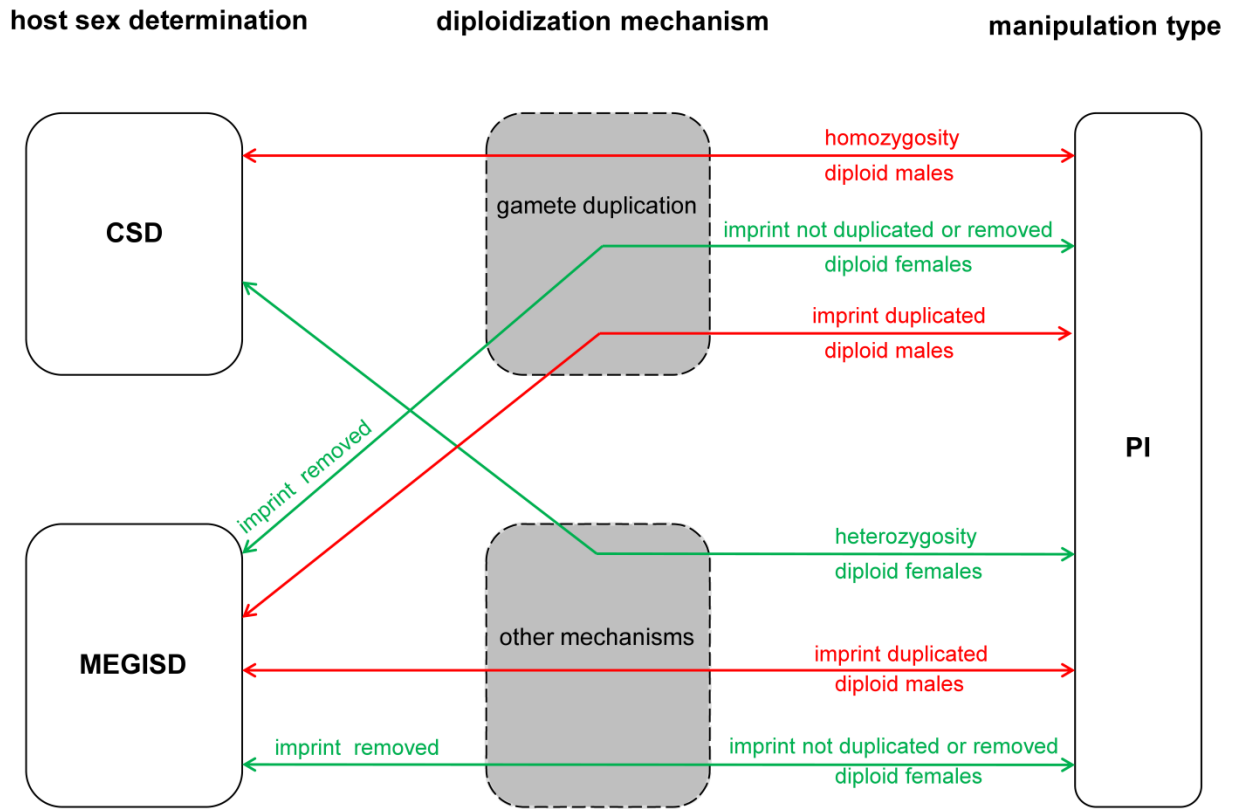


Fig. 1.



930
 931
 932
 933
 934
 935
 936
 937
 938

Fig. 2.



939

940

Fig. 3.

941

942

943

944

945

946

Table 1. Association between endosymbionts, arthropod host orders and host sex determination. Data are summarized from Kageyama et al. [2012].

Manipulation phenotype	Endosymbiont	Arthropod host order	Host sex determination (number of species reported)
Cytoplasmic incompatibility (CI)	<i>Wolbachia</i> <i>Cardinium</i>	Coleoptera	XY or XO male heterogamety (7)
		Diptera	XY or XO male heterogamety (18)
		Hymenoptera	haplodiploidy (9)
		Hemiptera	XY male heterogamety (3)
		Lepidoptera	ZW or ZO female heterogamety (5)
		Orthoptera	XO or XY male heterogamety (6)
		Isopoda	ZW female heterogamety (2)
		Trombidiformes	haplodiploidy (6)
		Mesostigmata	unknown (1)
		Parthenogenesis (PI)	<i>Wolbachia</i> <i>Cardinium</i> <i>Rickettsia</i>
Thysanoptera	haplodiploidy (1)		
Trombidiformes	haplodiploidy (2)		
Male killing (MK)	<i>Wolbachia</i> <i>Spiroplasma</i> <i>Rickettsia</i> <i>Arsenophonus</i> <i>Flavobacteria</i> Microsporidia parasites unknown virus	Coleoptera	XY male heterogamety (4) ZW female heterogamety (4) Unknown (3)
		Diptera	XY male heterogamety (14)
		Pseudoscorpiones	XO male heterogamety (1)
		Hemiptera	XO male heterogamety (1)
		Lepidoptera	ZW or ZO female heterogamety (13)
		Hymenoptera	Haplodiploidy (1)
		Feminization (FM)	<i>Wolbachia</i> <i>Cardinium</i> Microsporidia parasites <i>Gasteromermis</i> f factor (unknown)
Hemiptera	XO male heterogamety (1)		
Hymenoptera	haplodiploidy (2)		
Trombidiformes	haplodiploidy (3)		
Isopoda	ZW female heterogamety (2)		
	XY male heterogamety (1)		
	unknown (2)		
Ephemeroptera	unknown (1)		
Amphipoda	unknown (4)		

947

948