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1 Chromosomal resolution reveals symbiotic virus 2 colonization of parasitic wasp genomes

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15

16

17 Abstract

18 Most endogenous viruses, an important proportion of eukaryote genomes, are doomed to slowly
19 decay. Little is known, however, on how they evolve when they confer a benefit to their host.
20 Bracoviruses are essential for the parasitism success of parasitoid wasps, whose genomes they
21 integrated ~103 million years ago. Here we show, from the assembly of a parasitoid wasp
22 genome, for the first time at a chromosomal scale, that symbiotic bracovirus genes spread to
23 and colonized all the chromosomes. Moreover, large viral clusters are stably maintained
24 suggesting strong evolutionary constraints. Genomic comparison with another wasps revealed
25 that this organization was already established ~53 mya. Transcriptomic analyses highlight
26 temporal synchronization of viral gene expression, leading to particle production. Immune
27 genes are not induced, however, indicating the virus is not perceived as foreign by the wasp.
28 This recognition suggests that no conflicts remain between symbiotic partners when benefits to
29 them converge.

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34 **Main**

35 *Cotesia* wasps (Hymenoptera, Braconidae) are parasitoids of Lepidoptera. Female
36 wasps lay their eggs into caterpillars and larvae develop feeding on the host hemolymph.
37 Several *Cotesia* species are famous for their use as biological control agents to control insect
38 pests, such as *Cotesia flavipes* which is massively released over several million hectares of
39 sugarcane fields in Brazil^{1,2}. Parasitoid wasps evolved several strategies that increase parasitic
40 success, including a sensitive olfactory apparatus to locate their hosts^{3,4} and detoxification
41 mechanisms against plant toxic compounds accumulating in their host (Fig. 1). But the most
42 original strategy is the domestication of a bracovirus (BV) shared by over 46,000 braconid wasp
43 species⁵. Bracoviruses originate from a single integration event ~103 million years ago (mya)
44 of a nudivirus in the genome of the last common ancestor of this group⁶⁻⁹. Virus domestication
45 nowadays confers a benefit to the wasps that use BVs as virulence gene delivery systems¹⁰.
46 Indeed, virulence genes are introduced with wasp eggs into their hosts, causing inhibition of
47 host immune defenses¹⁰⁻¹². Imbedded in wasp DNA, the nudivirus genome has been extensively
48 rearranged¹³. Now BV sequences are differentiated as i.) genes involved in particles production
49 named “nudeviral genes”⁶, and ii.) proviral segments packaged as dsDNA circles in viral
50 particles¹³, encoding virulence genes involved in successful parasitism.

51 To understand the genetic underpinning of the endoparasitoid life-style and of BV
52 evolution since the original nudivirus capture event, we used a hybrid sequencing approach
53 combining 454 reads, illumina short reads, and chromosomal contact data (HiC), to obtain a
54 reference genome for *Cotesia congregata* at a chromosomal scale. These approaches allowed
55 the analysis of wasp genes potentially involved in the success of the endoparasitoid lifestyle
56 such as genes implicated in olfaction, detoxification and immunity. Moreover, the results
57 revealed the global distribution of bracoviral sequences in wasp chromosomes and the
58 identification of syntenies with genome scaffolds of *Microplitis demolitor* parasitoid wasp^{14,15},
59 which diverged from the *Cotesia* lineage 53 mya¹⁶. In addition, we sequenced draft genomes
60 of five *Cotesia* species (*C. rubecula*, *C. glomerata*, *C. vestalis*, *C. flavipes*, *C. sesamiae*) to
61 assess bracovirus molecular evolution between closely related species.

62 We discovered that, in discrepancy with the usual gene decay of endogenous viral
63 elements, bracovirus genes have spread in all wasp chromosomes, reaching a ~2.5-fold higher
64 number of genes than a pathogenic nudivirus. However strong evolutionary constraints have
65 maintained specialized chromosomal regions containing large virus gene clusters, resembling
66 “super gene” regions and concentrating 77% of virulence genes and half of the nudeviral genes,

67 respectively. Transcriptome analyses point to the major role of viral transcription in maintaining
68 the viral entity, however wasp immune response is not induced, suggesting no conflicts remain
69 in this ancient virus-wasp association.

70

71 **Results**

72 *Genome assembly, annotation and comparison*

73 First, a 207 Mb high-quality genome (scaffolds N50=1.1 Mb and N90=65 kb) was
74 obtained for *Cotesia congregata* using a combination of mate pair 454 pyrosequencing and
75 Illumina sequencing (Supplementary Table 1). Most of the assembly (86%) consisted of 285
76 scaffolds of over 100 kb. This genome was then re-assembled based on experimentally obtained
77 chromosomal contact maps. The HiC method yielded ten main scaffolds comprising >99% of
78 the previously obtained genome assembly (Supplementary Table 1 and 3; Supplementary Fig.
79 1 and 2), and corresponding to the ten chromosomes of *C. congregata*⁷. In addition, draft
80 genomes of five related *Cotesia* species – *C. rubecula*, *C. glomerata*, *C. vestalis*, *C. flavipes*
81 and *C. sesamiae* – were sequenced and assembled with Illumina shotgun sequencing reads
82 (Supplementary Table 1). They respectively resulted in scaffold N50 values of 13, 9, 15, 20 and
83 26 Kb and cumulative sizes of 216, 243, 176, 155 and 166 Mb, in agreement with cytometry
84 data (189-298 Mb) (Supplementary Table 2).

85 The genome of *C. congregata* comprised 48.7% of repeated DNA sequences including
86 34.7 % of known Transposable Elements (TEs) (Supplementary Fig. 3). Bracovirus proviral
87 segments comprised other TE sequences that had previously been annotated as bracovirus
88 genes: BV26 gene family corresponded to Sola2 elements (Supplementary Fig. 4;
89 Supplementary Table 5) and CcBV 30.5 to a *hAT*¹⁸. Moreover, the analysis suggested that the
90 BV7 gene family might also derive from TEs. Interestingly, some of these genes are highly
91 expressed in the host¹⁹ or likely participate in ecological adaptation²⁰. This indicates that, contrary
92 to a common paradigm in this field²¹, the virulence genes packaged in bracovirus particles do
93 not exclusively originate from the wasp cellular gene repertoire.

94 We annotated 14,140 genes in the genome of *C. congregata* (Methods and
95 Supplementary Table 7), which include more than 99% of 1,658 conserved insect genes (98 to
96 99 % of the genes for the other *Cotesia* species, Supplementary Fig. 5).

97 *Gene gains and losses in traits associated to the endoparasitoid lifestyle*

98 Expert annotation of chemoreceptor gene families identified 243 odorant receptor (OR),
99 54 gustatory receptor (GR) and 105 ionotropic receptor (IR) genes in *C. congregata*. These
100 numbers are in line with those of other parasitoid wasps, but are lower than in ants²²
101 (Supplementary Table 10). Phylogenetic analyses showed *C. congregata* ORs belong to 15 of
102 the 18 OR lineages (Fig. 2) described in Apocrita²³ and revealed independent OR gene
103 expansions in *N. vitripennis* and in Braconidae (Fig. 2). The most spectacular Braconidae-
104 specific expansions occurred in five clades each harboring at least 25 genes in *C. congregata*
105 (Fig. 2; Supplementary Table 10). Highly duplicated OR genes were found in 6 clusters of at
106 least 10, and up to 19, tandemly arrayed genes (Supplementary Fig. 16). Within Braconidae,
107 many duplications occurred in the ancestors of *Cotesia* but OR copy numbers varied
108 significantly between species (Fig. 2). This illustrates the highly dynamic evolution of OR gene
109 families within parasitoid wasps and between *Cotesia* species, which have different host ranges.

110 All the genes involved in detoxification in insects were identified in *C. congregata* and
111 are largely conserved within *Cotesia* (Supplementary Table 12 and 13). For instance, each
112 species harbors conserved numbers of UDP-glucosyltransferases (UGTs) and slightly different
113 numbers of glutathione-S-transferases (GSTs). In contrast, carboxylesterases (CCEs) and
114 cytochrome P450 (P450s) numbers vary widely with *C. flavipes* and *C. sesamiae* harboring few
115 representatives (respectively 22-24 CCEs and 49 P450s), compared to the 32 CCEs of *C.*
116 *rubecula* and the 70 P450s found in *C. congregata*, which are both exposed to plant toxic
117 compounds (Supplementary Table 12 and 13). *Cotesia* specific P450 families were identified
118 in the clan 3 and 4, both of which are often associated to adaptation to plant compounds and
119 insecticides²⁴ (Supplementary Table 12 and 13). Altogether *Cotesia* appear equipped for
120 detoxification although no spectacular gene expansion was observed.

121 The complete genome annotation of *C. congregata* revealed 102 nudiviral gene copies
122 that have colonized all the chromosomes. This number is similar to that of pathogenic
123 nudiviruses²⁵, which is an unexpected result given that endogenous viral elements usually
124 undergo gene loss in the course of evolution²⁶. Here this surprisingly high number of nudiviral
125 genes can be explained by the fact that gene losses have been balanced by expansions of gene
126 families. At least 25 of the 32 nudivirus core genes involved in essential viral functions²⁷ have
127 been retained in the wasp genome, with the notable absence of the nudiviral DNA polymerase
128 (Supplementary Table 8). The loss of this major replication gene may have prevented viral
129 escape^{10,25,28}. The *fen* genes, generally involved in DNA replication, form a gene family with six
130 tandem copies that is found specifically in the *Cotesia* lineage (Fig. 3B). The most spectacular

131 expansion concerns the *odv-e66* gene family with 36 genes in 10 locations (Fig. 2B; Fig. 3C),
132 in contrast to the one or two copies typically found in nudivirus genomes²⁹. This expansion
133 occurred both before and after the divergence between *C. congregata* and *M. demolitor* since
134 we found tandemly duplicated copies in homologous loci of the two species or in *C. congregata*
135 only (Fig. 3D). In baculoviruses, *odv-e66* encodes a viral chondroitinase³⁰ involved in digesting
136 the peritrophic membrane lining the gut, thus allowing access to target cells during primary
137 infection. Different ODV-E66 proteins may similarly allow BVs to cross various host barriers,
138 and BV infection to spread to virtually all Lepidoptera tissues^{31,32}.

139 *Genomic architecture and synteny of bracovirus genes*

140 Chromosome scale genome assembly of *C. congregata* provides the first comprehensive
141 genomic organization of a bracovirus within the genome of a wasp. Half of the single copy
142 nudiviral genes are located in the nudiviral cluster, which comprises 25 genes and is amplified
143 during bracovirus production but not packaged within the particles³³. Comparison with the
144 scaffolds of *M. demolitor* showed overall gene content conservation as well as conserved
145 syntenic blocks in the genomic regions flanking nudiviral sequences, over ~53 million years of
146 evolution. This confirms that the nudiviral clusters of both species are orthologous (Fig. 3) and
147 likely derive from a genome fragment of the nudivirus that originally integrated in the ancestor
148 wasp genome. This striking stability suggests that major evolutionary constraints maintain these
149 genes together. The other nudiviral genes are dispersed in the wasp genome, although not
150 evenly, as more loci are located in the 4 smaller chromosomes. Orthology with *M. demolitor*
151 could be identified for 20 nudiviral gene regions (Fig. 3; Supplementary Fig. 9), indicating they
152 were already dispersed in the last common ancestor of both wasps and have stayed in the same
153 loci. Altogether, this showed that nudivirus gene loss and dispersion occurred during the first
154 period of wasp-bracovirus association (100 to 53 mya).

155 The expansion of virulence genes is another aspect of wasp genome colonization. In *C.*
156 *congregata*, the 249 virulence genes encoded by proviral segments are found in 5 regions of
157 the genome located on three chromosomes. However, 77% of these genes clustered in a single
158 region, which comprised four physically linked proviral loci (PL1, PL2, PL4, PL10)
159 interspersed by wasp genes (Fig. 3). This major virulence gene coding region, named
160 “macrolocus”¹³, spans half of chromosome 5 short arm and is almost comparable (2 Mb, 177
161 genes) to the Major Histocompatibility Complex (MHC) involved in mammalian immunity (~4
162 Mb, ~260 genes)³⁴. Orthology relationships could be inferred between the PL1 in *C. congregata*

163 macrolocus and the largest proviral region (comprising 11 segments) of *M. demolitor*¹⁴ (Fig. 3)
164 but the macrolocus expanded specifically in the *Cotesia* lineage since *M. demolitor* does not
165 have regions corresponding to PL2, PL4 and PL10. Further syntenies were found between 5
166 isolated proviral loci, showing they pre-date wasp lineage split (Fig. 3) whereas the 3 proviral
167 loci in the long arm of chromosome C9 and C10 appear as a genuine novelty of the *Cotesia*
168 lineage. The homologous relationships of PLs contrasts with their gene content divergence³⁵,
169 which show high turnover probably because of their role as virulence factors and coevolution
170 with the hosts²⁰.

171 *Strong conservation of the bracoviral machinery*

172 The DNA circles packaged in bracovirus particles are produced following the genomic
173 amplification of replication units (RU) that span several proviral segments of PLs^{33,36}. Detailed
174 genomic analyses of *C. congregata* data led to the identification of a specific sequence motif at
175 each RU extremity and confirmed the presence of circularization motifs^{13,37} on all proviral
176 segments at the origin of packaged circles indicating the conservation of the viral machinery
177 whatever the localization of viral sequences. (Fig. 4; Supplementary Fig. 10).

178 Over 100 million years of evolution, the conservation of viral functions in microgastrid
179 wasps must have endured through several bouts of natural selection. Synonymous to
180 nonsynonymous substitution ratio analyses on orthologous nudiviral genes in *Cotesia* showed
181 most of the nudiviral genes (65 genes among the 79 tested genes), are evolving under
182 conservative selection, that is however less stringent than on the insect genes used to assess
183 genome completeness (Fig. 4; Supplementary Table 8). This selection is notably strong for
184 genes involved in viral transcription, such as the RNA polymerase subunits (*lef-4*, *lef-8*, *lef-9*,
185 *p47*), which most likely control nudiviral gene expression and, consequently, particle
186 production⁶⁸. In contrast, genes involved in infectivity (homologues of baculovirus *pif* genes)
187 appear less conserved (Supplementary Table 8). This might reflect divergence occurring during
188 host shifts, through adaptation of virus envelope proteins to particular host cell receptors. The
189 large *odv-e66* gene family and duplicated genes (*p6.9_2*, *pif-5_2*, *I7a*) similarly displayed less
190 stringent to relaxed selection (Fig. 4; Supplementary Table 8), which might be conducive to
191 mutational space exploration for adaptation by neo-functionalization or sub-functionalization³⁸.
192 Virulence genes encoded by proviral segments globally displayed lower conservative selection
193 than nudiviral genes (Fig. 4), as expected for genes interacting with host defenses and involved
194 in evolutionary arms race or adaptation to new hosts²⁰.

195 *Synchronized nudiviral transcription precedes bracovirus production*

196 Bracovirus particle production only occurs in the calyx region of the ovaries, and starts
197 before wasp emergence at day 5. Detailed RNAseq analysis of wasp ovaries and venom glands
198 during pupal development showed 91 (out of 102) nudiviral genes were only expressed in the
199 ovaries and could not be detected in the venom gland, which highlights strong ovarian tissue
200 specificity. The onset of nudiviral gene expression was detected from day 2 of pupal
201 development, with the expression of the RNA polymerase subunits (Fig. 5), reaching a peak on
202 day 4 and declining in later stages (Fig. 5). The other nudiviral genes followed roughly the same
203 expression pattern, low or undetectable at day 2, high at day 3 and reaching a peak at day 5
204 (Fig. 5). This pattern concurs with the hypothesis that the nudiviral RNA polymerase controls
205 the expression of the other nudiviral genes and therefore bracovirus particle production.
206 Notably, 12 nudiviral genes reached very high mRNA levels (at day 5) and are among the top
207 50 of most expressed genes in *C. congregata*. Three genes from the nudiviral cluster (including
208 a major capsid component vp39) are by far the 3 most expressed of all wasp genes. Altogether,
209 it is remarkable that the expression of almost all nudiviral genes remains strongly synchronized
210 during pupal ovarian development. Considering the age of the nudivirus wasp association, this
211 suggests strong evolutionary constraints.

212 *Immune gene expression during bracovirus production*

213 After 100 million years of endogenous evolution within the wasp genome, one can
214 question whether the bracovirus is considered as a regular part of the wasp or is still perceived
215 as non-self, in which case its production should trigger an immune response. However, whether
216 viral production interacts in any way with the wasp immune system was totally unknown.
217 Globally, the annotation of immune-related genes indicated *C. congregata* has an arsenal of
218 258 immune genes that are potentially involved in recognition, signal transduction, different
219 signaling pathways, melanization and effector functions (Supplementary Table 9), in
220 accordance with the recently reported annotation of *C. Vestalis* immune genes³⁹. We identified
221 all members of the Toll, IMD, Jak/STAT and RNA interference pathways found in
222 Hymenoptera (Supplementary Table 9).

223 In contrast to the sharp increase in nudiviral gene expression, no significant changes in
224 immune genes expression could be detected in the ovaries during pupal development (Fig. 6).
225 In particular, expression of the genes involved in antiviral immunity (encoding members of the
226 RNA interference, Jak/STAT or Imd/JNK pathways) was high in ovaries, even at stages before

227 particle production is observable (ovaries stages 2 to 4), but hardly fluctuated during the course
228 of ovary development, even at day 5, when massive particle production becomes apparent by
229 TEM (Fig. 5A). Thus, no immune response appears to be induced or repressed at the cellular
230 level as a response to high level nudiviral genes expression and particles production. This
231 strongly suggests the bracovirus is not perceived as foreign by wasp ovarian cells (Fig. 1).

232

233 Discussion

234 To investigate the genome features related to the endoparasitoid lifestyle, we sequenced
235 the genomes of 6 *Cotesia* species, and obtained an assembly at the chromosomal scale for *C.*
236 *congregata*. This approach provided insights into essential functions of the wasps, such as
237 olfaction, detoxification and immunity, as well as on the genomic evolution of the bracovirus.
238 Large OR gene diversifications are often associated to host localization and acceptance. Indeed,
239 female wasps rely on sensitive olfactory apparatus to locate their hosts from volatile cues plants
240 emit in response to herbivore attacks³⁴ and to assess caterpillar quality before oviposition⁴⁰.
241 Interestingly, OR copy numbers varied significantly during the evolution of the genus *Cotesia*
242 (Fig. 2). The high dynamics of OR repertoire might point to the need for more specific
243 recognition of chemical cues from the host and its food plant.

244 In contrast, no comparable diversification was observed in the detoxification arsenal,
245 even though *Cotesia* larvae can be exposed to various toxic phytochemicals while feeding on
246 the hemolymph of caterpillar hosts (i.e. potential exposure of *C. congregata* to insecticidal
247 nicotine when parasitizing *Manduca sexta* feeding on tobacco; *C. rubecula*, *C. glomerata* and
248 *C. vestalis* are potentially exposed to glucosinolates by developing in hosts consuming
249 crucifers; and *C. flavipes* to phenylpropanoids and flavonoids when parasitizing hosts on
250 sugarcane). This surprisingly low diversification of the detoxification arsenal could suggest that
251 wasp larvae may not be as exposed to toxic compounds as expected due to direct excretion of
252 these chemicals by the host larvae^{41,42}.

253 *Cotesia* wasps face different immune challenges during their lifetime. While feeding on
254 nectar the adult might be exposed to similar environmental challenges as honey bees.
255 Development inside the caterpillar host could on the one hand shield wasp larvae from
256 pathogens but on the other hand expose them to opportunistic infections, because parasitism
257 alters caterpillar immune defenses. Lastly, metamorphosis coincides with the production of
258 bracovirus particles, against which wasp antiviral responses had so far not been investigated.

259 Insects were recently shown to recognize their obligate bacterial symbionts as foreign and to
260 exert strong immune control, as documented for *Sitophilus oryzae* Primary Endosymbiont
261 (SOPE)⁴³. As the immunity gene arsenal of *C. congregata* is comparable to that of the honey
262 bee, this wasp is most probably able to mount an immune response against pathogens, including
263 viruses. However, the transcriptomic approach did not reveal any significant difference in
264 immune gene expression between the ovaries of different pupal stages, although massive
265 amounts of bracovirus particles are produced from day 5. This lack of anti-viral gene induction
266 suggests the wasp no longer perceives the bracovirus as potentially pathogenic but rather
267 considers it as a regular secretion.

268 With the ancestral integration of a nudivirus genome, the parasitoid wasp gained a series
269 of viral functions: including viral transcription, viral DNA replication, particle morphogenesis
270 and infectivity. Whereas the function of viral DNA replication has been lost¹⁰, thus impeding
271 autonomous virus re-emergence, the other viral functions have been reorganized for virulence
272 gene transfer via bracovirus particles. Chromosomal scale resolution of *C. congregata* genome
273 showed bracovirus genes have colonized all the chromosomes with a nearly 2.5 fold increase
274 of in the total number of virus genes compared to the genome of a pathogenic nudivirus. This
275 contrasts sharply with the decay of most viruses integrated into eukaryote genomes that do not
276 provide a benefit to their host. Bracovirus dispersion occurred between 100 and 53 mya, as 25
277 viral loci are orthologous between *Cotesia* and *Microplitis* (Fig. 3; Supplementary Fig. 5). Yet,
278 the organization of many bracovirus genes in clusters suggests strong evolutionary constraints
279 maintain these genes together. In the case of the nudiviral cluster, which encodes major capsid
280 components (VP39, 38K) on chromosome 7, DNA amplification, as a single unit³³, might be
281 essential to the mass production of bracoviral particles that are injected into parasitized hosts.
282 This could explain the counter selection on the dispersion or loss of these clustered nudivirus
283 genes since the separation of the *Cotesia* and *Microplitis* lineages. The genomic organization
284 stasis is reminiscent of bacterial symbiont genomes, which underwent major rearrangements
285 specifically during the initial steps of association⁴⁴. In the case of the particularly large
286 macrolocus which comprises 77% of virulence genes in *Cotesia* genome, clustering could
287 facilitate the evolution of new virulence genes copies by duplications¹³, and thereby wasp
288 adaptation against host resistance or to new hosts^{10,45}. This organization may also promote the
289 transmission of bracovirus virulence genes as a block of co-evolved genes as shown for
290 supergenes involved in butterfly wing patterns⁴⁶ and ant social behavior⁴⁷.

291 Remarkably, despite their dispersed locations in the wasp genome, bracovirus genes

292 remain synchronically expressed and under conservative selection, thus enabling the production
293 of infectious virus particles. This striking conservation of the viral machinery highlights the
294 paramount importance of the production of viral particles allowing virulence gene transfer to a
295 host, in the evolutionary history of the wasp. Although high nudiviral gene expression and
296 massive particle production underline the continuing existence of a virus entity, it is now
297 considered as a regular secretion by the wasp. Thus, contrary to a vision of obligatory
298 mutualism as an unstable alliance in which partners can become opponents or cheaters^{48,49}, this
299 very ancient association shows conflicts disappear when the benefits of the partners converge.

300

301 **Methods**

302 **Sampling**

303 The *C. congregata* laboratory strain was reared on its natural host, the tobacco hornworm, *M.*
304 *sexta* (Lepidoptera: Sphingidae) fed on artificial diet containing nicotine as previously
305 described⁵⁰. *C. sesamiae* isofemale line came from individuals collected in the field in Kenya
306 (near the city of Kitale) and was maintained on *Sesamia nonagrioides*⁵¹. *C. flavipes* individuals
307 originated from the strain used for biological control against *Diatraea saccharalis* in Brazil⁵².
308 *C. glomerata*, *C. rubecula* and *C. vestalis* laboratory cultures were established from individuals
309 collected in the vicinity of Wageningen in Netherlands, and reared on *Pieris brassicae*, *Pieris*
310 *rapae* and *Plutella xylostella* larvae, respectively^{53,54}. To reduce the genetic diversity of the
311 samples prior to genome sequencing, a limited number of wasps were pooled; for example,
312 only haploid males from a single virgin female were used for Illumina sequencing of *C.*
313 *congregata* genome, ten female and male pupae originating from a single parent couple were
314 used to generate *C. glomerata* genome, five male pupae originating from a single *C. rubecula*
315 virgin female for *C. rubecula* genome and 40 adult males and 8 adult females from multiple
316 generations of *C. vestalis* cultured in the Netherlands were used. DNAs were extracted from
317 adult wasps and stored in extraction buffer following two protocols. *C. congregata*, *C. sesamiae*
318 and *C. flavipes* DNA were extracted using a Qiamp DNA extraction kit (Qiagen) with RNase
319 treatment following the manufacturer's instructions and eluted in 200 µl of molecular biology
320 grade water (5PRIME). *C. glomerata*, *C. rubecula* and *C. vestalis* DNA was extracted using
321 phenol-chloroform.

322

323 **Genome sequencing and assembly**

324 *Cotesia congregata* genome was sequenced combining two approaches: (i) single-end reads
325 and MatePair libraries of 3Kb, 8Kb and 20Kb fragments on 454 GS FLX Titanium platform
326 (Roche) and (ii) paired-end reads of 320bp fragments with HiSeq2000 platform (Illumina). *C.*
327 *glomerata*, *C. rubecula* and *C. vestalis* DNA libraries were prepared using insert sizes of 300
328 and 700 bp. For *C. sesamiae* and *C. flavipes* libraries an insert size of 400 bp was selected.
329 These libraries were sequenced in 100 bp paired-end reads on a HiSeq2000 platform (Illumina)
330 at the French National Sequencing Institute (CEA/Genoscope, France) and at the Sequencing
331 Facility of the University Medical Center (Groningen, Netherlands). Reads were then filtered
332 according to different criteria: low quality bases ($Q < 20$) at the read extremities, bases after the
333 second N found in a read, read shorter than 30 bp and reads matching with phiX (Illumina intern
334 control) were removed using in-house software (<http://www.genoscope.cns.fr/fastxtend>) based
335 on the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) as described in
336 (<https://www.nature.com/articles/sdata201793>).

337 The *C. congregata* genome was generated by *de novo* assembly of 454 reads using GS De Novo
338 Assembler from the Newbler software package v2.8⁵⁵. The consensus was polished using the
339 Illumina data as described in ([https://bmcbgenomics.biomedcentral.com/articles/10.1186/1471-](https://bmcbgenomics.biomedcentral.com/articles/10.1186/1471-2164-9-603)
340 [2164-9-603](https://bmcbgenomics.biomedcentral.com/articles/10.1186/1471-2164-9-603)). Gaps were filled using GapCloser module from the SOAPdenovo assembler
341 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3626529/>). The genomes of *C. sesamiae*, *C.*
342 *flavipes*, *C. glomerata*, *C. rubecula* and *C. vestalis* were assembled with Velvet v1.2.07⁵⁵ using
343 the following parameters: `velveth k-mer 91 -shortPaired -fastq -separate, velvetg -clean yes` and
344 specific adapted values for `-exp_cov` and `-cov_cutoff` for each species. The genome size was
345 estimated using *k*-mer distribution of raw reads on all *Cotesia* species with 17-mer size using
346 Jellyfish v1.1.11⁵⁶.

347

348 **Chromosome scale assembly of *C. congregata* genome**

349 *HiC library preparation*

350 The individual wasps had their gut removed and were immediately suspended after sampling
351 in 30 mL of 1X tris-EDTA buffer and formaldehyde at 3% concentration, then fixed for one
352 hour. 10 mL of glycine at 2.5 M was added to the mix for quenching during 20 min. A
353 centrifugation recovered the resulting pellet for -80°C storage and awaiting further use. The
354 libraries were then prepared and sequenced (2×75 bp, paired-end Illumina NextSeq with the
355 first ten bases acting as tags), as previously described⁵⁷ using the *DpnII* enzyme.

356 *Read processing and Hi-C map generation*

357 The HiC read library was processed and mapped onto *DpnII* fragments of the reference
358 assembly using HiCbox (available at <https://github.com/kozullab/HiCbox>) with bowtie2⁵⁸ on
359 the back-end (option --very-sensitive-local, discarding alignments with mapping quality below
360 30). Fragments were then filtered according to size and coverage distribution, discarding
361 sequences shorter than 50 bp or below one standard deviation away from the mean coverage.
362 Both trimmed contact maps were then recursively sum-pooled four times by groups of three,
363 yielding bins of $3^4=81$ fragments.

364 *Contact-based re-assembly*

365 The genome was re-assembled using an updated version of GRAAL (dubbed instaGRAAL) for
366 large genomes on the aforementioned contact maps for 1,000 cycles, as described in⁵⁹. Briefly,
367 the program modifies the relative positions and/or orientations of sequences according to
368 expected contacts given by a polymer model. These modifications take the form of a fixed set
369 of operations (swapping, flipping, inserting, merging, etc.) on the 81-fragment bins. The
370 likelihood of the model is maximized by sampling the parameters with an MCMC method.
371 After a number of iterations, the contact distribution converges and the global genome structure
372 ceases to evolve, at which point the genome is considered reassembled. The process yielded
373 eleven main scaffolds comprising >99% of the bin sequences.

374 *Polishing and post-assembly processing*

375 Each scaffold was independently polished by reconstructing the initial contig structure
376 whenever relocations or inversions were found. In addition, previously filtered sequences were
377 reintegrated next to their original neighbors in their initial contig when applicable. The
378 implementation is part of instaGRAAL polishing and available at
379 <https://github.com/kozullab/instaGRAAL> (run using the --polishing mode).

380 *Assembly validation*

381 We performed the validation with QUAST-LG⁶⁰, an updated version of the QUAST analyzer
382 tailored for large genomes. The initial assembly from Illumina short reads was used as
383 reference. The assessed metrics include genomic feature completeness, Nx and NGx statistics
384 as well as global and local misassemblies. In addition, each assembly was assessed for ortholog
385 completeness with BUSCO v3⁶¹. The reference dataset used for expected gene content was
386 pulled from the OrthoDB (version 9) database for Hymenoptera, comprising 4,415 orthologs.

387

388 **Genome annotations**

389 *Transposable element annotation*

390 Genome annotation was first done on the *C. congregata* reference genome and then exported
391 on the genomes of the five other *Cotesia* species. First, the transposable element annotation was
392 realized following the REPET pipeline comprising a *de novo* prediction (TEdenovo) and an
393 annotation using TE libraries (TEannot)⁶². This annotation was exported into GFF3 files used
394 as mask for the gene annotation.

395 *Automated gene annotation*

396 The automated gene prediction and annotation of *C. congregata* genome was done using
397 Gmove (<https://github.com/institut-de-genomique/Gmove>) integrating different features based
398 on (i) the mapping of Hymenoptera proteins from all hymenopteran genomes available on
399 NCBI (Supplementary Table 6) and UniProt Hymenoptera, (ii) the mapping of RNA-Seq data
400 from *C. congregata*, *C. glomerata*, *C. vestalis* and *C. rubecula*^{63,64}, and (iii) *ab initio* genes
401 predictions using SNAP⁶⁵. The automated annotation of the five other *Cotesia* species was
402 performed using MAKER⁶⁶ using the same features as for the annotation of *C. congregata* but
403 also including the output automated annotation of *C. congregata*.

404 *Automated gene functional annotation*

405 The functional annotation was performed using blastp from BLAST+ v2.5.0⁶⁷ to compare the
406 *C. congregata* proteins to the NCBI non-redundant database (from the 29/06/2014). The ten
407 best hits below an e-value of 1e-08 without complexity masking were conserved. Interproscan
408 v5.13-52.0⁶⁸ was used to analyze protein sequences seeking for known protein domains in the
409 different databases available in Interproscan. Finally, we used Blast2GO⁶⁹ to associate a protein
410 with a GO group.

411 *Specialist gene annotation*

412 The automated annotations were followed by manual curations, corrections and annotations
413 realized by specialists of each gene family of interest through Apollo⁷⁰. The genomes were
414 available to this consortium through the web portal: <https://bipaa.genouest.org/is/parwaspdb/>.

415 *Genome completeness evaluation*

416 The completeness of the genomes and annotations were evaluated using Benchmarking
417 Universal Single-Copy Orthologs BUSCO v3⁶¹ using the insecta_odb9 database composed of
418 1658 genes.

419 Contigs were searched for similarities against the nonredundant NCBI nucleotide (nt) (release
420 November 2019) and the Uniref90 protein (release November 2019) databases using
421 respectively blastn v2.7.1+⁶⁷ and diamond v0.9.29.130⁷¹. For both tasks, e-value cutoff was set
422 to 10⁻²⁵. Taxa were inferred according to the highest-scoring matches sum across all hits for
423 each taxonomic rank in the two databases. Sequencing coverage was deduced after mapping
424 Illumina paired reads to the assembly with Bowtie2 v2.3.4.2⁵⁸. Contigs were then displayed
425 with Blobtools v1.1.1⁷² using taxon-annotated-GC-coverage plots.

426

427 **Orthologous genes identification, alignment and phylogeny**

428 Orthologous genes between all genes annotated in the six *Cotesia* species and the four
429 outgroups (*Microplitis demolitor*, *Nasonia vitripennis*, *Apis mellifera* and *Drosophila*
430 *melanogaster*) were identified using OrthoFinder v1.14⁷³. Universal single-copy ortholog genes
431 from BUSCO v3⁶¹ were extracted for the six *Cotesia* species and the four outgroups, aligned
432 using MAFFT v7.017⁷⁴ and concatenated. The species phylogeny was performed on this
433 alignment composed of 1,058 orthologous for a length of 611 kb using PhyML program⁷⁵ with
434 the HKY85 substitution model, previously determined by jModelTest v2.1⁷⁶ and the branch
435 support were measured after 1,000 bootstrap iterations. The cluster of orthologous genes was
436 used to determine the phylogenetic level of each gene represented in Fig. 2. as follows: genes
437 shared by all species are called shared by all; genes shared by at least nine species among the
438 ten studied species without phylogenetic logical are named "shared by some"; genes shared by
439 only Hymenoptera species and without orthologous gene in *D. melanogaster* are considered as
440 "Hymenoptera specific"; genes shared only by Microgastrinae are named "Microgastrinae
441 specific"; genes shared only by *Cotesia* species and without orthologous genes in any of the
442 outgroup are considered as "Cotesia specific".

443

444 **Synteny analyses**

445 The different synteny analyses were performed on the orthologous genes identified by
446 OrthoFinder v1.14⁷³ and by reciprocal blastp from BLAST+ v2.2.28⁶⁷ on the annotated proteins

447 (e-value below $10e^{-20}$). The correspondence between the genes, the localizations on the scaffold
448 and the figures were realized thanks to a custom R script (R Core Team 2013).

449

450 **Evolution of gene families**

451 For OR, P450 and odv-e66 genes manually annotated genes from the reference genome of *C.*
452 *congregata* were used along with orthologous genes from the five other *Cotesia* species, *M.*
453 *demolitor*⁷⁷, *N. vitripennis*⁷⁸, *A. mellifera*⁷⁹ to create a phylogeny of each family among
454 Hymenoptera. Protein sequences were first aligned with MAFFT v7.017⁷⁴ and the maximum-
455 likelihood phylogeny was performed with PhyML⁷⁵ using the JTT+G+F substitution model
456 for OR and using HKY80 substitution model for P450 and odv-e66 genes. The branch support
457 was assess using aLRTfor OR and 1,000 bootstraps for P450 and odv-e66 genes.. The trees
458 were then rooted to Orco (OR-coreceptor) clade for OR and t the midpoint for the other. The
459 gene gains and losses along the phylogeny for the different gene families of interest were
460 identified with NOTUNG v2.9^{80 81}.

461

462 **Evolution of single copy genes**

463 To determine evolutionary rates within *Cotesia* genus, single-copy orthologous gene clusters
464 (BUSCO, nudiviral and virulence genes) were first aligned using MACSE⁸² to produce reliable
465 codon-based alignments. From these codon alignments, pairwise dN/dS values were estimated
466 between *C. congregata* and *C. sesamiae*, the two most diverging species in the *Cotesia*
467 phylogeny, with PAML v4.8 using the YN00 program⁸³. dN/dS of the different gene categories
468 of interest were then compared using a Kruskal–Wallis test, and Nemenyi-Tests for multiple
469 comparisons were realized with the R package. For the nudiviral genes the dN/dS values were
470 calculated using genes from the 6 species. Orthologous genes from the six *Cotesia* species were
471 aligned as described before and codeml (implemented in PAML v4.8) was used to estimate the
472 M0 dN/dS (free ratio model). This model was compared to a neutral model for which the dN/dS
473 is fixed to 1.

474

475 **RNA-Seq analyses**

476 *Sample preparation, extraction and sequencing*

477 The ovaries and venom glands were extract from females at five pupal stages, i.e. days 2, days
478 3, days 4, days 5 and at emergence, corresponding to the number of days after the creation of
479 the cocoon and identified following body melanization⁸⁴. Ovaries were pooled by groups of 20
480 pairs and venom glands by 100 and in duplicates for each condition. Samples were stored in
481 buffer provided in the extraction kit by adding 2-mercaptoethanol to reduce RNA degradation.
482 Extractions were performed using QIAGEN RNeasy kit following manufacturer's
483 recommendations. Library preparation and sequencing were performed at the Genescope using
484 a paired-end strategy with HiSeq2000 platform (Illumina).

485 *Analyses*

486 The pair-end reads from *C. congregata* ovary and venom gland libraries were mapped on the
487 reference genome using TopHat2⁸⁵ with default parameters. Then, featureCounts program from
488 the Subread package⁸⁶ was used to determine fragment counts per genes using default
489 parameters.

490 To analyze gene expression, the raw fragment counts of ovaries and venom glands samples
491 were first converted to counts per million (CPM) using the edgeR⁸⁷ R-implemented package
492 (R-Core Team 2017). Expressed genes were filtered based on a CPM > 0.4 (corresponding to
493 raw count of 15) in at least two of the libraries incorporated in the analysis (Supplementary Fig.
494 6 A) and subsequent normalization was performed on CPMs using the edgeR TMM method for
495 Normalization Factor calculation⁸⁸ (Supplementary Fig. 6 B). The reproducibility of replicates
496 was then assessed by Spearman correlation of gene expression profiles based on filtered and
497 normalized CPMs (Supplementary Fig. 6 C).

498 To examine differential expression between ovary stages and with venom glands a quasi-
499 likelihood negative binomial generalized log-linear model was fitted to the data after estimation
500 of the common dispersion using edgeR. Then, empirical Bayes quasi-likelihood F-tests were
501 performed to identify differentially expressed (DE) genes under chosen contrasts⁸⁹. Finally, F-
502 test p-values were adjusted using False Discovery Rate (FDR) method⁹⁰. Genes were
503 considered as DE whether FDR < 0.05 and fold-change (FC) of expressions between compared
504 conditions was higher or equal to 1.5. Four contrasts were designed between the five successive
505 ovary stages and a control contrast was tested between ovaries and venom glands at wasp
506 emergence stage.

507

508 **Data availability**

509 The datasets generated during the current study are available from the National Center for
510 Biotechnology Information (NCBI) at the following BioProject ID: PRJEB36310 for genome
511 raw reads; PRJNA594477 for transcriptome raw reads. Genome database (genomes and
512 annotated genes) are available on the web site BIPAA (Bioinformatic Platform for Agrosystem
513 Arthropods) <https://bipaa.genouest.org/is/parwaspdb/>. Custom scripts are available from
514 https://github.com/JeremyLGauthier/Script_Gauthier_et_al._2020_NEE.

515

516

517 **Figure Legends**

518 **Fig. 1. A.** Major traits involved in the parasitoid koinobiont lifestyle and genome content of six
519 *Cotesia* species. First OLFACTION plays an important role in the detection of the plant
520 (tobacco) attacked by caterpillars and host (*M. sexta*) larvae acceptance by adult wasps. Once
521 the host is accepted, the wasp injects its eggs bathed in ovarian fluid filled with bracovirus
522 particles. Bracovirus particles infect host cells, from which expression of bracovirus
523 VIRULENCE GENES alter host immune defenses, allowing wasp larvae development (the
524 eggs laid in the host body would otherwise be engulfed in a cellular sheath of hemocytes). As
525 the host ingests plant toxic compounds, such as nicotine, while feeding, wasp larvae consuming
526 the hemolymph containing these compounds rely on DETOXIFICATION to complete their life
527 cycle. But in these species associated with endogenous viruses the most important trait for
528 parasitism success consists in BRACOVIRUS MORPHOGENESIS during wasp
529 metamorphosis, using genes originating from a nudivirus ancestrally integrated in the wasp
530 genome. As massive production of virus particles occurs within wasp ovaries, WASP
531 IMMUNITY may be induced during particles production; d2, d3, d4, d5 refer to developmental
532 stages of *C. congregata* larvae⁸⁴. **B** Pictures of the six *Cotesia* species sequenced (credit H. M.
533 Smid and R. Copeland). **C** Phylogeny of these species based on 1,058 single-copy orthologous
534 insect genes including the Microgastrinae *Microplitis demolitor* and outgroups (*N. vitripennis*,
535 *A. mellifera*, and *D. melanogaster*). Black dots highlight branches with at least 90% support
536 from maximum-likelihood analysis (1,000 bootstraps). **D** Distribution of shared genes at
537 several phylogenetic levels. Full protein-coding gene sets were included to identify orthologous
538 gene groups. The “shared by some” category refers to genes shared by at least nine species
539 among the 10 studied. Note that the lower number of genes for *C. congregata* probably reflects
540 the higher quality of the genome assembly obtained.

541 **Fig. 2. Gene family extensions in *Cotesia*.** A Maximum-likelihood phylogeny of the OR
542 family in *C. congregata* and four other Hymenoptera species. The dataset included 243 amino
543 acid sequences from *C. congregata* (blue), 203 sequences from *M. demolitor* (red), 216
544 sequences from *N. vitripennis* (orange), 162 sequences from *A. mellifera* (yellow). The tree was
545 rooted using the Orco (OR-coreceptor) clade. Circles indicate nodes strongly supported by the
546 approximate likelihood-ratio test (black circles $aLRT \geq 0.95$; white circles $0.90 \geq aLRT \leq 0.95$).
547 The scale bar represents 0.5 expected amino acid substitutions per site. ORs of the five
548 Hymenoptera species are distributed into 18 OR subfamilies previously described in ²³
549 delineated in grey. **B** Copy number dynamics of OR (olfaction) P450 (detoxification) and *Odv-*
550 *e66* genes, note that the later are found specifically in bracovirus associated wasps since they
551 derive from the ancestrally integrated nudivirus. Estimated numbers of gene gain and loss
552 events are shown on each branch of the species tree. The size of OR repertoires in common
553 ancestors is indicated in the boxes. The lack of phylogenetic resolution for closely related
554 *Cotesia* OR genes precluded any comprehensive analysis of gene gains and losses.

555 **Fig. 3. Synteny of nudiviral genes loci and Proviral Loci (PL) between *C. congregata* and**
556 ***M. demolitor*.** A *C. congregata* chromosome map with the position of 24 nudiviral genes loci.
557 **B** Comparisons between nudiviral gene regions of *C. congregata* and *M. demolitor*. Synteny
558 between the two species has been characterized by at least two hymenopteran (non-viral)
559 orthologous genes in the vicinity of homologous nudiviral gene(s) of both species. Genome
560 scaffolds are represented in black. Red boxes indicate nudiviral genes and white boxes refer to
561 hymenopteran genes. 1. the *vp91* region is orthologous indicating the position of this gene was
562 inherited from their common ancestor 53 mya; 2.: the *fen* region is also orthologous but an
563 expansion occurred specifically in *Cotesia* lineage giving rise to six copies; 3.: the organization
564 of the nudiviral cluster encoding in particular capsid genes has remained strikingly similar with
565 the same viral genes in the same order (except p6-9-2) in both species indicating strong
566 evolutionary constraints. **C** *C. congregata* chromosome map with the position of gene loci
567 corresponding to the highly expanded *odv-e66* nudiviral gene family. **D** Comparison of two
568 *odv-e66* loci showing that expansion occurred before (cluster 7) and after (cluster 4) the
569 separation of both species **E** *C. congregata* chromosome map with the position of Proviral Loci
570 (PL) encoding virulence genes packaged in bracovirus particles. Note the concentration of loci
571 (successively PL10-PL1-PL2 and PL4) in a 2 Mb region termed "macrolocus" and representing
572 half of the chromosome 5 short arm. **F** Comparison of *C. congregata* and *M. demolitor* PL.
573 Numbers 1 to 37 and letters correspond to the different dsDNA circles present in CcBV and

574 MdBV particles produced from the PL. Blue boxes indicate virulence genes while white boxes
575 refer to hymenopteran genes and the red box to a nudiviral *odv-e66* gene located between PL1
576 and PL2. Ø indicates the absence of orthologs PL in the *M. demolitor* genome.

577 **Fig. 4. A** *C. congregata* chromosome map with the location of bracovirus loci: nudiviral gene
578 loci are shown in red, nudiviral *odv-e66* gene loci in hatched red and Proviral Loci (PL) in blue.
579 The sizes of the circles correspond to the relative number of genes in each locus **B** taxon-
580 annotated GC content-coverage plot of *C. congregata* scaffolds. Each circle represents a
581 scaffold in the assembly, scaled by length, and colored by taxonomy assignment to Braconidae
582 and Polydnviridae. The x-axis corresponds to the average GC content of each scaffold and the
583 y-axis corresponds to the average coverage based on alignment of illumina reads. **C** Measure
584 of selection pressure on hymenopteran conserved genes, nudiviral genes and virulence genes.
585 Pairwise evolutionary rates (dN/dS) of single-copy orthologous BUSCO genes, nudiviral genes,
586 different copies from the expanded *odv-e66* nudiviral gene family and virulence genes of *C.*
587 *congregata* and *C. sesamiae*. Letters above boxes indicate significant differences determined
588 by Kruskal–Wallis test ($H= 296.8$, 2 d.f., $P<0.001$) followed by *post-hoc* comparisons. **C**
589 Schematic representation of the genomic amplification during the production of viral particles
590 in the wasp ovaries. Replication Unit Motifs (RUM) are the motifs that constitute the
591 extremities of the molecules amplified during particle production. Direct Repeat Junctions
592 (DRJ), at the extremities of each segment are used during the excision/circularization process
593 to produce packaged dsDNA circles from the amplified molecules. Host Integration Motifs
594 (HIM) are motifs used during the integration of bracovirus circles in host genome. For each of
595 these motifs an alignment of a representative set of sequence comprising five motifs from *C.*
596 *congregata* and *M. demolitor* are represented (all alignments are reported in Supplementary
597 Fig. 10).

598 **Fig. 5. Gene expression of nudiviral genes in the ovaries during *C. congregata* nymphal**
599 **development.** **A** Pictures of wasp developmental stages studied and characteristic electron
600 micrographs of ovarian cells involved in particle production. From d2 to d4 stage, cells that
601 will produce particles show enlarged nuclei with chromatin condensation (left panel). From d5
602 massive particle production begins, particle assembly occurs at the periphery of a zone of
603 electron dense material in the nucleus named "virogenic stroma" (middle panel). In newly
604 emerged wasp nuclei are completely filled with bracovirus particles (right panel). Credit: Juline
605 Herbinière. **B** Unsupervised hierarchical clustering based on gene expression in ovaries and

606 venom glands. Ov2, Ov3, Ov4, Ov5 refer to the different ovaries across wasp developmental
607 stages. Ove and vg refer to ovaries and venom glands of adult wasps. The colored squares
608 associated with the clustering tree indicate the viral functions to which different nudiviral genes
609 are supposed to contribute based on those of their baculovirus homologues. Heatmap of
610 expression levels of 95 nudiviral genes is shown in the middle panel. Bold names highlight the
611 genes that are validated as significantly differentially expressed between two consecutive stages
612 using the statistical analysis and dots represent the four different comparisons studied between
613 ovary stages (Ov2 vs. Ov3, Ov3 vs. Ov4, Ov4 vs. Ov5 and Ov5 vs. Ove). Black, red and green
614 dots indicate similar, increased and reduced expressions between consecutive developmental
615 stages respectively. The increase of some nudivirus genes expression between d2 and d3
616 visualized on the heat map was not validated statistically for all of them because in one of the
617 d2 duplicates (shown in C) nudiviral genes expression had already reached high levels.
618 Underlined genes show higher expression in venom glands compared to ovaries (Ove) note that
619 27a and 17b are not nudiviral genes but wasp genes, the products of which have been identified
620 in *Chelonus inanitus* bracovirus particles. **C** Unsupervised hierarchical clustering of gene
621 expression from the two replicates of Ov2 ovary stage, that are very different regarding
622 nudiviral gene expression levels, although dissected nymphae presented a similar coloration
623 pattern, the left one representing a slightly earlier stage from the analysis of the whole set of
624 wasp genes. Note that the genes within the box are already expressed at a high level in the
625 earlier stage, including all of the genes involved in nudiviral transcription (shown in green)
626 except *lef5*, in accordance with the hypothesis that the nudiviral RNA polymerase complex
627 controls the expression of the other genes (*lef-5* is associated to the complex but not a part of
628 it).

629 **Fig. 6. Gene expression of immune genes in the ovaries during *C. congregata* nymphal**
630 **development.** Gene expression of antiviral immunity genes during *C. congregata*
631 development. The heatmaps show the expression of genes involved in **A** RNAi, **B** Imd, **C** Toll
632 and **D** Jak-STAT pathways across the developmental stages of ovaries (Ov2, Ov3, Ov4, Ov5,
633 Ove) and in venom glands (vg). The trees on the left are unsupervised hierarchical clustering
634 of expression values. Boxplots represent overall expression of each pathway in ovaries and
635 venom glands. Bold names highlight the genes that are differentially expressed between two
636 stages and dots represent the four different comparisons studied between consecutive ovary
637 stages (Ov2 vs. Ov3, Ov3 vs. Ov4, Ov4 vs. Ov5 and Ov5 vs. Ove). Black, red and green dots
638 indicate similar, increased and reduced expressions between consecutive developmental stages

639 respectively. Note that no particular trend appears correlated to bracovirus particles production
640 which occurs massively from Ov5 onward.

641

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648

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716

717 **Ethics declarations**

718 Competing interests

719 The authors declare no competing interests.

720

721 **Additional information**

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724

725 **Supplementary information**

726 [Cotesia_genomes_Supplementary_data.pdf](#)

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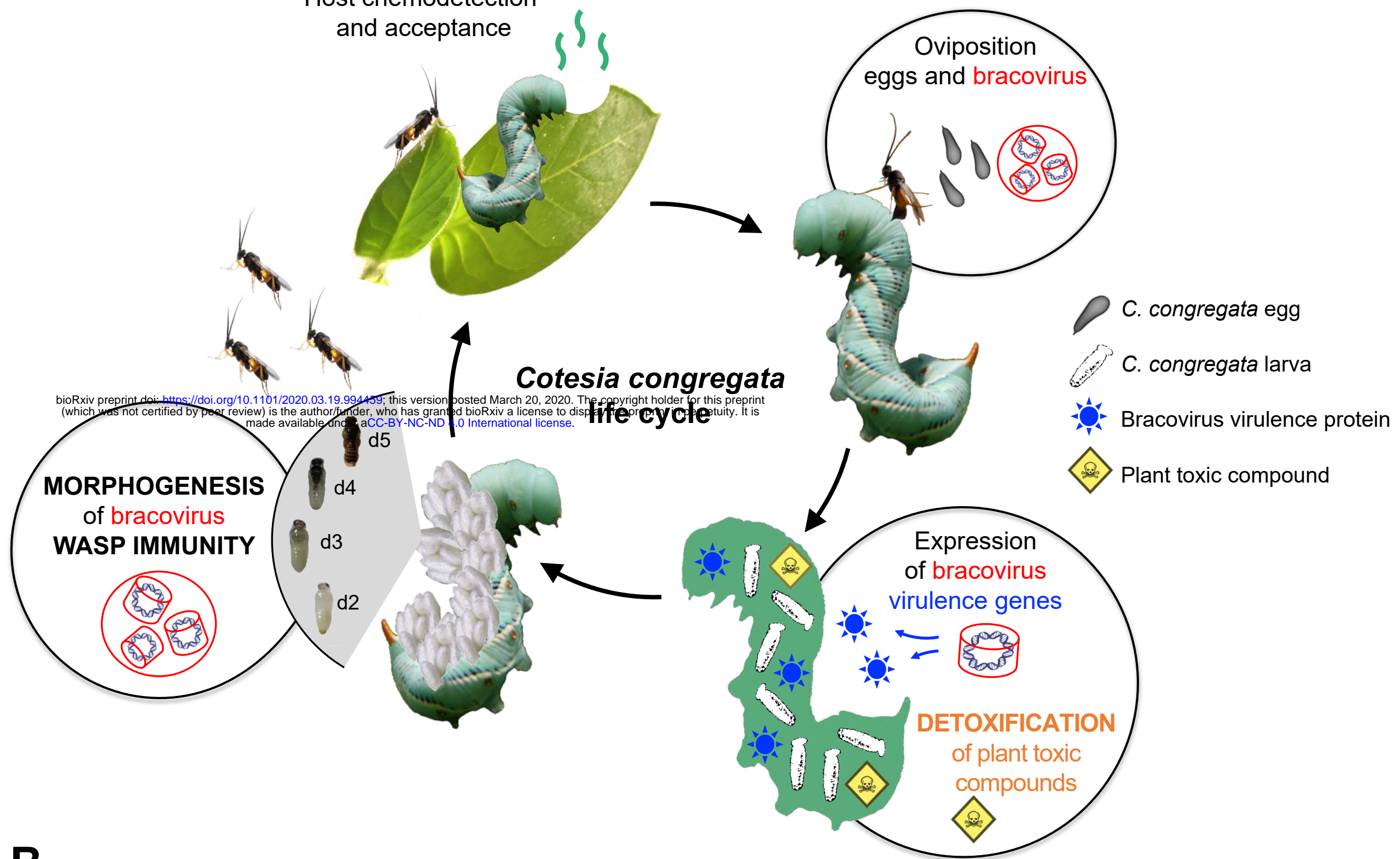
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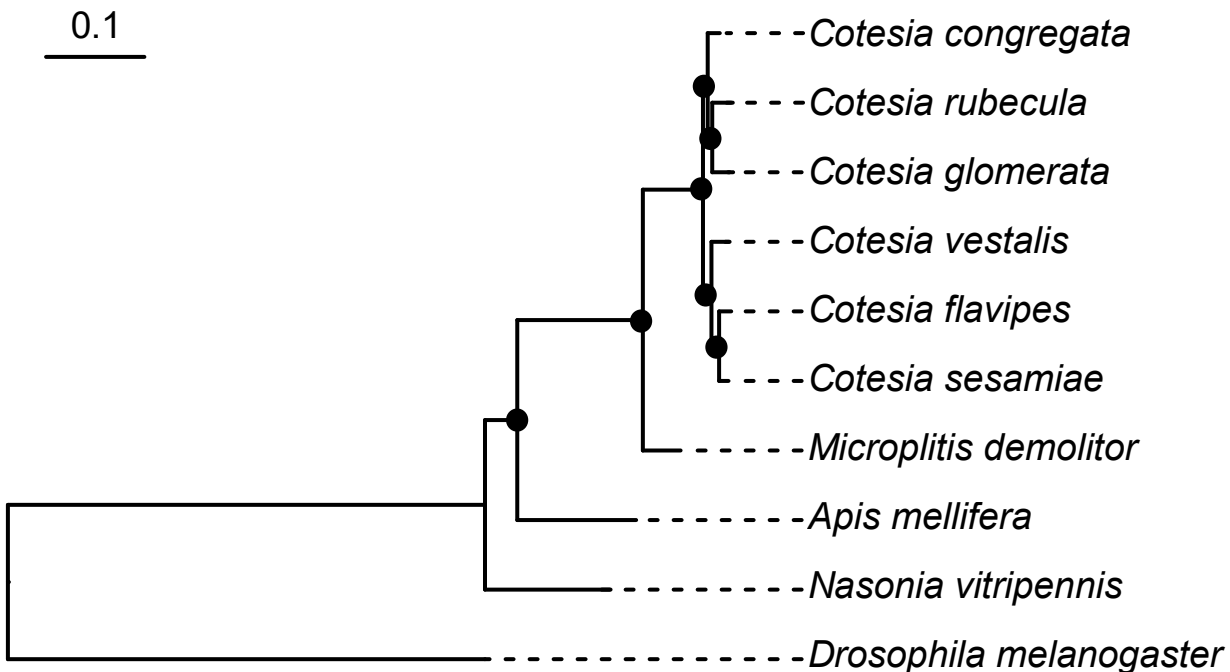
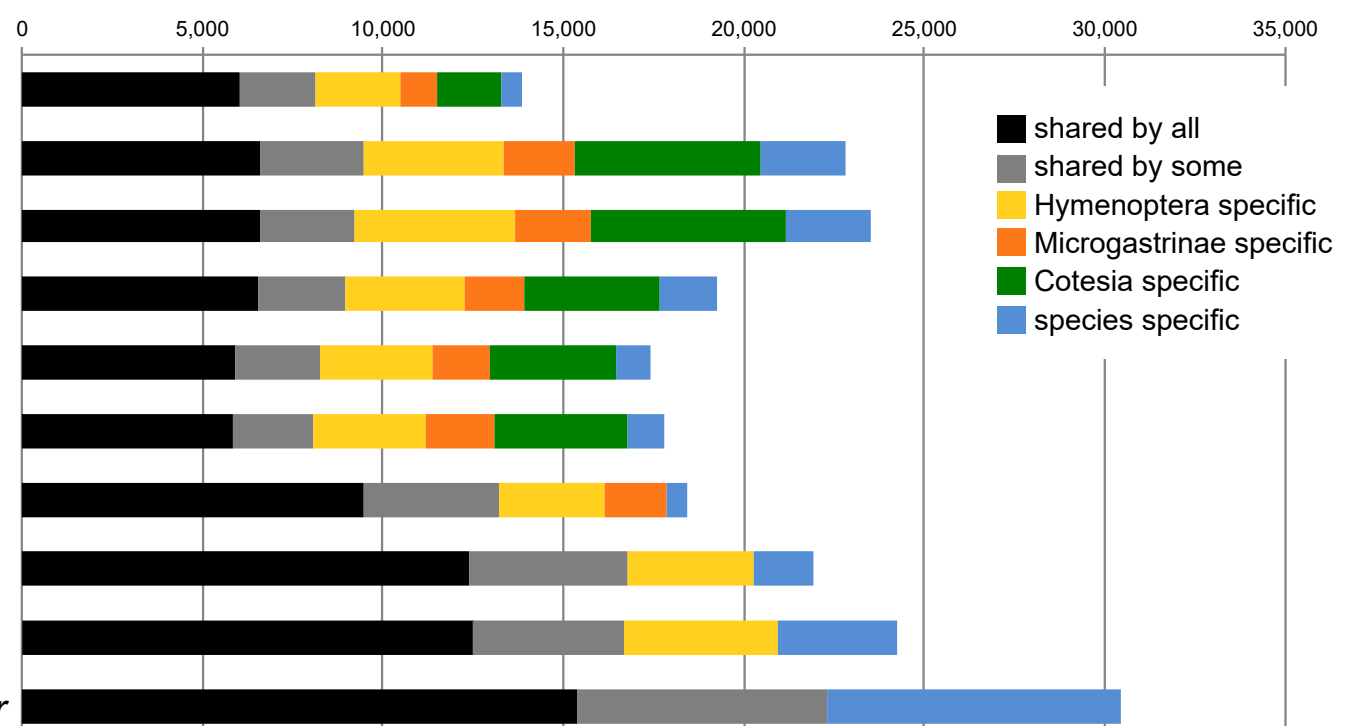
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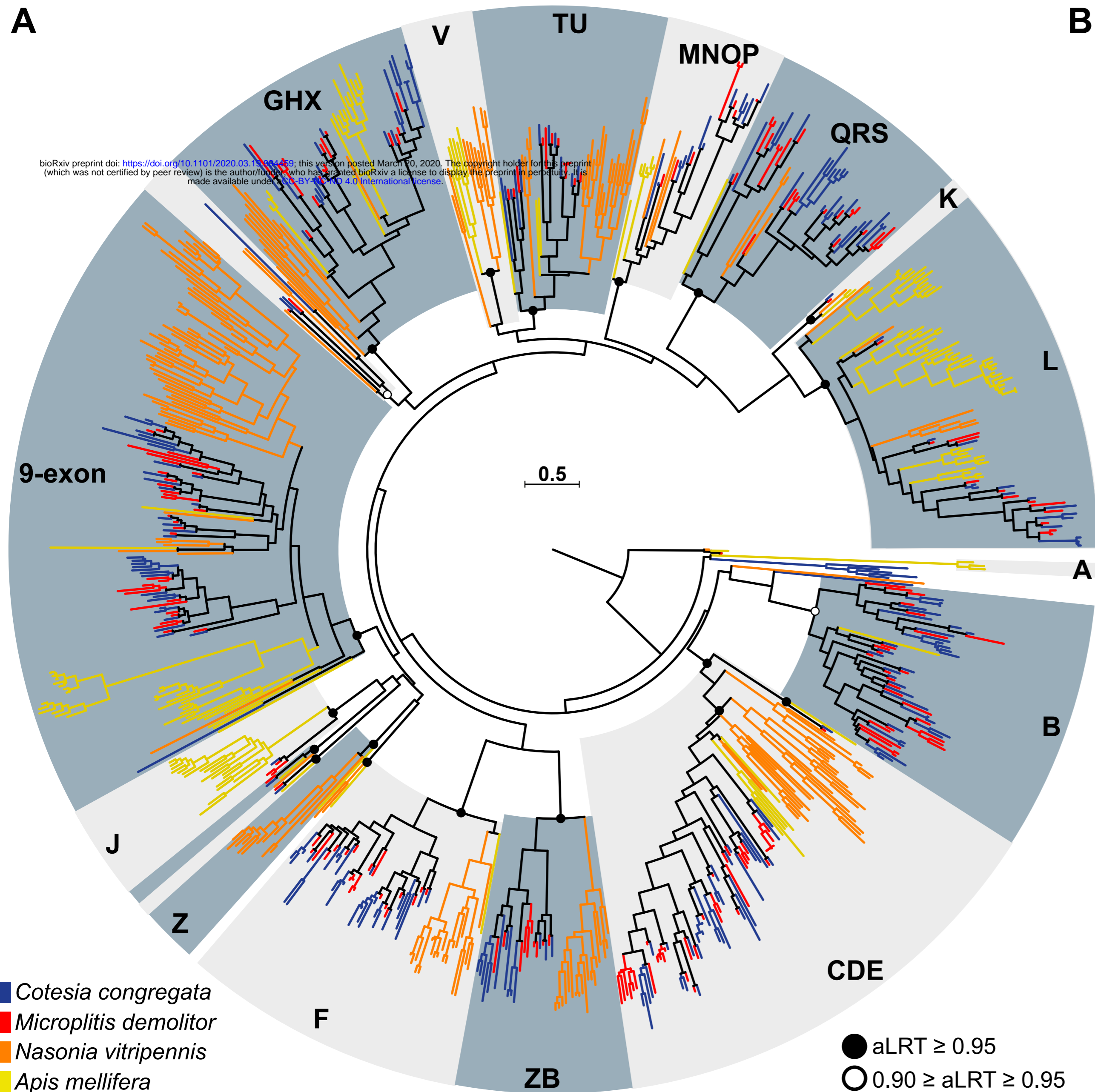
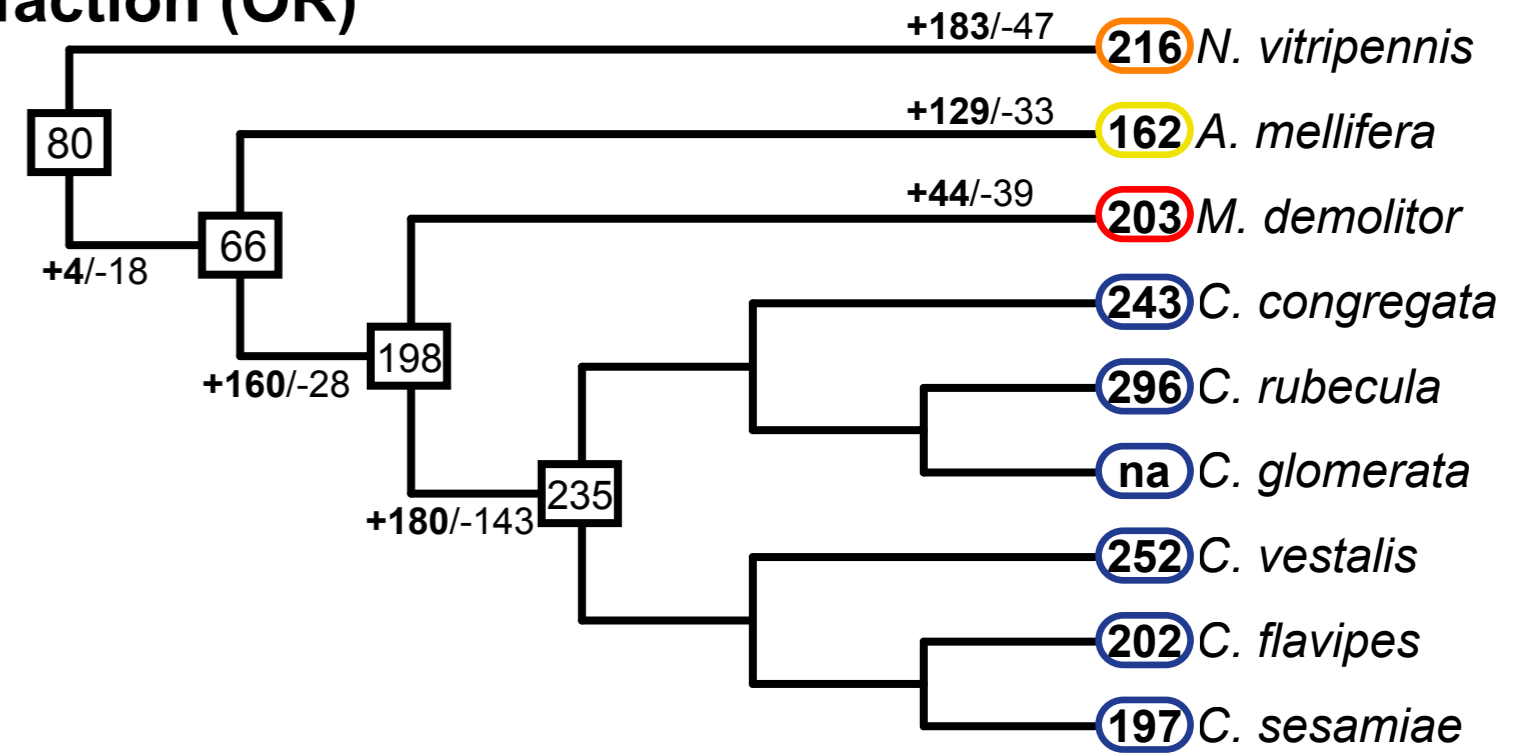
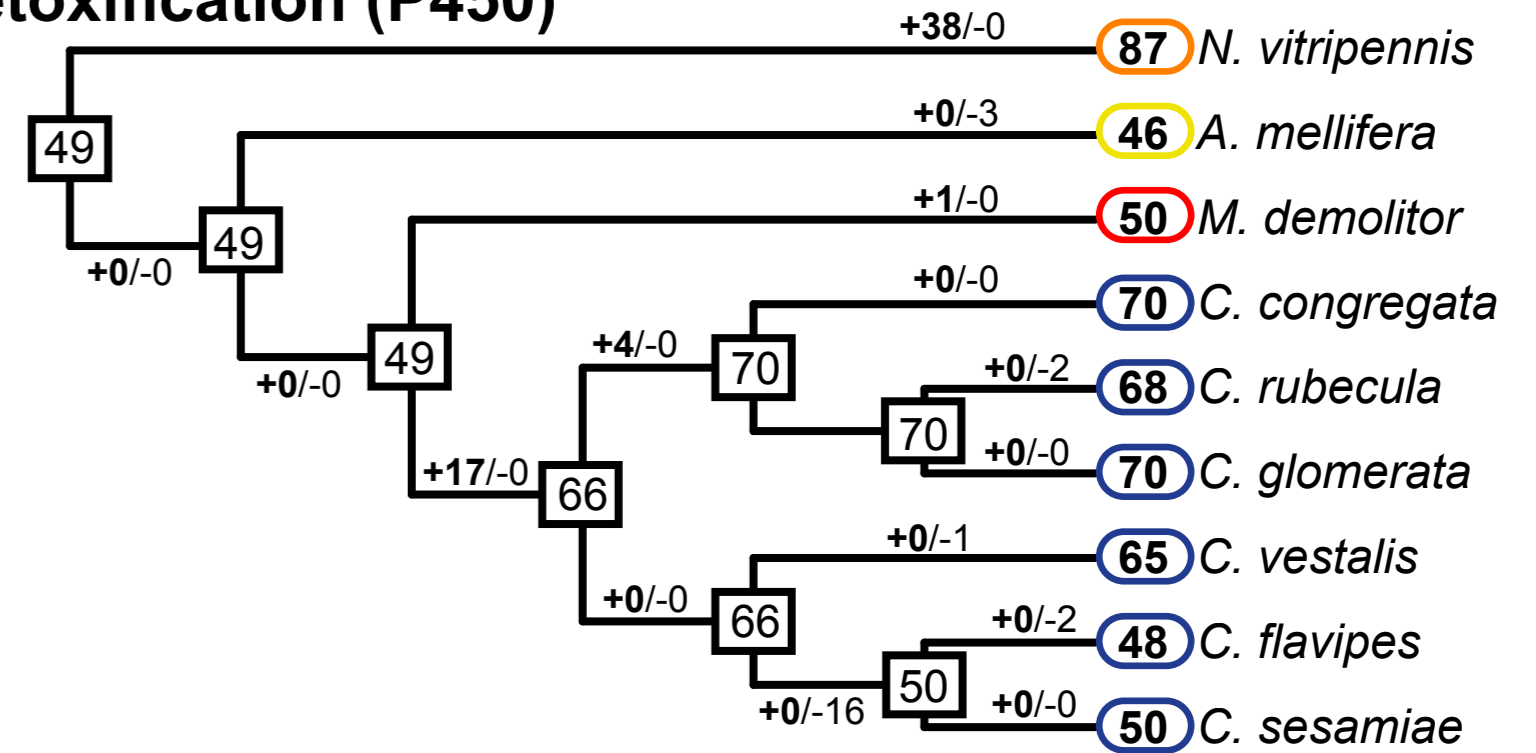
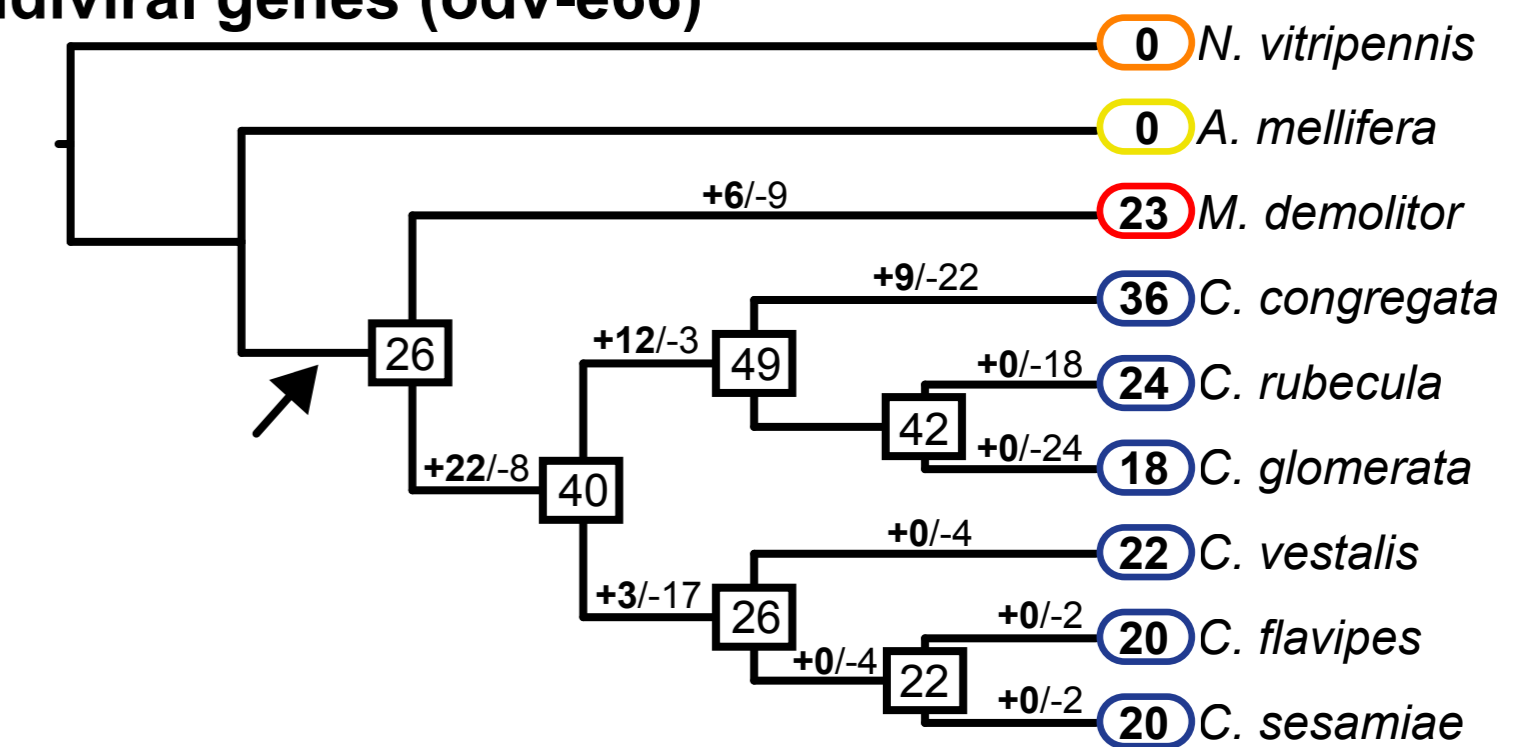
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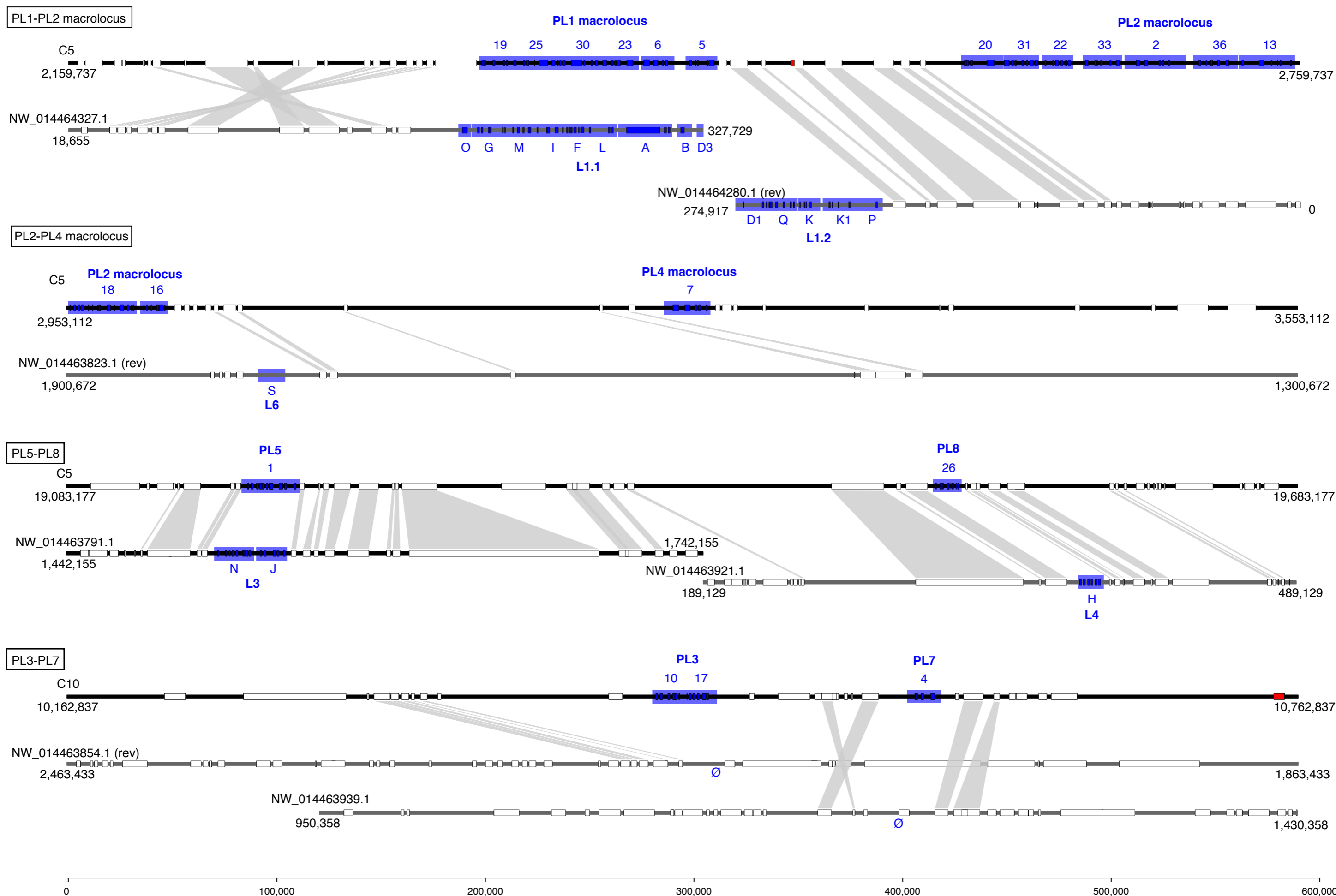
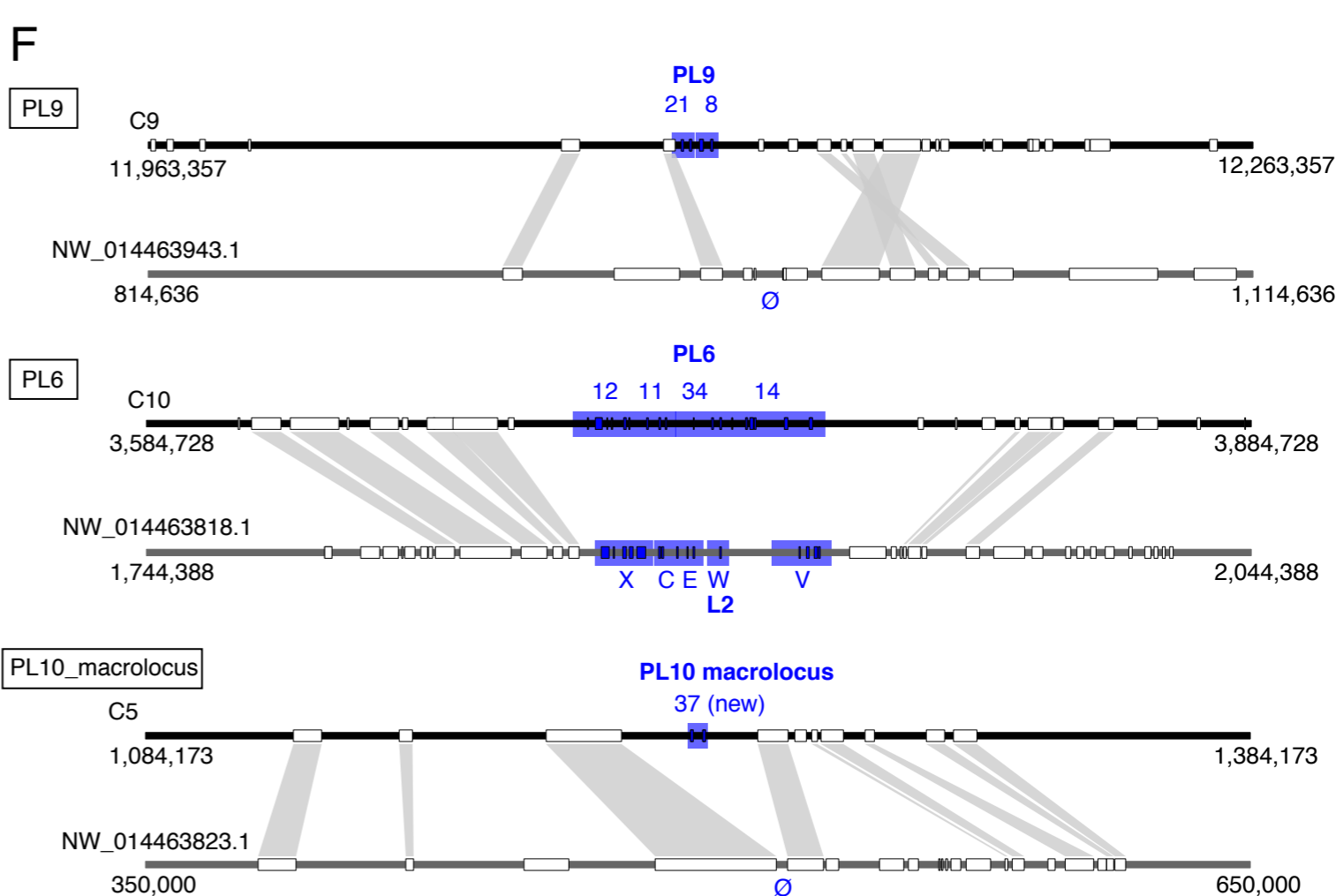
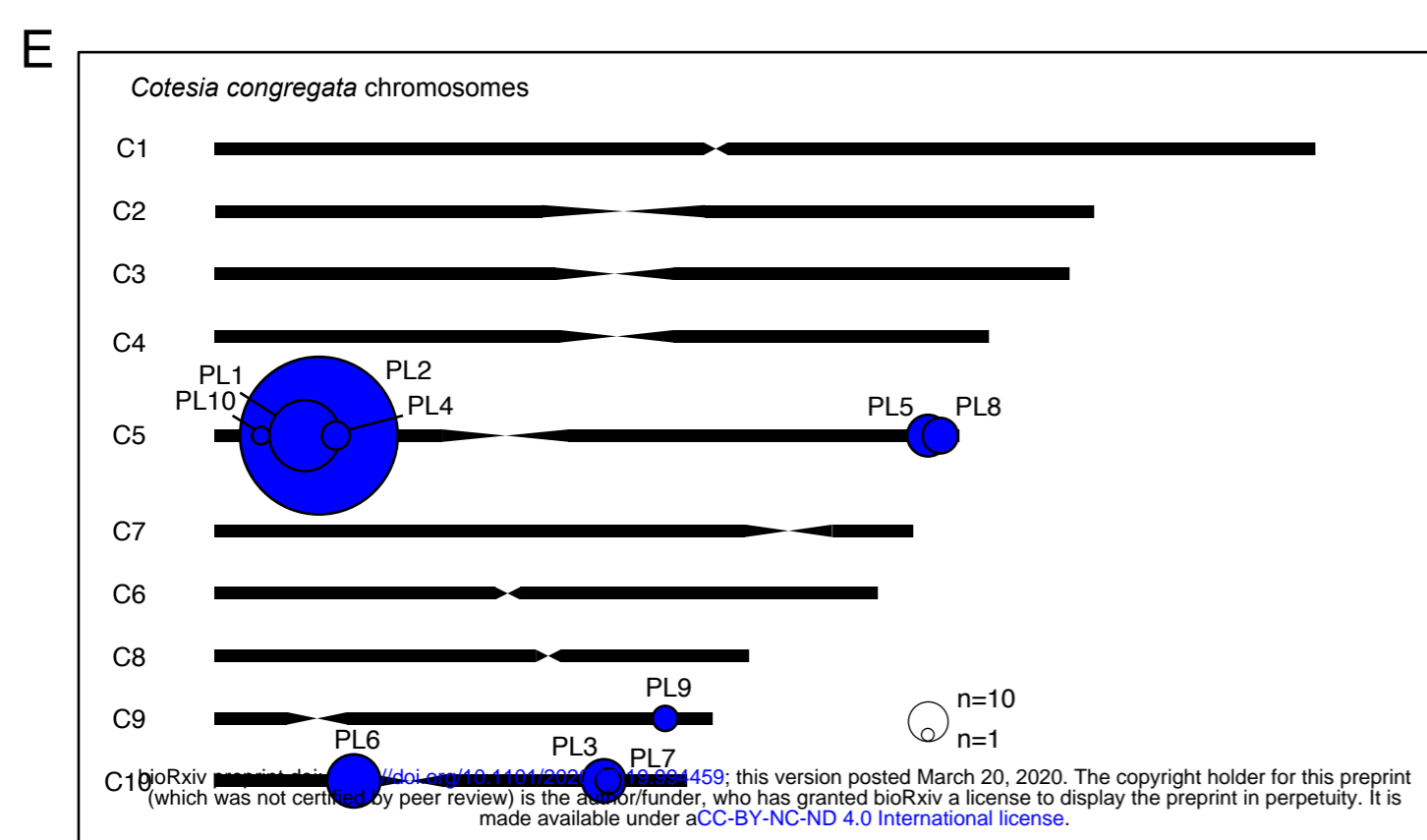
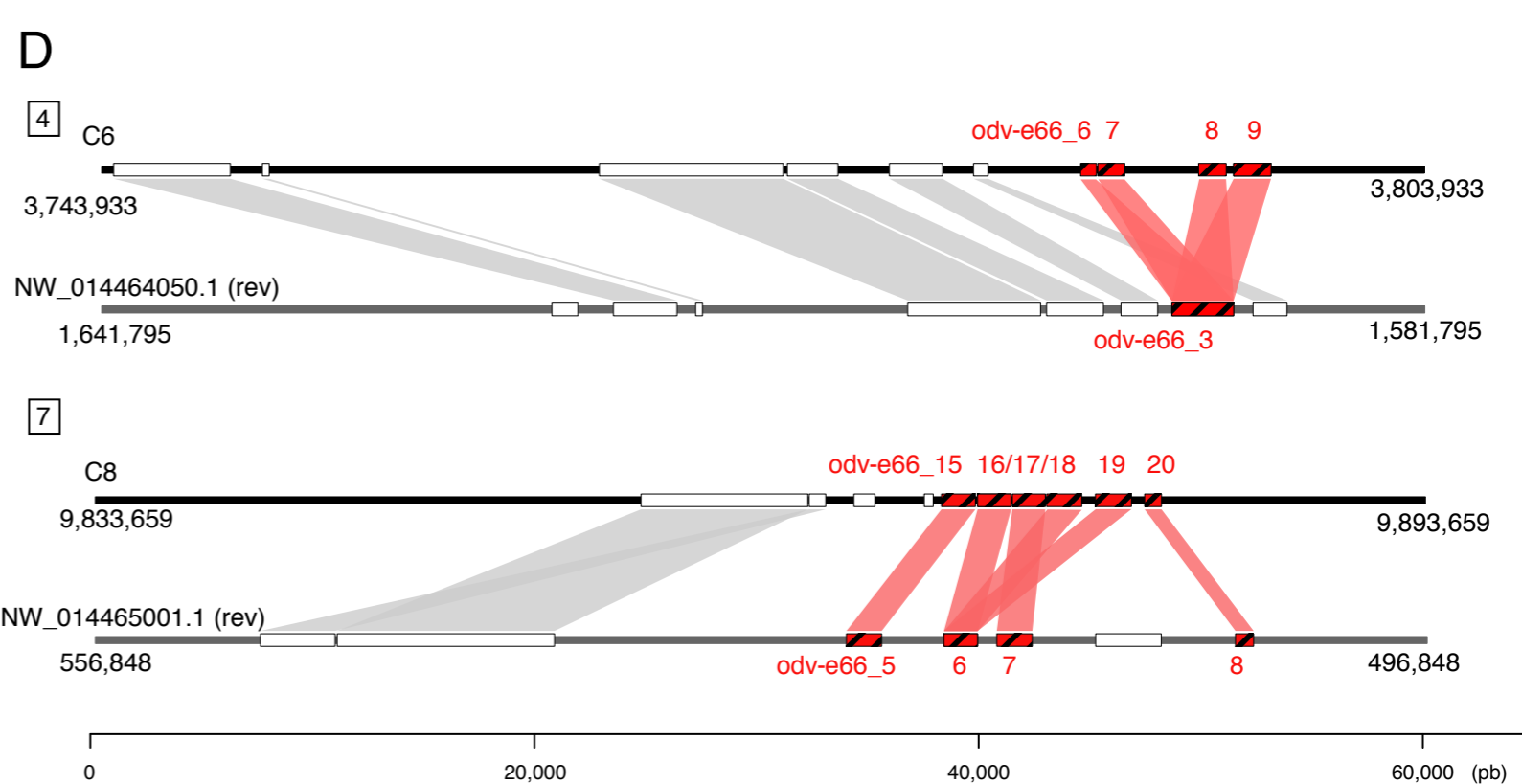
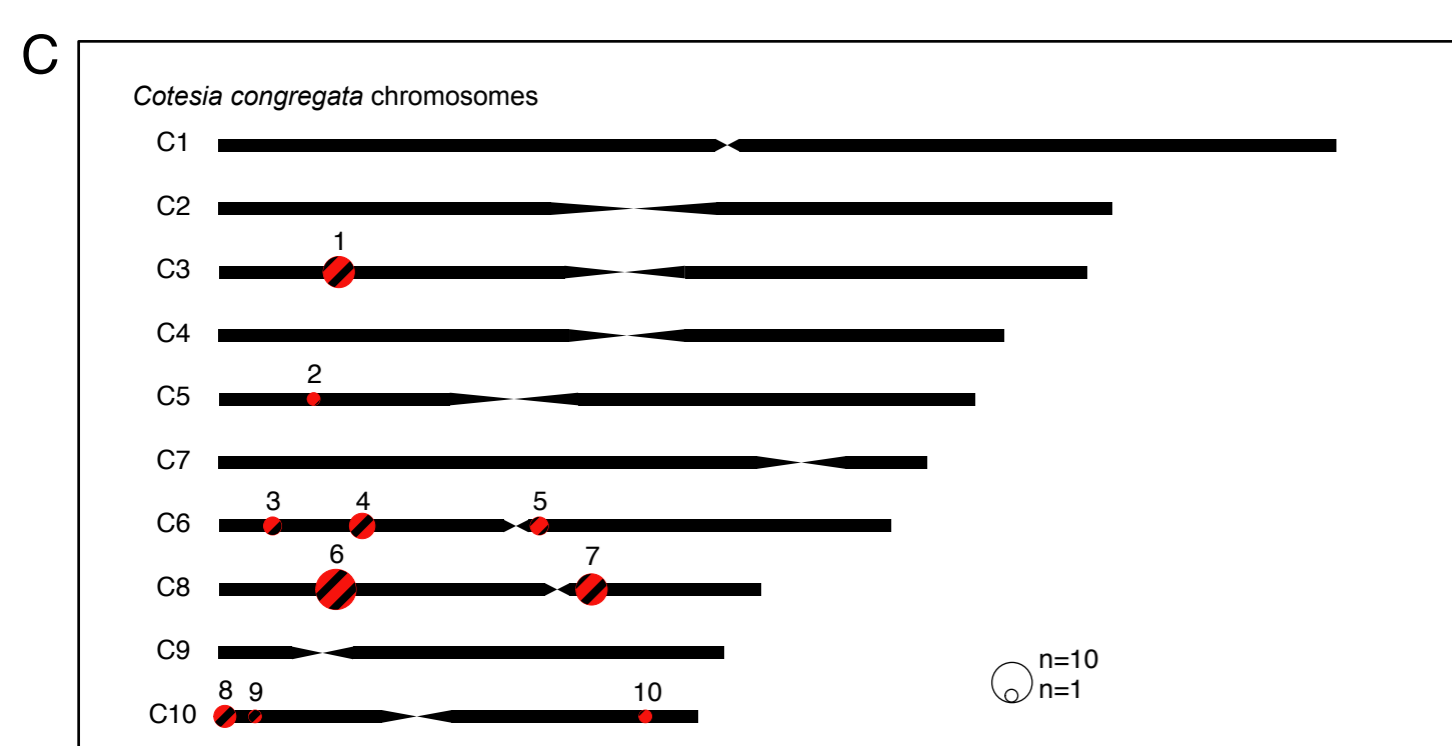
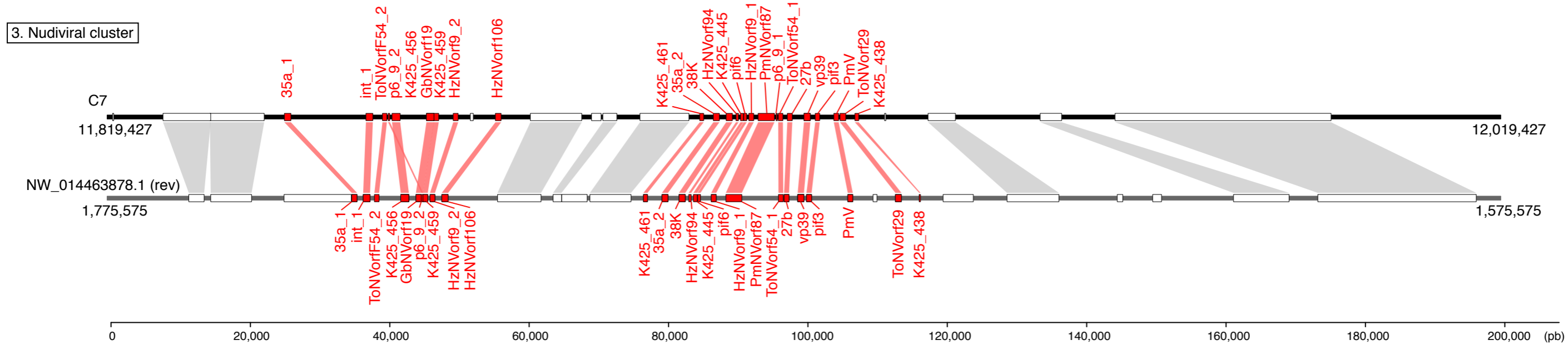
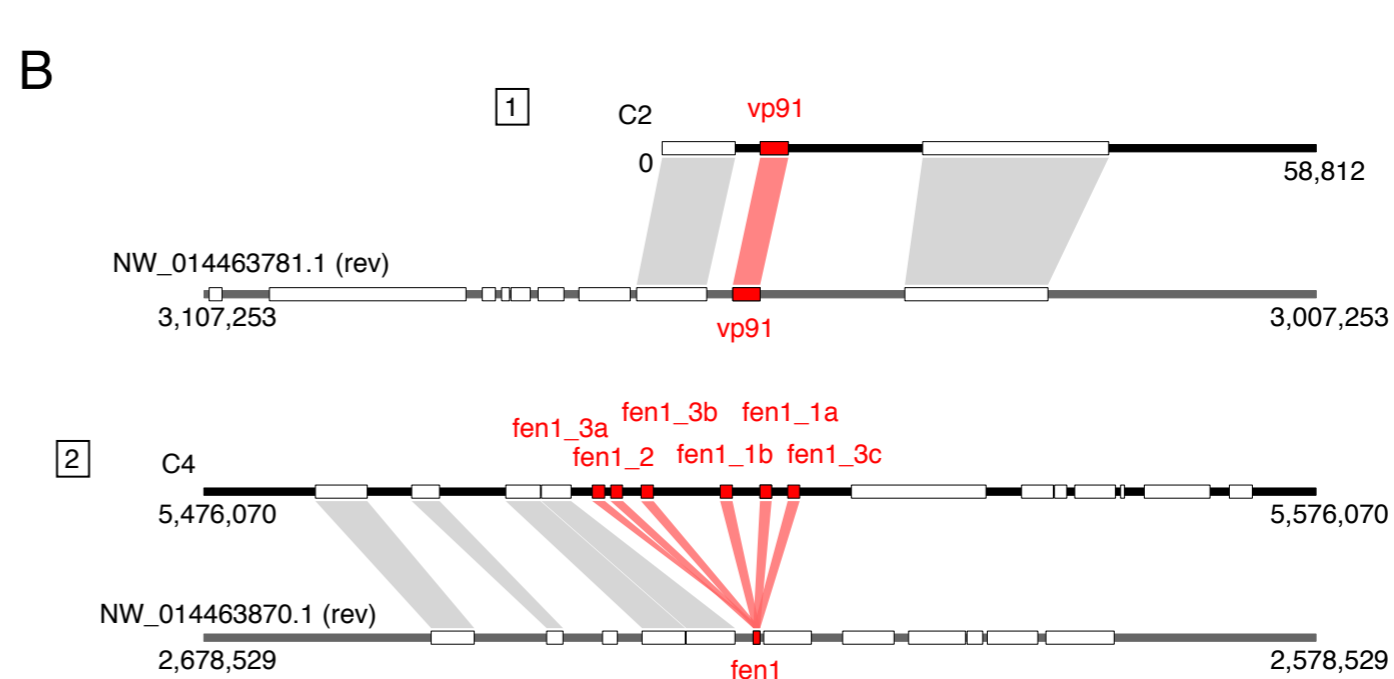
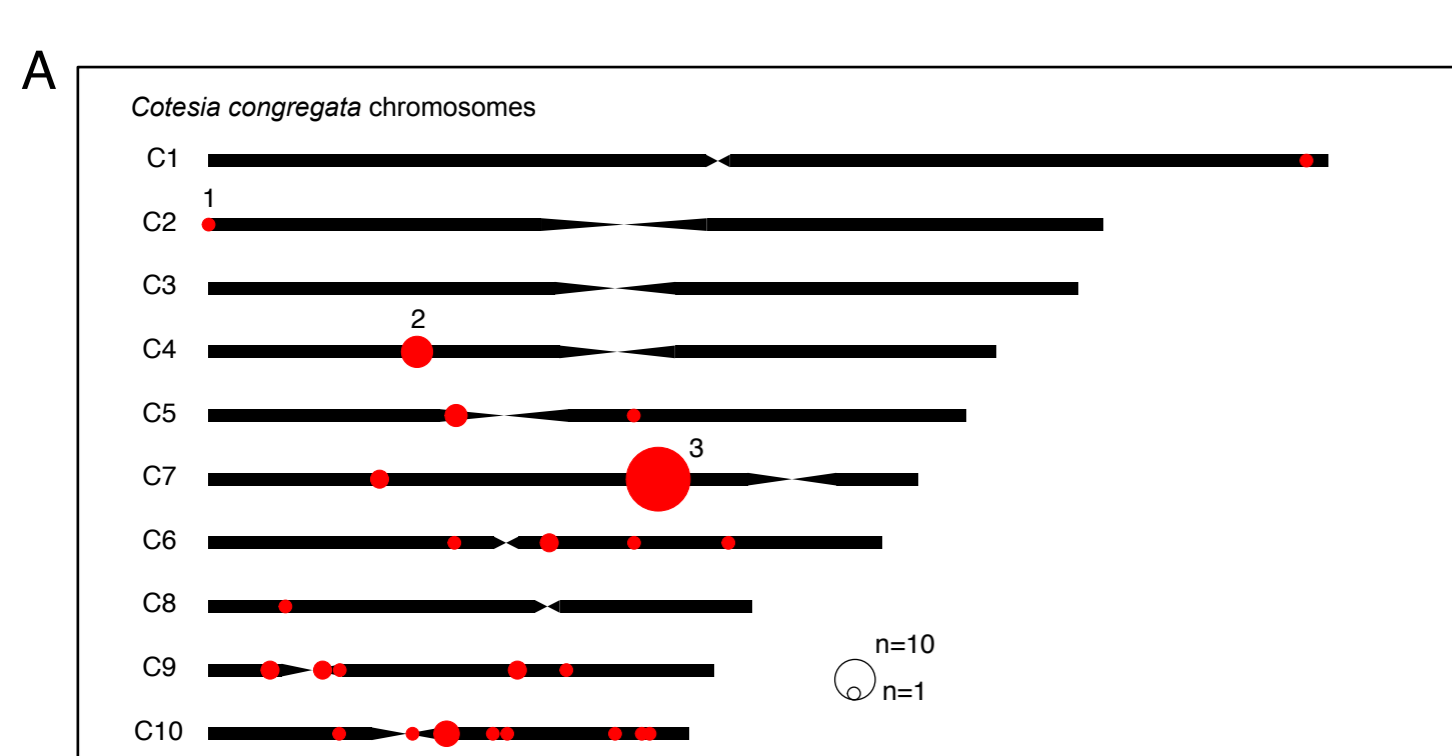
OLFACTION
Host chemodetection
and acceptance

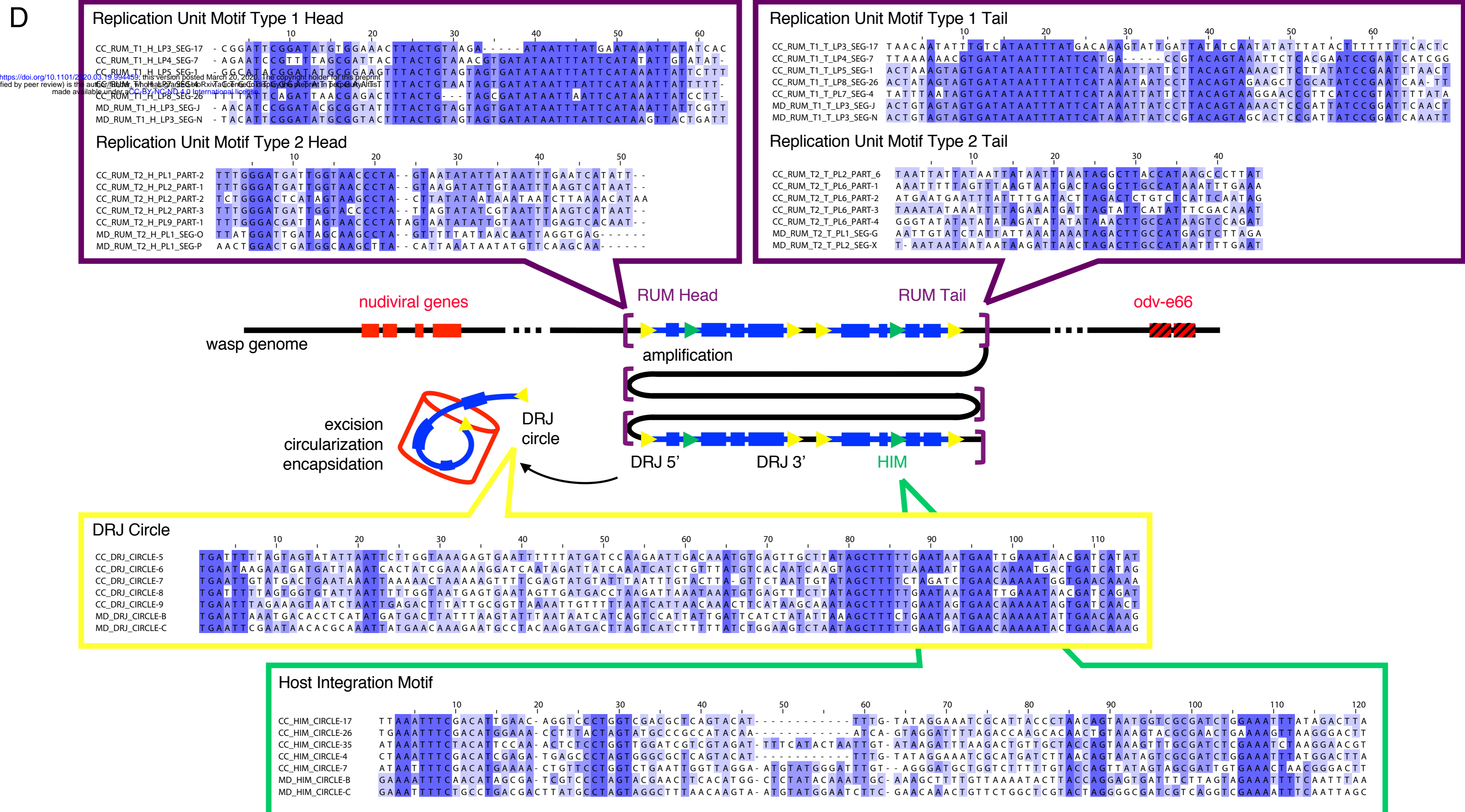
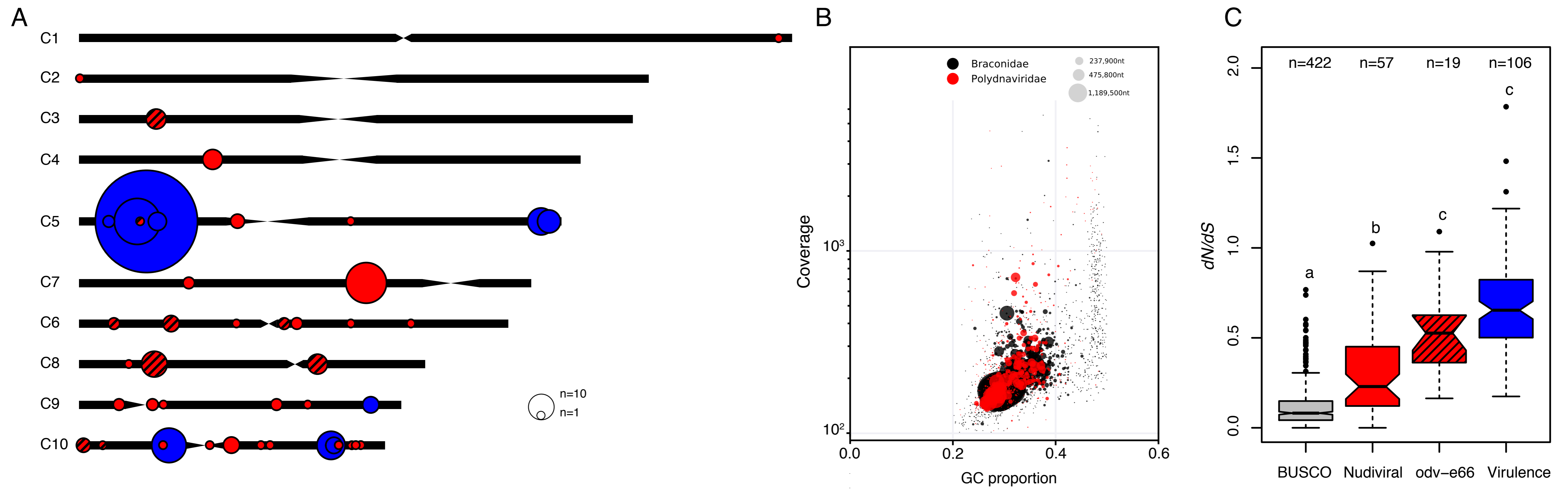
**B***Cotesia congregata**Cotesia rubecula**Cotesia glomerata**Cotesia vestalis**Cotesia flavipes**Cotesia sesamiae***C**

0.1

**D**

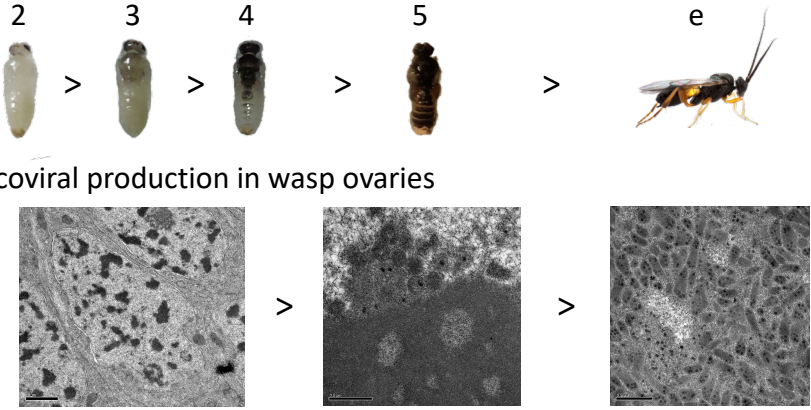
A**B****Olfaction (OR)****Detoxification (P450)****Nudiviral genes (odv-e66)**





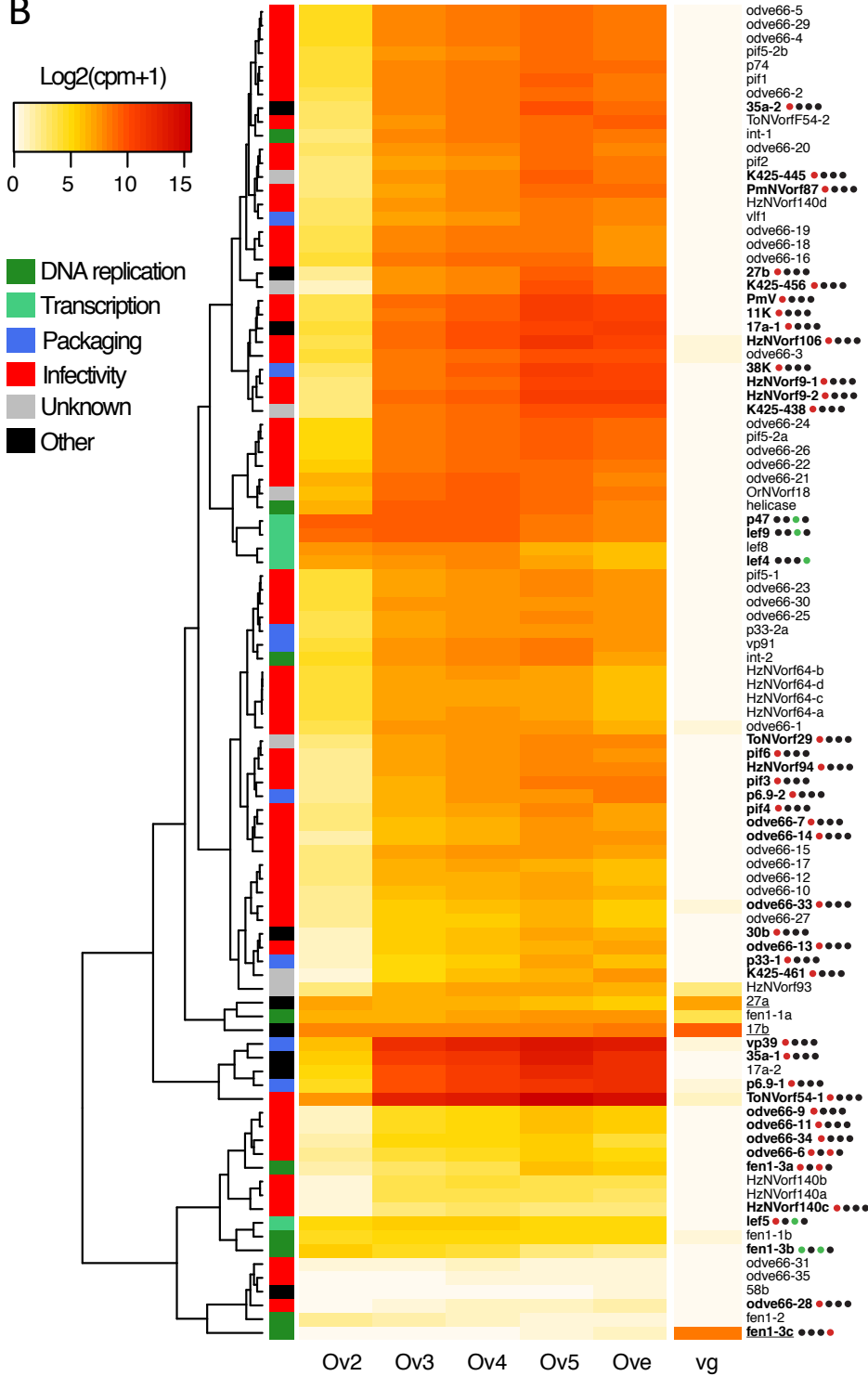
A

Wasp metamorphosis



Bracoviral production in wasp ovaries

B



C

