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1 **Investigating bracovirus chromosomal integration and inheritance in**
2 **lepidopteran host and non-target species**

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18

19 **Abstract**

20 Bracoviruses (BVs) are domesticated viruses found in braconid parasitoid wasp genomes. They
21 are composed of domesticated genes from a nudivirius, coding viral particles in which wasp
22 DNA circles are packaged. BVs are viewed as possible vectors of horizontal transfer of genetic
23 material (HT) from wasp to their hosts because they are injected, together with wasp eggs, by
24 female wasps in their host larvae, and because they undergo massive chromosomal integration
25 in multiple host tissues. Here, we show that chromosomal integrations of the *Cotesia typhae*
26 BV (CtBV) persist up to the adult stage in individuals of its natural host, *S. nonagrioides*, that
27 survived parasitism. However, while reproducing host adults can bear an average of nearly two
28 CtBV integrations per haploid genome, we were unable to retrieve any of these integrations in
29 500 of their offspring using Illumina sequencing. This suggests either that host gametes are
30 less targeted by CtBVs than somatic cells or that gametes bearing BV integrations are non-
31 functional. We further show that CtBV can massively integrate into the chromosomes of other
32 lepidopteran species that are not normally targeted by the wasp in the wild, including one
33 which is at least 100 million years divergent from the natural host. Cell entry and chromosomal
34 integration of BVs are thus unlikely to be major factors shaping wasp host range. Together, our
35 results shed new light on the conditions under which BV-mediated wasp-to-host HT may occur
36 and provide information that may be helpful to evaluate the potential risks of uncontrolled HT
37 associated with the use of parasitoid wasps as biocontrol agents.

38 **Introduction**

39 Horizontal transfer (HT) of genetic material involves transmission of DNA by means other than
40 reproduction (Keeling & Palmer, 2008). Widespread in prokaryotes, HT is also increasingly
41 recognized as an important evolutionary process in eukaryotes (Aubin et al., 2021; Boto, 2014;
42 Van Etten & Bhattacharya, 2020). Many studies report remarkable examples of HT of genes
43 with important consequences in multiple lineages (Danchin et al., 2016; Simion et al., 2021;
44 Wybouw et al., 2016; Xia et al., 2021; Li et al. 2022). In addition, large scale studies of
45 transposable elements (TEs) inferred dozens to thousands of horizontal transfers of
46 transposable elements in various multicellular eukaryotes such as plants (Baidouri et al.,
47 2014), insects (Peccoud et al., 2017; Heringer et al. 2022; Wallau et al. 2016), and vertebrates
48 (Zhang et al., 2020). Given the profound impact TEs have on genome evolution, HT of TEs can
49 be viewed as a key process shaping eukaryote genomes (Gilbert & Feschotte, 2018).
50 Contrasting with our detailed knowledge of HT in prokaryotes, the mechanisms and possible
51 vectors of HT remain elusive in eukaryotes. The finding that viruses and extracellular vesicles
52 can carry host genetic material suggests that they may accidentally act as vectors of HT (Gilbert
53 & Cordaux, 2017; Ono et al., 2019). Here we further evaluate the conditions and frequency at
54 which a specific type of viruses, the bracoviruses (BVs) of Braconidae endoparasitoid wasps
55 (Hymenoptera), may mediate HT between wasps and lepidopterans.

56 Braconidae endoparasitoid wasps lay their eggs in insect hosts, mostly lepidopterans (see the
57 picture of Fig. 1 as an example), which are used by wasp larvae as substrate and food source.
58 In addition to their eggs, females of many Braconidae wasp species inject BVs into their host,
59 which interfere with host developmental and immunological pathways, facilitating the
60 development of wasp embryos (Beckage & Drezen, 2011; Strand & Burke, 2014). Genes

61 encoding structural components of these viruses originate from domestication of viral genes
62 that became integrated into the genome of braconid wasp ancestors about 100 million years
63 ago (labeled 1c in Fig. 1) (Gauthier et al., 2018; Herniou et al., 2013). Bracovirus structural
64 genes, of nudiviral origin, are specifically expressed in the calyx of the ovaries, resulting in the
65 production of viral particles into which circular double stranded DNA molecules are packaged
66 (labeled 2 in Fig. 1). These packaged DNA circles result from amplification, excision and
67 circularization of so-called “proviral segments” (labeled 1a and 1b in Fig. 1), which contain
68 wasp genes and other genes of unknown evolutionary origin (Bézier et al., 2013; Drezen et al.,
69 2014; Herniou et al., 2013). Circularization takes place in calyx cells *via* site-specific
70 recombination at Direct Repeat Junction (DRJ) motifs, which are conserved between proviral
71 segments and between Braconidae species (Beck et al., 2011; Desjardins et al., 2008; Gauthier
72 et al., 2021; Gruber et al., 1996; Muller, Chebbi, et al., 2021; Pasquier-Barre et al., 2002; Savary
73 et al., 1997). A remarkable feature of BVs is that once released in host’s cells, DNA circles
74 containing a specific motif called Host Integration Motif (HIM) integrate massively into the host
75 genome (labeled 3 in Fig. 1) (Beck et al., 2011; Chevignon et al., 2018). We use the term “HIM-
76 containing circles” to refer to these circles and we call other circles, which do not contain HIM
77 “circles devoid of HIM”. Integration involves site-specific recombination between HIMs and the
78 host genome, likely mediated by BV-encoded enzymes. In the wasp *Cotesia typhae*, it was
79 shown that the 16 HIM-containing circles (out of 27 circles) undergo chromosomal integration
80 in at least four host tissues/organs (hemocytes, fat body, ganglionic chain, and head), yielding
81 an average of 12 to 85 integrated circles per host haploid genome (Muller, Chebbi, et al., 2021).
82 Integration involves linearization of DNA circles through two DNA double strand breaks within
83 HIMs and loss of a small \approx 50-bp region lying between the two breaks (labeled 3 in Fig. 1). The

84 role played by BV DNA circles integration in wasp-host interactions remains to be
85 characterized. So far, it appears that integration does not enhance expression of circle-borne
86 genes (Chevignon et al., 2014, 2018), nor does it enhance persistence of DNA circles
87 throughout wasp development (Muller, Chebbi, et al., 2021).

88 Systematic and massive DNA circle integration in host genomes during parasitism may
89 facilitate wasp-to-host HT with long-term consequences. Some of BV circle sequences have
90 been found in various lepidopteran genomes that clearly result from HT from wasp to
91 lepidopterans (Gasmi et al., 2015; Schneider & Thomas, 2014). Remarkably, some BV genes
92 acquired by *Spodoptera* species have been domesticated and now play a role in anti-bacterial
93 immune response (Di Lelio et al., 2019; Gasmi et al., 2015). Thus, BV DNA circles can integrate
94 into the genome of the host germline and some host individuals surviving parasitism can
95 transmit these integrations to their offspring (Drezen et al., 2017). These studies also
96 demonstrated that horizontally transferred BV genes can be an important source of genetic
97 novelty and adaptation in lepidopterans.

98 Integration in the host germline is consistent with the known tropism of BV particles for a large
99 range of tissues (Beck et al., 2011; Wyder et al., 2003) and the capacity of BV DNA circles to
100 integrate in the genome of various cell types (Muller, Chebbi, et al., 2021), although at different
101 levels depending on the species and tissues. Survival of parasitized lepidopterans is a rather
102 common phenomenon in nature, as the success of endoparasitoid wasps' development often
103 strongly varies depending on the geographical origin of host populations (Gitau et al., 2007).
104 To which extent resistant hosts retain BV DNA integrated in their genome at the adult stage,
105 and in which proportion these wasp sequences can be transmitted vertically to the next host

106 generation has never been investigated. Yet, given that some endoparasitoid wasps are used
107 as biocontrol agents (Polaszek & Walker, 1992), measuring the frequency of such possible HT
108 is relevant not only from a basic science point of view, but also to evaluate the risks of
109 uncontrolled HT potentially associated with strategies that use exotic wasps bearing BV.

110 Another possible way chromosomally integrated BV circles may be vertically transmitted in
111 host populations is through accidental parasitism of a non-permissive host species, in which
112 by definition parasitism fails. In laboratory conditions, endoparasitoid wasps are indeed able
113 to lay their eggs into species not known to be targeted in the wild, with varying degrees of
114 success (Harwood et al., 1998). Whether BV DNA circle integration occurs in non-natural hosts,
115 supporting such hosts as possible facilitators of wasp-to-host HT of BV DNA, has never been
116 investigated so far.

117 In this study, we first evaluate the frequency under which integration of BV DNA circles in host
118 germline followed by transmission to the next generation may occur in the wasp-host system
119 involving the wasp *Cotesia typhae* and its host, the Mediterranean corn borer *Sesamia*
120 *nonagrioides* (Lepidoptera: Noctuidae). *S. nonagrioides* is a major pest of corn in
121 Mediterranean regions (Bosque-Perez & Schulthess, 1998; Kankonda et al., 2018; Moyal et al.,
122 2011). *C. typhae* (Fernandez-Triana) (Hymenoptera: Braconidae) is a newly described species
123 closely related to *Cotesia sesamiae* found in east Africa (Kaiser et al., 2015, 2017). The two
124 wasp species are reproductively isolated and although the former is a generalist
125 endoparasitoid, *C. typhae* is a specialist at both host (*S. nonagrioides*) and plant (*Typha*
126 *domingensis*) levels. Due to such a specialization, in addition to inducing a high host death rate
127 during parasitism, *C. typhae* is currently under study as a potential biological control agent

128 against *S. nonagrioides* in Europe (Kaiser et al., 2015, 2017). We then assessed whether *C.*
129 *typhae* BV DNA circles integrate into the genome of three other stemborer lepidopteran
130 species: two Noctuidae (*Globia sparganii* and *Nonagria typhae*) and one Crambidae (*Chilo*
131 *phragmitella*). These species are normally not targeted in the wild by *C. typhae* but since their
132 distribution overlaps with that of *S. nonagrioides* in France and they share similar ecological
133 niches (Galichet et al., 1992; Teder & Tammaru, 2002; Tewksbury et al., 2002), they might
134 suffer non-target effects during a putative biocontrol campaign, the consequences of which
135 are important to assess (Louda et al., 2003; Sands & Driesche, 2000).

136 **Materials and methods**

137 **Parasitism and sequencing of 500 *S. nonagrioides* larvae and two adults**

138 Sixty-two French *S. nonagrioides* larvae at the L4/L5 stage were each parasitized by a different
139 *C. typhae* female. *S. nonagrioides* larvae used for this experiment came either from the wild
140 (collected in France at Sorbets and Coublucq) or from a strain reared at EGCE since 2010 which
141 initially comes from the southwest of France and which is refreshed yearly with French
142 individuals from the wild. In order to maximize the number of resistant *S. nonagrioides*, we
143 chose to parasitize them with the *C. typhae* strain “Makindu”, which shows low virulence on
144 French *S. nonagrioides* larvae, with about 60% of individuals resisting to parasitism (Benoist et
145 al., 2017). This “Makindu” wasp strain is reared at EGCE since 2015, but it initially comes from
146 10 individuals who emerged from *S. nonagrioides* collected in 2010-2011 from the wild in the
147 Makindu locality (2.278S, 37.825E; South-East Kenya). An isofemale line was then produced
148 and maintained by parasiting at each generation three-weeks-old larvae from the Makindu *S.*

149 *nonagrioides* strain, on which the wasp virulence is higher than on the french strain used for
150 the experiments of this article (Benoist et al., 2020).

151 Oviposition events were all confirmed by visual observation. Here, 37 larvae (59.7%) resisted
152 to parasitism, *i.e.* no wasp pupae emerged from them and they reached adult stage. We
153 obtained a total of 20 males and 17 females, but because of important delays in the
154 development of some individuals, we were able to form 15 couples only. A total of 13 females
155 involved in these couples produced some eggs (sometimes less than 10 eggs), but most of
156 these eggs were either unfertilized or non-viable. Numerous and viable eggs leading to the
157 development of offspring could be recovered for only five females (Suppl. Fig. 1). The names
158 of these F1 offspring refer to their mothers: F1-Adult1, F1-Adult2, F1-Adult3, F1-Adult4, and
159 F1-Adult5. We let all these five progenies develop until the larval stage L3/L4 and we sacrificed
160 them by freezing at -80°C. We then randomly picked a total of 500 caterpillars for the
161 experiment. For technical reasons (high mortality of eggs in some progenies because of mold
162 in our rearing tubes), we obtained less than 100 caterpillars for some couples, impeding us to
163 sample identical numbers of individuals from each crossing. We ended up with 110 offspring
164 individuals for F1-Adult1, 80 for F1-Adult2, 115 for F1-Adult3, 70 for F1-Adult4, and 125 for F1-
165 Adult5 (Suppl. Fig. 1).

166 A piece from each of these caterpillars was then cut with a razor blade and weighed. Pieces of
167 equal weight were pooled five by five for each set of offspring, *i.e.* 100 pools of 5 offspring.
168 The rest of the bodies were individually frozen at -80°C to be able to identify which individuals
169 would bear the eventual BV integration identified in pools of F1 (see below). We extracted
170 DNA from the 100 pools with the kit DNeasy Purification (QIAGEN). We then pooled these DNA

171 extractions 20 by 20 with equimolar ratio, leading to five libraries composed of 100 caterpillars
172 each (Suppl. Fig. 1): libraries PoolF1-A to PoolF1-E. We sub-contracted Novogen to build a
173 paired-end library (2 x 150 bp; insert size = 350 bp) for each of these five libraries. Each library
174 was then sequenced on an Illumina platform to produce a targeted amount of 100 Gbp each.
175 In addition, the DNA of two resistant *S. nonagrioides* adults was also sequenced. One of these
176 adults (Adult-1) is a female who produced offspring (progeny included in this experiment
177 under the name F1-Adult1), whereas the other (Adult-6) is a female who did not produce any
178 offspring. DNA was extracted from the thorax of these two resistant *S. nonagrioides* females,
179 using the DNeasy Purification kit (QIAGEN) after their natural death. Library construction and
180 sequencing was done by Novogen following the same protocol as the one used for the five
181 other libraries, except that the targeted number of bases produced was 50 Gbp (*i.e.*, a depth
182 of about 50X on the *S. nonagrioides* genome).

183 **Parasitism of non-target species**

184 *C. typhae* females from the strain “Kobodo” reared at EGCE since 2019, also as an isofemale
185 line but from six parasitized caterpillars collected in the field at Kobodo locality (0.679S,
186 34.412E; West Kenya), were set to parasitize two caterpillars from the bulrush wainscot
187 *Nonagria typhae* (Lepidoptera: Noctuidae), five from the Webb's wainscot *Globia sparganii*
188 (Lepidoptera: Noctuidae), and eight from the Reed Veneer *Chilo phragmitella* (Lepidoptera:
189 Crambidae). Each of these caterpillars was parasitized by a different wasp, and oviposition was
190 confirmed by visual observation. These non-natural host species were sampled in the wild in
191 different regions in France in June 2020. The caterpillars of *N. typhae* and *G. sparganii* were
192 collected in Saint Michel en l'Herm (46.354N, 1.260W), and the caterpillars of *C. phragmitella*
193 in Sacy-le-Grand (49.347N, 2.537E). *C. typhae* larvae emerged from four out of these 15

194 parasitized non-natural hosts, all from *C. phragmitella*. Irrespective of whether parasitism was
195 successful or not, all parasitized individuals died between five and 26 days post parasitism, all
196 at late larval or pre-pupal stage. Using the kit NucleoBond AXG20 (Macherey-Nagel), we
197 extracted DNA from the whole body of three larvae, from which no wasp larvae emerged: one
198 *N. typhae* larva, one *G. sparganii* larva, and one *C. phragmitella*, which died 17, 19, and 25
199 days post-parasitism, respectively. We sub-contracted Novogen to build a paired-end library
200 (2 x 150 bp; insert size = 350 bp) for these three samples for sequencing on an Illumina
201 platform to produce a targeted amount of 100 Gb each.

202 **Genome assemblies**

203 The genome of *C. typhae* and *S. nonagrioides* were retrieved from GenBank under accession
204 numbers JAAOIC0000000000 (Muller, Chebbi, et al., 2021) and JADWQK0000000000 (Muller,
205 Ogereau et al., 2021). The genomes of *N. typhae*, *G. sparganii*, and *C. phragmitella* were *de*
206 *novo* assembled for this study with the following procedure: (i) raw Illumina reads produced
207 from parasitized larvae were mapped against the *C. typhae* genome with Bowtie2 in order to
208 remove all reads from *C. typhae*, (ii) the filtered reads were assembled with MaSuRCA v4.0.1
209 with all parameters set to default, except for jellyfish hash size (JF_SIZE = 20000000000), and
210 we activated USE_LINKING_MATES since we did not use any long-reads, (iii) any trace of *C.*
211 *typhae* genome remaining in the assembly were identified by similarity search (blastn)
212 between the newly assembled genomes and *C. typhae* genome. We filtered out from the
213 assemblies all contigs with more than 99% identity with a *C. typhae* contig on more than 90%
214 of its length. This last step led to remove four contigs from *N. typhae*, none from *G. sparganii*,
215 and 24 from *C. phragmitella*. We also ran purge_dups on *C. phragmitella*, whose assembly

216 initially harbored 9.5% of duplicated BUSCO (Guan et al., 2020). We assessed the quality of
217 these three new genomes with Busco v5.0.0 using 5286 lepidopteran core genes.

218 **Sequencing data processing**

219 To search for the presence of BV sequences integrated into *G. sparganii*, *N. typhae*, *C.*
220 *phragmitella* and *S. nonagrioides* genomes, we developed the workflow WorkflowBowBlast
221 (github/HeloiseMuller/WorkflowBowBlast). This workflow proceeds all the data the same way
222 as described in Muller, Chebbi, et al. (2021), in which we characterized and quantified the
223 genome-wide patterns of *C. typhae* BV (CtBV) integration in various tissues of parasitized *S.*
224 *nonagrioides*. We activated all the tools of the workflow (integrity with fastqc v.0.11.8,
225 trimmomatic v.0.38, bowtie2 v2-2.3.4.2, coverage with bedtools v.2.26.0 and blastn v.2.6.0)
226 with all default parameters. We used the appropriate lepidopteran genomes (*S. nonagrioides*,
227 *N. typhae*, *G. sparganii*, or *C. phragmitella*) for the first reference genome and *C. typhae* for
228 the second optional genome. For the analyses, we used two outputs of WorkflowBowBlast.
229 First, we used the bedtools genomecov output of WorkflowBowBlast that indicates the
230 sequencing depth at each position of the *C. typhae* genome. We then used CovWindows
231 (github/HeloiseMuller/CovWindows) to get sequencing depth on each CtBV DNA
232 (corresponding to any of the three CtBV forms) from this file. WorkflowBowBlast also
233 automatically gives the average sequencing depth on both genomes, and the percentage of
234 the genomes covered at least once by Illumina reads. Second, we used the two blastn outputs
235 of WorkflowBowBlast, one on each genome (Lepidoptera and wasp), to identify chimeric reads
236 involving *C. typhae* proviral segments and the lepidopteran DNA using an R pipeline (Peccoud
237 et al., 2018). This pipeline, specifically adapted to find chromosomally integrated BV in Muller,
238 Chebbi, et al. (2021), identifies a read as chimeric if (i) at least 16 bases align only on the first

239 reference genome, and a minimum of 16 other bases align only on the second reference
240 genome; (ii) less than 10% of the read length maps to neither reference genome; (iii) no more
241 than 20 bases align simultaneously on both reference genomes; and (iv) no more than 5 bases
242 are inserted between the two genomes at the integration point. The two latter filters imply
243 that aligned read regions are allowed to overlap by up to 20 bp or to be separated by at most
244 5 bp. After this pipeline, we filtered out chimeric reads resulting from PCR duplicates, except
245 when comparing the number of chimeric reads to the sequencing depth, since PCR duplicates
246 are not filtered out in the latest.

247 In addition to our ten samples (five libraries of 100 pooled *S. nonagrioides* offspring, two adult
248 *S. nonagrioides* females, one *G. sparganii* larva, one *N. typhae* larva, and one *C. phragmitella*
249 larva), we also processed in the exact same way two samples that we generated in earlier
250 studies and used here for comparison: Caterpillar and Control. Both samples were sequenced
251 in the same conditions as our ten samples. Caterpillar corresponds to a *S. nonagrioides*
252 caterpillar parasitized by *C. typhae* and sequenced seven days post parasitism, for which no
253 wasp larvae were present (sample called “whole body” in the original publication (Muller,
254 Chebbi, et al., 2021)). This sample was included to compare the number of CtBV chromosomal
255 integrations in the natural host to those in the non-natural hosts. Control corresponds to the
256 Illumina trimmed reads used to assemble the genome of non-parasitized *S. nonagrioides*
257 (Muller, Ogereau, et al., 2021). We included this last sample in order to estimate the
258 proportion of reads that would map on *C. typhae* genome when *S. nonagrioides* did not
259 undergo any contact with *C. typhae*. In addition, this control also allowed us to determine that
260 our pipeline returned no false positive *S. nonagrioides*-*C. typhae* chimeras. All analyses and
261 figures were done with R v4.0.5.

262 **Estimations and normalization of integration events**

263 Integration events of CtBV DNA into host genomes were counted for each sample, as each
264 position in the host genome supported by at least one read (*i.e.*, CtBV-host junctions
265 supported by more than one read were counted as one integration event). We estimated the
266 average number of integration events per haploid genome with $IPMH * G/k$, where IMPH is the
267 normalized number of Integration events Per Million of Host reads, G is the genome size of the
268 host in megabases (1,021Mb for *S. nonagrioides*), and k is the read length (150bp).

269 When assessing the transmission of CtBV from resistant parasitized moths to their offspring,
270 we did not sequence 100% of the 1000 gametes we sampled (we sampled 500 offspring, and
271 each inherited 2 gametes from their resistant parents). Thus, we had to estimate the number
272 of gametes we sequenced (noted S). To do this, we calculated an upper limit, which is the
273 maximum number of gametes we might have sequenced, with: $S = \sum_{k=1}^n (dk * pk)$, where d
274 is the sequencing depth in library k (n=5 libraries), and p is the proportion of the genome
275 sequenced on average in one offspring for library k. To estimate p, we randomly sampled one
276 hundredth reads of the library (because each library contains 100 offspring), and we calculated
277 the proportion of the host genome covered when looking only at this subset of reads. This
278 proportion is an estimation of the coverage of one of the 100 offspring of the library. We
279 repeated this sampling ten times and we calculated the average, *i.e.* the proportion of genome
280 sequenced for one individual on average in library k (p_k).

281 **Results**

282 **Wasp cells do not persist in adult *S. nonagrioides* surviving to parasitism by *C. typhae***

283 In a previous study, we showed that seven days after a female *C. typhae* was oviposited into
284 larvae of its host *S. nonagrioides*, CtBV DNA persisted under three forms in parasitized
285 caterpillars: the circular form, the chromosomally integrated form, and the proviral segments
286 located in the wasp genome of wasp teratocyte cells present in the caterpillars (Muller, Chebbi,
287 et al., 2021). Teratocytes are cells released from the wasp embryonic membranes into the host
288 when eggs hatch. They are involved in various functions, including suppressing the host
289 immune response (Buron & Beckage, 1997). Since insects undergo important cellular changes
290 during metamorphosis (Tettamanti & Casartelli, 2019), we wondered whether some CtBV DNA
291 and/or other wasp DNA can persist at the adult stage in host individuals surviving to parasitism,
292 in one form or another. Addressing this question is important to assess the extent to which
293 transmission of germline-integrated CtBV and/or free CtBV to host offspring can occur. For this
294 end, we Illumina-sequenced the whole genome of two adult *S. nonagrioides* out of the 37 that
295 resisted to parasitism in our experiment. Both sequenced adult *S. nonagrioides* were female,
296 but while Adult-1 produced many offspring, Adult-6 did not produce any.

297 To assess the extent to which wasp cells may persist in adult hosts resisting to parasitism, we
298 looked whether the wasp genome was covered by the Illumina reads. For this, we mapped
299 trimmed Illumina reads obtained from the two *S. nonagrioides* females on the whole genomes
300 of both *C. typhae* and *S. nonagrioides*. Average sequencing depths over the *S. nonagrioides*
301 genome were in the targeted range (41X and 49X) and 97% of the moth genome was
302 sequenced at least once for the two individuals (Fig. 2). By contrast, average sequencing

303 depths over the *C. typhae* genome were very low (0.5X and 0.86X) and reads mapped to only
304 a small fraction of the wasp genome (<1% and 7.4%) (Fig. 2). To assess whether these
305 sequencing depth and coverage, although low, indicate the presence of residual amounts of
306 wasp cells, we compared these statistics to a control dataset corresponding to trimmed
307 Illumina reads generated in an earlier study aiming at sequencing the genome of non-
308 parasitized *S. nonagrioides* individuals that were never in contact with *C. typhae* (Muller,
309 Ogereau, et al., 2021). For this dataset, we obtained an average sequencing depth of 0.43X
310 over the *C. typhae* genome with less than 1% of the wasp genome being covered at least once.
311 The average sequencing depth on the genome of *S. nonagrioides* was 38.9X. This control
312 indicates that in a *S. nonagrioides* individual devoid of any *C. typhae* DNA, we can expect an
313 average sequencing depth on the latter at about 1.1% of the former, and less than 1% of the
314 wasp genome is expected to be covered at least once. For Adult-1, the average depth over the
315 *C. typhae* genome was 1.23% of the one on *S. nonagrioides* and less than 1% of the wasp
316 genome was covered, which is close to what we obtained for the control dataset. This suggests
317 that teratocytes and other wasp cells were absent in this individual. However, Adult-6 clearly
318 had higher amounts of wasp DNA than expected in the absence of *C. typhae* DNA, with an
319 average depth on the *C. typhae* genome at 1.77% of the one computed on the *S. nonagrioides*
320 genome, and 7.4% of the wasp genome covered at least once. Although these figures could be
321 interpreted as being due to the presence of small amounts of wasp cells in this *S. nonagrioides*
322 adult individual, we found that the wasp genome regions covered by sequencing reads were
323 very small, although *C. typhae* scaffolds are large (83% of these regions were between 100 and
324 300 bp). This suggests that the wasp DNA sequenced from this adult individual is highly
325 fragmented and unlikely to originate from complete whole wasp genomes. Thus, while the

326 origin of this wasp DNA remains to be determined, our results tend to indicate that teratocytes
327 and other wasp cells are unlikely to persist in host adult individuals surviving to parasitism.

328 **Persistence of CtBV DNA in adults *S. nonagrioides* surviving to parasitism by *C. typhae***

329 We next focused on evaluating whether CtBV DNA may persist in adult *S. nonagrioides*
330 surviving to parasitism, in one form or another. We found that the average sequencing depth
331 over the 27 CtBV circles was higher than the average depth on the entire wasp genome in the
332 two *S. nonagrioides* adult individuals (3.7X versus 0.5X in Adult-1 and 12.46X versus 0.86X in
333 Adult-6). This suggests that contrary to wasp cells, CtBV persisted in adult hosts, in one form
334 or another. To assess whether some of the CtBV circles persisted in their chromosomally
335 integrated form, we looked for chimeric reads between CtBV and *S. nonagrioides*. We found
336 43 and 303 chimeric reads corresponding to HIM-mediated integration of the 16 HIM-
337 containing CtBV segments in Adult-1 and Adult-6, respectively. Thus, the 16 HIM-containing
338 CtBV segments were able to persist at least in their integrated forms at the adult stage in both
339 adult hosts (Fig. 3). More precisely, if we consider only independent integration events (IEs),
340 *i.e.* counting only once chimeric reads with the exact same integration coordinate, we found
341 41 HIM-mediated IEs in Adult-1 and 297 HIM-mediated IEs in Adult-6. In order to compare
342 samples, we normalized the number of IEs with the number of host reads (Integration events
343 Per Million reads of Host, or IPMH). Adult-1 had 0.29 IPMH and Adult-6 had 1.77 IPMH (Fig.
344 3). From this normalized number of IEs, we can also express the average number of IE per
345 haploid genome (see materials and methods), which allowed us to estimate 1.97 and 12.03 IEs
346 per haploid genome for Adult-1 and Adult-6, respectively.

347 We then compared the normalized number of IEs of both adult *S. nonagrioides* to the one
348 found in the whole body of a parasitized caterpillar in which no wasp embryos were observed
349 seven days after oviposition (called “Caterpillar”) (Muller, Chebbi, et al., 2021). We observed
350 that the number of integrated CtBV circles can be quite high in adults, since Adult-6 is in the
351 same order of magnitude as in a caterpillar (Fig. 3b). This raises the question of whether some
352 CtBV circles might be integrated into the germline genome of *S. nonagrioides* and transmitted
353 to the next generation of moth.

354 Before addressing this question, we investigated whether CtBV could also persist under their
355 circular forms. Based on the current knowledge of the system, factors involved in bracovirus
356 replication are not injected in the host (Louis et al., 2013), so bracoviruses are only known to
357 replicate in the calyx of the wasp, not in the caterpillar. This is why, one can expect bracovirus
358 circles to be degraded after some times in the caterpillar. However, Muller, Chebbi, et al. (2021)
359 found that these circles still persist in high amounts in *S. nonagrioides* caterpillars seven days
360 post-parasitism, and Chevignon et al. (2018) found a persistence of bracovirus circles in
361 *Manducta sexta* 12 days post-parasitism. To determine whether CtBV can persist under circular
362 form in adult moths, we measured the sequencing depth over circles devoid of HIM, for which
363 no or very few integrations have been found in the present study (zero and one IE in total in
364 Adult-1 and Adult-6, respectively). While in Adult-1 all CtBV circles devoid of HIM had a
365 sequencing depth close to the one of the *C. typhae* genome (0.5X), in Adult-6 at least two CtBV
366 devoid of HIM were sequenced at a depth markedly higher than the *C. typhae* genome (0.86X):
367 CtBV S20/33 (4.8X) and S23 (5.9X) (see the two red diamonds in Fig. 4b). Although limited, the
368 quantity of persisting S20/33 and S23 is as high as that of three HIM-containing CtBV (S10, S11,

369 and S14), indicating that at least some CtBV circles can persist up to the adult stage of the host
370 and during its entire lifespan (Adult-6 died 46 days post-oviposition).

371 **No vertical transmission of CtBV circles by *S. nonagrioides* adults surviving to parasitism by**
372 ***C. typhae*.**

373 To assess the extent to which CtBV DNA persisting in adults *S. nonagrioides* can be transmitted
374 vertically to the next generation of moth, we screened for the presence of CtBV in F1
375 individuals resulting from crosses of *S. nonagrioides* individuals that had survived parasitism.
376 In total we Illumina-sequenced 500 offspring individuals produced by five couples. Each couple
377 was made of a male and a female *S. nonagrioides* that both survived parasitism. The DNA of
378 the 500 individuals was distributed in five libraries each containing 100 individuals (Suppl. Fig.
379 1).

380 The obtained sequencing depth on the *S. nonagrioides* genome was between 105 and 143X
381 depending on the library, and 98.7% to 99.5% of the moth genome was covered at least once
382 in the five libraries (Fig. 2). As expected, the sequencing depth and coverage of the *C. typhae*
383 genome were very low for the five libraries (1.2% to 1.3% of the sequencing coverage on the
384 host, and <1% of the wasp genome covered at least once). These figures are similar to those
385 obtained for the control dataset of reads produced from *S. nonagrioides* larvae that never
386 encounter *C. typhae* (1.1% of the sequencing coverage on the host, and <1% of the wasp
387 genome covered). Thus, as expected, no wasp whole genome was transmitted to moth F1
388 offspring individuals.

389 Importantly, the average sequencing depth on the 27 CtBV circles was close to zero (between
390 0 and 0.01X) for the five libraries of offspring DNA, suggesting that no CtBV DNA, in any forms,

391 were sequenced (Fig. 2A). In agreement with this result, we could not identify any chimeric
392 read between CtBV and *S. nonagrioides*. Thus, we could not identify any vertical transmission
393 of CtBV, neither in their circular nor integrated forms, to any of the 500 offspring individuals
394 we sequenced. This result provides a way to estimate an upper limit of the frequency at which
395 vertical transmission of bracoviruses may be transmitted in host populations. We estimated
396 that among the 1000 gametes transmitted to these offspring, we did not sequence more than
397 374 gametes (see methods). This gives a probability to detect the vertical transmission of a
398 CtBV lower than 1/374 gametes. This probability might have been different, if we had chosen
399 a different sampling strategy. Here we chose to sequence 100 offspring individuals from five
400 couples but we cannot tell whether sequencing more offspring from less couples, or
401 sequencing less offspring from more couples could have increased our chances to find a
402 transmission.

403 **Chromosomal integration of CtBV in non-target species**

404 In the context of biocontrol, it is worth to evaluate the possible consequences, in terms of BV
405 chromosomal integration, of the introduction of an exotic biocontrol agent on autochthonous
406 lepidopteran species that are not present in the native area of the wasp. Since such species
407 have never interacted with the introduced wasp, they may be less permissive hosts and thus
408 more likely to survive parasitism (Mahmoud et al., 2012). In turn, chromosomal integration of
409 BV DNA in such non- or less- permissive species may increase the likelihood that germline-
410 integrated BV DNA are transmitted through host generations (Drezen et al., 2017). Yet, the
411 capacity of BV circles to undergo chromosomal integration in species not known to be naturally
412 targeted by an endoparasitoid wasp has never been studied.

413 Here, we investigated whether CtBV can undergo chromosomal integration in three stemborer
414 lepidopteran species (*Nonagria typhae* [Noctuidae], *Globia sparganii* [Noctuidae], and *Chilo*
415 *phragmitella* [Crambidae]), the geographical distribution of which overlaps with that of *S.*
416 *nonagrioides* in France (Galichet et al., 1992; Teder & Tammaru, 2002; Tewksbury et al., 2002).
417 Among the two *N. typhae*, the five *G. sparganii*, and the eight *C. phragmitella* individuals that
418 were parasitized by *C. typhae* for this experiment, only four *C. phragmitella* yielded wasp
419 cocoons, yet all hosts died before reaching the adult stage. For each species, we Illumina-
420 sequenced one individual in which no wasp cocoon developed. To study CtBV infection in these
421 three caterpillars, we first assembled their reference genomes, which are not available in
422 public databases. Before doing so, we filtered out all reads mapping to the genome of *C. typhae*
423 (see materials & methods). The quality of the assemblies we obtained was heterogenous, with
424 a higher completeness for *N. typhae* (647Mb) and *G. sparganii* (972Mb) (93.6% and 82.1% of
425 complete BUSCO, respectively) than for *C. phragmitella* (942Mb; 67.4% complete BUSCO)
426 (Table 1). These assemblies all turned out to be sufficient to test for chromosomal integration.
427 Looking at sequencing depth and coverage over the whole *C. typhae* genome in the three
428 samples revealed different patterns. While virtually no wasp whole genome was sequenced in
429 *G. sparganii* (0.5X), it was deeply sequenced in *N. typhae* and *C. phragmitella* (88X and 166X,
430 respectively), and almost entirely covered at least once (99.3% in the two last samples) (Fig.
431 2). These figures indicate the absence of wasp cells in *G. sparganii* and their presence in *N.*
432 *typhae* and *C. phragmitella*. This further suggests that although no cocoons emerged from *N.*
433 *typhae* and *C. phragmitella* larvae before they died, wasp embryos were in fact able to start
434 their development in these two species.

435 Interestingly, we found that as observed when sequencing a parasitized *S. nonagrioides* larvae,
436 the total sequencing depth over CtBV DNA (in any form) was higher than that over the whole
437 wasp genome for the three species not normally targeted by *C. typhae* (Fig. 2). Subtracting the
438 whole wasp genome from this total CtBV DNA depth (*i.e.*, subtracting the proviral segment
439 form) provides an estimate of the depth at which circular plus integrated forms of CtBV were
440 sequenced: 11.8X, 20.5X and 107.6X in *G. sparganii*, *N. typhae* and *C. phragmitella*,
441 respectively (Fig. 2a). Moreover, we identified HIM-mediated integration events (IEs) of CtBV
442 in the three samples, with 120, 592 and 1114 IEs in *G. sparganii*, *N. typhae* and *C. phragmitella*,
443 respectively (Fig. 3). The normalized number of integration events was the highest for *C.*
444 *phragmitella*, in which it is even higher than in the natural host *S. nonagrioides* (Fig. 3). It is
445 noteworthy that sequencing depths over non-integrated CtBV circles (circles for which we did
446 not identify any chimeric read) are in the same range as those over integrated CtBV circles for
447 the three non-natural host species (Fig. 4c-e). This indicates that circles can persist in non-
448 natural host species at similar levels in their non-integrated forms and in their integrated
449 forms, even up to 25 days post-oviposition (for *C. phragmitella*).

450 We finally compared the molecular signatures associated with CtBV chromosomal integration
451 in the three non-target species to those characterized in the natural host *S. nonagrioides*. We
452 have shown that almost all integrations in parasitized *S. nonagrioides* involve HIM-containing
453 circles (99%) and are HIM-mediated (96.5%) (Muller, Chebbi, et al., 2021). In non-target
454 species, we found similar signatures in *N. typhae* and *G. sparganii*, with 98.9% and 96.8% of
455 the integration events involving HIM-containing circles, and 92.8% and 97.6% of these
456 integrations occurring within the HIM, respectively. The pattern was markedly different in *C.*
457 *phragmitella*, with only 91.6% of the integration events involving HIM-containing circles, and

458 only 74.3% of them occurring within the HIM. This high number of integrations outside the
459 HIM motif in *C. phragmitella* is not due to just one or few circles integrating differently, but to
460 a common tendency of most circles: 14 circles out of the 16 HIM-containing circles have
461 significantly more integration events outside HIM in *C. phragmitella* than in *S. nonagrioides*
462 (Fisher test: $p.value < 0.05$; see Fig. 5). Such differences may be explained by the fact that host
463 factors are involved in BV integration and that these factors are expected to diverge
464 substantially in *C. phragmitella*, the non-target species the most distantly related to *S.*
465 *nonagrioides*. This would be consistent with a recent study showing that host integrases are
466 involved in BV integration (Wang et al., 2021).

467 **Discussion**

468 In this study, we have characterized the presence of chromosomally-integrated and non-
469 integrated forms of BV circles in *S. nonagrioides* adult individuals surviving parasitism by the
470 endoparasitoid wasp *C. typhae*. We found that the two forms of CtBV can persist to the host
471 adult stage in numbers that are similar to those identified in larval stages (Muller, Chebbi, et
472 al., 2021), despite the tissue remodeling and other physiological changes undergone during
473 metamorphosis by *S. nonagrioides* (Tettamanti & Casartelli, 2019). Interestingly, we found that
474 circular forms of HIM-containing CtBV persist in higher amounts at adult stage than CtBV
475 circles devoid of HIM (sequenced at 12.2X and 1.2X on average respectively, see Fig. 4). In our
476 earlier study (Muller, Chebbi, et al., 2021), we did not observe differences of persistence levels
477 between HIM-containing CtBV circles and those devoid of HIM at seven days post oviposition
478 (their sequencing depths were similar). The present results indicate that most DNA circles are
479 lost/degraded, between seven days post-parasitism and the adult stage. A study monitoring

480 DNA circles persistence in a permissive host from seven days post parasitism to the end of the
481 wasp larvae development would shed additional light on segment copy number and
482 persistence during development of wasp offspring, and therefore to some extent on the role
483 of bracovirus circle integration. It is also striking that Adult-6, the adult with the highest
484 number of CtBV integrations, did not produce any offspring, nor even any eggs, whereas Adult-
485 1, which suffered less CtBV integration events, is the mother of hundreds of caterpillars (some
486 of them being included in our analysis). We cannot conclude here on a possible link between
487 the number of CtBV integrations and host fertility because of a lack of replicates. In the same
488 way, it is possible that the reason why the majority of resisting adult couples (10 out of 15) did
489 not produce any offspring is because of the effect of CtBV integration. Yet, these adults were
490 not crossed as pools of many male and female individuals as routinely implemented in our lab,
491 but as couples (*i.e.*, each made of one male and one female individual), which we previously
492 noticed tends to decrease the likelihood of successful breeding, even when using non-
493 parasitized individuals. It is thus difficult to assess whether parasitism or breeding conditions
494 or both had an effect on breeding success. This question certainly deserves to be addressed in
495 the future as it would help to better understand the possible risks of transmission of integrated
496 CtBV circles to the next host generation, and thus of horizontal transfer with possible long-
497 term consequences on the host.

498 We tested for the possibility that CtBV DNA persisting in adult *S. nonagrioides* may be
499 transmitted to F1 individuals resulting from crossings between host individuals surviving to
500 parasitism. We were unable to identify any CtBV DNA in 500 of these F1. This indicates that
501 the probability of transmission of CtBV DNA from one host generation to the next is lower than
502 our detection limit. Our previous study showed that CtBV integrated in all *S. nonagrioides*

503 tissues studied, which did not include gonads (Muller, Chebbi, et al., 2021), and we here report
504 an average of 1.97 IEs per haploid genome in Adult-1 (the adult who produced offspring).
505 Based on these findings, one could expect most gametes to harbor at least one IE.
506 Nonetheless, the lack of detection of transmission we obtain in the present study means that
507 at least a significant number of gametes do not harbor any IE. Although we found 1.97 IEs per
508 haploid genome on average, we actually have no way to estimate the variance, that would
509 indicate whether IEs are equitably shared by all cells or whether some cells carry most IEs.
510 Regardless of the variance, gametes might be less subject to integration than other tissues
511 because they may be less accessible. In agreement with this, Wang et al. (2021) measured
512 lower amounts of circular CvBV in testis than in hemocytes of *Plutella xylostella*. Another
513 possibility is that CtBV may integrate in the germline at rates comparable to other tissues but
514 it may impede proper gametogenesis and/or negatively impact the functionality of gametes.
515 Sequencing gonads of parasitized hosts would provide important information on this aspect.
516 Although we can only estimate an upper limit probability of transmission for resistant
517 individuals, the probability of transmission at the population scale (including all parents, non-
518 surviving, and sterile individuals) must be much lower. Indeed, out of the 62 parasitized
519 caterpillars included in this study, 25 died before reaching adult stage, and 27 did not produce
520 any offspring. Thus, the probability of transmission was equal to zero for 52 out of 62
521 parasitized *S. nonagrioides* in our experiment. It is well known that such transmission can occur
522 in some species, as several cases of bracovirus-derived sequences resulting from ancient HT
523 from wasp to lepidopterans have been characterized (Di Lelio et al., 2019; Gasmi et al., 2015;
524 Schneider & Thomas, 2014). Yet, it is interesting to note that in spite of presumably being
525 heavily targeted by *C. typhae* and other endoparasitoid wasps in the wild since many

526 generations, *S. nonagrioides* does not contain any trace of bracovirus sequences in its
527 (germline) genome (Muller, Chebbi, et al., 2021). Thus, the real probability of BV transmission
528 in host populations is likely to be much lower than the upper limit we were able to estimate
529 here. Although our upper limit estimate will be interesting for future studies aiming at
530 investigating this rate, it is better to consider it as not so informative in the context of
531 biocontrol strategies. Indeed, at the scale of biocontrol, even a low probability could lead to
532 numerous BV transmissions since a large number of *S. nonagrioides* caterpillars (and
533 potentially other lepidopteran species not known to be targeted by *C. typhae* in the wild)
534 would be parasitized every year. Future experiments should aim at refining estimates of the
535 frequency of BV transmission to better inform policy makers in the context of wasp-based
536 biocontrol strategies, as well as to better understand what impact wasp-to-lepidopteran HT
537 may have had on the evolution of lepidopteran genomes.

538 Our study also shows that CtBV circles can persist as extrachromosomal molecules and
539 undergo chromosomal integration in parasitized larvae of three lepidopteran species (*N.*
540 *typhae*, *C. phragmitella*, *G. sparganii*) that are not naturally targeted by *C. typhae*, at rates
541 comparable to those measured in *S. nonagrioides* (the native host). This shows that CtBV cell
542 entry and chromosomal integration can occur in a wide range of lepidopteran species,
543 including a Crambidae which diverged at least 100 million years ago from *S. nonagrioides*.
544 Thus, these two processes are unlikely to play a major role in shaping the host range of
545 Braconid wasps. This is in line with a study that showed that bracovirus gene expression levels,
546 rather than cell entry and persistence of BV DNA, are more likely to be a key factor underlying
547 host permissiveness (Bitra et al., 2016).

548 The extent to which the three non-natural host species included here are permissive to *C.*
549 *typhae* is under study in our laboratory. It depends on ecological, behavioral and chemical
550 factors that condition their attractiveness and acceptance as host. Yet, the outcome of the
551 present study – only 4 host larvae out of 15 yielded wasp cocoons – together with other
552 unpublished preliminary results suggest that these three species are much less permissive
553 than *S. nonagrioides*. This suggests that the capacity of BV to enter in host cells and undergo
554 chromosomal integration may not be used as a good proxy for evaluating wasp permissive host
555 range. In the context of using *C. typhae* as a biocontrol agent, our results are important
556 because they show that autochthonous lepidopteran species occurring in the area in which
557 the wasp could be released can suffer BV chromosomal integration and thus be affected in one
558 way or another. This is, however, considering that interaction between *C. typhae* and these
559 other autochthonous lepidopteran species in the wild are timely and ecologically possible,
560 which remains to be assessed. On a more basic level, the fact that BV DNA can undergo
561 chromosomal integration in a wide range of non-natural hosts, likely to be less permissive than
562 native hosts, supports the idea that parasitizing non-natural hosts may increase the propensity
563 of wasp-to-host HT of BV DNA with long term consequences (Drezen et al., 2017).

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570

571 **Data Accessibility and Benefit-Sharing**

572 **Data accessibility statement**

573 All raw sequencing reads produced during this study are available in NCBI under accession
574 number PRJNA816685. The two previously published datasets that we used in this study are
575 available under accession numbers SRX9975807 (Illumina trimmed reads used for the
576 assembly of *S. nonagrioides* reference genome (Muller, Ogereau, et al., 2021)) and

577 SAMN18533545 (“whole body” sample corresponding to a parasitized caterpillar for which no
578 wasp larvae were present seven days post parasitism (Muller, Chebbi, et al., 2021)). Here, we
579 called the former “Control” and the latter “Caterpillar”. The R scripts used for the analyses and
580 to produce the figures of this project are available on GitHub (github/HeloiseMuller/Chimera).

581 **Benefit-Sharing statement**

582 *C. typhae* individuals used in this study originated from the International Centre of Insect
583 Physiology and Ecology under the juridical framework of material transfer agreement CNRS
584 072057/IRD 302227/00. Access to genetic resources of the non-target lepidopteran species
585 collected in France and benefit sharing have been done under the juridical framework of the
586 Ministère de la Transition Ecologique (NOR: TRE2103218S/428, decision of March 26th, 2021).

587 **Tables and Figures**

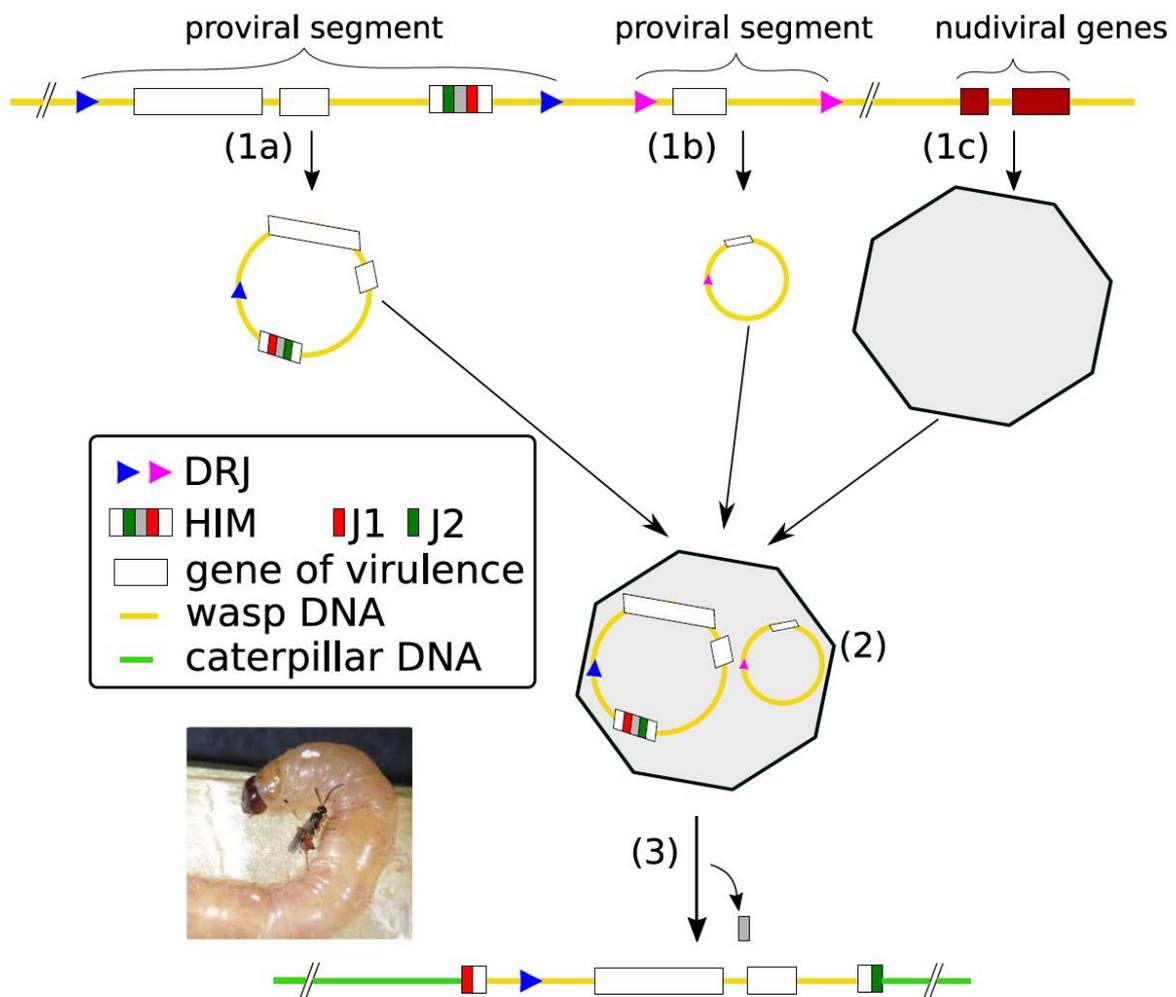
588 **Table 1. Characteristics of the three *de novo* assemblies produced in this study.**

	#Contigs	Genome size assembly (Mb)	N50 (kb)	Complete BUSCO (duplicated)	Missing BUSCO
<i>N. typhae</i>	72,086	647	29.6	93.6% (1.9%)	2.0%
<i>G. sparganii</i>	304,227	972	8.5	82.1% (3.3%)	7.1%
<i>C. phragmitella</i>	293,788	942	4.7	67.4% (5.6%)	19.0%

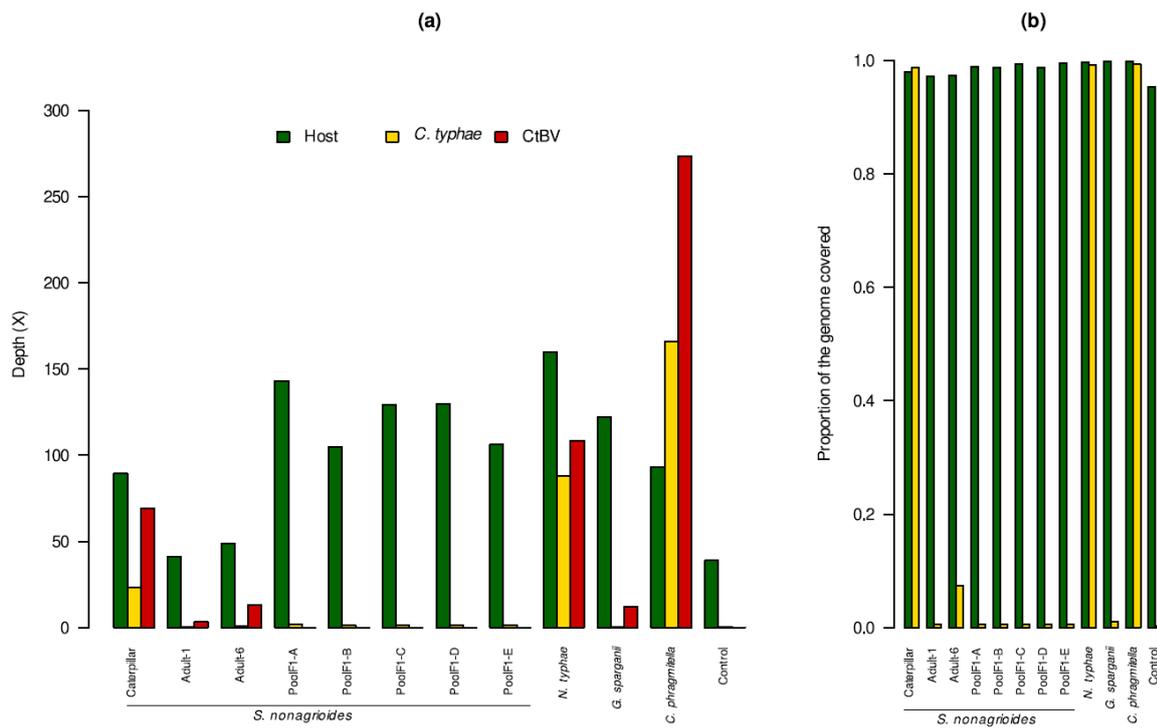
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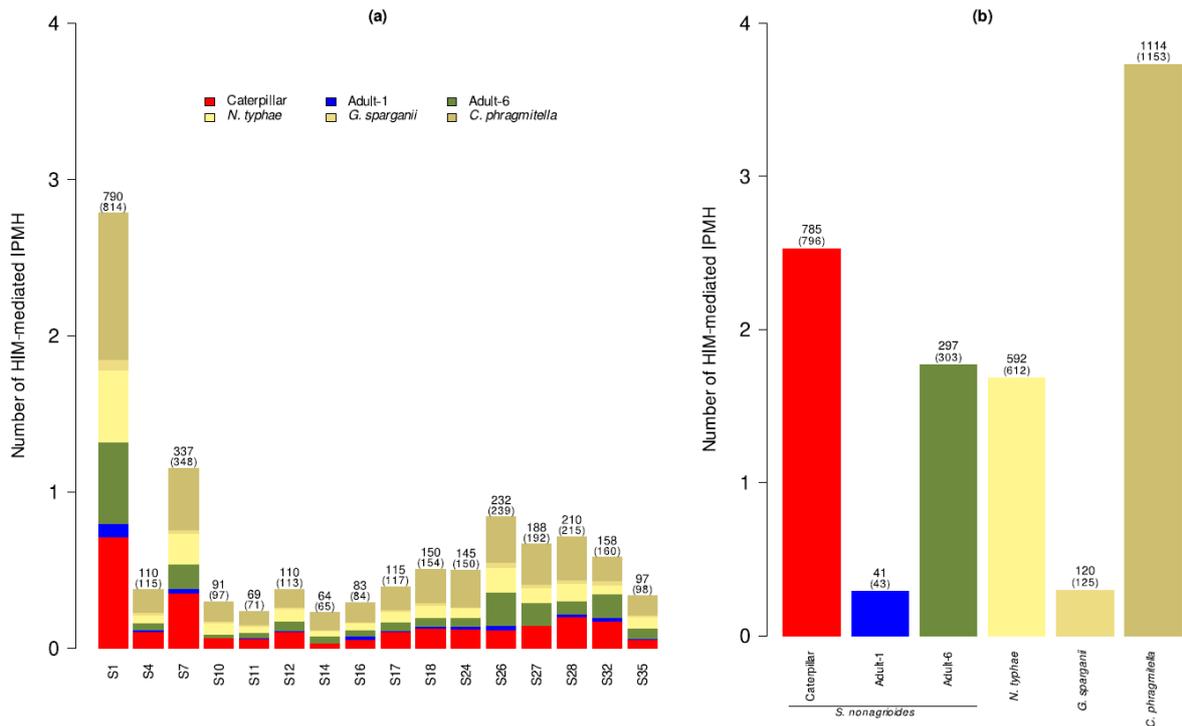
591 **Figure Captions**



592
 593 **Figure 1. Structure of bracoviruses.** In the wasp genome, (1a) is a HIM-containing segment,
 594 (1b) is a segment devoid of HIM, and (1c) corresponds to genes of viral origin. White rectangles
 595 are virulence genes. In the wasp calyx, (1a) and (1b) form DNA circles thanks to their DRJ,
 596 whereas (1c) form viral particles. In (2), DNA circles are packaged into viral particles, which are
 597 injected in the caterpillar host at the same time as the wasp eggs (see the picture as an
 598 example, where *C. typhae* is parasiting *S. nonagrioides*). In (3), the HIM-containing segment
 599 integrates via its HIM into the caterpillar genome, losing a ~50bp region located between J1
 600 and J2.

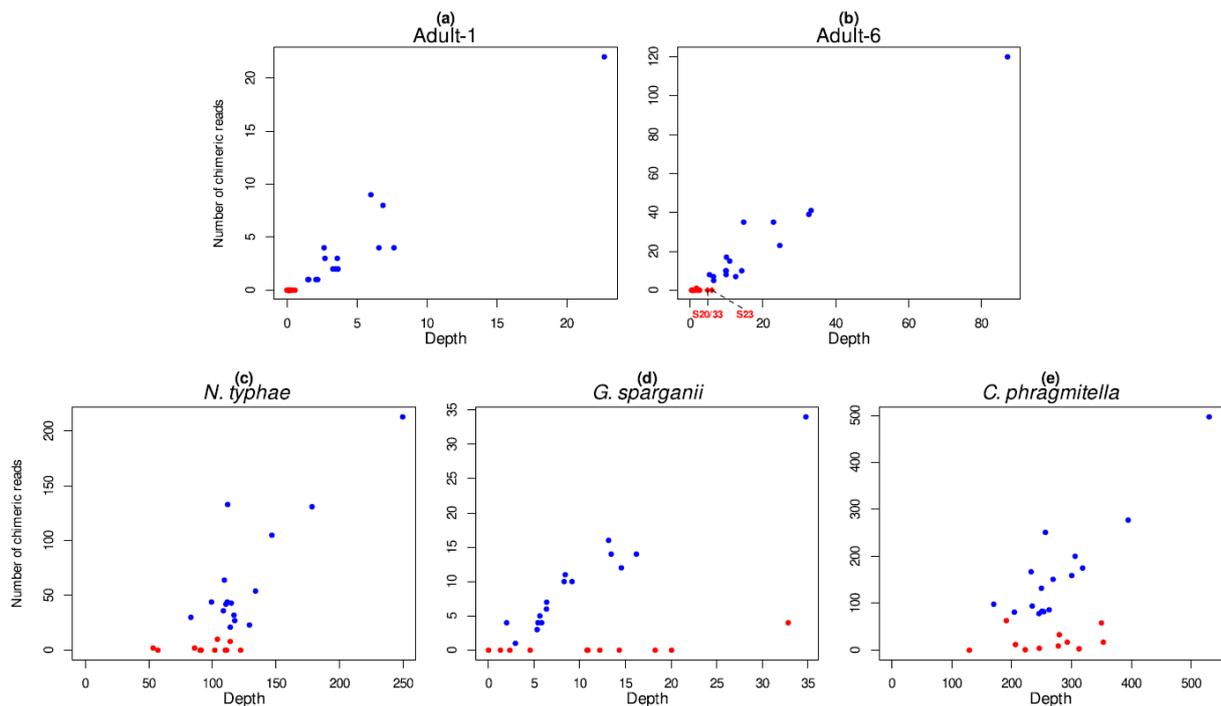


601
 602 **Figure 2. Sequencing depth and coverage over the genome of the wasp (*Cotesia typhae*), the**
 603 **bracovirus (CtBV) and the various moth hosts. (a)** Barplot of the average sequencing depths.
 604 **(b)** Barplot of the proportion of the genomes of *C. typhae* and the hosts covered at least once.
 605 The green, yellow and red colors indicate the sequencing statistics over the whole genome of
 606 the host (*S. nonagrioides*, *N. typhae*, *G. sparganii*, or *C. phragmitella* depending on the
 607 samples), the whole genome of *C. typhae*, and the 27 CtBV DNA, respectively. The sequencing
 608 metrics are given for the ten samples produced for this study, but also for two other samples:
 609 Caterpillar is the sample corresponding to the whole body of a *S. nonagrioides* caterpillar
 610 sequenced seven days post-parasitism, and Control corresponds to the Illumina reads used for
 611 the assembly of the reference genome of *S. nonagrioides*. This sample has never been in
 612 contact with *C. typhae*.



613
 614 **Figure 3. Number of HIM-mediated CtBV integration events per millions reads mapped on**
 615 **the host genome (IPMH) for each sample and CtBV circle. Absolute numbers of HIM-mediated**
 616 **integration events (IEs) and chimeric reads (in parentheses) are shown on top of each bar**
 617 **(without PCR duplicates). (a) Barplot comparing HIM-containing segments. (b) Barplot**
 618 **comparing samples.**

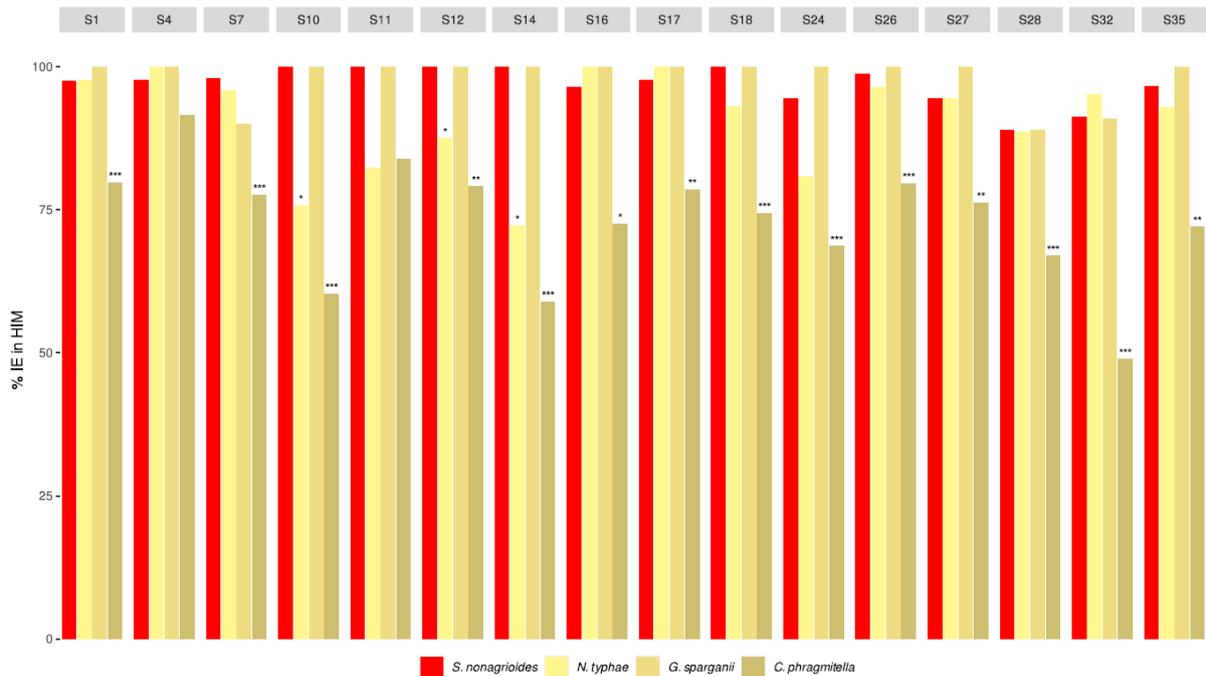
619
 620



621
 622 **Figure 4. Sequencing depth over CtBV DNA as a function of the number of chimeric reads.**
 623 Since all reads are taken into account to calculate the sequencing depth, all chimeric reads are
 624 also taken into account in this plot: the ones that result from a HIM-mediated integration or
 625 not, and also the PCR duplicates. Each dot shows a CtBV (whose depth can result from
 626 sequencing its proviral segment, its circular form or its integrated form), in blue for HIM-
 627 containing CtBV, and in red for CtBV devoid of HIM. Results are shown by sample, with *S.*
 628 *nonagrioides* Adult-1 in **(a)**, *S. nonagrioides* Adult-6 in **(b)** where the two red diamonds
 629 represent S20/33 and S23, *N. typhae* in **(c)**, *G. sparganii* in **(d)**, and *C. phragmitella* in **(e)**.

630

631



632
 633 **Figure 5. Percentage of HIM-mediated integration events (IEs) for the 16 HIM-containing**
 634 **circles.** Data of the two adults *S. nonagrioides* and the sample Caterpillar are merged and
 635 compared to the three species not normally targeted by the wasp in the wild. Stars show
 636 whether the percentage of integration events which are HIM-mediated for the segment of
 637 interest is significantly different between the non-target species and *S. nonagrioides* (Fisher
 638 test; *: p.value<0.05, **: p.value<0.01; ***: p.value<0.001).

639
 640 **Supplementary Figure 1. Experimental design to obtain offspring of surviving parasitized**
 641 **caterpillars.** In black are represented caterpillars of unidentified sex, in red the female adults,
 642 in green the male adults, and in yellow *C. typhae* (the square represents an adult in oviposition,
 643 and ovals represent wasp larvae). Although most moth couples produced some eggs, they lead
 644 to offspring only for 5 couples. Details of the experiment are shown for the progeny of Adult-
 645 1 (F1-Adult1), yet all the offspring were processed the same way. The DNA extractions
 646 (composed of 5 caterpillars each) were pooled 20 by 20 with equimolar ratio, leading to five
 647 libraries composed of 100 caterpillars each: library PoolF1-A to PoolF1-E. PoolF1-A was
 648 composed of 20 pools of F1-Adult5, PoolF1-B (shown on the figure as an example) of 20 pools
 649 of F1-Adult1, PoolF1-C of 16 pools of F1-Adult2 plus 1 pool of F1-Adult5 plus 3 pools of F1-
 650 Adult3, PoolF1-D of 20 pools of F1-Adult3 and PoolF1-E of 14 pools of F1-Adult4 plus 4 pools
 651 of F1-Adult5 plus 2 pools of F1-Adult1.

652
 653 **Author contributions**
 654 T.F., C.G., L.K., F.M., and H.M. designed the study; T.F. and H.M. performed lab work; T.F.
 655 conducted field work and provided samples; C.H. and H.M performed data analysis with input

656 from C.G. and F.M.; C.G. and H.M. wrote the manuscript, with input from E.H., F.M., and all
657 other coauthors.