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).

Endosymbionts. The taxonomic assignment of each contigs show that most of them (96%) match with arthropods indicating no (or very few) DNA contamination (Supplementary Figure 1C,D). Unlike in the human louse, no evidence for an obligate endosymbiont was detected in the bee louse. Imms (1942) discussed the possibility that the bee louse larvae have gut microbes that facilitate the digestion of the beeswax. We cannot rule out this hypothesis, but our results show that such microbiota, if present, likely do not persist in the adult stage.

Gene content. The annotation of the bee louse genome yielded 11,221 protein-coding genes. This number is higher than in *A. mellifera* and *P. humanus* where 9,935 and 10,773 protein-coding genes are found, respectively, but lower than that of *D. melanogaster* (13,968 protein-coding genes). The Annotation Edit Distance (AED), which measures the congruence between gene annotation and its supporting evidence was ≤ 0.5 for 96.7% of our gene models, indicating the near completeness of our annotation. Similarly, 79.79% of the corresponding proteins had a Pfam domain, which is another indication of annotation completeness since it varies between 57% and 75% in most eukaryotes.

Synteny. Orthologous genes were used to map *B. coeca* scaffolds to their corresponding Muller's elements in *D. melanogaster*. The alignment showed strong consistency indicating persistent synteny between the two lineages (Figure 1D). However, given the short length of the scaffolds it was difficult to assess how much collinearity and rearrangement events took place since their divergence. There is no karyotypic map of *B. coeca* so it remains difficult at this stage to infer the chromosomal number from the assembly alone.

Mitochondrial genome. The mitochondrial genome consisted of a single scaffold of 19 kb (Figure 1E) unlike in the human louse, which has multiple fragments inside the mitochondria. Compared to conserved gene content and collinearity of the *D. melanogaster* and *A. mellifera* mitogenomes (Crozier and Crozier 1993), the mitochondrial gene content of the bee louse was incomplete, lacking the *ND1* gene, included several duplications of the *ND2* and *ND4L* genes that are truncated and/or display frameshifts and had a rearrangement between the *COX1* and *COX2* genes. The nuclear genomes contained 165 mitochondrial DNA insertions (NUMTs) distributed on 88 contigs and totalizing 278 kb (~0.09% of the genome). The proportion of NUMTs in the bee louse approaches that of the honey bee (0.08%, (Behura 2007)) but exceeds that of the genus *Drosophila* (0.03%, (Rogers and Griffiths-Jones 2012).

Transposable elements. The large genome size of the bee louse despite its low gene content compared to *D. melanogaster* suggest an increase in repetitive sequences. RepeatModeler and RepeatMasker analyses indicated that nearly 41.41% of the *B. coeca* genome consist of such sequences, compared to 22.15% and 11.14% in *D. melanogaster* and *A. mellifera*, respectively (Supplementary Table 1). There is a far larger proportion of long interspersed nuclear elements (LINEs) retrotransposons in *B. coeca* (15.18%) compared to only 2.38% and none in *D. melanogaster* and *A. mellifera*, respectively. Long terminal repeat (LTR) elements on the other hand were fewer in *B. coeca* (0.57%) and *A. mellifera* (0.17%) than in *D. melanogaster* (6.99%).

Because host-parasite relationships have repeatedly be invoked as a factor that may favor horizontal transfer of transposable elements (TE) (Gilbert *et al.* 2010; Ortiz *et al.* 2015; Venner *et al.* 2017), we searched for evidence of such transfers between *B. coeca* and *A. mellifera* (Supplementary Text 1). We found one TE, *Famar1-like* element, previously described in the earwig *Forficula auricularia* (Barry *et al.* 2004a) that shows high similarity between *B. coeca* and *A. mellifera* but was absent in *D. melanogaster*, highly suggestive of acquisition through horizontal transfer (Supplementary Figure 2). However, phylogenetic analysis of multiple copies of this TE extracted from 37 widely divergent animal species (Supplementary Figure 3) shows that it was most likely acquired through independent transfer events in *B. coeca* and *A. mellifera*, and that the tight ecological connection between the two species are unlikely to have played a direct role in these transfers (Supplementary Text 1).

Gene family evolution

Families with excess losses. We identified gene families that underwent reduction or extension as compared to *D. melanogaster* using DAVID (Sherman *et al.* 2022). Underrepresented families (27 families with False Discovery Rate (FDR) < 0.05) showed a striking parallelism with the honey bee, with three of the most underrepresented families being similar (given in Honey bee Genome Sequencing Consortium 2006; Supplementary Table 2). These included InterPro-defined families such as Peptidase S1 (IPR001254), Ecdysteroid kinase-like (IPR004119) and Zinc finger AD-type (IPR012934).

Peptidases play a major role in detoxification and digestion of protein-rich diets. Their reduction in both the honey bee and the bee louse could be due to the overprotectiveness of their mutual habitat, the hive, and/or the low protein content of

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

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

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

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

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

(Müggenburg 1892), and the bee louse demonstrates negative phototaxis, indicating a certain degree of light perception (Kaschef 1959). In agreement with reduced vision in the bee louse, we found three out of the seven rhodopsin genes, which are responsible for colored vision and the positive phototaxis of *D. melanogaster*. Two of the three genes, *Rh1* and *Rh6*, are expressed in the ommatidia and are sensitive to light with long wavelengths (Senthilan and Helfrich-Förster 2016), whereas the third one, *Rh7*, is expressed in the brain and regulates light-dependent circadian entrainment (Ni *et al.* 2017). The role of these opsins in light detection despite the absence of ommatidia is unclear. Remarkably, *Rh1*, *Rh6* and *Rh7* are structurally required in mechanosensory bristles to control larval locomotion (Zanini *et al.* 2018), and *Rh1* and *Rh6* also detect temperature (Leung and Montell 2017), whereas *Rh1* and *Rh7* detect low concentrations of a bitter plant component (Leung *et al.* 2020). Therefore, the retention of these rhodopsins in the bee louse could mainly be due to their unconventional functions. On the other hand, the red-sensitive rhodopsin *Rh2*, which is exclusively expressed in the ocelli and used for horizon detection in *D. melanogaster* (Mishra *et al.* 2021), is among those which were lost in the bee louse, in agreement with the loss of the ocelli in the bee louse.

Olfaction. Odorant receptors (ORs) are essential to detect volatile chemical signals from the environment. In most *Drosophila* species, there are nearly 61 ORs, whereas in the honey bee this family has expanded to reach 160 (Robertson and Wanner 2006). We found 29 ORs in the bee louse in addition to the Orco ortholog. Of these four had no direct orthologs in *D. melanogaster*, but the remaining 25 genes were orthologous to 21 genes in *D. melanogaster* and 12 were duplicates specific to the bee louse lineage (Figure 2A; Supplementary Figure 3). Judging from the response of those ORs to different volatiles in *D. melanogaster* as curated in the DOOR database (Münch and Galizia 2016) and assuming potential conservation of function, we can divide the bee louse ORs into three categories. First, 11 ORs respond to different components of the honey bee workers alarm and mandibular gland pheromones, e.g., 2-heptanol, propyl acetate, 2-heptanone, 1-hexanol, butyl acetate, isopentyl acetate, etc. These included *Or13a* (x2 paralogues), *Or42a*, *Or42b/Or59b*, *Or43a*, *Or47b*, *Or49a/Or85f*, *Or67b* (x4), *Or74a*, *Or85b/Or85c*, and *Or85e* (x2). Second, 12 ORs respond to different floral, pollen and nectar aromas, such as acetophenones, ethylguaiacol, geranyl acetate or fenchone, but most importantly to benzaldehyde, a major volatile of honey (Machado *et al.* 2020; Starowicz *et al.* 2021). These included *Or30a*, *Or45b* (x2), *Or59a*, *Or67b* (x4), *Or82a*, *Or85e* (x2) and *Or94a*. Note

that the multiple *B. coeca* paralogs orthologous to *D. melanogaster Or67b* and *Or85e* genes belong to both categories. Third, one OR, *Or67d*, which is involved in sexual pheromone 11-cis-vaccenyl acetate perception (Ha and Smith 2006), is present in the bee louse. Three among the ORs that were lost in the bee louse compared to other drosophilids, *Or49b*, *Or56a* and *Or98a*, are responsible to *D. melanogaster* response to the anti-ovarian honey bee queen mandibular pheromones 9-oxo-2-decenoic acid (9ODA) and 10-hydroxy-2-decenoic acid (10HDA) (Galang *et al.* 2019). Their loss most likely protected the capacity of the bee louse to reproduce in the hive.

Taste. Gustatory receptors (GRs) allow detecting soluble chemical signals. There are nearly 68 GRs in *D. melanogaster*, that respond mostly to sweet, bitter and carbon dioxide (CO_2) tastes (Weiss *et al.* 2011). Unlike their expanded OR family, the honey bee has only 10 GRs, of which 7 are orthologous to sweet *Drosophila* GRs (Robertson and Wanner 2006). This is likely due to the bees' food reliance on sweet floral nectars and honey. We found 13 GRs in the bee louse, with no duplications (Figure 2B; Supplementary Figure 4). These GRs could be classified according to their *D. melanogaster* orthology into three categories. First, 6 GRs belong to the sweet class, namely *Gr43a*, *Gr61a* and *Gr64a,b,e,f* which usually respond to sucrose, maltose and fructose and other major honey sugars. Second, 5 GRs belong to the bitter class, namely *Gr33a*, *Gr57a*, *Gr66a*, *Gr93a* and *Gr94a*, suggesting that nearly 50 mostly bitter tasting drosophilid GRs were lost in the bee louse. Note that in *D. melanogaster*, *Gr33a* and *Gr66a*, are potentially involved in sexual pheromones detection (Lacaille *et al.* 2007; Moon *et al.* 2009). Third, 2 GRs, *Gr21a* and *Gr63a*, which detects CO_2 odor in *D. melanogaster* (Jones *et al.* 2007) are present in the bee louse. Those GRs are absent in the honey bee despite its ability to perceive CO_2 (Stange and Diesendorf 1973). High CO_2 concentrations are probably characteristic of largely populated hives and induce fanning response in bees (Seeley 1974). The quasi-blind bee louse may therefore use CO_2 concentrations to detect the bees in the dark hive environment.

Discussion

That the enigmatic bee louse is indeed a drosophilid (Winkler *et al.* 2022), a lineage within the most investigated insect family with more than 100 fully sequenced genomes (Kim *et al.* 2021), is undoubtedly one of the most exciting discoveries in Dipteran phylogeny. How could a fly with a typical drosophilid genome become partly ecologically

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

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

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

et al. 2007), suggesting that the detection of the host odors could be a common strategy among phylogenetically distant social inquilines and parasites. We also noted that the bee louse has multiple copies related to *Or67b* which responds to several components of bee alarm pheromones, such as 1-hexanol, 2-heptanone, 1-butanol and 3-methyl-1-butanol, as well as to benzaldehyde, the major honey volatile, in *D. melanogaster* (Münch and Galizia 2016), suggesting a possible dual ancestral function that might have facilitated association with the honey bees. The multiple copies related to *Or67b* are remarkable since the copy number expansion of this gene was associated with the evolution of herbivory and strong plant-association in the drosophilid genus *Scaptomyza* (Goldman-Huertas *et al.* 2015; Matsunaga *et al.* 2022). Therefore, the bee louse might have evolved from a flower-breeding or plant-associated lineage within the Drosophilidae, a lifestyle that has recurrently evolved in this family (Yassin 2013).

Whereas major molecular convergences could exist between the inquiline and its social host, divergent strategies to adapt to the eusocial lifestyle requirements are still needed. The loss of the three odorant receptors, *Or56a*, *Or49b* and *Or98a*, that respond in *D. melanogaster* to the honey bee queen's pheromones which "sterilize" the bee workers, mainly 9-oxo-2-decenoic acid (9ODA) and possibly 9-hydroxy-2-decenoic acid (9HDA) (Galang *et al.* 2019), is a notable example. The queen's pheromones elicit anti-ovarian response in other insects including *D. melanogaster* mostly through the activation of the neurons bearing these receptors. Therefore, a *sine qua non* condition for reproducing in a beehive is to protect against the effects of those pheromones, with the loss of the responding receptors being a preliminary and effective strategy. However, this raises the question of how the bee lice recognize the queen, which they preferentially infest (Imms 1942). It is therefore possible that higher sensitivity to worker pheromones, as suggested by the repertoire of retained odorant receptors (see above), may help to mostly discriminate the workers hence facilitating the recognition of the queen, who has a rudimentary sting gland. Another possibility is that strong CO_2 emission by the court surrounding the queen may be an indicator of her location, as could be suggested from the retention of the two CO_2-smelling gustatory receptors, *Gr21a* and *Gr63a*, whose orthologs are absent in the honey bee. It also remains unclear how the bee lice sexually communicate in the hive given the predominance of chemical camouflage; the hydrocarbon profile of bee lice from different colonies mimicked that of the colony of origin (Martin and Bayfield 2014). The retention of some chemosensory genes whose

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

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

Materials and Methods

Sample collection and genomic library preparation

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

Genome sequencing and assembly

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

using the SRE XS from Circulomics (https://www.circulomics.com/, Baltimore, Maryland, USA). The SQK-LSK110 kit from Oxford Nanopore Technology (Lu *et al.* 2016; https://nanoporetech.com/) was then used to prepare the samples for nanopore sequencing following manufacturer's protocol. The library was loaded and sequenced on a R9.4.1 flow cell (ref FLO-Min106) for sequencing. Raw data were basecalled using Guppy v5.0.11 and the "sup" algorithm. Illumina paired-end sequencing was performed by Novogene Company Limited (https://en.novogene.com, Cambridge, UK) on the same DNA library.

We used MaSuRCA v4.0.3 (Zimin *et al.* 2017) to produce the hybrid assembly of our genome, using the Cabog assembler. We obtained a final assembly size of 309,35Mb in 2477 contigs, with a N50 of 347211 pb. The completeness of the assembly was estimated to 95,8% with Busco v5.0 on the *diptera_odb10* dataset (C:95.8%[S:94.6%,D:1.2%],F:0.7%,M:3.5%,n:3285).

Estimation of genome size, endosymbionts detection and mitogenome assembly

K-mers frequencies within short-read data were obtained with KMC 3 (Kokot *et al.* 2017). Genome size and ploidy were inferred using GenomeScope v2.0 with k-mer size = 21 and Smudgeplot (Ranallo-Benavidez *et al.* 2020). Contig taxonomy was performed using Blobtools (Laetsch and Blaxter 2017) with Diamond as search engine (Buchfink *et al.* 2015) against the UniProt database using a local copy of the NCBI TaxID file for the taxonomic assignation of the best hit. Minimap2 (Li 2018) was used for read mapping.

Genome annotation

The *B. coeca* genome was annotated using Maker v2.31.10 (Cantarel *et al.* 2008), following the protocol given in Muller *et al.* (2021), wherein multiple rounds of Maker supported by the training of the SNAP v.2006-07-28 (Korf 2004) and Augustus v.3.3.3 (König *et al.* 2016) gene finding and prediction tools, were conducted. Transcriptome of *B. coeca* (NCBI accession no. SRR2046564; 1KITE Consortium; (Bayless *et al.* 2021)) and proteomes of five *Drosophila* species, namely *D. innubila* (Hill *et al.* 2019), *D. albomicans* (Mai *et al.* 2020), *D. bipectinata* (Kim *et al.* 2021), *D. melanogaster* (Adams *et al.* 2000) and *D. virilis* (Clark *et al.* 2007) were used to guide the annotation. Protein-Protein BLAST 2.9.0+ (Altschul *et al.* 1997) (-evalue 1e-6 -max_hsps 1 -max_target_seqs 1) was then used to assess putative protein functions in *B. coeca* by comparing the protein sequences given

by Maker to the protein sequences from the annotated genome of *D. melanogaster*. The colocalization of *B. coeca* and their *D. melanogaster* orthologs on their respective contigs and chromosomal arms was analyzed to test for synteny.

Transposable elements annotation and transfer

Transposable elements were identified in each species following a two-step protocol. First, we used RepeatModeler v 2.0.1 (Flynn *et al.* 2020) with default parameters to generate a *de novo* library of repetitive regions. RepeatMasker v 4.0.9 (Flynn *et al.* 2020) was then run with the newly generated library and the options -a (create a .align output file) and -s (slow search; more sensitive) to create a summary of the families of transposable elements found in each reference genome along with the percentage of the genome they represent. Horizontal transfer analyses protocols of the *Famar1*-like element are given in Supplementary Text 1.

Gene family evolution

The Database for Annotation, Visualization and Integrated Discovery DAVID 2021 (Sherman *et al.* 2022) was used to test for gene ontology (GO) terms enrichments among lists of *D. melanogaster* orthologs annotated on the *B. coeca* genome (see above). The program was either fed with the list of *D. melanogaster* genes that were absent or duplicated in the *B. coeca* genome to test for under- and overrepresented gene families, respectively, compared to *D. melanogaster* genome, as denoted by the False Discovery Rate (FDR) correction for multiple tests. Each analysis was conducted for biological processes, molecular function, and cytological components.

Chemosensory superfamilies evolution

Protein sequences of the odorant (ORs) and gustatory (GRs) receptors of *A. mellifera* and *D. melanogaster* were obtained from Robertson and Wanner (2006) to which we added the annotated ORs and GRs protein sequences of *B. coeca*. Sequences were aligned using Molecular Evolutionary Genetic Analysis (MEGA X) software package (Kumar *et al.* 2018), which was also used to infer a maximum-likelihood phylogenetic tree for each family. We used iToL v4 (Letunic and Bork 2019) to visualize the trees. *B. coeca* protein sequences are given in Supplementary Datasets 2 and 3 for ORs and GRs, respectively.

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Conflicts of interest

The authors declare no conflicts of interest.

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Legends of figures

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

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

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

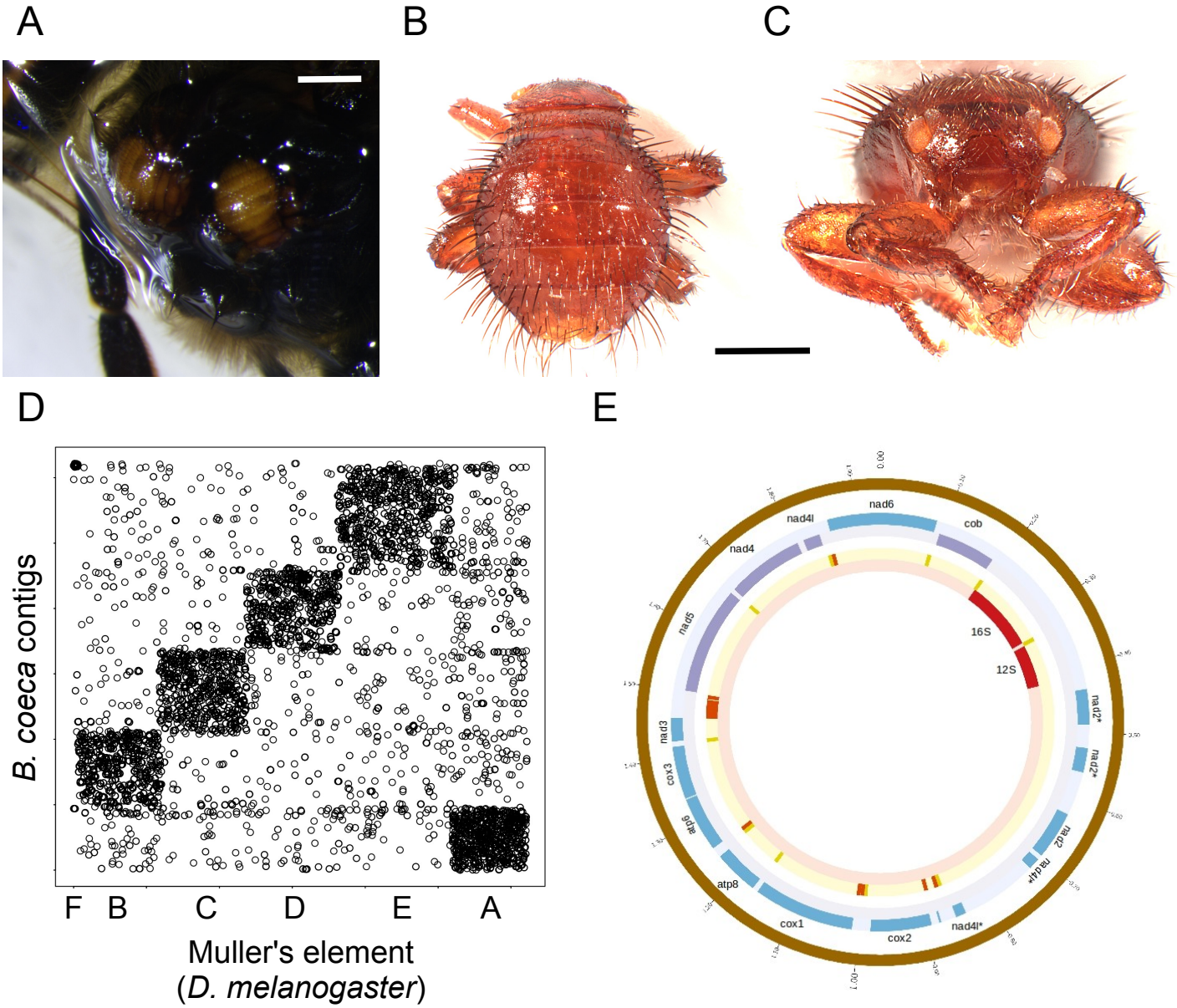
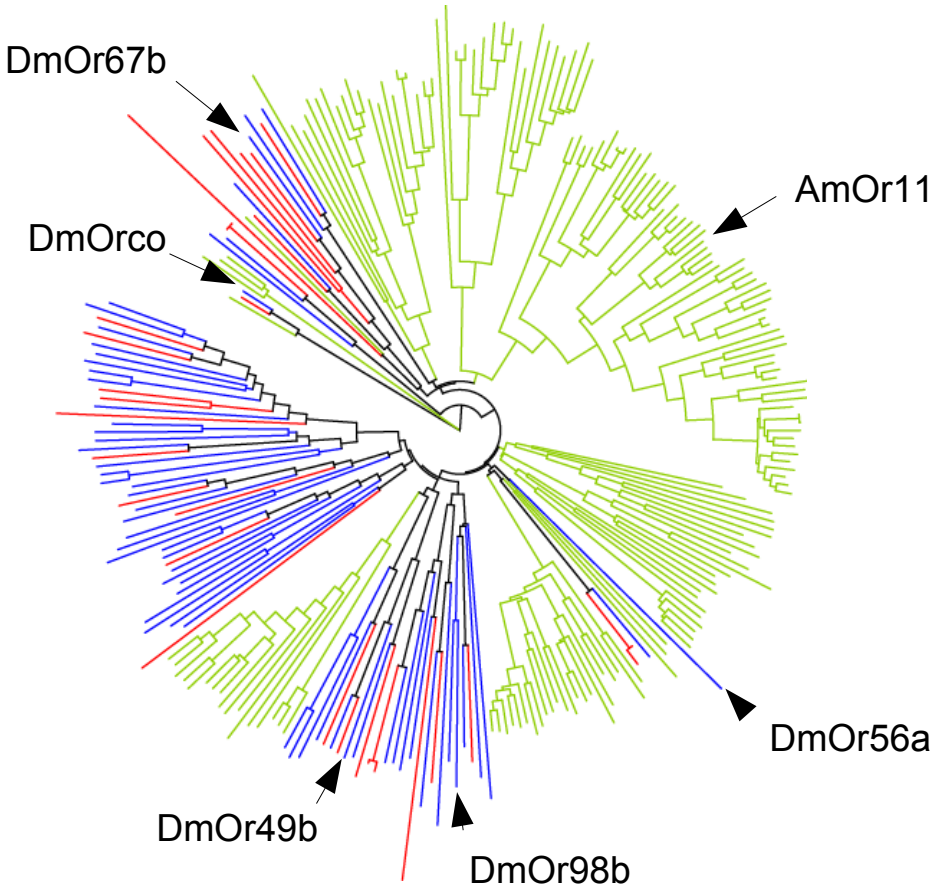


Figure 2

A



B