Spontaneous parthenogenesis in the parasitoid wasp *Cotesia typhae*: low frequency anomaly or evolving process?

Claire Capdevielle Dulac[1, *], Romain Benoist[1], Sarah Paquet[1], Paul-André Calatayud[1], Julius Obonyo[2], Laure Kaiser[1], Florence Mougel[1]

[1] *Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France.*
[2] *icipe, International Center of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya*

** Corresponding author: claire.capdevielle-dulac@egce.cnrs-gif.fr*

Abstract

Hymenopterans are haplodiploids and unlike most other Arthropods they do not possess sexual chromosomes. Sex determination typically happens via the ploidy of individuals: haploids become males and diploids become females. Arrhenotoky is believed to be the ancestral reproduction mode in Hymenopterans, with haploid males produced parthenogenetically, and diploid females produced sexually. However, a number of transitions towards thelytoky (diploid females produced parthenogenetically) have appeared in Hymenopterans, and in most cases populations or species are either totally arrhenotokous or totally thelytokous. Here we present the case of *Cotesia typhae* (Fernandez-Triana), a Braconidae that produces parthenogenetic females at a low frequency. The phenotyping of two laboratory strains and one natural population showed that this frequency is variable, and that this rare thelytokous phenomenon also happens in the wild. Moreover, mated females from one of the laboratory strains produce a few parthenogenetic daughters among a majority of sexual daughters. The analysis of daughters of heterozygous virgin females allowed us to show that a mechanism of automixis with central fusion is very likely at play in *C. typhae*. This mechanism allows some parts of the genome to remain heterozygous, especially at the chromosomes' centromeres, which can be advantageous depending on the sex determination system involved. Lastly, in most species, the origin of thelytoky is either bacterial or genetic, and an antibiotic treatment as well as PCR experiments did not demonstrate a bacterial cause in *C. typhae*. The unusual case of low parthenogenetic frequency described in this species constitutes another example of the fascinating diversity of sex determination systems in Arthropods.

Introduction

Sexual reproduction is the most widespread reproductive strategy among pluricellular organisms and especially in animals. In contrast with its predominance, this reproductive mode appears costly due, for instance, to the necessity to detect and attract a partner, escape sexually transmitted diseases or avoid predation during mating. Because they share parenthood with their mate, sexual individuals transmit two-fold less their genetic material to their progeny compared to asexual counterparts. The ubiquity of sex despite such disadvantages led to the definition of the so-called "paradox of sex" (Meirmans et al., 2012; Otto, 2009).

Numerous cases of evolution toward asexual reproduction or parthenogenesis have been reported, notably within arthropod taxa (The Tree of Sex Consortium, 2014). Parthenogenesis can produce either males (arrhenotoky) or females (thelytoky) from unfertilized eggs, but only the last case strictly coincides with asexual reproduction. It is also referred to as parthenogenesis *sensu stricto*.

Thelytoky has been observed in almost all basal Hexapoda and non-holometabolous insect taxa (Vershinina and Kuznetsova, 2016) as well as in many holometabolous insect species (Gokhman and Kuznetsova, 2018). This wide taxonomic range illustrates the frequent transition from sexual to asexual taxa that arose independently in various lineages. This scattered distribution hides a global low percentage of parthenogenesis: thelytokous species represent less than 1% of the Hexapoda (Gokhman and Kuznetsova, 2018). The proportion of asexual lineages is also highly heterogenous among taxa. Liegeois et al. (2021) detected frequencies between 0 and 6.7% among families of mayflies. Van der Kooi et al. (2017) reported frequencies ranging from 0 to 38% among genera of haplodiploid arthropods.

Transition from sexual to asexual reproductive mode requires bypassing genetic and developmental constraints, a challenge that may be easier to face in some taxa. In most species with a haplodiploid sex determination system, males develop from unfertilized eggs and are haploid while females develop from fertilized eggs leading to a diploid state. In such cases, embryonic development is initiated independently from egg fertilization, a trait probably favoring the evolution toward thelytoky (Vorburger, 2014). The variable frequency of asexual reproduction even among haplodiploid lineages indicates that other factors allowing the transition toward this reproductive mode remain to be identified (van der Kooi et al., 2017).

The multiple and independent acquisitions of asexual reproduction are associated with numerous mechanisms to restore diploid state and produce females (Rabeling and Kronauer, 2013; Vorburger, 2014), illustrated in figure 1B. Apomixis is based on mitosis and induces clonal reproduction. The same clonal issue may also occur when endoreplication precedes meiosis, resulting in recombination between identical chromosomes (Ma and Schwander, 2017). In automixis, meiosis occurs and is followed by different diploid restoration processes. Two meiosis products may assemble to generate a diploid cell: i) fusion of non-sister products separated during the first *reductional* division in central fusion or ii) fusion of sister cells produced during the second *equational* division in terminal fusion. The restoration of diploidy may also result from gamete duplication involving either fusion of mitosis products or chromosomal replication without cellular division. In some lineages, the restoration of diploidy may operate during embryogenesis *via* endomitosis (Little et al., 2017; Pardo et al., 1995). The consequences of thelytoky in terms of heterozygosity are variable depending on the mechanism: from complete homozygosity in one generation under gamete duplication to completely preserved heterozygosity in apomixis, with intermediate levels of homozygosity in terminal and central fusion. According to the biology of species and to the necessity to maintain heterozygosity, each mechanism may be favored or not.

Three main origins of thelytoky have been described: hybridization, bacterial endosymbiosis and genetic mutation (Tvedte et al., 2019). Hybridization, joining genomes from two distinct species, leads to improper chromosome pairing and dysfunctional meiosis that may promote asexuality (Morgan-Richards and Trewick, 2005). Endosymbiotic origin is the most widely studied cause of parthenogenesis (Ma and Schwander, 2017). Up to date, only bacteria have been evidenced as parthenogenesis inducers, but it is likely that other microorganisms could be involved. Most of the described causative agents belong to the genera *Wolbachia*, *Rickettsia* and *Cardinium*, endosymbionts also known to induce cytoplasmic incompatibility or feminization of male embryos. The particularity of endosymbiont induced parthenogenesis resides in its partial or total reversibility. Thelytokous species treated with antibiotics or heat may revert to sexual reproduction, although often performing less well than true sexual counterparts (Stouthamer et al., 1990). The genetic origin of thelytoky has often been suggested when antibiotic or heat treatment had no effect, but the precise identification of loci responsible for parthenogenesis has only been conducted in a few

species (Chapman et al., 2015; Jarosch et al., 2011; Lattorff et al., 2005; Sandrock and Vorburger, 2011).

The frequency of thelytoky within species also varies between taxa (Gokhman and Kuznetsova, 2018; Vershinina and Kuznetsova, 2016). Some species are described as obligate thelytokous when this mode of reproduction is the only one observed. Alternatively, thelytoky appears cyclic in some species where asexual generations alternate with sexual ones (Neiman et al., 2014). In other cases, polymorphism in the reproductive mode is observed either between populations (Foray et al., 2013; Leach et al., 2009) or within populations (Liu et al., 2019). Even in such polymorphic situations, thelytokous females produce female only progeny, albeit low frequency of males may be reported allowing to maintain rare events of sexual reproduction (Pijls et al., 1996).

Spontaneous occurrence of parthenogenesis has also been described in species reproducing via a sexual mode and qualified as tychoparthenogenesis (Ball, 2001; Pardo et al., 1995). Tychoparthenogenesis is characterized by a low hatching rate and a weak survival probability of the offspring (Little et al., 2017). It is typically considered as a dead-end accidental phenomenon in species adapted to sexual reproduction, although it may also correspond to an intermediate state in the evolution toward asexuality (van der Kooi and Schwander, 2015).

Cotesia typhae (Fernandez-Triana; Hymenoptera, Braconidae) is a gregarious endoparasitoid wasp native to Eastern Africa (Kaiser et al., 2017, 2015). It is specialized to one host, the corn stemborer *Sesamia nonagrioides* (Lefèbvre, Lepidoptera, Noctuidae). *Cotesia typhae* reproduces sexually and fertilized females typically lay 70-100 eggs in the first host encountered, among which about 70% develop into females and 30% into males (Benoist et al., 2020b). At least in laboratory conditions, sister-brother mating (sib-mating) currently occurs indicating that inbreeding is not detrimental to this species. A genetic survey was conducted on this parasitoid wasp to compare two laboratory strains initiated from wild individuals sampled in two distant Kenyan localities, Kobodo and Makindu (Benoist et al., 2020a). The study led to the construction of a genetic map, based on crosses between the two strains. The phenotyping of the progenies obtained from these controlled crosses revealed an extremely variable sex-ratio, ranging from 100% to as low as 5% of females. Such a phenomenon could result from poorly mated females but also from rare thelytokous events in the progeny of unfertilized females. This last hypothesis was validated in a preliminary experiment allowing virgin females to oviposit. Among the numerous males emerging from the parasitized hosts, a few females were detected.

The aim of this study is to describe the low frequency thelytoky phenomenon in *Cotesia typhae*. We measured the percentage of females able to produce daughters parthenogenetically as well as the proportion of thelytokous daughters to arrhenotokous sons in their progeny. These ratios were obtained for both laboratory strains but also for the wild population from Kobodo. We measured the ploidy level of the thelytokous daughters to test whether they result from male feminization or from diploid restoration. Taking advantage of the available genetic map, we investigated the mechanism responsible for thelytoky. The identified markers were also used to detect the presence of thelytokous daughters in the progeny of fertilized females. Finally, we tested the endosymbiotic origin of thelytoky and its reversibility by supplying wasps with antibiotics for successive generations and testing for the presence of well-known endosymbionts using barcoding markers.

Material and Methods

Biological material

135 Two separate *Cotesia typhae* parasitoid strains were obtained from adults that emerged from
136 naturally parasitized *Sesamia nonagrioides* caterpillars collected in the field at two localities in Kenya
137 (Kobodo: 0.679S, 34.412E; West Kenya; 3 caterpillars collected in 2013 and Makindu: 2.278S,
138 37.825E; South-East Kenya; 10 caterpillars collected in 2010-2011). Isofemale lines were initiated in
139 2016 and inbred rearings have been subsequently kept for more than 80 generations at the
140 Evolution, Génomes, Comportement et Ecologie laboratory (EGCE, Gif-sur-Yvette, France), where
141 cross experiments and phenotyping were performed. The phenotyping of the wild population was
142 performed on individuals that emerged from naturally parasitized *Sesamia nonagrioides* caterpillars
143 collected in the field in 2020 at Kobodo (see above). The *S. nonagrioides* host strain used was
144 initiated from caterpillars collected at Makindu (see above) and Kabaa (1.24S, 37.44E). The rearing
145 protocol of *C. typhae* and *S. nonagrioides* is detailed in Benoist et al. (2020b).

146

147 **Phenotyping the strains/populations for the thelytokous character**

148 In this study, the phenotyping consists of counting the number of males and females in the offspring
149 of virgin females, to quantify the thelytoky phenomenon. To obtain virgin females, individual
150 cocoons were isolated from cocoon masses and kept in tubes with a moistened cotton wool ball and
151 a drop of honey at 27°C until the emergence of the adults. The virgin females were then each
152 allowed to oviposit in one *S. nonagrioides* caterpillar, and the number of males and females in their
153 offspring was counted after the development of the new *C. typhae* generation.

154

155 **Flow cytometry for ploidy analysis**

156 Flow cytometry analysis was performed on one control female from a mixed cocoon mass (produced
157 by a fertilized female), two control males, and five parthenogenetic females (produced by a virgin
158 female), all coming from the Makindu laboratory strain, to determine their ploidy. The individuals
159 were frozen in liquid nitrogen and processed in the Imagerie-Gif Platform of Institute for Integrative
160 Biology of the Cell (I2BC), CNRS, Gif-sur-Yvette according to the protocol in (Bourge et al., 2018).

161

162 **Fecundity assessment of parthenogenetic females**

163 Cocoon masses resulting from the eggs laid by *C. typhae* Makindu virgin females in *S. nonagrioides*
164 caterpillars were divided in smaller cocoon packs to spot parthenogenetic females more easily
165 among the males after the emergence of the adults. Adults were left together for one day with water
166 and honey to allow mating, and eleven parthenogenetic females were then allowed to oviposit in *S.*
167 *nonagrioides* caterpillars. After the emergence of the resulting offspring, the number of males and
168 females was counted for each one of them and the sex-ratio was calculated for comparison with that
169 obtained from fertilized females from the control Makindu laboratory strain.

170

171 **Identifying the thelytoky mechanism in *Cotesia typhae***

172 To find out which thelytoky mechanism is at play in the *C. typhae* Makindu laboratory strain, virgin
173 heterozygous females are needed to analyse the recombination patterns of their offspring. Indeed,
174 according to the mechanism, the female offspring will be more or less heterozygous, as explained in
175 introduction (Figure 1B). To obtain virgin heterozygous females, six controlled crosses were

176 performed between the Makindu and Kobodo laboratory strains, 3 in each direction (Figure 1A).
177 Prior to this, cocoons had been isolated from masses of each strain, in order to obtain virgin males
178 and females for the crosses. Cocoons were then isolated from the masses resulting from the crosses,
179 leading to the emergence of virgin F1 heterozygous females. 57 of these females were allowed to
180 oviposit in *S. nonagrioides* caterpillars, and the offspring of the 57 resulting cocoon masses were
181 sexed and counted. Six females and four males from the two parental strains (including the
182 individuals used for the initial crosses), five F1 heterozygous females and the nine parthenogenetic
183 females obtained through this experiment were kept for DNA extraction and genotyping, in order to
184 analyse the recombination patterns resulting from parthenogenesis.

185 DNA was extracted from the 24 individuals using the NucleoSpin® Tissue from Macherey Nagel,
186 following the manufacturer's instructions.

187 To analyse the recombination patterns of the nine parthenogenetic females, we genotyped 63 SNP
188 (Single Nucleotide Polymorphism) markers, having different alleles between the Makindu and
189 Kobodo strains, and being distributed along the 10 chromosomes of the genetic map of *Cotesia*
190 *typhae*, (Benoist et al., 2020a). Four chromosomes contained more markers than the others to
191 investigate the recombination patterns along chromosomes. For these four chromosomes, the
192 markers were chosen to have about 10cM between two successive markers when possible. The
193 genetic position of all the markers used in this present study are given in Supplementary Table 1.

194 The HRM (High Resolution Melt) technique was used to genotype 61 of the markers. This technique
195 is based on the analysis of melt curves of DNA fragments after amplification by PCR. The melt curves
196 are different according to the nucleotide composition of the DNA fragments, and therefore allow to
197 discriminate between homozygotes and heterozygotes at a given SNP. For each SNP marker, a 10µL
198 mix was made with about 1ng of DNA, 0.2µM of each primer, and 5µL of Precision Melt Supermix
199 (Bio-Rad), completed with water. The PCR protocol was 95°C for 2 minutes, followed by 40 cycles of
200 95°C for 10 seconds, 60°C for 30 seconds, 72°C for 30 seconds, followed by a complete denaturation
201 of 30 seconds at 95°C before performing the melt curve. The melt curve was performed on a CFX96™
202 Real-Time System (Bio-Rad), and was started by an initial step of 1 minute at 60°C, followed by 10
203 seconds of every 0.2°C increment between 65°C and 95°C. The raw data resulting from the melt
204 curves were analysed with the uAnalyze v2.1 software (Dwight et al., 2012) in order to infer the
205 females' genotypes at each SNP marker.

206 The two remaining SNP markers (8225nov and 21770nov) were genotyped using allele specific PCR.
207 For this method, two parallel amplifications were performed on individuals, one of the primers of the
208 couple being a common primer, and the other one being a specific primer, either to the Makindu, or
209 Kobodo allele. About 1ng of DNA was mixed with 1X buffer, 3mM $MgCl_2$, 0.4mM dNTP, 0.4µM of
210 each primer, 1U GoTaq® Flexi DNA Polymerase (Promega), and completed with water. The PCR
211 programme was 5 minutes at 95°C, followed by 40 cycles of 95°C for 1 minute, 50°C or 55°C for 1
212 minute (50°C for 8225nov and 55°C for 21770nov), 72°C for one minute and a final elongation of 5
213 minutes at 72°C. The PCR products were then run on a 2% agarose gel to check which PCR were
214 positive and therefore infer the genotypes.

215 All the primers used were designed for this study and their sequences are given in Supplementary
216 Table 2.

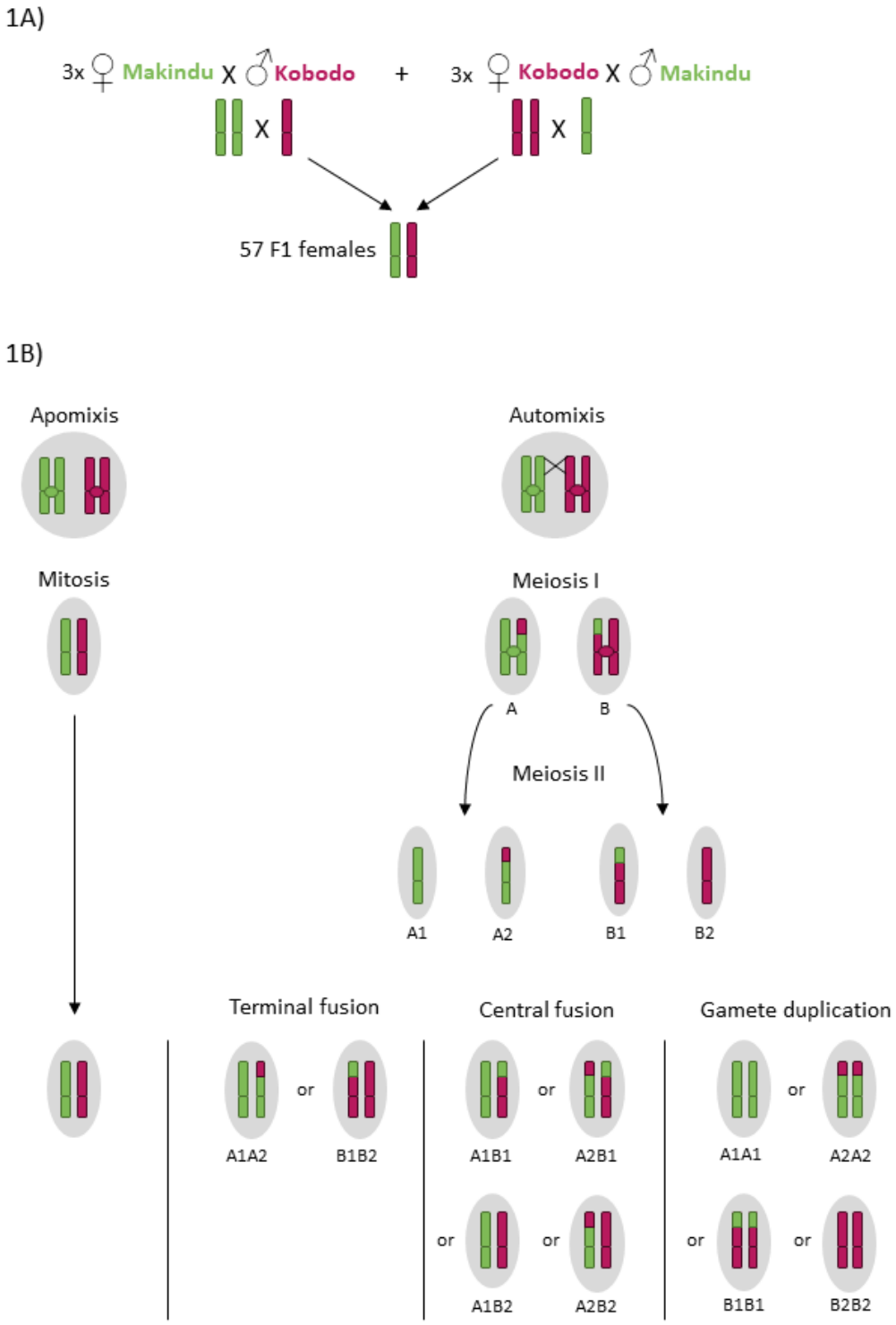


Figure 1: A) Crosses performed in this study in order to obtain virgin heterozygous females. B) Expected genotypes' patterns of parthenogenetic daughters according to the thelytoky mechanism

220 involved. The patterns can vary from complete loss of heterozygosity (under gamete duplication) to
221 complete maintenance of heterozygosity (under apomixis).

222

223 **Looking for thelytoky in mated females**

224 To see if mated females produce parthenogenetic daughters as well as sexual daughters, 40 crosses
225 between Makindu virgin females and Kobodo males were performed, according to the protocol
226 described in the previous section. By genotyping the daughters of these crosses with a SNP marker
227 differing between the Makindu and Kobodo strains, we can deduce if they were produced sexually (if
228 heterozygous at the marker) or through parthenogenesis (if homozygous for the Makindu allele at
229 the marker).

230 Out of the 40 crosses, only the ones leading to a mix of male and female offspring were kept in our
231 analysis, leading to a total of 1861 daughters and 1803 sons. To genotype the 1861 daughters, a
232 direct PCR method was used, instead of a classic DNA extraction. For each female, the abdomen was
233 removed (because the presence of gametes could hinder the genotyping) and the rest of the body
234 was placed in 20µL of Dilution Buffer and 0.5µL of DNA Release Additive (Thermoscientific). The
235 tubes were kept at room temperature for 5 minutes then placed at 98°C for 2 minutes. One
236 microliter of this mix was used as template for the PCR performed for the HRM genotyping (see
237 above) of one SNP marker, 27068nov. All the females that had a clear Makindu homozygote
238 genotype and all the females presenting an uncertain genotype were withdrawn from their buffer
239 and their DNA was properly extracted with the NucleoSpin® Tissue kit from Macherey Nagel. They
240 were then genotyped at 2 more markers (8225nov and 21770nov) to confirm their status, by an
241 allele-specific PCR method. The three markers used here for genotyping were used previously for the
242 identification of the thelytoky mechanism, and the protocol is the same as described above.

243

244 **Search for a bacterial cause of thelytoky in *Cotesia typhae***

245 To find out if the cause of thelytoky in *C. typhae* could be bacterial, we first performed PCR with
246 primers designed to amplify several micro-organisms known to manipulate sex in insects. Ten virgin
247 Makindu females that produced parthenogenetic daughters and two virgin Makindu females that
248 didn't produce daughters were tested with 8 primers sets, taken from (Foray et al., 2013), except for
249 one primer set, specific to *Wolbachia*, taken from (Casiraghi et al., 2005). The primers' sequences, Tm
250 used for PCR, and their original publication are given in Supplementary Table 3. About 1ng of DNA
251 was mixed with 1X buffer, 3mM $MgCl_2$, 0.4mM dNTP, 0.4µM of each primer, 1U GoTaq® Flexi DNA
252 Polymerase (Promega), and completed with water. The PCR programme was 5 minutes at 95°C,
253 followed by 40 cycles of 95°C for 1 minute, Tm for 1 minute, 72°C for one minute and a final
254 elongation of 5 minutes at 72°C. The PCR products were then run on a 1% agarose gel to check for
255 positive amplification.

256 The amplified fragments obtained with the *Arsenophonus* primer set were sequenced with the
257 BigDye™ Terminator v1.1 Cycle Sequencing Kit (ThermoFisher Scientific), following the
258 manufacturer's protocol. After the identification of the bacteria *Pantoea dispersa* through
259 sequencing, 8 virgin Kobodo females that didn't produce any parthenogenetic daughters were also
260 tested with this primer set. Since our Kobodo laboratory strain doesn't undergo thelytoky, this test
261 was performed to check if *Pantoea dispersa* could be the causative agent of thelytoky in *C. typhae*.

262 To complete this experiment, we performed an antibiotic treatment on the Makindu laboratory
263 strain to remove any potential sex manipulating bacteria in *C. typhae* females. Rifampicin was added
264 in the host caterpillars' artificial diet, at a final concentration of 2g/L, for 4 *C. typhae* generations. This
265 treatment has previously been shown to eliminate *Wolbachia* bacteria in a close species, *Cotesia*
266 *sesamiae* (Mochiah et al., 2002). The phenotyping results after this first treatment being ambiguous,
267 it was continued for 7 more generations, with tetracyclin added for the 4 last generations, also at a
268 final concentration of 2g/L. At that point, 79 virgin *C. typhae* females were phenotyped, according to
269 the phenotyping protocol previously described.

270

271 **Results**

272 **Phenotypes of the different strains/populations**

273 We phenotyped two laboratory strains for the thelytokous character, Makindu and Kobodo, and a
274 wild population, coming from the Kobodo locality. The numbers of available virgin females obtained
275 for phenotyping were the following: 99 from 13 different cocoon masses for the Makindu strain, 40
276 from 7 cocoon masses for the Kobodo strain, and 29 from 6 cocoon masses for the Kobodo wild
277 population. The results (Table 1) are very contrasted between these three populations, since the
278 number of parthenogenetic females is null in the Kobodo isofemale strain, intermediate in the
279 Kobodo wild population (28% of virgin females produced daughters), and high in the Makindu
280 isofemale strain (68% of virgin females produced daughters). The thelytokous phenotype is therefore
281 present in the wild and is not a laboratory artefact, but was apparently lost in the Kobodo laboratory
282 strain, or present at a frequency too low to be detected. Unfortunately, the wild Makindu population
283 does not exist anymore and could not be tested in this study.

284

		N	Number of offspring with parthenogenetic females	Total number of males in the N offspring	Total number of parthenogenetic females in the N offspring	Mean number [min ; max] of parthenogenetic females per offspring
Makindu isofemale strain		99	67 (68%)	10657	225 (2%)	2.3 [0;8]
Kobodo isofemale strain		40	0	5405	0	0
Kobodo wild population		29	8 (28%)	2132	8 (0.4%)	0.3 [0;1]
Makindu strain after antibiotic treatment	After 4 generations	55	15 (27%)	5886	17 (0.3%)	0.3 [0;2]
	After 11 generations	79	13 (16%)	8272	16 (0.2%)	0.2 [0;4]

285 Table 1: Results of the thelytoky phenotyping of the different strains/populations. The phenotyping is
286 based on the number and frequency of daughters produced parthenogenetically (parthenogenetic
287 females) in the offspring of virgin *C. typhae* females. "N" is the number of virgin females tested.

288 **Ploidy of the daughters of virgin females**

289 One daughter from the progeny of a fertilized female and 2 males, all belonging to the Makindu
290 laboratory strain, were processed by flow cytometry as respective controls for diploid and haploid
291 *Cotesia typhae* genomes. Five parthenogenetic daughters of virgin females were then processed,
292 resulting in an estimated genome size identical to the control female and twice that of the control
293 males (Table 2). We can therefore conclude that *C. typhae* parthenogenetic females are diploid and
294 not the result of feminization of haploid eggs.

Sample	Size (pg)	Size (Mpb)	Ploidy
Control female	0.47	458.27	Diploid
Control male 1	0.26	251.84	Haploid
Control male 2	0.25	241.12	Haploid
Parthenogenetic female 1	0.48	467.51	Diploid
Parthenogenetic female 2	0.48	473.24	Diploid
Parthenogenetic female 3	0.49	475.28	Diploid
Parthenogenetic female 4	0.49	475.91	Diploid
Parthenogenetic female 5	0.5	484.26	Diploid

295 Table 2: Genome size estimated by flow cytometry. Parthenogenetic females have the same genome
296 size as the control female, corresponding to about twice the males' haploid genome size.

297

298 **Fecundity of parthenogenetic females**

299 Eleven parthenogenetic females (issue from virgin Makindu mothers) were randomly allowed to
300 mate with their brothers and were used to parasitize eleven caterpillars. Out of these eleven
301 females, 4 had male only offspring and 7 had a mixed offspring. The number of offspring per female
302 and the sex-ratio are indicated in Table 3. No significant difference of the offspring size and sex ratio
303 was observed between the control and parthenogenetic female datasets (p-value obtained following
304 Mann-Whitney rank test was 0.559 for offspring number and 0.07 for sex-ratio). The fecundity of
305 parthenogenetic females is therefore equivalent to that of the control females.

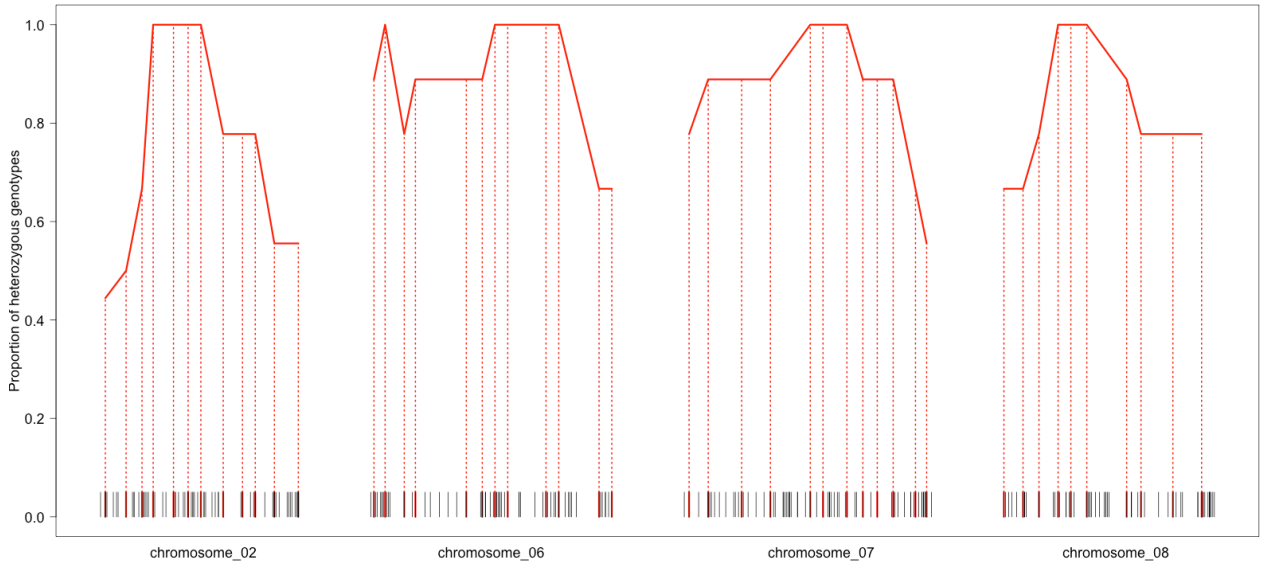
	N	Mean number of offspring per mated female ± Standard Error	Mean female sex-ratio (when mated) ± Standard Error
Control Makindu strain[a]	41	59 ± 4.2	0.78 ± 0.03
Parthenogenetic females	7	54.3 ± 9.2	0.65 ± 0.12

306 Table 3: Comparison of the fecundity between Makindu parthenogenetic females and control
307 females of the same laboratory strain. a: data from (Benoist et al., 2017).

308 **Thelytoky mechanism occurring in *Cotesia typhae***

309 The genotyping of the 63 SNP markers first confirmed that fathers and mothers of the initial crosses
310 between the Makindu and Kobodo laboratory strains were homozygotes for their strain's alleles. The
311 57 virgin F1 daughters resulting from these crosses were thus heterozygous at the SNP markers,
312 which was confirmed for the 5 F1 daughters that were genotyped. Each of these females successfully
313 parasitized a host larva, and from the 57 resulting offspring, six contained parthenogenetic females
314 (originating from 4 of the initial 6 crosses, 2 in each cross direction), corresponding to a total of 9 F2
315 parthenogenetic females for 6653 males. These 9 females were genotyped for the 63 SNP. The
316 genotypes and the deduced recombination events are presented in Supplementary Table 1. The
317 recombination patterns of the 4 chromosomes genotyped with a higher density of markers are
318 shown in Figure 2.

319 For six of the females, a mixture of heterozygous and homozygous markers was observed, with a
320 surplus of heterozygotes (280 heterozygous genotypes for 94 homozygous genotypes). The number
321 and pattern of heterozygous markers for these females indicates a mechanism of automixis with
322 central fusion. Indeed, the central parts of the chromosomes maintain a heterozygous state while
323 there is a recombination gradient leading to more homozygous genotypes towards the extremities of
324 the chromosomes (Fig. 2). On average, nine recombination events per genome were detected for
325 these six females with a minimum value of five events and a maximum of 16 events detected. Based
326 on the density of the markers characterized, these results are consistent with the genetic length
327 measured by Benoist et al. (2020a).



328

329 Figure 2: Proportion of heterozygous females (out of nine) for each genotyped SNP marker. The
330 results are only shown for the 4 chromosomes for which a higher number of markers were
331 genotyped. The black segments on the x axis are indicative of the genetic position of all the markers
332 of the genetic map (Benoist et al., 2020a) and the red segments with the dotted lines correspond to
333 the positions of the markers genotyped in this study. The occurrence of homozygous and
334 heterozygous states along the chromosomes is congruent with a mechanism of automixis. The
335 observation of 100% heterozygosity in the central part of the chromosomes suggests that diploidy is
336 restored through central fusion and is indicative of the position of each chromosome's centromere.

For the other 3 parthenogenetic females, all the 63 markers were heterozygous, revealing no detection of recombination event on the 10 chromosomes. We estimated the probability of such an observation under the hypothesis that a unique mechanism of central fusion occurs. For each chromosome, we calculated a mean number of recombinations based on the nine parthenogenetic females. Assuming that the number of recombinations on a chromosome follows a Poisson distribution, we can estimate the probability of zero recombination for each chromosome based on the mean number estimate. It varies according to the genetic length of the chromosome and to the density of markers genotyped: it was estimated between 0.29 for chromosome 2 and 0.8 for chromosome 9. Multiplying the probability over the ten chromosomes, we calculated a probability of 0.0025 to observe an entirely heterozygous parthenogenetic daughter. Using this individual probability, we estimated that the probability to detect three out of nine parthenogenetic daughters showing no recombination events on the 10 chromosomes was 1.6×10^{-6}. This hypothesis is very unlikely, therefore we suspect another mechanism could also be at play in causing thelytoky in *Cotesia typhae*. It is interesting to note that we observed both patterns (partial homozygosity and complete heterozygosity) in the offspring resulting from initial crosses of both directions. Moreover, one of the F1 heterozygous females displayed both patterns in her progeny.

Presence of parthenogenetic females among the daughters of mated females

Among the 40 crosses between Makindu females and Kobodo males, 35 had mixed offspring comprising both males and females, and were kept in our analysis, leading to a total of 1861 females and 1803 males. All 1861 females were genotyped for one SNP marker. Females resulting from fecundation should be heterozygous while parthenogenetic females should be homozygous for the Makindu allele. In total, we found 14 homozygous females, which were confirmed by the genotyping of 2 other markers. These 14 females correspond to 0.77% of the parthenogenetic offspring (males resulting from arrhenotoky representing 99.33%) and originate from 10 different mothers (29% of the 35 mothers) (Table 4). Even though the percentage of parthenogenetic females found is much smaller than in the offspring of virgin females, this finding shows that the female progeny of mated females can come from a mixture of parthenogenesis and sexual reproduction.

	N	Number of parthenogenetic offspring (males plus parthenogenetic females)	Number of parthenogenetic females among the parthenogenetic offspring	Mean number [min ; max] of parthenogenetic females in offspring when present
Makindu virgin females	99	10882	225 (2%)	2.3 [1;8]
Makindu mated females	35	1817	14 (0.77%)	1.4 [1;3]

Table 4: Comparison of the frequency of the thelytokous character between virgin and mated Makindu females. In each case, the number of parthenogenetic daughters is presented as a percentage of the total number of parthenogenetic offspring, mainly composed of males obtained from arrhenotoky. “N” is the number of offspring analysed.

370 **Origin of thelytoky in *Cotesia typhae***

371 In order to find out if thelytoky in *Cotesia typhae* has a bacterial origin, we extracted DNA from
372 Makindu virgin mothers that produced daughters and used primers to try to amplify the DNA of six
373 different micro-organisms known for sex manipulation in insects: *Wolbachia, Ricketssia, Cardinium,*
374 *Arsenophonus, Spiroplasma* and *Microsporidia* (Foray et al., 2013). Only one primer set led to a solid
375 amplification, the one designed to amplify *Arsenophonus* 23S. After sequencing the amplified
376 fragment, the bacterium was identified not as *Arsenophonus* but as *Pantoea dispersa*, for which no
377 mention in relation to thelytoky was found in literature. We then tried to amplify this same
378 bacterium from the DNA of Kobodo virgin mothers, who don't produce any daughters: *Pantoea*
379 *dispersa* was present in all the samples as well. This makes it unlikely for this bacterium to be
380 responsible for thelytoky in *C. typhae*.

381 After rearing parasitized caterpillars for four generations on a rifampicin diet, we phenotyped the
382 Makindu strain again. 55 virgin females, coming from 5 different cocoon masses, were allowed to
383 parasitize *Sesamia nonagrioides* caterpillars. Fifteen of these virgin females produced daughters,
384 leading to a total of 17 daughters for 5886 sons (Table 1). Another phenotyping was performed on 79
385 females from 10 different cocoon masses, after 11 generations of a rifampicin diet (with tetracyclin
386 added for the last 4 generations). Thirteen of these virgin females produced daughters, leading to a
387 total of 16 daughters for 8272 sons (Table 1). The percentage of thelytokous females is thus smaller
388 than the one observed before antibiotic treatment but not null.

389

390 **Discussion**

391 The phenotypic survey presented here confirms the biological reality of low frequency asexual
392 production of females in the haplo-diploid Hymenoptera *Cotesia typhae*. The process has been
393 observed in a significant number of progenies from both an inbred laboratory strain and a natural
394 population. It has been shown to occur in the progeny of virgin as well as fertilized females, despite
395 concerning only a small fraction of the individuals from a cocoon mass.

396 This configuration of low frequency thelytoky is unusual. As a matter of fact, the expression thelytoky
397 is even defined by some authors as a "parthenogenetic mode where females produce only females
398 from unfertilized eggs" (Vershinina and Kuznetsova, 2016). Among illustrated examples of
399 parthenogenesis *sensu stricto*, even when facultative, asexual production of females involves the
400 whole progeny. The case described here is somewhat closer to what is called tychoparthenogenesis,
401 based on the frequency of birth of parthenogenetic female eggs (Whiting, 1945).
402 Tychoparthenogenesis is defined as "kind of occasional thelytoky characterized by the spontaneous
403 hatching of a small proportion of eggs laid by virgin females" (Pardo et al., 1995). It has been mainly
404 described in diplodiploid species where embryonic development is induced by sperm fertilization. In
405 such species, developmental constraints and inbreeding depression prevent successful hatching of
406 unfertilized eggs in most of the cases (Little et al., 2017). In haplodiploid species, unfertilized eggs
407 hatch with a high frequency because they naturally produce males in species reproducing sexually. It
408 is thus difficult to classify *C. typhae* as a tychoparthenogenetic species.

409 A better description of its asexual reproductive mode would require the comparative study of the
410 hatching success of fertilized diploid eggs, unfertilized diploid eggs and unfertilized haploid eggs. As
411 *C. typhae* is an endoparasitoid species, such estimates are difficult to obtain. The low proportion of
412 asexual daughters seems not however to be detrimental to the wasp as progeny numbers were
413 similar between those without, and those including thelytokous daughters. Benoist et al. (2020b)

414 showed that the number of oviposited eggs was close to the number of emerging adults. The survival
415 ratio deduced from these two estimates is similar between the two laboratory strains Kobodo and
416 Makindu, however thelytoky was demonstrated only in the Makindu strain. Taken together, these
417 data suggest that the phenomenon most likely results from rare events rather than from low
418 developmental success. Furthermore, the daughters produced parthenogenetically turned out to be
419 viable and fertile, indicating that low frequency thelytoky may be either neutral or beneficial but not
420 disadvantageous.

421 The question remains as to whether the phenomenon is accidental or an ongoing evolutionary
422 process due to its adaptive benefit (van der Kooi and Schwander, 2015). Studying the occurrence of
423 parthenogenesis among Ephemeroptera, Liegeois et al. (2021) suggested that asexual reproduction
424 was selectively advantageous in many species from this insect order despite its associated low
425 hatching success. The benefit derives from the short adult life and the low dispersal ability that
426 reduce the probability of encountering a reproductive partner. The fitness of sexually reproducing
427 individuals may consequently be reduced under certain circumstances. As in mayflies, *C. typhae* has
428 an adult life limited to a few days (between two and three days in laboratory conditions, Kaiser et al.,
429 2017). However, it is gregarious and mating between sisters and brothers emerging from the same
430 cocoon mass is observed in rearing conditions. Female access to male fertilization should thus be
431 facilitated. Whether sib-mating occurs in natural conditions is actually unknown but it may be
432 favoured by the endophytic mode of life of its larval host, because cocoon masses are formed inside
433 plant stems. The dispersal behaviour of males and females is also unknown but is likely to be limited
434 to short distances, as it was estimated to be at most 64m for the close relative *Cotesia flavipes*
435 (Dinardo-Miranda et al., 2014; Sallam et al., 2001). Sib-mating has been shown to be avoided in
436 several studies. In *Cotesia glomerata*, it was demonstrated that females mated with males from the
437 same cocoon mass in only 27.5% of the cases (Gu and Dorn, 2003). In *Venturia canescens*, studies of
438 mating behaviour showed that sib-mating was limited by a male-biased dispersal (Collet et al., 2020).
439 If *C. typhae* males behave similarly in nature, females may face limited access to copulation. Another
440 potential restricted access to fertilization concerns sperm limitation. A mated female may meet some
441 difficulties due to sperm incompatibility or a delay post-mating where sperm is not available. When
442 the sex ratio is highly biased, males have to mate with numerous females and may undergo
443 temporarily or definitively sperm depletion (Boivin, 2013). Preliminary experiments revealed that *C.*
444 *typhae* males were able to fertilize ten females successively with no detectable impact on the
445 progeny's sex-ratio (data not shown). However, some of the mated females (whatever their mating
446 rank was in the protocol) produced male-only offspring, suggesting that sperm transfer and/or usage
447 is sometimes difficult. Whether it results from restricted access to males or to sperm itself, sperm
448 limitation may favour expansion of asexual reproduction. Further experiments are needed to
449 estimate mating and fertilization success of *Cotesia typhae* in natural conditions.

450 Beyond reproductive strategy itself, parthenogenesis has been shown to be associated with
451 ecological characteristics that may favour or prevent its evolution. Two opposite ecological trends
452 have been described co-occurring with asexual reproduction expansion: the "general purpose
453 genotype" (GPG) where asexual lineages are observed on broader ecological niches than their sexual
454 counterparts and the "frozen niche variation" (FNV) where parthenogenetic species or populations
455 have far more restricted niches than sexual ones (Tvedte et al., 2019). Exploring a wide dataset of
456 haplodiploid arthropods reproducing exclusively parthenogenetically (obligate parthenogenesis), van
457 der Kooi et al. (2017) concluded that GPG was the most common situation. They showed that most
458 parthenogenetic species have broader ecological and geographical range than close relative sexual
459 species but also that transition toward parthenogenesis was more likely for species exhibiting a wide
460 distribution. Nevertheless, numerous cases of ecological specialization of asexual lineages have been

461 described, such as *Venturia canescens*. In this polymorphic species, two kinds of populations live in
462 sympatry: parthenogenetic populations found in stable anthropic habitats (bakeries and granaries)
463 and sexual ones associated with natural and more instable resources (Schneider et al., 2002).
464 Interestingly, before being characterized as a new species, *Cotesia typhae* was first identified as a
465 specialized clade (only one host insect, *Sesamia nonagrioides,* mainly found on one host plant, *Typha*
466 *domingiensis*) of the parasitoid species *Cotesia sesamiae*. According to Branca et al. (2019), some
467 populations of *C. sesamiae* are less specialized than others. Studying the existence of thelytokous
468 reproduction in those populations would be informative about the possible link between emerging
469 parthenogenesis and specialization.

470 Regarding the mechanism involved in thelytokous reproduction, we faced an unexpected result as
471 data strongly suggest that two different processes may co-occur: automixis with central fusion and
472 apomixis. More surprisingly, the two supposed mechanisms were observed to co-occur in the
473 progeny of a single female (K4M1) and independently of the cross direction to obtain F1 virgin
474 mothers (Kobodo female x Makindu male or Makindu female x Kobodo male). Unfortunately, this
475 result is supported by small sample size due to the scarcity of the phenomenon. We may wonder
476 whether a unique mechanism, distinct from those already described, could explain such a result. Ma
477 and Schwander (2017) describe for example an unusual process where meiosis is inverted (sister
478 chromatids separate before homologs) followed by terminal fusion. However, the resulting progeny
479 of such a process is 100% heterozygous, a result that does not differ from apomixis. Another
480 mechanism presented in the same review implies an endoreplication preceding meiosis. Assuming
481 such a process occurs in *C. typhae*, and hypothesizing that recombination, and consequently
482 segregation during the first division, may arise either between identical or between homologous
483 chromosomes, some intermediate situations are expected. Once again, it does not reconcile the
484 clear-cut figure we observe with individuals entirely heterozygous suggesting zero recombination
485 between homologs and individuals for which recombination is observed for almost all homologs. To
486 better understand the mechanism underlying thelytoky in *C. typhae*, a cytological approach of
487 meiosis and parthenogenesis would be necessary.

488 Despite the lack of a unified mechanism to explain the genotypic profile observed in the F2 progenies
489 obtained, we can confirm that recombination occurred, at least in cases attributable to central
490 fusion, and that these recombination events were as frequent as those observed in sexual
491 reproduction (Benoist et al., 2020a). By contrast, severe reductions of recombination rates were
492 observed associated with parthenogenetic reproduction in the literature. For example,
493 recombination is reduced by up to 10-fold in the Cap bee, *Apis mellifera capensis*, a social parasite of
494 honeybee which reproduces parthenogenetically *via* automixis with central fusion (Baudry et al.,
495 2004). In the little fire ant *Wasmannia auropunctata*, sexual populations coexist with asexual
496 populations in which reproductive queens are produced by automictic parthenogenesis with central
497 fusion. In the asexual populations, recombination rate is reduced by a factor 45 compared to the
498 sexual populations (Rey et al., 2011). The reduction of recombination rate is assumed to mitigate the
499 potential deleterious impact of thelytoky: under automixis with central fusion, heterozygosity is
500 preserved unless recombination occurs (Figure 1). In species affected by inbreeding depression, a
501 homozygosity increase would be detrimental and could be advantageously limited by a low
502 recombination rate. As the molecular mechanism involved in restoration of diploidy and
503 recombination are probably distinct, the situation observed in Cap bee and little fire ant may result
504 from a long-term evolutionary process. If the phenomenon described in *C. typhae* is recent, it may
505 explain the unchanged recombination rate.

506 Otherwise, the inbreeding impact could be meaningless in *C. typhae*. Hymenoptera are haplodiploid
507 and could thus be less sensitive to inbreeding because most of the deleterious alleles are purged at
508 the haploid state in males. However, their sex determination system may be highly compelling
509 regarding homozygosity and ability to reproduce *via* thelytoky (Vorburger, 2014). The most common,
510 and likely ancestral, sex determination system is governed by the genotype at one (sl-CSD: single
511 locus Complementary Sex Determination) or few loci (ml-CSD: multi locus CSD) (Heimpel and de
512 Boer, 2008). Under such a determinism, individuals that are heterozygous at least at one of these
513 CSD loci develop as diploid females while hemizygous or homozygous individuals at all CSD loci
514 develop as haploid or diploid males respectively. In most hymenopteran species, diploid males have a
515 low survival rate and/or are often sterile. Enhanced homozygosity due to thelytoky may be very
516 costly when it results in diploid male production (de Boer et al., 2015, 2012; van Wilgenburg et al.,
517 2006; Zhou et al., 2007). Other sex determination systems have been described in Hymenoptera that
518 are less sensitive to homozygosity. In Paternal Genome Elimination, haploid males initially develop
519 from a fertilized egg from which the paternal genome is expulsed secondarily (Heimpel and de Boer,
520 2008). This mechanism can be discarded for *Cotesia typhae* as virgin females are able to produce
521 haploid sons. Genome imprinting has been studied in *Nasonia vitripennis* and relies on epigenetic
522 landmarks deposited by the mother in the oocyte that will be modified by paternal genome
523 expression to allow female development (van de Zande and Verhulst, 2014).

524 Within the *Cotesia* genus, sex determination has been investigated in a few species. The CSD system
525 has been demonstrated in three species: *C. glomerata* where sex is determined by a single locus
526 (Zhou et al., 2006), *C. vestalis* and *C. rubecula* where sex determination is encoded by at least two
527 different loci (de Boer et al., 2012, 2008). The sl-CSD system was discarded for *C. flavipes* and *C.*
528 *sesamiae* (Niyibigira et al., 2004), the closest relative to *C. typhae*. The two strains of *C. typhae*
529 studied here have been reared for more than 80 generations in laboratory conditions starting from
530 an isofemale line, using full-sisters mated with their brothers to generate each generation. Each new
531 generation has subsequently been produced from at most 20 different females, leading to highly
532 inbred strains. Despite this, the sex-ratio in the rearing strains remains female biased, indicating that
533 either several CSD loci or another mechanism, such as genome imprinting, are involved in sex
534 determination. Whatever the process responsible for sexual identity, it is less sensitive to
535 homozygosity increase than sl-CSD and should induce a lower selective pressure on recombination
536 rate than observed in *Apis mellifera capensis* or the ant *Wasmannia auropunctata*.

537 The bacterial origin of thelytoky in *C. typhae* could not be either confirmed or completely discarded
538 in the present study as an intermediate state (in terms of frequency of parthenogenesis) was
539 observed following antibiotic treatment. The knowledge of the genetic mechanism could give some
540 clues about the origin of parthenogenesis as endosymbionts have been mainly shown to favour
541 gamete duplication. However, detailing specific interactions reveals a more complex picture.
542 *Cardinium* is able to feminize diploid males (Giorgini et al., 2009) but also to induce automixis with
543 central fusion (Zchori-Fein and Perlman, 2004). *Wolbachia* is mainly known to induce gamete
544 duplication (Leach et al., 2009; Ma and Schwander, 2017) but it has also been described to promote
545 apomixis (Weeks and Breeuwer, 2001). *Rickettsia* has also been shown to trigger functional apomixis
546 (Adachi-Hagimori et al., 2008). Furthermore, the list of endosymbionts is probably partial and in most
547 of the documented examples of parthenogenesis endosymbiotically determined, the cytological
548 mechanism remains unknown. Evidence that microorganisms can promote all processes of
549 parthenogenesis will probably arise from future research. The examples of demonstrated genetic
550 determinism of thelytoky are rare and only concern automixis with central fusion. This is the case for
551 the Cape honeybee (Verma and Ruttner, 1983) and for the wasp *Venturia canescens* (Beukeboom
552 and Pijnacker, 2000). However, Tsutsui et al. (2014) described an apomixis mechanism in the

553 parasitoid wasp *Meteorus pulchricornis* for which they proposed a genetic origin of thelytoky. Even
554 more than for endosymbiont origin, genetic determinism of parthenogenesis requires thorough
555 investigations to determine whether it is restricted to a few cytological mechanisms. Anyway, the
556 clearly evidenced mechanism of autoximis with central fusion in *C. typhae* does not allow to settle
557 between genetic and endosymbiont origin as this mechanism is common to both situations.

558

559 **Conclusion**

560 In this study, we described an unusual example of low frequency thelytokous reproduction within a
561 sexually reproducing species. We showed that, despite its scarcity, this phenomenon is biologically
562 significant, occurring in a laboratory strain but also in natural populations, and concerning virgin but
563 also mated females that produced mixed progenies composed of arrhenotokous males, rare
564 parthenogenetic females and mostly sexually determined females. Two distinct mechanisms were
565 described to produce parthenogenetic females, apomixis and automixis with central fusion. This
566 unfixed mechanism associated with the low frequency of the phenomenon could reflect an
567 accidental process, but may also be consistent with an emerging and evolving property of this
568 parasitoid species.

569 The results concerning the origin (genetic or endosymbiotic) need to be consolidated, notably
570 through the inspection of ovaries and search for the presence of microorganisms. Those further
571 investigations would benefit from an increased frequency of thelytoky, therefore we will try in the
572 future to select strains showing higher frequency of asexual reproduction.

573

574 **Acknowledgments**

575 The present work has benefited from the I2BC Cytometry platform (Université Paris-Saclay, CEA,
576 CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France) with the help
577 of Mickaël Bourge. We thank Rémi Jeannette and Sylvie Nortier for insect rearing at Gif, and Daniel
578 Couch for proofreading.

579 This work was supported by the French National Research Agency (project Cotebio ANR-17-CE32-
580 0015), and by the authors' operating grants from IRD, CNRS and *icipe*. R. Benoist was funded by the «
581 Ecole doctorale 227 MNHN-UPMC Sciences de la Nature et de l'Homme: évolution et écologie ».

582 All experimentations were realized under the juridical frame of a Material Transfer Agreement signed
583 between IRD, icipe and CNRS (CNRS 072057/IRD 302227/00) and the authorization to import Cotesia
584 in France delivered by the DRIAAF of Ile de France (IDF 2017-OI-26-032)

585

586 **Data availability**

587 Raw data for Figure 2, and Tables 1, 3 and 4 are available at https://zenodo.org/record/5785373

588

589 **References**

590 Adachi-Hagimori, T., Miura, K., Stouthamer, R., 2008. A new cytogenetic mechanism for bacterial
591 endosymbiont-induced parthenogenesis in Hymenoptera. Proc. R. Soc. B. 275, 2667–2673.
592 https://doi.org/10.1098/rspb.2008.0792

593 Ball, S.L., 2001. Tychoparthenogenesis and mixed mating in natural populations of the mayfly
594 Stenonema femoratum. Heredity 87, 373–380.

595 Baudry, E., Kryger, P., Allsopp, M., Koeniger, N., Vautrin, D., Mougel, F., Cornuet, J.M., Solignac, M.,
596 2004. Whole-Genome Scan in Thelytokous-Laying Workers of the Cape Honeybee (Apis mellifera
597 capensis): Central Fusion, Reduced Recombination Rates and Centromere Mapping Using Half-Tetrad
598 Analysis. Genetics 252, 243–252.

599 Benoist, R., Capdevielle-Dulac, C., Chantre, C., Jeannette, R., Calatayud, P., Drezen, J., Dupas, S., Le
600 Rouzic, A., Le Ru, B., Moreau, L., Van Dijk, E., Kaiser, L., Mougel, F., 2020a. Quantitative trait loci
601 involved in the reproductive success of a parasitoid wasp. Mol Ecol 29, 3476–3493.
602 https://doi.org/10.1111/mec.15567

603 Benoist, R., Chantre, C., Capdevielle-Dulac, C., Bodet, M., Mougel, F., Calatayud, P.A., Dupas, S.,
604 Huguet, E., Jeannette, R., Obonyo, J., Odorico, C., Silvain, J.F., Le Ru, B., Kaiser, L., 2017. Relationship
605 between oviposition, virulence gene expression and parasitism success in Cotesia typhae nov. sp.
606 parasitoid strains. Genetica 145, 469–479. https://doi.org/10.1007/s10709-017-9987-5

607 Benoist, R., Paquet, S., Decourcelle, F., Guez, J., Jeannette, R., Calatayud, P.-A., Le Ru, B., Mougel, F.,
608 Kaiser, L., 2020b. Role of egg-laying behavior, virulence and local adaptation in a parasitoid's chances
609 of reproducing in a new host. Journal of Insect Physiology 120, 103987.
610 https://doi.org/10.1016/j.jinsphys.2019.103987

611 Beukeboom, L.W., Pijnacker, L.P., 2000. Automictic parthenogenesis in the parasitoid Venturia
612 canescens (Hymenoptera: Ichneumonidae) revisited 43, 6.

613 Boivin, G., 2013. Sperm as a limiting factor in mating success in Hymenoptera parasitoids. Entomol
614 Exp Appl 146, 149–155. https://doi.org/10.1111/j.1570-7458.2012.01291.x

615 Bourge, M., Brown, S.C., Siljak-Yakovlev, S., 2018. Flow cytometry as tool in plant sciences, with
616 emphasis on genome size and ploidy level assessment. Genetics & Applications 2, 1–12.

617 Branca, A., Le Ru, B., Calatayud, P.-A., Obonyo, J., Musyoka, B., Capdevielle-Dulac, C., Kaiser-Arnauld,
618 L., Silvain, J.-F., Gauthier, J., Paillusson, C., Gayral, P., Herniou, E.A., Dupas, S., 2019. Relative
619 Influence of Host, Wolbachia, Geography and Climate on the Genetic Structure of the Sub-saharan
620 Parasitic Wasp Cotesia sesamiae. Front. Ecol. Evol. 7, 309. https://doi.org/10.3389/fevo.2019.00309

621 Casiraghi, M., Bordenstein, S.R., Baldo, L., Lo, N., Beninati, T., Wernegreen, J.J., Werren, J.H., Bandi,
622 C., 2005. Phylogeny of Wolbachia pipientis based on gltA, groEL and ftsZ gene sequences: clustering
623 of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the
624 Wolbachia tree. Microbiology 151, 4015–4022. https://doi.org/10.1099/mic.0.28313-0

625 Chapman, N.C., Beekman, M., Allsopp, M.H., Rinderer, T.E., Lim, J., Oxley, P.R., Oldroyd, B.P., 2015.
626 Inheritance of thelytoky in the honey bee Apis mellifera capensis. Heredity 114, 584–592.
627 https://doi.org/10.1038/hdy.2014.127

628 Collet, M., Amat, I., Sauzet, S., Auguste, A., Fauvergue, X., Mouton, L., Desouhant, E., 2020. Insects
629 and incest: Sib-mating tolerance in natural populations of a parasitoid wasp. Mol Ecol 29, 596–609.
630 https://doi.org/10.1111/mec.15340

631 de Boer, J.G., Groenen, M.A., Pannebakker, B.A., Beukeboom, L.W., Kraus, R.H., 2015. Population-
632 level consequences of complementary sex determination in a solitary parasitoid. BMC Evol Biol 15,
633 98. https://doi.org/10.1186/s12862-015-0340-2

634 de Boer, J.G., Kuijper, B., Heimpel, G.E., Beukeboom, L.W., 2012. Sex determination meltdown upon
635 biological control introduction of the parasitoid *Cotesia rubecula?* Evol Appl 5, 444–454.
636 https://doi.org/10.1111/j.1752-4571.2012.00270.x

637 de Boer, J.G., Ode, P.J., Rendahl, A.K., Vet, L.E.M., Whitfield, J.B., Heimpel, G.E., 2008. Experimental
638 support for multiple-locus complementary sex determination in the parasitoid Cotesia vestalis.
639 Genetics 180, 1525–35. https://doi.org/10.1534/genetics.107.083907

640 Dinardo-Miranda, L.L., Fracasso, J.V., Costa, V.P. da, Lopes, D.O.T., 2014. Dispersal of Cotesia flavipes
641 in sugarcane field and implications for parasitoid releases. Bragantia 73, 163–170.
642 https://doi.org/10.1590/brag.2014.023

643 Dwight, Z.L., Palais, R., Wittwer, C.T., 2012. uAnalyze: Web-Based High-Resolution DNA Melting
644 Analysis with Comparison to Thermodynamic Predictions. IEEE/ACM Trans. Comput. Biol. and Bioinf.
645 9, 1805–1811. https://doi.org/10.1109/TCBB.2012.112

646 Foray, V., Helene, H., Martinez, S., Gibert, P., Desouhant, E., 2013. Occurrence of arrhenotoky and
647 thelytoky in a parasitic wasp Venturia canescens (Hymenoptera: Ichneumonidae): Effect of
648 endosymbionts or existence of two distinct reproductive modes? Eur. J. Entomol. 110, 103–107.
649 https://doi.org/10.14411/eje.2013.014

650 Giorgini, M., Monti, M.M., Caprio, E., Stouthamer, R., Hunter, M.S., 2009. Feminization and the
651 collapse of haplodiploidy in an asexual parasitoid wasp harboring the bacterial symbiont Cardinium.
652 Heredity 102, 365–371. https://doi.org/10.1038/hdy.2008.135

653 Gokhman, V.E., Kuznetsova, V.G., 2018. Parthenogenesis in Hexapoda: holometabolous insects. J
654 Zool Syst Evol Res 56, 23–34. https://doi.org/10.1111/jzs.12183

655 Gu, H., Dorn, S., 2003. Mating system and sex allocation in the gregarious parasitoid Cotesia
656 glomerata. Animal Behaviour 66, 259–264. https://doi.org/10.1006/anbe.2003.2185

657 Heimpel, G.E., de Boer, J.G., 2008. Sex determination in the hymenoptera. Annual review of
658 entomology 53, 209–30. https://doi.org/10.1146/annurev.ento.53.103106.093441

659 Jarosch, A., Stolle, E., Crewe, R.M., Moritz, R.F.A., 2011. Alternative splicing of a single transcription
660 factor drives selfish reproductive behavior in honeybee workers (Apis mellifera). Proceedings of the
661 National Academy of Sciences 108, 15282–15287. https://doi.org/10.1073/pnas.1109343108

662 Kaiser, L., Fernandez-Triana, J., Capdevielle-Dulac, C., Chantre, C., Bodet, M., Kaoula, F., Benoist, R.,
663 Calatayud, P.-A., Dupas, S., Herniou, E.A., Jeannette, R., Obonyo, J., Silvain, J.-F., Le Ru, B., 2017.
664 Systematics and biology of Cotesia typhae sp. n. (Hymenoptera, Braconidae, Microgastrinae), a
665 potential biological control agent against the noctuid Mediterranean corn borer, Sesamia
666 nonagrioides. ZK 682, 105–136. https://doi.org/10.3897/zookeys.682.13016

667 Kaiser, L., Le Ru, B.P., Kaoula, F., Paillusson, C., Capdevielle-Dulac, C., Obonyo, J.O., Herniou, E.A.,
668 Jancek, S., Branca, A., Calatayud, P., Silvain, J., Dupas, S., 2015. Ongoing ecological speciation in
669 *Cotesia sesamiae* , a biological control agent of cereal stem borers. Evol Appl 8, 807–820.
670 https://doi.org/10.1111/eva.12260

Lattorff, H.M.G., Moritz, R.F.A., Fuchs, S., 2005. A single locus determines thelytokous parthenogenesis of laying honeybee workers (Apis mellifera capensis). Heredity 94, 533–537. https://doi.org/10.1038/sj.hdy.6800654

Leach, I.M., Pannebakker, B.A., Schneider, M.V., Driessen, G., van de Zande, L., Beukeboom, L.W., 2009. Thelytoky in Hymenoptera with Venturia canescens and Leptopilina clavipes as Case Studies, in: Schön, I., Martens, K., Dijk, P. (Eds.), Lost Sex. Springer Netherlands, Dordrecht, pp. 347–375. https://doi.org/10.1007/978-90-481-2770-2_17

Liegeois, M., Sartori, M., Schwander, T., 2021. Extremely Widespread Parthenogenesis and a Trade-Off Between Alternative Forms of Reproduction in Mayflies (Ephemeroptera). Journal of Heredity 112, 45–57. https://doi.org/10.1093/jhered/esaa027

Little, C.J., Chapuis, M.-P., Blondin, L., Chapuis, E., Jourdan-Pineau, H., 2017. Exploring the relationship between tychoparthenogenesis and inbreeding depression in the Desert Locust, *Schistocerca gregaria*. Ecol Evol 7, 6003–6011. https://doi.org/10.1002/ece3.3103

Liu, Q., Zhou, J., Zhang, C., Ning, S., Duan, L., Dong, H., 2019. Co-occurrence of thelytokous and bisexual Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae) in a natural population. Sci Rep 9, 17480. https://doi.org/10.1038/s41598-019-53992-8

Ma, W.-J., Schwander, T., 2017. Patterns and mechanisms in instances of endosymbiont-induced parthenogenesis. J. Evol. Biol. 30, 868–888. https://doi.org/10.1111/jeb.13069

Meirmans, S., Meirmans, P.G., Kirkendall, L.R., 2012. The Costs Of Sex: Facing Real-world Complexities. The Quarterly Review of Biology 87, 19–40. https://doi.org/10.1086/663945

Mochiah, M.B., Ngi-Song, A.J., Overholt, W.A., Stouthamer, R., 2002. Wolbachia infection in Cotesia sesamiae (Hymenoptera: Braconidae) causes cytoplasmic incompatibility: implications for biological control. Biological Control 25, 74–80. https://doi.org/10.1016/S1049-9644(02)00045-2

Morgan-Richards, M., Trewick, S.A., 2005. Hybrid origin of a parthenogenetic genus? Molecular Ecology 14, 2133–2142. https://doi.org/10.1111/j.1365-294X.2005.02575.x

Neiman, M., Sharbel, T.F., Schwander, T., 2014. Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. J. Evol. Biol. 27, 1346–1359. https://doi.org/10.1111/jeb.12357

Niyibigira, E.I., Overholt, W.A., Stouthamer, R., 2004. Cotesia flavipes Cameron (Hymenoptera: Braconidae) does not exhibit complementary sex determination (ii) Evidence from laboratory experiments. Appl. Entomol. Zool. 39, 717–725. https://doi.org/10.1303/aez.2004.717

Otto, S.P., 2009. The Evolutionary Enigma of Sex. The American Naturalist 174, S1–S14. https://doi.org/10.1086/599084

Pardo, M.C., López-León, M.D., Cabrero, J., Camacho, J.P.M., 1995. Cytological and developmental analysis of tychoparthenogenesis in Locusta migratoria. Heredity 75, 485–494. https://doi.org/10.1038/hdy.1995.165

Pijls, J.W.A.M., van Steenbergen, H.J., van Alphen, J.J.M., 1996. Asexuality cured: the relations and differences between sexual and asexual Apoanagyrus diversicornis. Heredity 76, 506–513. https://doi.org/10.1038/hdy.1996.73

710 Rabeling, C., Kronauer, D.J.C., 2013. Thelytokous Parthenogenesis in Eusocial Hymenoptera. Annu.
711 Rev. Entomol. 58, 273–292. https://doi.org/10.1146/annurev-ento-120811-153710

712 Rey, O., Loiseau, A., Facon, B., Foucaud, J., Orivel, J., Cornuet, J.-M., Robert, S., Dobigny, G., Delabie,
713 J.H.C., Mariano, C.D.S.F., Estoup, A., 2011. Meiotic Recombination Dramatically Decreased in
714 Thelytokous Queens of the Little Fire Ant and Their Sexually Produced Workers. Molecular Biology
715 and Evolution 28, 2591–2601. https://doi.org/10.1093/molbev/msr082

716 Sallam, M.N., Overholt, W.A., Kairu, E., 2001. Dispersal of the exotic parasitoid Cotesia flavipes in a
717 new ecosystem. Entomologia Experimentalis et Applicata 98, 211–217.
718 https://doi.org/10.1046/j.1570-7458.2001.00776.x

719 Sandrock, C., Vorburger, C., 2011. Single-Locus Recessive Inheritance of Asexual Reproduction in a
720 Parasitoid Wasp. Current Biology 21, 433–437. https://doi.org/10.1016/j.cub.2011.01.070

721 Schneider, M.V., Beukeboom, L.W., Driessen, G., Lapchin, L., Bernstein, C., Van Alphen, J.J.M., 2002.
722 Geographical distribution and genetic relatedness of sympatrical thelytokous and arrhenotokous
723 populations of the parasitoid Venturia canescens (Hymenoptera): Thelytoky and arrhenotoky in
724 Venturia canescens. Journal of Evolutionary Biology 15, 191–200. https://doi.org/10.1046/j.1420-
725 9101.2002.00394.x

726 Stouthamer, R., Pinto, J.D., Platner, G.R., Luck, R.F., 1990. Taxonomic Status of Thelytokous Forms of
727 Trichogramma (Hymenoptera: Trichogrammatidae). Annals of the Entomological Society of America
728 83, 475–481. https://doi.org/10.1093/aesa/83.3.475

729 The Tree of Sex Consortium, 2014. Tree of Sex: A database of sexual systems. Sci Data 1, 140015.
730 https://doi.org/10.1038/sdata.2014.15

731 Tsutsui, Y., Maeto, K., Hamaguchi, K., Isaki, Y., Takami, Y., Naito, T., Miura, K., 2014. Apomictic
732 parthenogenesis in a parasitoid wasp *Meteorus pulchricornis* , uncommon in the haplodiploid order
733 Hymenoptera. Bull. Entomol. Res. 104, 307–313. https://doi.org/10.1017/S0007485314000017

734 Tvedte, E.S., Logsdon, J.M., Forbes, A.A., 2019. Sex loss in insects: causes of asexuality and
735 consequences for genomes. Current Opinion in Insect Science 31, 77–83.
736 https://doi.org/10.1016/j.cois.2018.11.007

737 van de Zande, L., Verhulst, E.C., 2014. Genomic Imprinting and Maternal Effect Genes in Haplodiploid
738 Sex Determination. Sex Dev 8, 74–82. https://doi.org/10.1159/000357146

739 van der Kooi, C.J., Matthey-Doret, C., Schwander, T., 2017. Evolution and comparative ecology of
740 parthenogenesis in haplodiploid arthropods. Evolution Letters 1, 304–316.
741 https://doi.org/10.1002/evl3.30

742 van Wilgenburg, E., Driessen, G., Beukeboom, L., 2006. Single locus complementary sex
743 determination in Hymenoptera: an “unintelligent” design? Front Zool 3, 1.
744 https://doi.org/10.1186/1742-9994-3-1

745 van der Kooi, C.J., Schwander, T., 2015. Parthenogenesis: Birth of a New Lineage or Reproductive
746 Accident? Current Biology 25, R659–R661. https://doi.org/10.1016/j.cub.2015.06.055

747 Verma, S., Ruttner, F., 1983. Cytological analysis of the thelytokous parthenogenesis in the Cape
748 honeybee (Apis mellifera capensis Escholtz). Apidologie 14, 41–57.
749 https://doi.org/10.1051/apido:19830104

750 Vershinina, A.O., Kuznetsova, V.G., 2016. Parthenogenesis in Hexapoda: Entognatha and non-
751 holometabolous insects. J Zoolog Syst Evol Res 54, 257–268. https://doi.org/10.1111/jzs.12141

752 Vorburger, C., 2014. Thelytoky and Sex Determination in the Hymenoptera: Mutual Constraints. Sex
753 Dev 8, 50–58. https://doi.org/10.1159/000356508

754 Weeks, A.R., Breeuwer, J.A.J., 2001. *Wolbachia* –induced parthenogenesis in a genus of
755 phytophagous mites. Proc. R. Soc. Lond. B 268, 2245–2251. https://doi.org/10.1098/rspb.2001.1797

756 Whiting, P.W., 1945. The Evolution of Male Haploidy. The Quarterly Review of Biology 20, 231–260.
757 https://doi.org/10.1086/394884

758 Zchori-Fein, E., Perlman, S.J., 2004. Distribution of the bacterial symbiont Cardinium in arthropods:
759 Molecular Ecology 13, 2009–2016. https://doi.org/10.1111/j.1365-294X.2004.02203.x

760 Zhou, Y., Gu, H., Dorn, S., 2007. Effects of inbreeding on fitness components of Cotesia glomerata, a
761 parasitoid wasp with single-locus complementary sex determination (sl-CSD). Biological Control 40,
762 273–279. https://doi.org/10.1016/j.biocontrol.2006.11.002

763 Zhou, Y., Gu, H., Dorn, S., 2006. Single-locus sex determination in the parasitoid wasp Cotesia
764 glomerata (Hymenoptera: Braconidae). Heredity 96, 487–92. https://doi.org/10.1038/sj.hdy.6800829

765