The genome of the bee louse fly reveals deep convergences in the evolution of social inquilinism

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Abstract

The nests of social insects often harbor a rich fauna of intruders, known as inquilines. Close relatedness between the host and the inquiline prevails due to potential genetic predispositions but how phylogenetically distant inquilines adapt to their hosts remains unclear. Here, we analyzed the genome of the wingless and blind bee louse fly *Braula coeca*, an inquiline of the Western honey bee *Apis mellifera*. We found that unlike many parasites, such as the human louse, the bee louse genome did not show significant erosion or strict reliance on an endosymbiont, likely due to a relatively recent age of inquilinism. However, a strikingly parallel evolution in a set of gene families was observed between the honey bee and the bee louse. Convergences included genes potentially involved in metabolism and immunity, and the loss of nearly all bitter-tasting gustatory receptors in agreement with life in a protective hive and a major diet of honey, pollens, and beeswax. Vision-related and odorant receptor genes also exhibited rapid losses. Only genes whose orthologs in the closely related *Drosophila melanogaster* respond to components of the honey bee alarm pheromones or floral aroma were retained, whereas the losses included orthologous receptors responsive to the anti-ovarian honey bee queen pheromone. These results establish a new model for the study of major morphological and neuroethological transitions and indicate that deep genetic convergences between phylogenetically distant organisms can underlie the evolution of social inquilinism.

Keywords: parasitism; morphological evolution; behavioral adaptations; gene family evolution; Drosophila.

Introduction

Inquilinism is a form of interspecific interactions wherein an organism, the inquiline, inhabits the "home" of another organism (Hegner 1926). Examples abound in nature (Moser 1964; Luczkovich *et al.* 1991; Sanver and Hawkins 2000; Kneitel and Miller 2002), and ancient cases are even present in the fossil record (Landman *et al.* 2014). The relationship between the inquiline and its host can range from commensalism to parasitism depending on the overlap in and availability of resources and space. Factors favoring the evolution of inquilinism greatly depend on the inquiline's capacity to hide its presence from the host. This is particularly true in the case of social inquilines, which constitute the most frequent case of inquilinism. wherein the efficient nest cleaning and care for the offspring performed by social organization are often fatal for the intruders. Therefore, phylogenetic relatedness with the host was suggested to play a major role in facilitating social inquilinism, since the inquiline would share some common genetic factors promoting camouflage, known as Emery's rule (Cini *et al.* 2019). Although recent phylogenetic studies have revised and sometimes rejected Emery's rule at the specific level (Huang and Dornhaus 2008; Lopez-Osorio *et al.* 2015; Romiguier *et al.* 2018; but see Savolainen and Vepsäläinen 2003; Degueldre *et al.* 2021), most cases of social inquilines remain related to the same genus (Jansen *et al.* 2010; Cardinal *et al.* 2010), tribe (Schrader *et al.* 2021), family (Ronquist 1994; Cardinal *et al.* 2010), or even order (Gilbert *et al.* 2012). The genetic basis underlying inquilines belonging to distinct orders or across wide phylogenetic distances is still less understood.

The hive of the Western honey bee *Apis mellifera* comprises several parasites and inquilines that are attracted by the hive's rich resources (honey, pollen, beeswax, and the bees themselves) and its clean and protective shelter (Winston 1987). These include several insects belonging to distinct orders such as wax moths, hive beetles, and endoparasitoid flies. None of these has endured as profound morphological changes that even its affiliation at the order-level was confounded, as the apterous and quasi-blind bee louse fly, *Braula coeca* (Figure 1A-C). The female lays eggs in honey (not brood) cells, and the hatched larvae eat pollen and wax, where they borrow tunnels in which they pupate without forming true puparia (Skaife 1922; Imms 1942). Following emergence, the adults attach to the body of worker bees, migrating from one individual to another until reaching the queen. There, they move to the head, stimulate regurgitation and imbibe from her mouth the honey and nectar (Skaife 1922; Imms 1942). The bee louse is considered an

inquiline kleptoparasite with potential negative effects on bee colony health due to galleries in bee combs and the facilitation of transmitting serious pathogenic viruses to the bees (Avalos *et al.* 2019).

The phylogenetic positioning within the Diptera of the family Braulidae, which contains the bee louse, has long been puzzling due to its modified morphology (Grimaldi and Underwood 1986). Interestingly, recent phylotranscriptomic and phylogenomic analyses show the bee louse to constitute a basal lineage within the Drosophilidae (Bayless *et al.* 2021; Winkler *et al.* 2022). This proximity to *Drosophila melanogaster*, the most investigated insect at the genetic, developmental, and neurobiological levels, makes the bee louse a unique model for the study of the genomic changes underlying major morphological and ecological shifts. We present here an annotated assembly of the bee louse *B. coeca* genome and compare the evolution of its genomic architecture and gene content with those of *D. melanogaster* (Adams *et al.* 2000), the honey bee *A. mellifera* (Weinstock *et al.* 2006), as well as to its homonym and morphologically-similar the ectoparasitic human louse *Pediculus humanus* (Kirkness *et al.* 2010). The comparisons revealed striking evidence of cross-order genomic parallelism and shed new light on the evolution of social inquilinism between phylogenetically distant organisms.

Results

Genome architecture

We sequenced whole genome from a pooled sample of 15 unsexed *Braula coeca* individuals, all collected from the same geographical location, the Island of Ouessant in France. We used a hybrid approach to assemble a draft genome using both long-read Oxford Nanopore Technology (ONT) and short-read Illumina sequencing (see Methods).

Size. The final assembly of the bee louse showed a size of 309 Mb, an N50 of 347227 bp and a GC content of 34.95%. BUSCO gave a score of 95.8% of the Dipteran conserved single-copy orthologs with 1.3% of duplicated genes. Analysis of heterozygous k-mers pairs distribution indicated that the genome is diploid (Supplementary Figure 1A) and genome size prediction using k-mers distribution spectra predict a genome size of 308 Mb, concordant with the assembly size (Supplementary Figure 1B). The bee louse genome is therefore larger than the genomes of the honey bee (227 Mb), *Drosophila melanogaster* (143 Mb), and the ectoparasitic human louse (108 Mb).

104 **Endosymbionts.** The taxonomic assignment of each contigs show that most of them
105 (96%) match with arthropods indicating no (or very few) DNA contamination
106 (Supplementary Figure 1C,D). Unlike in the human louse, no evidence for an obligate
107 endosymbiont was detected in the bee louse. Imms (1942) discussed the possibility that
108 the bee louse larvae have gut microbes that facilitate the digestion of the beeswax. We
109 cannot rule out this hypothesis, but our results show that such microbiota, if present,
110 likely do not persist in the adult stage.
111 **Gene content.** The annotation of the bee louse genome yielded 11,221 protein-coding
112 genes. This number is higher than in *A. mellifera* and *P. humanus* where 9,935 and 10,773
113 protein-coding genes are found, respectively, but lower than that of *D. melanogaster*
114 (13,968 protein-coding genes). The Annotation Edit Distance (AED), which measures the
115 congruence between gene annotation and its supporting evidence was ≤ 0.5 for 96.7% of
116 our gene models, indicating the near completeness of our annotation. Similarly, 79.79%
117 of the corresponding proteins had a Pfam domain, which is another indication of
118 annotation completeness since it varies between 57% and 75% in most eukaryotes.
119 **Synteny.** Orthologous genes were used to map *B. coeca* scaffolds to their corresponding
120 Muller's elements in *D. melanogaster*. The alignment showed strong consistency
121 indicating persistent synteny between the two lineages (Figure 1D). However, given the
122 short length of the scaffolds it was difficult to assess how much collinearity and
123 rearrangement events took place since their divergence. There is no karyotypic map of *B.*
124 *coeca* so it remains difficult at this stage to infer the chromosomal number from the
125 assembly alone.
126 **Mitochondrial genome.** The mitochondrial genome consisted of a single scaffold of 19
127 kb (Figure 1E) unlike in the human louse, which has multiple fragments inside the
128 mitochondria. Compared to conserved gene content and collinearity of the *D.*
129 *melanogaster* and *A. mellifera* mitogenomes (Crozier and Crozier 1993), the
130 mitochondrial gene content of the bee louse was incomplete, lacking the *ND1* gene,
131 included several duplications of the *ND2* and *ND4L* genes that are truncated and/or
132 display frameshifts and had a rearrangement between the *COX1* and *COX2* genes. The
133 nuclear genomes contained 165 mitochondrial DNA insertions (NUMTs) distributed on
134 88 contigs and totalizing 278 kb (~0.09% of the genome). The proportion of NUMTs in
135 the bee louse approaches that of the honey bee (0.08%, (Behura 2007)) but exceeds that
136 of the genus *Drosophila* (0.03%, (Rogers and Griffiths-Jones 2012).

137 **Transposable elements.** The large genome size of the bee louse despite its low gene
138 content compared to *D. melanogaster* suggest an increase in repetitive sequences.
139 RepeatModeler and RepeatMasker analyses indicated that nearly 41.41% of the *B. coeca*
140 genome consist of such sequences, compared to 22.15% and 11.14% in *D. melanogaster*
141 and *A. mellifera*, respectively (Supplementary Table 1). There is a far larger proportion of
142 long interspersed nuclear elements (LINEs) retrotransposons in *B. coeca* (15.18%)
143 compared to only 2.38% and none in *D. melanogaster* and *A. mellifera*, respectively. Long
144 terminal repeat (LTR) elements on the other hand were fewer in *B. coeca* (0.57%) and *A.*
145 *mellifera* (0.17%) than in *D. melanogaster* (6.99%).
146 Because host-parasite relationships have repeatedly be invoked as a factor that
147 may favor horizontal transfer of transposable elements (TE) (Gilbert *et al.* 2010; Ortiz *et*
148 *al.* 2015; Venner *et al.* 2017), we searched for evidence of such transfers between *B. coeca*
149 and *A. mellifera* (Supplementary Text 1). We found one TE, *Famar1-like* element,
150 previously described in the earwig *Forficula auricularia* (Barry *et al.* 2004a) that shows
151 high similarity between *B. coeca* and *A. mellifera* but was absent in *D. melanogaster*, highly
152 suggestive of acquisition through horizontal transfer (Supplementary Figure 2). However,
153 phylogenetic analysis of multiple copies of this TE extracted from 37 widely divergent
154 animal species (Supplementary Figure 3) shows that it was most likely acquired through
155 independent transfer events in *B. coeca* and *A. mellifera*, and that the tight ecological
156 connection between the two species are unlikely to have played a direct role in these
157 transfers (Supplementary Text 1).
158
159 *Gene family evolution*
160 **Families with excess losses.** We identified gene families that underwent reduction or
161 extension as compared to *D. melanogaster* using DAVID (Sherman *et al.* 2022).
162 Underrepresented families (27 families with False Discovery Rate (FDR) < 0.05) showed
163 a striking parallelism with the honey bee, with three of the most underrepresented
164 families being similar (given in Honey bee Genome Sequencing Consortium 2006;
165 Supplementary Table 2). These included InterPro-defined families such as Peptidase S1
166 (IPR001254), Ecdysteroid kinase-like (IPR004119) and Zinc finger AD-type (IPR012934).
167 Peptidases play a major role in detoxification and digestion of protein-rich diets.
168 Their reduction in both the honey bee and the bee louse could be due to the
169 overprotectiveness of their mutual habitat, the hive, and/or the low protein content of

170 their food, nectar and honey. The Ecdysteroid kinase-like (EcKL) family is also suspected
171 to include proteins involved in detoxification (Scanlan *et al.* 2020). We also noted a
172 functional affinity between two underrepresented families involved in the formation of
173 the exoskeleton: the Insect cuticle protein (IPR000618) in the honey bee and Chitin-
174 binding domain (IPR002557) in the bee louse. Cuticles could act as barriers against
175 environmental toxins, which may not be highly encountered in the hive. Remarkably, *B.*
176 *coeca* is unique among Cyclorrhaphan Diptera as its pupa, similarly to the honey bee's
177 (Winston 1987), is contained in the unmodified cuticle of the third instar larva, and no
178 sclerotized puparium is formed (Skaife 1922; Imms 1942).

179 Significant biological processes terms (FDR < 0.05) were mostly related to
180 proteolysis and lipid metabolism (e.g., fatty-acyl-co-A biosynthesis), whereas cellular
181 components terms were associated with the membranes and extracellular space in
182 agreement with a biased loss of genes potentially involved with metabolism,
183 detoxification and/or immunity (Supplementary Table 2).

184 **Families with excess gains.** We did not find a similar important overlap in
185 overrepresented gene families between the bee louse and the honey bee (5 families with
186 FDR < 0.05; Supplementary Table 3). Expanding gene families unique to the honey bee
187 included 7TM odorant receptor (IPR004117), Ankyrin (IPR002110), Yellow/royal jelly
188 protein (IPR003534) and LysR substrate-binding (IPR005119) (Weinstock *et al.* 2006).
189 We found one family, Pleckstrin homology domain (IPR001849), that was
190 overrepresented in both species. This domain is a part of several lipases that are known
191 to be involved in wax ingestion. Indeed, larvae of the bee louse mine the beeswax (Imms
192 1942), whereas honey bee workers use their salivary lipases while chewing the wax to
193 form the hive combs (Kurstjens *et al.* 1985).

194 Gene Ontology (GO) biological and cellular terms with significant enrichment at
195 FDR < 0.05 associated with morphological (e.g., imaginal disc-derived leg morphogenesis,
196 autophagy, and dorsal closure) and/or neurological (e.g., dendrite morphogenesis, axon
197 guidance, sensory perception of sound, and neuromuscular junction) developments
198 (Supplementary Table S3). The bee louse duplicated genes hence may play a role in the
199 evolution of the particular morphologies that helped adaptation to the phoretic lifestyle.

200 **Vision.** The species Latin name refers to the early assumption that the bee louse was blind
201 due to the reduction of the eye size and the loss of the ocelli. However, thin optic nerves
202 connect the brain to the rudimentary eyes, which lack ommatidia and pigments

203 (Müggenburg 1892), and the bee louse demonstrates negative phototaxis, indicating a
204 certain degree of light perception (Kaschef 1959). In agreement with reduced vision in
205 the bee louse, we found three out of the seven rhodopsin genes, which are responsible for
206 colored vision and the positive phototaxis of *D. melanogaster*. Two of the three genes, *Rh1*
207 and *Rh6*, are expressed in the ommatidia and are sensitive to light with long wavelengths
208 (Senthilan and Helfrich-Förster 2016), whereas the third one, *Rh7*, is expressed in the
209 brain and regulates light-dependent circadian entrainment (Ni *et al.* 2017). The role of
210 these opsins in light detection despite the absence of ommatidia is unclear. Remarkably,
211 *Rh1*, *Rh6* and *Rh7* are structurally required in mechanosensory bristles to control larval
212 locomotion (Zanini *et al.* 2018), and *Rh1* and *Rh6* also detect temperature (Leung and
213 Montell 2017), whereas *Rh1* and *Rh7* detect low concentrations of a bitter plant
214 component (Leung *et al.* 2020). Therefore, the retention of these rhodopsins in the bee
215 louse could mainly be due to their unconventional functions. On the other hand, the red-
216 sensitive rhodopsin *Rh2*, which is exclusively expressed in the ocelli and used for horizon
217 detection in *D. melanogaster* (Mishra *et al.* 2021), is among those which were lost in the
218 bee louse, in agreement with the loss of the ocelli in the bee louse.
219 **Olfaction.** Odorant receptors (ORs) are essential to detect volatile chemical signals from
220 the environment. In most *Drosophila* species, there are nearly 61 ORs, whereas in the
221 honey bee this family has expanded to reach 160 (Robertson and Wanner 2006). We
222 found 29 ORs in the bee louse in addition to the Orco ortholog. Of these four had no direct
223 orthologs in *D. melanogaster*, but the remaining 25 genes were orthologous to 21 genes
224 in *D. melanogaster* and 12 were duplicates specific to the bee louse lineage (Figure 2A;
225 Supplementary Figure 3). Judging from the response of those ORs to different volatiles in
226 *D. melanogaster* as curated in the DOOR database (Münch and Galizia 2016) and assuming
227 potential conservation of function, we can divide the bee louse ORs into three categories.
228 First, 11 ORs respond to different components of the honey bee workers alarm and
229 mandibular gland pheromones, e.g., 2-heptanol, propyl acetate, 2-heptanone, 1-hexanol,
230 butyl acetate, isopentyl acetate, etc. These included *Or13a* (x2 paralogues), *Or42a*,
231 *Or42b/Or59b*, *Or43a*, *Or47b*, *Or49a/Or85f*, *Or67b* (x4), *Or74a*, *Or85b/Or85c*, and *Or85e*
232 (x2). Second, 12 ORs respond to different floral, pollen and nectar aromas, such as
233 acetophenones, ethylguaiacol, geranyl acetate or fenchone, but most importantly to
234 benzaldehyde, a major volatile of honey (Machado *et al.* 2020; Starowicz *et al.* 2021).
235 These included *Or30a*, *Or45b* (x2), *Or59a*, *Or67b* (x4), *Or82a*, *Or85e* (x2) and *Or94a*. Note

236 that the multiple *B. coeca* paralogs orthologous to *D. melanogaster Or67b* and *Or85e* genes
237 belong to both categories. Third, one OR, *Or67d*, which is involved in sexual pheromone
238 11-cis-vaccenyl acetate perception (Ha and Smith 2006), is present in the bee louse. Three
239 among the ORs that were lost in the bee louse compared to other drosophilids, *Or49b*,
240 *Or56a* and *Or98a*, are responsible to *D. melanogaster* response to the anti-ovarian honey
241 bee queen mandibular pheromones 9-oxo-2-decenoic acid (9ODA) and 10-hydroxy-2-
242 decenoic acid (10HDA) (Galang *et al.* 2019). Their loss most likely protected the capacity
243 of the bee louse to reproduce in the hive.
244 **Taste.** Gustatory receptors (GRs) allow detecting soluble chemical signals. There are
245 nearly 68 GRs in *D. melanogaster*, that respond mostly to sweet, bitter and carbon dioxide
246 (CO_2) tastes (Weiss *et al.* 2011). Unlike their expanded OR family, the honey bee has only
247 10 GRs, of which 7 are orthologous to sweet *Drosophila* GRs (Robertson and Wanner
248 2006). This is likely due to the bees' food reliance on sweet floral nectars and honey. We
249 found 13 GRs in the bee louse, with no duplications (Figure 2B; Supplementary Figure 4).
250 These GRs could be classified according to their *D. melanogaster* orthology into three
251 categories. First, 6 GRs belong to the sweet class, namely *Gr43a*, *Gr61a* and *Gr64a,b,e,f*
252 which usually respond to sucrose, maltose and fructose and other major honey sugars.
253 Second, 5 GRs belong to the bitter class, namely *Gr33a*, *Gr57a*, *Gr66a*, *Gr93a* and *Gr94a*,
254 suggesting that nearly 50 mostly bitter tasting drosophilid GRs were lost in the bee louse.
255 Note that in *D. melanogaster*, *Gr33a* and *Gr66a*, are potentially involved in sexual
256 pheromones detection (Lacaille *et al.* 2007; Moon *et al.* 2009). Third, 2 GRs, *Gr21a* and
257 *Gr63a*, which detects CO_2 odor in *D. melanogaster* (Jones *et al.* 2007) are present in the
258 bee louse. Those GRs are absent in the honey bee despite its ability to perceive CO_2 (Stange
259 and Diesendorf 1973). High CO_2 concentrations are probably characteristic of largely
260 populated hives and induce fanning response in bees (Seeley 1974). The quasi-blind bee
261 louse may therefore use CO_2 concentrations to detect the bees in the dark hive
262 environment.
263
264 **Discussion**
265 That the enigmatic bee louse is indeed a drosophilid (Winkler *et al.* 2022), a lineage
266 within the most investigated insect family with more than 100 fully sequenced genomes
267 (Kim *et al.* 2021), is undoubtedly one of the most exciting discoveries in Dipteran
268 phylogeny. How could a fly with a typical drosophilid genome become partly ecologically

269 like a bee and partly morphologically like a louse? Our cross-order comparisons of the bee
270 louse to its homonyms shed significant light on the genomic basis of these spectacular
271 convergences.

272 The genome of the human louse is among the smallest sequenced insect genomes
273 (Kelley *et al.* 2014). Loss of significant portions of genomic and gene contents is a
274 characteristic of obligate parasites specializing on specific hosts or inhabiting extreme
275 environments. Indeed, the gene content of the bee louse genome approached that of the
276 human louse, but the ~309 Mb-long genome of the bee louse is longer than that of most
277 drosophilid species, even being slightly longer from the largest genome in this family
278 (~304 Mb-long; Kim *et al.* 2021). Besides, the human louse has an obligatory bacterial
279 endosymbiont that may compensate the loss of its genes (Kirkness *et al.* 2010) but no
280 evidence for such associations is present in the bee louse. These differences may mainly
281 indicate the relative recency of the shift to inquilinism in the bee louse compared to the
282 230 million years (myr) of specialization in true lice (Misof *et al.* 2014). *Megabraula*, the
283 closest relative to the genus *Braula*, is an inquiline of the giant honey bee *Apis laboriosa*,
284 whose divergence from the Western honey bee *A. mellifera* is around 23 myr ago
285 (Grimaldi and Underwood 1986; Cardinal *et al.* 2010). The crown age of the Drosophilidae
286 and the divergence time between *Apis* and its closest pollen-basket (corbiculate) bees are
287 estimated at 66-70 myr ago (Cardinal *et al.* 2010; Suvorov *et al.* 2021). This indicates that
288 association between braulids and *Apis* has arisen between 70 to 20 myr ago.

289 The origin of social inquilinism requires the evolution of multiple convergences
290 that can locate the host, deceive it to enter the colony, adapt to its social organization, and
291 remain undetected or not easily removed from the colony. These phenotypes, which are
292 often host-specific, evolve more easily when the inquiline and the host are
293 phylogenetically close (Cini *et al.* 2019). Despite the distance of the bee louse from honey
294 bees, gene family analysis provided strong evidence for convergent evolution mostly for
295 genes potentially involved in immunity, detoxification, metabolism and chemical
296 perception. Although *Braula* has lost nearly half of the typical drosophilids odorant
297 receptor repertoire, in contrast to the major expansion of this family in the honey bee, it
298 predominantly retained genes whose orthologs in *D. melanogaster* detect compounds of
299 the honey bee pheromones and/or floral aroma and honey odors. Low concentrations of
300 isopentyl acetate (IPA), the major component of the alarm pheromone, released by
301 unstressed workers at hive entries attract the parasitic hive beetle *Aethina tumida* (Torto

302 *et al.* 2007), suggesting that the detection of the host odors could be a common strategy
303 among phylogenetically distant social inquilines and parasites. We also noted that the bee
304 louse has multiple copies related to *Or67b* which responds to several components of bee
305 alarm pheromones, such as 1-hexanol, 2-heptanone, 1-butanol and 3-methyl-1-butanol,
306 as well as to benzaldehyde, the major honey volatile, in *D. melanogaster* (Münch and
307 Galizia 2016), suggesting a possible dual ancestral function that might have facilitated
308 association with the honey bees. The multiple copies related to *Or67b* are remarkable
309 since the copy number expansion of this gene was associated with the evolution of
310 herbivory and strong plant-association in the drosophilid genus *Scaptomyza* (Goldman-
311 Huertas *et al.* 2015; Matsunaga *et al.* 2022). Therefore, the bee louse might have evolved
312 from a flower-breeding or plant-associated lineage within the Drosophilidae, a lifestyle
313 that has recurrently evolved in this family (Yassin 2013).
314 Whereas major molecular convergences could exist between the inquiline and its
315 social host, divergent strategies to adapt to the eusocial lifestyle requirements are still
316 needed. The loss of the three odorant receptors, *Or56a*, *Or49b* and *Or98a*, that respond in
317 *D. melanogaster* to the honey bee queen's pheromones which "sterilize" the bee workers,
318 mainly 9-oxo-2-decenoic acid (9ODA) and possibly 9-hydroxy-2-decenoic acid (9HDA)
319 (Galang *et al.* 2019), is a notable example. The queen's pheromones elicit anti-ovarian
320 response in other insects including *D. melanogaster* mostly through the activation of the
321 neurons bearing these receptors. Therefore, a *sine qua non* condition for reproducing in a
322 beehive is to protect against the effects of those pheromones, with the loss of the
323 responding receptors being a preliminary and effective strategy. However, this raises the
324 question of how the bee lice recognize the queen, which they preferentially infest (Imms
325 1942). It is therefore possible that higher sensitivity to worker pheromones, as suggested
326 by the repertoire of retained odorant receptors (see above), may help to mostly
327 discriminate the workers hence facilitating the recognition of the queen, who has a
328 rudimentary sting gland. Another possibility is that strong CO_2 emission by the court
329 surrounding the queen may be an indicator of her location, as could be suggested from
330 the retention of the two CO_2-smelling gustatory receptors, *Gr21a* and *Gr63a*, whose
331 orthologs are absent in the honey bee. It also remains unclear how the bee lice sexually
332 communicate in the hive given the predominance of chemical camouflage; the
333 hydrocarbon profile of bee lice from different colonies mimicked that of the colony of
334 origin (Martin and Bayfield 2014). The retention of some chemosensory genes whose

335 orthologs are involved in sexual pheromone detection in *D. melanogaster*, such as *Or67d*,
336 *Gr33a* and *Gr66a* (Kurtovic *et al.* 2007; Lacaille *et al.* 2007; Moon *et al.* 2009) suggest that
337 pheromonal communications may still be present in the bee louse but perhaps at low
338 undetectable levels.

339 Small size, loss of wings and the evolution of strongly clinging legs are all
340 morphological adaptations that could prevent the honey bees getting rid of the bee lice.
341 All these adaptations are convergent with ectoparasitic true lice, and for some, such as
342 apterism, represent major recurrent changes that have responded to distinct pressures
343 throughout the history of insects (Roff 1990). We found intact most of the main wing
344 development genes whose mutations severely reduce the wing in *D. melanogaster*, such
345 as *wingless*, *apterous* or *vestigial*. This means that the major morphological changes more
346 likely resulted from regulatory changes of these core genes or modifications of other
347 genes. The regression of visual systems and their underlying genes in the bee louse
348 spending most of its life cycle in the bee hives is a common phenomenon in animals
349 inhabiting dark environments, such as fossorial mammals (Partha *et al.* 2017) and
350 cavefishes (Policarpo *et al.* 2021). With its genetic relatedness to *Drosophila* and
351 ecological association to *Apis*, two major laboratory models, and with new genomic tools
352 presented here, the bee louse *Braula coeca* is a promising model to address questions
353 related to deep convergences that are still difficult to approach in multiple highly
354 specializing animals.

355

356 **Materials and Methods**

357 *Sample collection and genomic library preparation*

358 Samples of *Braula coeca* were collected from honey bee colonies on the Island of Ouessant
359 in France and kindly provided to us by the *Association Conservatoire de l'Abeille Noire*
360 *Bretonne* (A.C.A.N.B.). Genomic DNA was extracted from 15 unsexed individuals
361 conserved in alcohol, using the Nucleobond AXG20 kit and buffer set IV from Macherey-
362 Nagel (ref. 740544 and 740604, https://www.mn-net.com, Düren, Germany).

363

364 *Genome sequencing and assembly*

365 We used a hybrid approach to assemble a draft genome of *B. coeca* using both long-
366 read Oxford Nanopore Technology (ONT) and short-read Illumina sequencing, as in Miller
367 et al. (2019). Before nanopore sequencing, a size selection was conducted on the DNA

368 using the SRE XS from Circulomics (https://www.circulomics.com/, Baltimore, Maryland,
369 USA). The SQK-LSK110 kit from Oxford Nanopore Technology (Lu *et al.* 2016;
370 https://nanoporetech.com/) was then used to prepare the samples for nanopore
371 sequencing following manufacturer's protocol. The library was loaded and sequenced on
372 a R9.4.1 flow cell (ref FLO-Min106) for sequencing. Raw data were basecalled using
373 Guppy v5.0.11 and the "sup" algorithm. Illumina paired-end sequencing was performed
374 by Novogene Company Limited (https://en.novogene.com, Cambridge, UK) on the same
375 DNA library.
376 We used MaSuRCA v4.0.3 (Zimin *et al.* 2017) to produce the hybrid assembly of
377 our genome, using the Cabog assembler. We obtained a final assembly size of 309,35Mb
378 in 2477 contigs, with a N50 of 347211 pb. The completeness of the assembly was
379 estimated to 95,8% with Busco v5.0 on the *diptera_odb10* dataset
380 (C:95.8%[S:94.6%,D:1.2%],F:0.7%,M:3.5%,n:3285).
381
382 *Estimation of genome size, endosymbionts detection and mitogenome assembly*
383 K-mers frequencies within short-read data were obtained with KMC 3 (Kokot *et al.*
384 2017). Genome size and ploidy were inferred using GenomeScope v2.0 with k-mer size =
385 21 and Smudgeplot (Ranallo-Benavidez *et al.* 2020). Contig taxonomy was performed
386 using Blobtools (Laetsch and Blaxter 2017) with Diamond as search engine (Buchfink *et*
387 *al.* 2015) against the UniProt database using a local copy of the NCBI TaxID file for the
388 taxonomic assignation of the best hit. Minimap2 (Li 2018) was used for read mapping.
389
390 *Genome annotation*
391 The *B. coeca* genome was annotated using Maker v2.31.10 (Cantarel *et al.* 2008),
392 following the protocol given in Muller *et al.* (2021), wherein multiple rounds of Maker
393 supported by the training of the SNAP v.2006-07-28 (Korf 2004) and Augustus v.3.3.3
394 (König *et al.* 2016) gene finding and prediction tools, were conducted. Transcriptome of
395 *B. coeca* (NCBI accession no. SRR2046564; 1KITE Consortium; (Bayless *et al.* 2021)) and
396 proteomes of five *Drosophila* species, namely *D. innubila* (Hill *et al.* 2019), *D. albomicans*
397 (Mai *et al.* 2020), *D. bipectinata* (Kim *et al.* 2021), *D. melanogaster* (Adams *et al.* 2000) and
398 *D. virilis* (Clark *et al.* 2007) were used to guide the annotation. Protein-Protein BLAST
399 2.9.0+ (Altschul *et al.* 1997) (-evalue 1e-6 -max_hsps 1 -max_target_seqs 1) was then used
400 to assess putative protein functions in *B. coeca* by comparing the protein sequences given

401 by Maker to the protein sequences from the annotated genome of *D. melanogaster*. The
402 colocalization of *B. coeca* and their *D. melanogaster* orthologs on their respective contigs
403 and chromosomal arms was analyzed to test for synteny.
404
405 *Transposable elements annotation and transfer*
406 Transposable elements were identified in each species following a two-step
407 protocol. First, we used RepeatModeler v 2.0.1 (Flynn *et al.* 2020) with default parameters
408 to generate a *de novo* library of repetitive regions. RepeatMasker v 4.0.9 (Flynn *et al.*
409 2020) was then run with the newly generated library and the options -a (create a .align
410 output file) and -s (slow search; more sensitive) to create a summary of the families of
411 transposable elements found in each reference genome along with the percentage of the
412 genome they represent. Horizontal transfer analyses protocols of the *Famar1*-like
413 element are given in Supplementary Text 1.
414
415 *Gene family evolution*
416 The Database for Annotation, Visualization and Integrated Discovery DAVID 2021
417 (Sherman *et al.* 2022) was used to test for gene ontology (GO) terms enrichments among
418 lists of *D. melanogaster* orthologs annotated on the *B. coeca* genome (see above). The
419 program was either fed with the list of *D. melanogaster* genes that were absent or
420 duplicated in the *B. coeca* genome to test for under- and overrepresented gene families,
421 respectively, compared to *D. melanogaster* genome, as denoted by the False Discovery
422 Rate (FDR) correction for multiple tests. Each analysis was conducted for biological
423 processes, molecular function, and cytological components.
424
425 *Chemosensory superfamilies evolution*
426 Protein sequences of the odorant (ORs) and gustatory (GRs) receptors of *A.*
427 *mellifera* and *D. melanogaster* were obtained from Robertson and Wanner (2006) to
428 which we added the annotated ORs and GRs protein sequences of *B. coeca*. Sequences
429 were aligned using Molecular Evolutionary Genetic Analysis (MEGA X) software package
430 (Kumar *et al.* 2018), which was also used to infer a maximum-likelihood phylogenetic tree
431 for each family. We used iToL v4 (Letunic and Bork 2019) to visualize the trees. *B. coeca*
432 protein sequences are given in Supplementary Datasets 2 and 3 for ORs and GRs,
433 respectively.

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Conflicts of interest

The authors declare no conflicts of interest.

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706 MaSuRCA mega-reads algorithm. Genome Res. 27: 787–792.
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708

709 **Legends of figures**

710

711 **Figure 1** – The bee louse fly (*Braula coeca*) morphology and genome. A) Two adults
712 attached to a honey bee worker preserved in alcohol. B) Dorsal view of an adult showing
713 the loss of the wings, halters and scutum, the reduction of the mesonotum and the
714 robustness of the legs. C) Frontal view of an adult showing the reduction of the eyes and
715 the loss of the ocelli. Scale bars = 1 mm in A and 0.5 mm in B and C. D) Orthologous genes
716 colocalization in *D. melanogaster* Muller's elements and *B. coeca* contigs demonstrating
717 the predominant conservation of synteny. E) The mitochondrial genome of *B. coeca*. Blue
718 and violet ribbons indicate the protein-encoding genes in sens and anti-sens respectively,
719 red and yellow ribbons indicate the rRNA and tRNA genes in sens and anti-sens
720 respectively. Names with asterix indicate the presence of inactivated paraloguous genes.

721

722 **Figure 2** – Maximum-likelihood phylogenetic tree for A) odorant receptors (ORs) and B)
723 gustatory receptors (GRs) of *A. mellifera* (green), *B. coeca* (red) and *D. melanogaster*
724 (blue). Genes commented in the text are labelled. For full labels see Supplementary
725 Figures 4 and 5. Sequences for *B. coeca* are given in Supplementary Datasets 2 and 3 for
726 ORs and GRs, respectively. Sequences for *A. mellifera* and *D. melanogaster* are from
727 Robertson and Wanner (2006). a.a. = amino acids.

728

729

730 **List of supplementary materials**

731

732 **Supplementary Dataset 1** – Sequence of all *Famar* and *Famar1*-like copies used to
733 reconstruct the phylogeny shown in Supplementary Figure 3. The sequence names
734 contain the name of the species, the Genbank accession number of the contig from which
735 the copy was extracted, the start and end position of the copy in the contig, as well as a
736 final number that allows making a correspondence with leaves in the tree shown in
737 Supplementary Figure 3.

738

739 **Supplementary Dataset 2** – Sequence of odorant receptors (ORs) of *Braula coeca* used
740 to reconstruct the phylogeny shown in Supplementary Figure 4.

741

742 **Supplementary Dataset 3** – Sequence of gustatory receptors (GRs) of *Braula coeca* used
743 to reconstruct the phylogeny shown in Supplementary Figure 5.

744

745 **Supplementary Figure 1** – Genomic features of *Braula coeca.* A) Ploidy estimation using
746 the coverage and the distribution of heterozygous k-mers pairs. B) Genome size
747 estimation using the k-mer profile spectrum. C) Taxonomic assignation of the contigs
748 according to their GC% and their coverages. D) Proportion of short-reads that mapped
749 onto the genome assembly (left) and onto the different contigs according to their
750 taxonomic assignments (right).

751

752 **Supplementary Figure 2** – Comparison of Famar1-like synonymous distance and
753 orthologous gene synonymous distances between *Braula coeca* and *Apis mellifera*. To
754 calculate the distribution of gene or gene fragment synonymous distances (dS) between
755 *B. coeca* and *A. mellifera* we selected best reciprocal blastp hits between single copy
756 BUSCO genes retrieved from the two species and calculated dS for each of them using the
757 approach described in Zhang *et al.* (2020). The red line indicates the 0.5% quantile of this
758 distribution (=1.76). The distribution is bimodal, with genes having highly saturated dS
759 values showing a peak centered on 9.99 and genes showing less saturated dS values
760 showing another peak around 2.5. We verified that genes showing less saturated values
761 correspond to highly genes that evolve under strong purifying selection and are thus
762 highly conserved between Hymenoptera and Diptera. The *Famar1*-like dS (green line, =

763 0.12) was calculated over the transposase open reading from of one copy of the element
764 extracted from the *A. mellifera* genome and another copy extracted from the *B. coeca*
765 genome.
766
767 **Supplementary Figure 3** – Phylogeny of *Famar1*-like copies from 38 animal species. The
768 ten *Famar1*-like copies showing the highest nucleotide identity to the *Famar* element
769 initially described in the earwig (Barry *et al.* 2004b) were retrieved using online blastn
770 (see Supplementary Text 1) and extracted from the genome of 37 animal species. Filled
771 circles indicate bootstrap value higher than 70%, with the diameter of the circle
772 proportional to each individual value. Name of the copies are composed of the name of
773 the species from which they were extracted and a unique number that allows making a
774 correspondence with sequences provided in Supplementary Dataset 1.
775
776 **Supplementary Figure 4** – Labelled maximum-likelihood phylogeny of odorant
777 receptors (ORs) of *A. mellifera* (green), *B. coeca* (blue), and *D. melanogaster* (red) show in
778 Figure 2A.
779
780 **Supplementary Figure 5** – Labelled maximum-likelihood phylogeny of gustatory
781 receptors (GRs) of *A. mellifera* (green), *B. coeca* (blue), and *D. melanogaster* (red) show in
782 Figure 2B.
783
784 **Supplementary Table 1** – Proportions of transposable elements in the genomes of *B.*
785 *coeca*, *D. melanogaster* and *A. mellifera*.
786
787 **Supplementary Table 2** – Gene Ontology (GO) enrichment for *D. melanogaster* genes that
788 are absent from *B. coeca* genome as inferred using DAVID 2021.
789
790 **Supplementary Table 3** – Gene Ontology (GO) enrichment for *D. melanogaster* genes that
791 have additional copies in *B. coeca* genome as inferred using DAVID 2021.
792
793 **Supplementary Text 1** – Analysis of horizontal transfer of transposable elements
794 between *B. coeca* and *A. mellifera*.
795

Figure 1

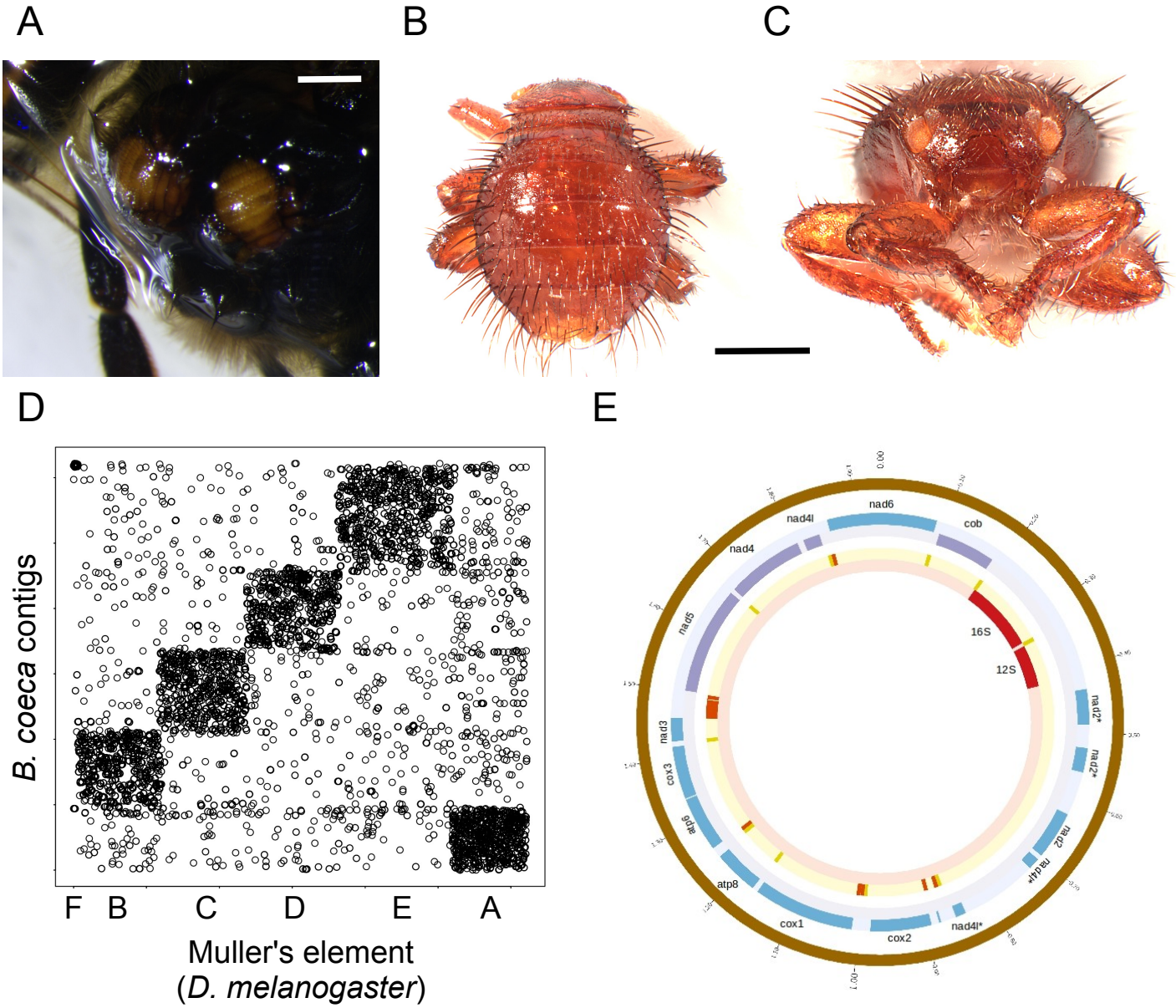
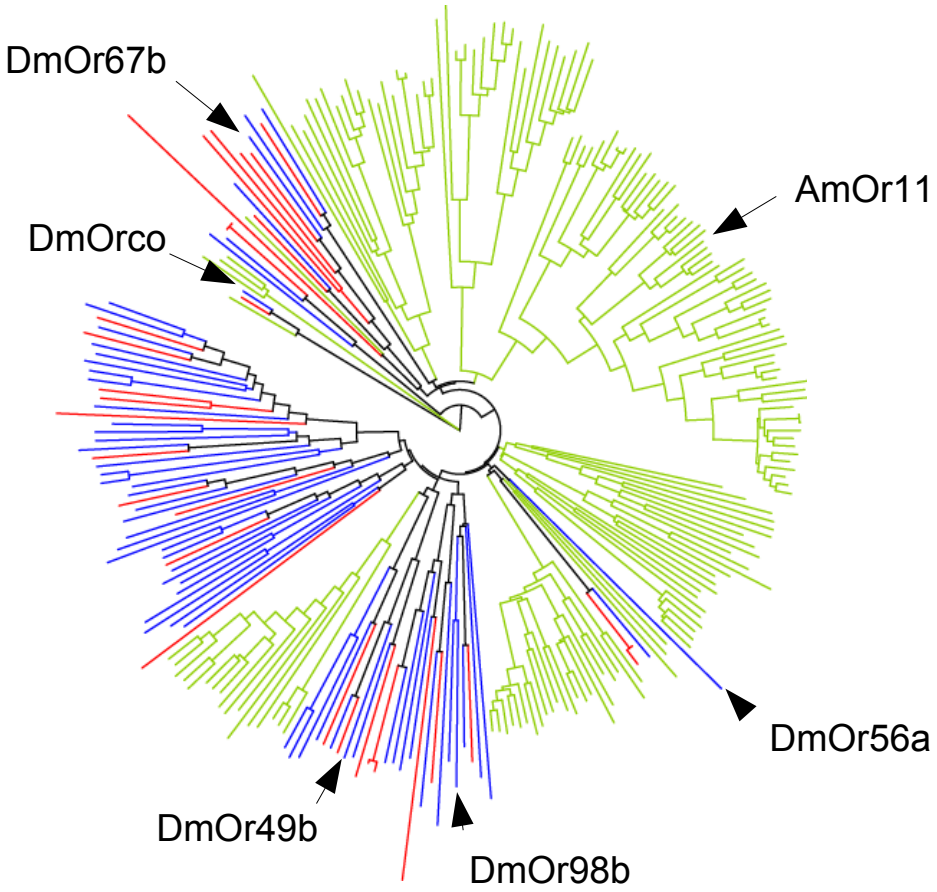
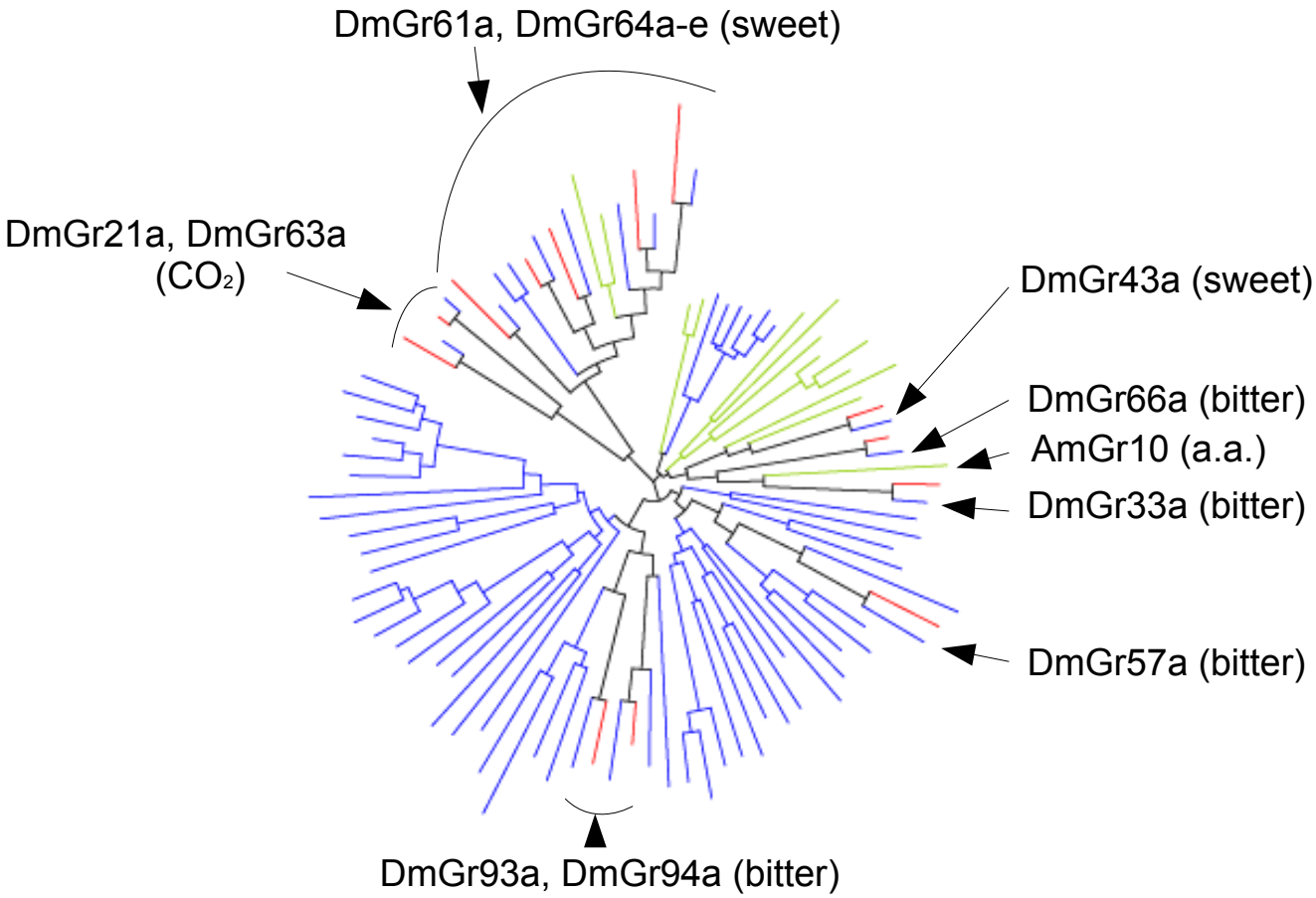

A
B
C
D
E
B. coeca contigs
F
B
C
D
E
A
Muller's element
(D. melanogaster)
nad6
cob
nad4l
nad4
nad5
16S
12S
nad2*
nad2*
nad2
nad4l*
nad4l*
nad3
cox3
atp6
atp8
cox1
cox2
nad4l*

Figure 2

A


DmOr67b
AmOr11
DmOrco
DmOr56a
DmOr49b
DmOr98b

B


DmGr61a, DmGr64a-e (sweet)
DmGr21a, DmGr63a
(CO_2)
DmGr43a (sweet)
DmGr66a (bitter)
AmGr10 (a.a.)
DmGr33a (bitter)
DmGr57a (bitter)
DmGr93a, DmGr94a (bitter)