

Thelytoky and sex determination in the Hymenoptera: mutual constraints

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1 **Abstract**

2

3 The Hymenoptera show a high propensity for transitions from arrhenotokous reproduction
4 (diploid females develop from fertilised eggs, haploid males from unfertilized eggs) to
5 thelytokous reproduction (diploid females develop from unfertilised eggs). However, the
6 evolution of thelytoky is frequently constrained by the sex determination system. Under the
7 ancestral system, complementary sex determination (CSD), the constraint results from the
8 production of diploid males by thelytokous females. The magnitude of this constraint
9 depends on the cytological mechanism of thelytoky, determining the rate at which
10 thelytokous lines lose heterozygosity, and on whether a single locus or multiple loci are
11 involved in CSD. In this review I discuss how diploid male production in the case of CSD or
12 other constraints in the case of alternative sex determination systems may impede transitions
13 to thelytoky, but I also show that under particular (and presumably rare) circumstances, the
14 production of diploid males will promote rather than hamper the evolution of thelytoky. I
15 further argue that the constraints between the evolution of thelytoky and sex determination
16 may be mutual, because once thelytoky has evolved, it can impact on the sex determination.
17 Finally, I encourage researchers to exploit the frequent occurrence of thelytoky as an
18 opportunity to learn more about the mechanisms of sex determination in the Hymenoptera.

19

20 Keywords: *automixis, complementary sex determination, diploid males, Hymenoptera,*
21 *thelytoky*

22

23 **Introduction**

24

25 Insects are the most diverse group of animals on our planet. Unsurprisingly for such a
26 huge group, insects comprise a mind-boggling variety of genetic systems [Normark, 2003].
27 As one of the largest orders within the insects, the Hymenoptera are remarkably
28 homogeneous in this respect. All show haplodiploidy, which in turn may explain a number of
29 important phenomena in this order, such as the evolution of eusociality, sex ratio
30 manipulation or the high tolerance to inbreeding [Hamilton, 1964; Werren, 1993; Shuker and
31 West, 2004]. Haplodiploidy is linked to the ability to bring unfertilized eggs to development
32 and to a sex determination system that does not rely on sex chromosomes [Kraaijeveld,
33 2009]. The former makes hymenopterans prone to transitions from arrhenotoky to thelytoky
34 and the latter may - depending on the cytological mechanism involved in thelytoky - lead to
35 interesting clashes with their sex determination [Engelstädter, 2008]. Here I discuss how the
36 sex determination system can constrain the evolution of thelytoky in the Hymenoptera via
37 different mechanisms that are mostly well understood, and I offer more speculative
38 arguments as to how thelytoky can influence the evolution of the sex determination system.
39 That is, I propose that the evolution of thelytoky and the evolution of sex determination may
40 be linked by mutual constraints in the Hymenoptera.

41

42 **Haplodiploidy as a pre-adaptation to thelytoky**

43

44 The potential for parthenogenetic reproduction is widespread in insects. It is latent even in
45 many groups that are diploid and generally reproduce sexually, such as locusts,
46 cockroaches or mayflies [Pardo et al., 1995; Ball, 2001; Corley et al., 2001]. The spontaneous
47 production of some diploid eggs that can develop into females without fertilization is referred

48 to as tytoparthenogenesis. It can be considered as a strategy that ensures some reproductive
49 success even when females are mate-limited [Gerritsen, 1980]. Tytoparthenogenesis has
50 also been proposed to be a starting point that may lead to the evolution of obligate
51 parthenogenesis through a positive feedback loop in that increasing rates of
52 tytoparthenogenesis skew the sex ratio towards females, which will further increase mate
53 limitation and thus selection for parthenogenetic reproduction [Schwander et al., 2010].
54 However, such transitions are often hampered by reduced fitness returns from
55 parthenogenetic reproduction [e.g. Corley and Moore, 1999]. This is typically due to
56 developmental constraints [Corley et al., 1999; Sekine and Tojo, 2010], which can be caused
57 by deviations from the maternal ploidy level, by inbreeding depression resulting from
58 increased homozygosity and by other effects [reviewed by Engelstädter, 2008]. In short,
59 many insects are capable of parthenogenesis, but most are not very good at it. This is
60 different in the Hymenoptera (and presumably other haplodiploids): Males develop from
61 unfertilized eggs (arrhenotoky); they are routinely produced by parthenogenesis and this has
62 been so for at least 200 million years [Grimaldi and Engel, 2005]. Clearly, hymenopterans are
63 good at it. As a consequence, hymenopterans are particularly prone to transitions from
64 arrhenotoky to thelytoky, the production of diploid daughters from unfertilized eggs. Such
65 transitions may be induced by *Wolbachia* and other bacterial endosymbionts that selfishly
66 manipulate their host's reproduction [Duron et al., 2008; Werren et al., 2008] or by thelytoky-
67 inducing alleles in the insect's own genome [Lattorff et al., 2005; Sandrock and Vorburger,
68 2011]. Whatever the actor, it can usurp a pre-existing cellular machinery that allows
69 successful development from unfertilized eggs.

70 A second reason why transitions to thelytoky occur readily in hymenopterans is the strong
71 selection against recessive deleterious mutations. Such mutations cannot accumulate under
72 haplodiploidy – at least if they are not female-limited – because they are not protected by a

73 wild-type allele in the haploid males. Males thus act as a filter against recessive deleterious
74 alleles, making hymenopterans more tolerant of inbreeding than diplodiploid insects [Werren,
75 1993]. This is important because thelytoky in hymenopterans is frequently achieved via some
76 form of automixis, which equates to uniparental inbreeding. Many species can even tolerate
77 its most extreme form, gamete duplication (Fig. 1), which leads to 100% homozygosity in the
78 offspring [Stouthamer and Kazmer, 1994]. However, thelytoky with automixis can be
79 detrimental if the sex determination system is such that some or all of the asexually produced
80 offspring develop into diploid males, which frequently have low fitness in hymenopterans
81 [Cook and Crozier, 1995; Holloway et al., 1999; Zayed and Packer, 2005]. This can also be
82 interpreted as a - potentially severe - form of inbreeding depression and is explained in more
83 detail below. Note that similar constraints may also apply to diplodiploid insects, particularly
84 those with female heterogamety (ZW sex chromosomes), in which gamete duplication would
85 lead to either ZZ (male) or WW (inviable) offspring [Cook and Butcher, 1999].

86

87 **Sex determination in the Hymenoptera**

88

89 Based on his studies of sex determination in the parasitoid wasp *Bracon hebetor*, Whiting
90 [Whiting, 1933, 1939, 1943] proposed a model of complementary sex determination (CSD)
91 governed by a single locus (sl-CSD). Under this model, zygotes develop into females if they
92 carry two different alleles at a proposed sex determination locus, but into males if they carry
93 one allele (hemizygotes) or two copies of the same allele (homozygotes). This model can be
94 extended to two or multiple loci (ml-CSD), under which heterozygosity at one or more loci
95 leads to female development, whereas individuals that are hemizygous or homozygous at all
96 sex determination loci develop into haploid or diploid males, respectively [Snell, 1935;
97 Crozier, 1971]. Both models predict the production of diploid males under inbreeding, albeit

98 at different rates. In a sl-CSD system, already the first generation of sib-matings should lead
99 to the occurrence of diploid males in half of the broods. In a ml-CSD system, several
100 generations of inbreeding may be required to observe diploid male production, depending on
101 the number of loci involved [Cook, 1993; de Boer et al., 2008].

102 The occurrence of CSD has been confirmed by experimental inbreeding for many species
103 [van Wilgenburg et al., 2006; Heimpel and de Boer, 2008] and has become a hallmark of
104 hymenopteran reproduction. Phylogenetic analyses do indeed support that CSD is the
105 ancestral mechanism of sex determination in the Hymenoptera, but they do not allow any
106 firm conclusions as to whether sl-CSD or ml-CSD was the ancestral state [Asplen et al.,
107 2009]. In the honey bee, which exhibits sl-CSD, the genetic regulation of sex determination
108 has been largely elucidated [Beye et al., 2003; Hasselmann et al., 2008; Gempe et al., 2009].
109 It turned out that the *csd* gene arose by gene duplication from the *feminizer (fem)* gene, which
110 is an ortholog of *transformer (tra)*, the principal sex determining gene in flies.

111 Heterozygosity at *csd* is required to produce the active proteins that induce female-specific
112 splicing of the *fem* mRNAs. The Fem protein then directs female-specific splicing of the
113 *doublesex (dsx)* gene and thus female development [Gempe and Beye, 2011].

114 However, CSD is not universal in the Hymenoptera. Many species and presumably some
115 entire clades such as the superfamilies Chalcidoidea and Cynipoidea do not produce any
116 diploid males under inbreeding and must therefore rely on other mechanisms of sex
117 determination [Heimpel and de Boer, 2008]. Unfortunately, current knowledge as to what
118 these are is still very limited. The only alternative mechanism that is reasonably well
119 understood was described in the chalcid wasp *Nasonia vitripennis*, in which a maternal effect
120 and genomic imprinting are implicated in sex determination [Verhulst et al., 2010].
121 Interestingly, this alternative mechanism revolves around the same principal gene. The
122 maternal copy of the *Nasonia vitripennis transformer (Nvtra)* gene is transcriptionally

123 silenced (imprinting), but a maternal input of *Nvtra* mRNA/protein to the egg is required
124 (maternal effect) to direct female-specific splicing of embryonic *Nvtra*, which is only
125 expressed in the presence of a paternally derived genome, i.e. in fertilized eggs. Just as in
126 other insects, sex-specific splicing of *tra* in turn regulates sex-specific splicing of *dsx* and
127 thus induces male or female development [Beukeboom and van de Zande, 2010; Verhulst et
128 al., 2010].

129

130 **Sex determination affects the evolution of thelytoky**

131

132 As introduced above, haplodiploidy predisposes hymenopterans to the evolution of
133 thelytoky, but the sex determination mechanism associated with haplodiploidy may put
134 constraints on such transitions. This is most obvious in the case of gamete duplication and
135 CSD. Independent of whether a single locus or multiple loci are involved in CSD, gamete
136 duplication would lead to complete homozygosity (Fig. 1) and thus to broods consisting
137 entirely of diploid males. Thelytokous parthenogenesis by gamete duplication is therefore
138 considered incompatible with CSD. Gamete duplication is observed when thelytoky is
139 induced by *Wolbachia* [Stouthamer and Kazmer, 1994; van Wilgenburg et al., 2006], which
140 leads to the prediction that CSD can prevent transitions from arrhenotoky to thelytoky by
141 infection with *Wolbachia*. This prediction is indeed supported by comparative evidence: even
142 though *Wolbachia* abounds in the Hymenoptera, it appears that parthenogenesis-inducing
143 strains (PI-*Wolbachia*) are either restricted to groups in which CSD remains unreported,
144 namely the Chalcidoidea and Cynipoidea [van Wilgenburg et al., 2006], or else to non-CSD
145 species in groups comprising CSD and non-CSD mechanisms of sex determination, such as
146 the Ichneumonoidea [Heimpel and de Boer, 2008; Asplen et al., 2009; Kremer et al., 2009]. It
147 would be premature, however, to conclude that CSD represents an absolute constraint to

148 microbe-induced thelytoky in general. For one thing, the cytogenetic details of
149 parthenogenesis-induction by *Wolbachia* have only been worked out for a handful of cases
150 [summarized in van Wilgenburg et al., 2006]. Considering the remarkable diversity of
151 reproductive manipulations exhibited by *Wolbachia* [Werren et al., 2008], it would not be too
152 surprising if a strain was discovered in the future that overcomes this constraint by an
153 alternative mechanism of parthenogenesis induction. A second reason for caution is that
154 additional microbial endosymbionts like *Cardinium* or *Rickettsia* are able to induce thelytoky
155 [Weeks et al., 2003; Hagimori et al., 2006]. In one such case, the *Rickettsia* inducing
156 thelytoky in the parasitoid wasp *Neochrysocharis formosa*, the cytological mechanism has
157 been shown to be functionally apomictic and would thus be fully compatible with CSD
158 [Adachi-Hagimori et al., 2008].

159 Forms of thelytoky other than gamete duplication are not or less constrained by CSD.
160 Apomictic or ameiotic parthenogenesis equates to cloning and produces offspring that retain
161 heterozygosity at all loci the mother was heterozygous for (Fig. 1). In species with CSD,
162 independent of the number of loci involved, an apomictic mother will produce daughters
163 only. The evolution of thelytoky by apomixis is thus unconstrained by CSD, yet it may be
164 constrained by sex determination mechanisms that rely on genomic imprinting (see below).

165 Other cytological mechanisms of thelytoky in the Hymenoptera involve the fusion of
166 nuclei after the second meiotic division and are referred to as automixis with terminal (fusion
167 of sister nuclei), central (fusion of non-sister nuclei) or random fusion (Fig. 1). In terms of the
168 loss of heterozygosity in offspring produced by thelytoky, these mechanisms lie between the
169 extreme cases of apomixis (all heterozygosity retained) and gamete duplication (all
170 heterozygosity lost). Therefore, the number of loci involved as well as their location in the
171 genome become important for whether sex determination by CSD can constrain the evolution
172 of thelytoky. In the absence of recombination, the genetic consequences of terminal fusion

173 would be equivalent to gamete duplication (complete homozygosity), and those of central
174 fusion would be equivalent to apomixis (heterozygosity retained), but under random fusion
175 there would be a 1/3 chance for every heterozygous locus to become homozygous in the
176 offspring. That is because each gene copy will be paired with equal probability to one of the
177 three other gene copies, one of which is identical to the focal copy [Oldroyd et al., 2008;
178 Engelstädter et al., 2011]. Yet recombination does of course occur during the first meiotic
179 division, which alters the genetic consequences of thelytoky by terminal or central fusion.
180 After a crossover event under terminal fusion, heterozygosity is lost between the centromere
181 and the chiasma, but may be retained between the chiasma and the telomere (Fig. 1). With
182 free recombination between the centromere and a heterozygous locus in the mother, the
183 overall probability for this locus to be homozygous in an offspring is 1/3 [Engelstädter et al.,
184 2011]. Thus, a thelytokous lineage with terminal fusion automixis is expected to show a
185 quick erosion of heterozygosity, starting from the centromeres out towards the telomeres of
186 each chromosome. This represents a severe constraint to the evolution of thelytoky in species
187 with CSD. After a crossover under central fusion, heterozygosity is maintained between the
188 centromere and the chiasma, but may be lost distal to the chiasma (Fig. 1). Again, the overall
189 probability for a heterozygous locus to become homozygous in the offspring is 1/3 with free
190 recombination. In contrast to terminal fusion, the loss of heterozygosity over time under
191 central fusion automixis is most rapid at the chromosome ends and progresses towards the
192 centromeres. Importantly, genomic regions that are sheltered from recombination, such as
193 those near the centromere or in chromosomal inversions, can retain heterozygosity
194 indefinitely under central fusion automixis. But note that at least in paracentric inversions,
195 heterozygosity can also be lost under central fusion if crossovers occur between centromere
196 and inversion [Sandrock et al., 2011]. Under random fusion automixis, finally, the average

197 probability of a heterozygous locus to become homozygous in the next generation (again 1/3)
198 is unaltered by recombination [Oldroyd et al., 2008].

199 Based on the above there is a clear ranking in terms of the rate at which thelytokous
200 lineages lose heterozygosity over time among the three modes of automixis: terminal fusion >
201 random fusion > central fusion. More importantly, central fusion automixis most readily
202 permits the long-term maintenance of heterozygosity at least in some regions of the genome.
203 That would make it the form of automictic thelytoky most compatible with CSD. So far there
204 is not enough information available for a comparative analysis, but it is unlikely to be a
205 coincidence that many genetically determined cases of thelytoky in groups with CSD exhibit
206 central fusion automixis, such as in several species of ants [Pearcy et al., 2006; Kellner and
207 Heinze, 2011; Rabeling et al., 2011; Kronauer et al., 2012; Rabeling and Kronauer, 2013], the
208 cape honeybee [Lattorff et al., 2005; Oldroyd et al., 2008], the ichneumonid parasitoid
209 *Venturia canescens* [Beukeboom and Pijnacker, 2000] or the braconid parasitoid of aphids,
210 *Lysiphlebus fabarum* [Belshaw and Quicke, 2003; Sandrock and Vorburger, 2011]. Terminal
211 fusion automixis has been reported for only three species [Comrie, 1938; Smith, 1941;
212 Rössler and Debach, 1973; Mateo Leach et al., 2009], and I am not aware of any documented
213 cases of random fusion automixis in the Hymenoptera. However, it would be premature to
214 exclude the occurrence of this form of automixis in the Hymenoptera, considering that it
215 would be difficult to distinguish it from terminal or central fusion. Central fusion automixis
216 has been observed in other animals such as hybrid fish [Lampert et al., 2007].

217 In some cases of central fusion automixis in the Hymenoptera, genetic marker data
218 confirmed that parts of the genome do maintain heterozygosity. In *L. fabarum*, for example,
219 about half of the available microsatellite loci lose heterozygosity quickly in thelytokous lines,
220 but the other half retain heterozygosity [Sandrock et al., 2011]. A thelytokous lineage of the
221 cape honey bee also retains partial heterozygosity at selectively neutral marker loci [Oldroyd

222 et al., 2011], although this may at least to some extent be a consequence of linkage to loci
223 under overdominant selection [Goudie et al., 2012].

224 Above I argued that the evolution of automictic thelytoky is least constrained by CSD if it
225 occurs by central fusion. A related prediction is that the evolution of automictic thelytoky is
226 less constrained by CSD if it involves more than a single CSD locus (ml-CSD). Virtually
227 nothing is known about the location of CSD loci in the few species for which ml-CSD could
228 be inferred [de Boer et al., 2008; de Boer et al., 2012], but under the simplest assumption that
229 these loci are distributed randomly across the genome, ml-CSD would increase the chance
230 that at least one locus is located in a region of the genome that is not or only rarely affected
231 by recombination. That, in turn, would decrease the load imposed by diploid male production
232 on the thelytokous line and increase its chances of persistence.

233

234 In addition to the number of CSD loci and their probability to be affected by
235 recombination, the fitness of diploid males is also important for the probability of automictic
236 thelytoky to evolve in haplodiploids with CSD. Engelstädter et al. [2011] addressed this
237 problem by modeling the spread of a single, recessive allele determining thelytoky in a sexual
238 haplodiploid population. That reflects the genetic basis of thelytoky in the cape honey bee
239 and in *L. fabarum* [Lattorff et al., 2005; Sandrock and Vorburger, 2011]. Under the common
240 assumption that diploid males are effectively sterile [Zayed and Packer, 2005], the production
241 of diploid males by thelytokous females did indeed impose a constraint on the spread of a
242 thelytoky-determining allele [Engelstädter et al., 2011]. However, at least in some species,
243 diploid males are viable and fertile [Cowan and Stahlhut, 2004; de Boer et al., 2007; Elias et
244 al., 2009; C. Sandrock & C. Vorburger, unpubl. data]. If that is the case, diploid male
245 production will promote rather than hamper the evolution of thelytoky, because these males
246 become vehicles for the effective spread of thelytoky-inducing alleles – a mechanism referred

247 to as contagious parthenogenesis [Simon et al., 2003; Engelstädter et al., 2011]. The
248 possibility that the production of fertile diploid males may aid the spread of thelytoky is a
249 very interesting aspect of genetically determined forms of automixis, but it is unlikely to be
250 realised in more than a few exceptional cases such as *L. fabarum* [Engelstädter et al., 2011;
251 Sandrock et al., 2011]. In general, the production of diploid males should be considered a
252 burden to the fitness of a thelytokous line. The strength of the constraint imposed by CSD on
253 the evolution of thelytoky is therefore proportional to the rate at which thelytokous lines lose
254 heterozygosity, that is highest under gamete duplication, lowest (absent) under apomixis, and
255 intermediate under the different forms of automixis with recombination. However, we should
256 not forget that the rate of recombination is a trait that can respond to selection [e.g. Kerstes et
257 al., 2012], and by extension the rate of diploid male production under automixis as well. If
258 transitions from arrhenotoky to thelytoky occur repeatedly and different thelytokous lines
259 compete within a population, those with a lower diploid male production will enjoy a higher
260 rate of increase and thus be favoured by selection. This may explain an interesting
261 observation in *L. fabarum*, a parasitoid species in which the genetic determination of
262 thelytoky by central fusion automixis allows the breeding of new thelytokous lines in the
263 laboratory. Such newly formed lines show a substantial diploid male production of up to one
264 third of the offspring [Engelstädter et al., 2011], whereas thelytokous lines occurring in the
265 field produce very few males, if any. Those males are typically haploid, indicating that they
266 are the result of occasional failures in the fusion of nuclei after the second meiotic division
267 and not of CSD homozygosity, although diploid males are rarely found in the field as well
268 [Sandrock et al., 2011]. Selection for reduced recombination may also explain the case of the
269 little fire ant, *Wasmannia auropunctata*, in which apomictic production of queens was initially
270 inferred from genotyping data [Fournier et al., 2005], but later found to occur by central
271 fusion automixis with dramatically reduced recombination rates [Rey et al., 2011].

272

273 Could sex determination systems other than CSD also constrain the evolution of thelytoky
274 in the Hymenoptera? The main obstacle to answering this question is the dearth of
275 information on the mechanisms of sex determination in species without CSD. For the one
276 alternative that is well understood, the maternal effect genomic imprinting mechanism
277 described from *Nasonia* [Verhulst et al., 2010], the answer is certainly yes. At first glance,
278 the requirement of a paternal genome for female development is an absolute constraint for
279 transitions to thelytoky. However, Beukeboom and van de Zande [2010] have argued that the
280 maternal imprint may occur during oogenesis, but not be copied on during the subsequent
281 divisions of the egg, which would allow thelytokous lineages to evolve independence from
282 the contribution of a paternal genome. This conjecture remains to be tested.

283

284 **Can thelytoky also influence the evolution of sex determination?**

285

286 While it is clear from the above that the mechanisms of sex determination can constrain
287 the evolution of thelytoky in the Hymenoptera, the opposite question has received less
288 attention. Can thelytokous reproduction have an impact on the sex determination system? The
289 model by Engelstädter et al. [2011] mentioned earlier illustrates a route by which this may
290 indeed occur, namely by eroding a ml-CSD system to a sl-CSD system.

291 It has been argued that ml-CSD can evolve from sl-CSD by one or several duplications of
292 the CSD locus and may be favoured by selection because it reduces the inbreeding load
293 resulting from diploid male production [Crozier, 1971; van Wilgenburg et al., 2006; de Boer
294 et al., 2008]. Analogously, I have argued above that ml-CSD may be conducive to the
295 evolution of thelytoky with automixis because it increases the probability that at least one
296 CSD locus occurs in a region of the genome that is sheltered from recombination. However,

297 as soon as an automictic line with two or more CSD loci has been formed, an increasing
298 number of loci will become homozygous over time until only one heterozygous CSD locus is
299 left [Engelstädter et al., 2011]. This would normally be the locus with the lowest probability
300 of becoming homozygous, by virtue of being close to a centromere or otherwise sheltered
301 from recombination. Thus, thelytoky with automixis can have a meltdown effect on ml-CSD
302 systems and reduce them to what is effectively sl-CSD. It is worth pointing out that this
303 process can be seen as conceptually related to the sex determination meltdown that may occur
304 in an arrhenotokous population with ml-CSD if a bottleneck is followed by inbreeding [de
305 Boer et al., 2012].

306 According to Normark [2003] transitions from arrhenotoky to thelytoky are at least in
307 principle reversible. However, this is likely to depend on the number of generations a lineage
308 has reproduced asexually. Over time such lineages can accumulate mutations in genes
309 important for sexual reproduction that will eventually preclude a return to sexual
310 reproduction [e.g. Zchori-Fein et al., 1992]. This is illustrated by the many cases of microbe-
311 induced thelytoky in which antibiotic curing of the symbiont cannot restore functional sexual
312 reproduction [Koivisto and Braig, 2003]. Nevertheless, in the light of the prediction that
313 automictic thelytoky would rapidly melt down ml-CSD to sl-CSD, it is tempting to consider
314 the intriguing possibility that episodes of asexual reproduction, to which hymenopterans are
315 so prone, may influence the evolution of their sex determination systems.

316

317 **Thelytoky as a window of opportunity to study sex determination**

318

319 I have pointed out conditions under which the induction of thelytoky by microbes or
320 genetic factors can clash with the sex determination of hymenopterans, and how thelytoky –
321 depending on the cytological mechanism involved – may in turn affect the sex determination

322 system. It is worth considering whether such dynamics could possibly be used as a window of
323 opportunity to study sex determination. It is difficult and labour-intensive, for example, to
324 experimentally distinguish non-CSD mechanisms of sex determination from ml-CSD,
325 because many generations of inbreeding are required to achieve a level of homozygosity that
326 allows ruling out CSD involving several loci based on the lack of diploid males among inbred
327 offspring [Cook, 1993; de Boer et al., 2008; Ma et al., 2013]. In principle, complete
328 homozygosity could be achieved more quickly if it was possible to introduce a strain of PI-
329 *Wolbachia* that induces thelytoky by gamete duplication in the species under investigation.
330 While it is possible to transfer *Wolbachia* horizontally to new species by microinjection
331 [Grenier et al., 1998], it is less predictable whether it will have the same phenotypic effect in
332 the new genetic background [Fujii et al., 2001; Veneti et al., 2005]. The feasibility of this
333 approach is thus currently uncertain.

334 Another potential opportunity is offered by the gradual erosion of heterozygosity under
335 automixis. According to the model by Engelstädter et al. [2011], this should lead to the
336 stepwise loss of heterozygosity at all but one CSD locus in species with ml-CSD, and hence
337 in a parallel increase in the proportion of diploid males produced. In species with a simple
338 genetic basis of automictic thelytoky, it may be possible to create new automictic lineages
339 from an outbred population by targeted breeding [Sandrock and Vorburger, 2011]. Similar to
340 multi-generation inbreeding experiments in arrhenotokous species, tracking the rate of
341 diploid male production in such newly formed automictic lines over generations might allow
342 inferences regarding the number of CSD loci a species possesses and the rates at which they
343 acquire homozygosity. Again, this is still a hypothetical scenario and its practical feasibility
344 remains to be tested.

345

346 **Conclusions**

347

348 Haplodiploidy in the Hymenoptera comes with the ability to initiate development of
349 unfertilized eggs and entails a low load of recessive deleterious mutations in the genome. As
350 a consequence, hymenopterans are prone to transitions from arrhenotoky to thelytoky, either
351 due to genetic mutation or to infection with parthenogenesis-inducing microbes. However,
352 the evolution of thelytoky can be constrained by the sex determination system. For the
353 ancestral mechanism of sex determination in the Hymenoptera, CSD, the constraint results
354 from the production of low-fitness diploid males when the cytological mechanism of
355 thelytoky involves some form of automixis and hence a loss of heterozygosity at the sex
356 locus (sl-CSD) or loci (ml-CSD). This constraint is absent for apomictic thelytoky. It is
357 therefore surprising that apomixis – generally a common form of thelytoky in insects – is
358 only rarely found in hymenopterans [Mateo Leach et al., 2009]. All other modes of thelytoky
359 are associated with a loss of heterozygosity, and the severity of the constraint imposed by
360 CSD on the evolution of thelytoky is proportional to the rate at which this loss occurs. It
361 follows the order central fusion automixis < random fusion automixis < terminal fusion
362 automixis < gamete duplication. Transitions to thelytoky by gamete duplication are fully
363 constrained by CSD because the loss of heterozygosity is complete, whereas central fusion is
364 the form of automixis most compatible with CSD, because it allows the long-term
365 maintenance of heterozygosity at least in some regions of the genome. Another mechanism of
366 sex determination by a maternal effect and genomic imprinting described from *Nasonia*
367 [Verhulst et al., 2010] is also likely to constrain the evolution of thelytoky because a paternal
368 genome is required for female development. However, more needs to be known about the
369 mechanism and timing of imprinting before it can be judged whether this constraint cannot be
370 circumvented. Additional mechanisms of hymenopteran sex determination are likely to be

371 discovered in the future, and it will be interesting to see what constraints, if any, those impose
372 on the evolution fo thelytoky.

373 Interestingly, constraints between sex determination and the evolution of thelytoky may be
374 mutual. Once thelytoky by automixis has evolved in a species with ml-CSD, for example, it
375 may in turn result in the meltdown of ml-CSD to sl-CSD [Engelstädter et al., 2011]. This
376 example shall highlight the more general aspect that the study of thelytoky – although
377 certainly of interest in its own right – may shed light on sex determination, since one of the
378 purposes of this review is to encourage researchers to think about how the frequent
379 occurrence of thelytoky in the Hymenoptera can be exploited to learn more about the
380 mechanisms of sex determination in this fascinating group of insects.

381

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383

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388

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572

573 **Figure captions**

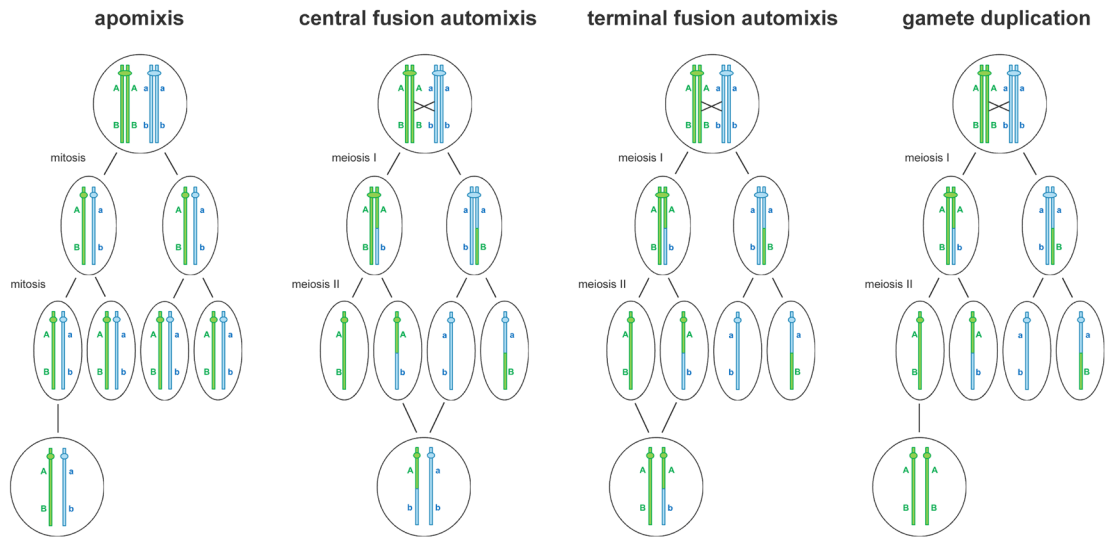
574

575 **Fig. 1.** Modes of thelytoky in the Hymenoptera. Under apomixis, all cell divisions are mitotic
576 and no recombination takes place. Under the other modes, crossing-over may occur during
577 the first meiotic division. The figure illustrates the fate of a heterozygous locus in the mother
578 that is (B/b) or is not (A/a) affected by recombination. One fusion product is illustrated as an
579 example, but four different products are possible under central fusion or gamete duplication,
580 and two under terminal fusion, depending on which nuclei fuse or duplicate, respectively. Not
581 shown in the figure is random fusion automixis, under which any two of the four nuclei could
582 fuse to form a diploid zygoid. This form of automixis might also occur in the Hymenoptera
583 but has so far not been documented unambiguously.

584

585 **Figure 1**

586



587