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Report

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Summary

Social insects nests harbor intruders known as inquilines,[1] which are usually related to their host.[2,3] However, distant non-social inquilines may also show convergences with their hosts,[4,5] though the underlying genomic changes remain unclear. We analyzed the genome of the wingless and blind bee louse fly *Braula coeca*, an inquiline kleptoparasite of the Western honey bee *Apis mellifera*.[6,7] Using large phylogenomic data, we confirmed recent accounts that the bee louse fly is a drosophilid,[8,9] and showed that it had likely evolved from a sap-breeder ancestor associated with honeydew and scale insects wax. Unlike many parasites, the bee louse fly genome did not show significant erosion or strict reliance on an endosymbiont, likely due to a relatively recent age of inquilinism. However, we observed a horizontal transfer of a transposon and a striking parallel evolution in a set of gene families between the honey bee and the bee louse fly. 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 nest and a diet of honey, pollen, and beeswax. Vision and odorant receptor genes also exhibited rapid losses. Only genes whose orthologs in the closely related *Drosophila melanogaster* respond to honey bee pheromones components or floral aroma were retained, whereas the losses included orthologous receptors responsive to the anti-ovarian honey bee queen pheromones. Hence, deep genomic convergences can underlie major phenotypic transitions during the evolution of inquilinism between non-social parasites and their social hosts.

Keywords: parasitism; inquilinism; phylogenomics; horizontal transposon transfer; gene family evolution; adaptation.

Results and Discussion

The bee louse fly Braula coeca *is an aberrant member of the Drosophilidae*

Among the several parasites and inquilines that are attracted by the rich resources and clean and protective shelter of the Western honey bee *Apis mellifera* nest, none has undergone as profound morphological changes 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 burrow tunnels in which they pupate without forming true puparia.[6,7] Following emergence, the adults attach to the body of worker bees, migrating from one individual to another until reaching the queen (Figure 1A). There, they move to the queen's head, stimulate regurgitation, and imbibe from her mouth honey and nectar.[6,7] The bee louse fly is considered an inquiline kleptoparasite with potential negative effects on honey bee colony health due to the galleries it makes in bee combs and the facilitation of transmitting serious pathogenic viruses to the bees.[10]

Ever since Réaumur's first description of the bee louse fly in 1740, and Nitzsch's creation of the genus *Braula* in 1818,[11,12] the positioning within the Diptera of the bee louse fly and affiliated species that were classified under the family Braulidae has been puzzling because of its aberrant morphology and unique adaptations to a social host. This family contains seven species belonging to the genera *Braula* and *Megabraula* that are all inquilines to honey bee species of the genus *Apis*. Recent phylogenetic analyses based on a transcriptome assembled from one adult fly and using 1,130 loci interestingly showed *Braula coeca*, the most widespread braulid, to constitute a basal lineage within the Drosophilidae that was sister to four genera of the subfamily Steganinae.[8,9] To reassess this hypothesis using a larger dataset, we sequenced the whole genome from a pooled sample of 15 unsexed *B. coeca* flies, all collected on Ouessant Island in Western France. We used a hybrid approach to assemble a genome using long-read Oxford Nanopore Technology (ONT) and short-read Illumina sequencing (see Methods). Benchmarking Universal Single-Copy Orthologs (BUSCO)[13] gave a score of 95.8% of the Dipteran conserved single-copy orthologs with 1.3% of duplicated genes. This value is higher than the recommended score of 90% for reference genomes[14]. Merqury[15] estimated an assembly completeness of 93.6% and a consensus quality value (QV) of 41, which exceeds the recommended threshold of QV40 for reference genome.[16] We assembled two genomes and one transcriptome of three additional steganine genera. We then built a supermatrix of 3,100 BUSCO genes (2,557,349 amino acids) that included 15 drosophilid species

(representative members of the four main radiations in the family),[17] and 5 species belonging to the superfamily Ephydroidea to which both the Drosophilidae and Braulidae belong (Table S1). The maximum-likelihood phylogenetic analysis of this large dataset reconfirmed the close-relatedness of *B. coeca* to the Drosophilidae. It further showed that it is a full member of the subfamily Steganinae (Figure 1D). The taxonomic priority principle should consider the family Drosophilidae, described in 1856,[18] a junior synonym for the family Braulidae, described in 1853.[19] However, the asymmetric size and scientific relevance of the two families argue against such a decision. We, therefore, opt for synonymizing the Braulidae with the Drosophilidae, referring hereafter to *Braula* and *Megabraula* as members of the subfamily Steganinae.

Inquilinism in the bee louse fly likely evolved from sap breeders associated with scale insects

To gain further insight into the history of the association between *Braula* and *Apis*, we mapped the predominant ecological habitats of ephydroid families on the phylogeny. The ancestral habitat of ephydroids was presumed to be rotting leaf molds.[20] From this, multiple specializations took place, including the exploitation of aquatic molds (and eventually algae) in the Ephydridae,[21] Mammal dung in Curtonotidae and Diastatidae,[21,22] and fermenting vegetables and fruits, sap and fungi, with specialization mostly on yeasts in the Drosophilidae[23] (Figure 1D). Bayesian reconstruction suggests the ancestral habitat of the Drosophilidae to be tree sap breeding (Figure 1D) with fungus- and fruit-breeding subsequently deriving and predominating in the genera *Leucophenga* and *Drosophila*, respectively. Remarkably, the deepest branches in the Steganinae and the Cryptochaetidae (the closest relative to the Drosophilidae) represent lineages whose larvae are predatory of scale insects and mealy bugs, e.g., *Acletoxenus formosus* and *A. indicus* on aleyrodoids, *Rhinoleucophenga brasiliensis* and *R. obesa* as well as *Cryptochaetidum iceryae* and *C. grandicorne* on coccoids.[24] In those lineages, adults are often seen to feed on the honeydew produced by the bugs, an abundant sugar-rich substrate sucked from plants' sap, while larvae take shelter and develop in the waxy secretions of these insects. This dependence on sugary substrate (honeydew) and development in a waxy environment could have predisposed *Braula*'s inquilinism in bee nests.

The bee louse fly inquilinism is relatively recent

To date *Braula* inquilinism, we inferred a fossil-calibrated phylogeny using 79 single-copy orthologs (63,192 amino acids) in 17 Acalyptrate dipteran and 25 Apocrite hymenopteran species (see Methods; Table S1; Figure 2A). Five non-ephydroid dipteran species with Ref-Seq assemblies were included in this analysis to correct for tree imbalance.[25] The divergence between *B. coeca* from its closest steganine relatives (node 1 in Figure 2A: 44.9 [37.8-53.8] million years (myr) [95% confidence interval]) overlapped with the origin of the Apidae (50.12 [42.9-64.5] myr) and with the transition from solitary to subsocial (2: 40.25 [32.4-47.8] myr) and primitively social habits (3: 30.98 [26.3-36.0] myr).[26] It is possible that the origin of the bee louse fly-apid interactions occurred at sap breeding sites, when early subsocial apids started to gather resin and other plant exudates, as well as scale insects honeydew, and stored them in their nests. As eusociality evolved (4: 23.8 [18.9-28.2] myr), the proportion of resin to secreted wax diminished, and some cells were also used to store nectar and honey for the brood.[27] A shift from the putatively ancestral dependence on honey and wax produced by scale insects to those produced by bees might have evolved by then. The transition to eusociality in the genus *Apis* required an important division of labor that involved the evolution of pheromonal control of the reproductive capacity of worker females by the queen and the evolution of trophallaxis.[27] Adaptation of *Braula* to the queen pheromone compounds that have anti-ovarian effects on a wide range of insects, including *Drosophila melanogaster*[28] and the exploitation of trophallaxis[7] could not have evolved before the advancement of eusociality (5: 17.1 [11.6-23.0] myr). The evolution of blindness and apterism should have constrained the dispersal of the bee lice, relating their speciation history to that of their hosts. Indeed, only seven bee louse fly species are known, of which five *Braula* species are restricted to the Western honey bee *A. mellifera*, and two *Megabraula* species are restricted to the giant honey bee *A. laboriosa* in the Himalayas.[29,30] The divergence between these *Apis* species, and presumably between *Braula* and *Megabraula*, is estimated at 6: 5.8 [2.8 -12.0] myr ago. Therefore, the evolution of the bee louse fly inquilinism has likely taken place during the Mid- to Late Miocene period between 5.8 and 17.1 myr ago (Figure 2A). We cannot rule out even a more recent origin if the ancestor of *Braula* or *Megabraula* has shifted from one *Apis* host to another, *i.e.* <5.8 myr ago.

The bee louse fly inquilinism was accompanied by a reduction of gene content but not genome size

Loss of significant portions of genomic and gene contents is a characteristic of obligate parasites specializing on specific hosts or inhabiting extreme environments. For example, the human body louse, *Pediculus humanus*, has one of the smallest genomes and the lowest numbers of genes in insects (108 megabases [Mb] and 10,773 protein-coding genes).[31] For *B. coeca*, we obtained a final assembly size of 309.35 Mb shared by 2,477 contigs with an N50 of 347,211 bp. This N50 estimate is typical of hybrid genome assemblies obtained using a pooled sample of wild-caught drosophilid flies from species with large genome sizes (> 300 Mb).[32] No evidence for polyploidy or other endosymbiont that could have biased the genome size estimate was detected (Figure S1). Genome size prediction using k-mers distribution spectra predicted a genome of 308 Mb, concordant with the assembly size (Figure S1). Such a genome size is significantly larger than the remaining drosophilid species (Student's *t* one-sample test $P < 1.3 \times 10^{-4}$; Shapiro-Wilk normality test $P = 0.55$). Phylogenetic analysis of genome size evolution indicates that the *B. coeca* genome likely retained the size of the ancestral Steganinae, *i.e.* a stronger reduction occurred in the Drosophilinae lineage containing *D. melanogaster* (Figure 2B; Figure S2; Table S1).

To determine the number of protein-coding genes, we used four rounds of Maker[33] supported by the training of the gene finding and prediction tools SNAP[34] and Augustus[35]. The annotation, made on the repeat-masked genome, yielded 10,349 protein-coding genes with an Annotation Edit Distance (AED) ≤ 0.5 for 96.4% of our gene models and a Pfam domain found in 83.66% of the proteins (BUSCO score = 91%). Using the same strategy, we annotated two steganine genomes, namely *Phortica variegata* and *Leucophenga varia*. The annotation yielded 11,067 (BUSCO score = 91%) and 13,160 (BUSCO score = 90.8%) protein-coding genes, respectively. The annotation of the ephydrid *Ephydra gracilis* genome yielded 9,154 protein-coding genes (BUSCO score = 68.9%) (Figure S2). *Ephydra* is particular among Ephydroidea in adapting to hypersaline waters and associated algal flora.[36] Given the current low knowledge of ephydrid genetics, whether their low gene content is due to their high specialization or an artifact of incomplete annotation, is hard to know. Regardless, the bee louse fly has the lowest number of protein-coding-genes compared to other drosophilids (Student's *t* one-sample

test $P < 1.5 \times 10^{-5}$; Shapiro-Wilk normality test $P = 0.48$) despite having a total genome size that is among the largest genomes in the family. Whereas the near-completeness of our *B. coeca* genome (~95%) might have reduced the number of annotated genes, low complexity hard-to-assemble genomic regions are usually mostly heterochromatic and poor in genes, e.g., centromeres, Y chromosomes, etc. A low gene content is also characteristic of bee genomes, compared to ants and wasps, with a remarkable trend of gene reduction within the family Apidae during the evolution of the genus *Apis* (Figure 2C; Figure S2; Table S1).

Transposable elements (TEs) expanded in the bee louse fly with one element horizontally transferred with the host

The bee louse fly's large genome size and low gene content suggest an increase in repetitive sequences. RepeatModeler and RepeatMasker analyses[37,38] indicated that nearly 41.34% of the *B. coeca* genome consists of such sequences, compared to 22.05% and 10.98% in *D. melanogaster* and *A. mellifera*, respectively (Figure 2D). Remarkably, half of the bee louse fly repetitive sequences consisted of long interspersed nuclear elements (LINEs) retrotransposons (14.94%). While LINEs are usually among the most abundant transposable elements after LTRs within the Drosophilidae,[39] their values did not exceed what was found in *B. coeca* (we found the highest percentage in *Leucophenga varia* with 5.54%). It is at present unclear what factors influence the diversity of transposable elements (TEs) landscapes among eukaryote species[40]. Nonetheless, this difference means that whereas the bee louse fly has likely retained the ancestral large genome size of the Drosophilidae, its TEs constitution has largely evolved.

Because host-parasite relationships have repeatedly been invoked as a factor that may favor horizontal transfer of TEs,[41] we searched for evidence of such transfers between *B. coeca* and *A. mellifera*. We found one TE, a DNA transposon *Famar1-like* element previously described in the earwig *Forficula auricularia*[42] that belongs to the Tc1-mariner superfamily. This element showed a high similarity between *B. coeca* and *A. mellifera* but was absent in all other drosophilid species for which a genome is available in GenBank, which is highly suggestive of an acquisition through horizontal transfer (Figure S2). Indeed, phylogenetic analysis of multiple copies of this TE extracted from 37 widely divergent animal species (Figure 2E) supported a direct transfer event between *B. coeca* and *A. mellifera*, although the directionality of the transfer cannot be inferred since

the elements from the two species form mutually-exclusive monophyletic clades. Remarkably, all elements found in the genomes of four *A. mellifera* subspecies, including *A. m. carnica*, *A. m. caucasia*, *A. m. mellifera* and *A. m. ligustica*, formed an exclusively monophyletic clade. The transfer time between *B. coeca* and *A. mellifera* has likely preceded the dispersion of this element among the subspecies or even their differentiation 0.77 myr ago[43] if the element was ancestral in *A. mellifera*. On the other hand, we did not find any trace of this element or any other related element in any other *Apis* species, indicating that the maximal time of horizontal transfer likely does not surpass 2.73 [0.70-8.9] myr ago, *i.e.* the time of divergence between *A. mellifera* and its closest-relative *A. cerana* (Figure 2A). The tight ecological connection between the bee louse fly and its host may have favored this transfer, as was suggested for blood- or sap-sucking insects.[44,45]

Gene families with excess losses show striking cross-order parallelism

Despite their deep divergence, we tested whether parallel changes could explain the reduction of protein-coding genes in both the honey bee and the bee louse fly. We used OrthoFinder[46] to cluster orthologous proteins from the 25 hymenopteran and 17 dipteran species. We identified 19,010 orthogroups. Of these, 935 showed significant size evolution among the 42 species when analyzed using CAFE5[47] and after applying an error model that accounted for misassemblies and misannotations. To classify those orthogroups into functional categories, we extracted groups that contained *D. melanogaster* orthologs for which a molecular function, *i.e.* a gene group, was assigned in the Flybase database[48] (see Methods). Of 1,078 gene groups, 136 significantly deviated from the birth-death model estimated by CAFE5.

After correction for multiple testing, 17 gene groups had significant losses in the bee louse fly with no group showing significant gain (Table 1). The reduction of most of these groups showed a striking parallelism with bees (Anthophila) in particular and hymenopterans in general (Table 1, Figure S3). The most significant groups were those involved in the chemical detection of taste (gustatory receptors and divergent ionotropic receptors) and odors (odorant receptors and odorant binding proteins). The remaining groups included those involved in recognition and signaling with a potential role in metabolism, immunity, and/or development such as C-type lectins, serine proteases, and Dorsal,[46] as well as ion and sugar transportations. Other groups are involved in

detoxification, such as cytochrome P450, GST-C, and carboxylases.[47] Indeed, bees have evolved a reduced repertoire of immunity and detoxification genes, likely due to the evolution of social behavior and their life in an overprotective and clean shelter, *i.e.* the nest.[49,50] Cytochrome P450 genes are more expressed in foraging workers than in the castes that remain in the nest (*i.e.* the queen and nurse workers).[51] The reduction of peptidases in both the honey bee and the bee louse fly could also be due to the low protein content of some of their food, *i.e.* nectar and honey. We also noted an underrepresentation of chitin-binding domain proteins and chitinases in the bee louse fly and the honey bee. Cuticles could act as barriers against environmental toxins, which may not be highly encountered in the nest. Remarkably, *B. coeca* is unique among cyclorrhaphan dipterans as its pupa, similarly to the honey bee's,[52] is contained in the unmodified cuticle of the third instar larva, and no sclerotized puparium is formed.[6,7] Whereas assembly and annotation errors can bias general estimates of gene losses, they should not specifically target the gene families that are ecologically relevant to both the host and the inquiline.

Honey and wax feeding drove the loss of almost all bitter-tasting gustatory receptors

The two most significantly evolving gene families in the bee louse fly, *i.e.* gustatory receptors (GRs) and divergent ionotropic receptors (IR-DIVs), allow the detection of soluble cues (Table 1). There are 60 GRs in *D. melanogaster*, of which 9 and 49 receptors respond primarily to sweet and bitter tastes, respectively, and 2 receptors respond to carbon dioxide (CO_2).[53] The three categories clustered into 35 orthogroups (Figure 3A), whose phylogenetic analysis indicates that the ancestral drosophilid repertoire consisted of 6 sweet, 26 bitter, and 2 CO_2 GRs assuming functional conservation of gustatory categories (Figure 3A). We identified 11 GRs in the bee louse fly with no duplications using InsectOR[54] and manual curation. These GRs could be classified according to their *D. melanogaster* orthologs into 3 sweet, 6 bitter, and 2 CO_2. That means that the *D. melanogaster* lineage disproportionally evolved more bitter receptors from the ancestral repertoire, whereas *B. coeca* disproportionally lost bitter receptors (Figure 3A). InsectOR inferred the number of GRs in the steganine species *L. varia* and *P. variegata* to be 21 and 26, respectively, further confirming that *B. coeca* has lost a significant portion of the ancestral GR repertoire (Figure S4). Honey bees have only 11 GRs, of which 7 are orthologous to sweet *Drosophila* GRs.[55] This is likely due to the bees' strong diet reliance

on sweet floral nectars and honey.[56] The loss of *B. coeca* bitter GRs and its retention of 2 ancestral sweet receptors is a strong convergence with its host.

Ionotropic receptors are another major class of chemoreceptors. They are divided into antennal IRs, which are conserved across insects and are most likely involved in olfaction, and divergent IRs (IR-DIVs), which evolve rapidly and are mostly involved in the taste perception of carboxylic and amino acids. Only divergent IRs showed a significant loss in *B. coeca* (Table 1). However, our knowledge about the function of the 42 *D. melanogaster* IR-DIVs is still limited.[57] We inferred the ancestral IR-DIV drosophilid repertoire to contain 29 receptors, of which only 9 were retained in *B. coeca*. Remarkably, whereas we found almost no direct orthologs between Diptera and Hymenoptera for IR-DIVs (Figure S3), bees are known to have few IRs in general[58] pointing to another possible taste convergence between the bee louse fly and its host.

One-fifth of ancestral odorant receptors was lost, including one receptor that is involved in anti-ovarian response in Drosophila melanogaster

Odorant receptors (ORs) are essential to detect volatile chemical cues from the environment. This family has expanded in the honey bee to reach 170.[59] However, only 9 of the honey bee genes have orthologs with *D. melanogaster*, and phylogenetic analysis indicates that this common OR repertoire has been gradually reduced during the evolution of *Apis* (Table 1, Figure S3). The 60 ORs of *D. melanogaster* clustered within 16 orthogroups (Figure 3B). We inferred the ancestral drosophilid OR repertoire to contain 44 ORs, with at least one representative for each orthogroup (Figure 3B). We identified in *B. coeca*, following InsectOR[54] and manual curation, 35 ORs in addition to *Orco*, *i.e.* one-fifth of the ancestral repertoire was lost. The number of ORs was 50 and 51 in the two closely related steganine species *L. varia* and *P. varia*, respectively (Figure S4). *Braula* ORs were direct orthologs to 18 genes in *D. melanogaster* (Figure 3B). Judging from the response of these orthologs to different volatiles in *D. melanogaster* as curated in the DoOR database[60] and assuming potential conservation of function, the retained bee louse fly ORs may respond to compounds produced by honey bee workers in a defense context (e.g., 1-hexanol, farnesol, 2-heptanone),[61] and/or of floral, pollen and nectar aromas, such as acetophenone and benzaldehyde, a major volatile of honey.[62,63] Two cases of tetraplications were observed. One case involved three recent duplications of genes orthologous to DmOr67b, a gene that is highly responsive in *D. melanogaster* to both

acetophenone and 1-hexanol. The second case involved three successive duplications of a gene orthologous to DmOr74a, which responds in *D. melanogaster* larvae to 1-nonanol and 1-heptanol, the latter being a major brood volatile,[64] and 1-hexanol, a component of the alarm pheromone.[65] Of these three duplications, two were unique to *B. coeca* compared to its closely-related steganine species (Figure S4). Low concentrations of isopentyl acetate, the main component of the alarm pheromone, released by unstressed workers at the nest entrances attract the parasitic nest beetle *Aethina tumida*,[66] suggesting that the detection of the host odors could be a common strategy among phylogenetically distant inquilines and parasites of social insects.

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. In honey bees, colony cohesion is driven by the volatile queen's mandibular pheromone (QMP), which "sterilizes" the bee workers.[67] This pheromone elicits an anti-ovarian response in other insects, including *D. melanogaster*.[28] An RNA interference (RNAi)-screen identified DmOr49b, DmOr56a, and DmOr98a to be potentially involved in the detection of the QMP compounds and the suppression of fecundity.[28,68] A *sine qua non* condition for a drosophilid to reproduce in a bee nest would, therefore, be to lose those receptors or to modify their response or effect. We found that the bee louse fly does not have an ortholog for DmOr98a, a receptor specific to the genus *Drosophila* (Figure S4). The bee louse fly has a pseudogene, orthologous to DmOr49b, that InsectOR identified. Orthologs of this *D. melanogaster* receptor are present and complete in all dipteran species, including *L. varia* and *P. variegata* (Figure S4). The bee louse fly had a receptor that we called BcOr22, which was orthologous to DmOr56a (Figure 4B). This last receptor is narrowly tuned in many *Drosophila* species to a single component, the mold volatile geosmin, whose perception also inhibits oviposition in *D. melanogaster*,[69] pointing to a possible conserved role in reproduction. Therefore, further functional analyses of the response of candidate ORs to various QMP compounds are required in both *D. melanogaster* and *B. coeca* to understand how modifications of these genes in *B. coeca* might have facilitated the evolution of the bee louse fly inquilinism.

Blindness and life in a dark nest were accompanied by the loss of multiple rhodopsins

The species Latin name of the bee louse fly refers to the assumption that it was blind due to the reduction of the eye size and the loss of the ocelli. In agreement with

reduced vision in the bee louse fly, we found only two out of the seven rhodopsin genes, which are responsible for colored vision and positive phototaxis in *D. melanogaster* and which were all present in the ancestral drosophilid repertoire. *D. melanogaster* orthologs of the *Rh1* and *Rh6* genes are expressed in the ommatidia and are sensitive to light.[70] The role of these opsins in light detection, despite the absence of ommatidia in the bee louse fly is unclear. Remarkably, *Rh1* and *Rh6* are structurally required in mechanosensory bristles to control larval locomotion.[71] They also detect temperature.[72] Therefore, the retention of these rhodopsins in the bee louse fly could mainly be due to their unconventional functions. On the other hand, the rhodopsin *Rh2*, which is exclusively expressed in the ocelli and used for horizon detection in *D. melanogaster* [73], is among those lost in the bee louse fly, in agreement with the loss of the ocelli. Regression of the visual system and its underlying opsin genes is common in animals inhabiting dark environments, such as fossorial mammals[74] and cavefishes,[75] representing a major example of deep convergences.

Apterism was not accompanied by the loss of major wing development genes

Small size, loss of wings, and the evolution of strongly clinging legs are all morphological changes that could prevent the honey bees from getting rid of the bee lice.[76] All these potential 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.[77] 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. Future developmental studies, specifically comparing the expression of wing and leg morphogenic genes between the bee louse fly and *D. melanogaster*, will definitively help shed light on the transcriptomic shifts underlying the major morphological changes of the bee louse fly.

Conclusion

That the enigmatic bee louse fly is indeed a drosophilid, a lineage within the most investigated insect family with more than 150 fully sequenced genomes, is undoubtedly one of the most exciting discoveries in dipteran phylogeny. How could a fly with an

ancestral drosophilid genome become ecologically adapted to bees and morphologically similar to lice? Our results show that a mosaic of deep convergences at the genomic level underlies the relatively recent and dramatic changes of the bee louse fly to nest inquilinism. This mosaicism involved deep convergences with the host, mostly in genes likely involved in immunity, detoxification, and chemical perception, as well as convergences with general features of fossorial animals in the visual systems. Future developmental studies may elucidate whether general morphologies, such as apterism and leg modifications, could also be shared between *Braula* and other ectoparasites. Due to its genetic relatedness to *Drosophila* and ecological association to *Apis*, two major laboratory models, the new genomic resources presented here can help establish the bee louse fly as a promising model to address questions related to deep convergences that are difficult to approach in multiple highly specializing animals.

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Author contributions

Conceptualization: H.B. and A.Y. ; Investigation: H.B., H.L., N.D., D.O., C.P., J.F., C.G., and A.Y.; Resources: H.L. and L.G.; Writing – Original draft: H.B., H.L., D.O., J.F., C.G., and A.Y.; Writing – Reviewing & Editing: H.B., J.C., C.G., F.M.P., F.R., J.C.S., and A.Y.; Visualization: H.B., J.F., C.G., and A.Y.; Supervision: H.B.; Funding Acquisition: H.B.

Declaration of interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly (Grammarly Inc.) in order to improve language and readability. After using this tool/service, the authors

reviewed and edited the content as needed and take full responsibility for the content of the publication.

Figures titles and legends

Figure 1. The bee louse fly (*Braula coeca*) is an inquiline of the Western honey bee (*Apis mellifera*) and has likely evolved from a sap-breeding drosophilid associated with scale insects. See also Table S1.

(A) Tens of *B. coeca* adults preferentially attached to the honey bee queen (© Etienne Minaud). Scale bar = 5 mm.

(B) Dorsal view of an adult showing the loss of the wings, halters, and scutum, mesonotum reduction and the legs' robustness. Scale bar = 0.5 mm.

(C) Frontal view of an adult showing the reduction of the eyes and the loss of the ocelli. Scale bar = 0.5 mm.

(D) Maximum likelihood phylogeny inferred from 3,100 conserved single-copy proteins (2,557,349 amino acids) showing the position of *B. coeca* (orange) in the subfamily Steganinae (light green) of the Drosophilidae (red). Outgroup species belong to the superfamily Ephydroidea (light blue). All internal nodes had an ultra-fast bootstrap value of 100% except * = 73%. Pie charts at internal nodes indicate the likelihood of ancestral breeding niches inferred from the predominant niches of terminal taxa.

Figure 2. Evolution of the bee louse fly inquilinism, its genome size, gene content, and transposable elements in the bee louse fly with evidence for horizontal transfer between the inquiline and its host. See also Table S1 and Figure S2.

(A) Fossil-calibrated maximum-likelihood phylogeny inferred from 79 conserved single-copy proteins (63,192 amino acids) demonstrating major stages in the evolution of the inquiline and its social host. All internal nodes had an ultra-fast bootstrap value of 100 (except when given), with a blue interval indicating a 95% confidence level of divergence time estimate inferred by MCMCTree. The red bar indicates the likely interval of the origin of the bee louse fly-*Apis* association. Labels 1-6 refer to the major stages mentioned in the text.

(B) Genome-size evolution. Red asterisk indicates the estimate for *B. coeca*.

(C) Gene content evolution. Red asterisk indicates the estimate for *B. coeca*.

(D) Proportions of transposable elements in the genomes of 42 dipteran and hymenopteran species. DNAs = DNA transposons, LCs = low complexity elements, LINEs = long interspersed nuclear elements, LTRs = long terminal repeats, SINEs = short interspersed nuclear elements, sRNAs = small RNAs, SRs = single repeats, and Unclass. = unclassified.

(E) Maximum-likelihood phylogeny of *Famar1*-like copies from 38 animal species. Filled circles indicate ultrafast bootstrap values higher than 90%.

Figure 3. Evolution of chemosensory receptors gene families in *Braula coeca* and *Drosophila melanogaster*. See also Figure S4.

(A) Maximum-likelihood phylogeny of gustatory receptors (GRs) with main taste categories color code given in a frame.

(B) Maximum-likelihood phylogeny of odorant receptors (ORs) with main ligands for each *D. melanogaster* receptor given in dark red. L = larva and A = adult expression.

For A and B, ultra-fast bootstrap values are given above nodes. Branches are colored according to orthogroups defined by OrthoFinder for 42 dipteran and hymenopteran species. Numbers in broken brackets before each orthogroup reflect the presumed ancestral gene content inferred by phytools.

STAR Methods

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Héloïse Bastide (heloise.bastide@universite-paris-saclay.fr).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Raw sequence data are deposited on NCBI Sequence Read Archive (SRA). Bioproject accession number is listed in the key resources table. Genome assemblies and all data associated to this study including translation of early taxonomic literature are deposited in Figshare. DOI is listed in the key resource table.

- All original code and commands for all programs have been deposited at Github depository. DOIs are listed in the key resource table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

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).

METHOD DETAILS

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.[78] Before nanopore sequencing, a size selection was conducted on the DNA using the SRE XS kit from Circulomics (https://www.circulomics.com/, Baltimore, Maryland, USA). The Ligation Sequencing kit SQK-LSK110 from ONT (https://nanoporetech.com/)[79] was then used to prepare the samples for nanopore sequencing following the manufacturer's protocol. The library was loaded and sequenced on an R9.4.1 flow cell (ref. FLO-Min106) for sequencing. Raw data were basecalled using Guppy v5.0.11 and the "sup" algorithm. The ONT raw data size was 4.4 Gb in 1,399,323 reads (mean read length 3,146 kb, longest read of 123.3 Mb), with an N50 of 4,677 kb. Phred scores ranged from 8 to 18, with a median of 13, as assessed by PycoQC.[80] Illumina paired-end sequencing was performed by Novogene Company Limited (https://en.novogene.com, Cambridge, UK) on the same DNA sample. The Illumina sequencing produced 119,719,537 paired 150 bp reads. Phred scores averaged 36 per read as analyzed by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We used MaSuRCA v4.0.3[81] 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 347,227 bp. The completeness of the assembly was estimated to 95.8% with BUSCO v5.0.0 on the

diptera_odb10 dataset (C:95.8%[S:94.5%,D:1.3%],F:0.7%,M:3.5%,n:3285), and to 93.6% using Merqury.

Estimation of genome size and endosymbionts detection

K-mers frequencies within short-read data were obtained with KMC 3.[82] Genome size and ploidy were inferred using GenomeScope v2.0 with k-mer size = 21 and Smudgeplot.[83] Contig taxonomy was performed using Blobtools[84] with Diamond as search engine[85] against the UniProt database using a local copy of the NCBI TaxID file for the taxonomic assignation of the best hit. Minimap2[86] was used for read mapping (Figure S1).

Genome annotation

The *B. coeca* genome was annotated using Maker v2.31.10,[33] following Muller et al.'s[87] protocol, wherein multiple rounds of Maker supported by the training of the SNAP v.2006-07-28[34] and Augustus v.3.3.3[35] gene finding and prediction tools, were conducted. RepeatModeler v2.0.1 was first used to identify the repeat-enriched regions that were masked by RepeatMasker v4.0.9 as implemented in Maker. Proteomes of five *Drosophila* species, namely *D. innubila*, *D. albomicans*, *D. bipectinata*, *D. melanogaster*, and *D. virilis* were obtained from NCBI and used to guide the annotation. Protein-Protein BLAST 2.9.0+[88] (-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 completeness of genome annotation was assessed using BUSCO at each round and the round with the highest score was retained.

Phylogenomic analysis of the Ephydroidea

Besides our *B. coeca* assembly, we obtained from NCBI repository genome assemblies for 12 species, transcriptome shotgun assemblies (TSA) for four species, and sequence read runs (SRR) for three species (Table S1). Paired-end DNA raw data of two species, namely *Rhinoleucophenga* cf. *bivisualis* and *Cacoxenus indagator* were assembled using MaSuRCA with default parameters. The transcriptome of *Acletoxenus* sp. was assembled using Trinity software package[89] on the Galaxy Europe website[90] following standard protocol[91]. BUSCO v.5.0 was used to assess the completeness of those assemblies and to extract single-copy BUSCO genes for all species. Protein sequences of 3,100 single and complete BUSCO genes were aligned using MAFFT[92] and concatenated into a single supermatrix (2,557,349 amino acids). A maximum-likelihood (ML) phylogeny was then inferred for the supermatrix using IqTREE 2[93] with 1,000 ultrafast bootstrap iterations[94]

and using the JTT+R substitution model inferred by ModelFinder[95] implemented by IqTree.

Reconstruction of the ancestral ecological niches

For each of the 20 ephydroid species we obtained a predominant ecological niche from the taxonomic literature.[20,23,24,96] Eight predominant niches were coded as discrete traits, and the Multistate program of the BayesTraits v.4 package[97] was used under the Reverse Jump MCMC model with 1,010,000 chain iterations and a burnin sample of 10,000.

Phylogenomic analysis of Diptera and Hymenoptera

The second phylogenomic analysis involved, besides *B. coeca*, 25 hymenopteran and 16 dipteran species for which an assembly can be downloaded from the NCBI Genome repository (Table S1). Protein sequences for all species but three, namely *Leucophenga varia*, *Phortica variegata*, and *Ephydra gracilis*, were obtained from NCBI. For these three species, we used the same four-round annotation procedure that we used for *B. coeca* to identify protein-coding genes and translate their sequences. We used BUSCO to assess the completeness of all annotated and downloaded genomes and their corresponding assemblies. OrthoFinder[46] was used to generate protein sequences of protein-coding-genes of the 42 species and to cluster these sequences into orthogroups. Only the longest isoform (*i.e.* the primary transcript) was used for genes with multiple isoforms. 79 orthogroups contained a single copy ortholog from each species, and their protein sequences were aligned using MAFFT and concatenated into a single supermatrix (63,192 amino acids). A maximum-likelihood (ML) phylogeny was then inferred for the supermatrix using IqTREE 2[93] with 1,000 ultrafast bootstrap iterations[94] and the JTT+R substitution model inferred by ModelFinder[95] implemented by IqTree.

MCMCTree[98] was used to date the inferred ML trees based on recently published fossil-calibrated phylogenies. First, two time points were obtained for the 42-species phylogeny. These included the divergence between ants and bees between 90-120 myr ago[99] and between *Scaptodrosophila* and *Drosophila* between 50-56 myr ago,[100] with a maximum root age for the ancestor of Hymenoptera and Diptera at 344 myr ago.[99]

Genome size and gene content evolution

Genome size and gene content (number of OrthoFinder generated protein-coding-genes after retaining the longest isoform for genes with multiple transcripts) inferred for each of the 42 dipteran and hymenopteran genomes were mapped on the phylogenetic

568 tree, and values at the ancestral nodes were inferred and visualized using the fastAnc
569 command in the R package Phytools v0.2.2.[101]
570 ***Transposons annotation and detection of Horizontal Transposon Transfer (HTT)***
571 Transposons were identified in the 42 dipteran and hymenopteran genomes
572 following a two-step protocol. First, we used RepeatModeler v2.0.1[37] with default
573 parameters to generate a *de novo* library of repetitive regions. RepeatMasker v 4.0.9[37] was
574 then run with the newly generated library and the options -a (create a .align output file)
575 and -s (slow search; more sensitive) to create a summary of the families of transposable
576 elements found in each genome along with the percentage of the genome they represent.
577 To detect possible HTT between *Braula coeca* and *Apis mellifera*, we used the *B. coeca*
578 whole genome as query to perform a blastn similarity search against the whole *A.*
579 *mellifera* genome (all default options, including "-task megablast"). All *B. coeca* genome
580 regions longer than 299 bp and aligning to *A. mellifera* with an e-value lower than 0.0001
581 were extracted and clustered at 80% nucleotide identity threshold with vsearch.[102] The
582 consensus sequence of each of the 50 resulting clusters were used as queries to perform
583 blastx searches on the non-redundant protein database of NCBI using Diamond.[85] A total
584 of eight consensus sequences had best hits to the *Famar1* element previously described
585 in the earwig *Forficula auricularia*, known to be also present in *A. mellifera* as a result of
586 horizontal transfer.[42,103] To verify that the *Famar1*-like element from *B. coeca* has indeed
587 been involved in HTT, we compared the *Famar1*-like synonymous distance (dS) to a
588 distribution of dS expected under vertical transmission since the last common ancestor
589 of *B. coeca* and *A. mellifera* following the approach developed in Zhang *et al*.[104] This
590 approach assumes that in case of HTT, TE dS should be much lower than dS expected
591 under vertical transmission. Briefly, we calculated the dS over the transposase open
592 reading frame between one copy of the *Famar1*-like element extracted from *C. coeca* and
593 another copy of this element from the *A. mellifera* genome. We then compared this
594 distance to the distribution of dS calculated over 2,179 alignments between single copy
595 BUSCO genes that produce best reciprocal hits in blastp similarity searches.[104] We found
596 that the *Famar1*-like dS (=0.12) fall below the 0.5% quantile (=1.76) of the distribution of
597 dS calculated for orthologous genes (Figure S2), confirming that the element has been
598 acquired through HTT in *B. coeca* and *A. mellifera*. To assess whether the tight ecological
599 interactions existing between *B. coeca* and *A. mellifera* might have favored direct transfer
600 of this element between the two species, we assessed how closely related are *B. coeca*

Famar1-like copies to those from *A. mellifera*. We first screened for the presence of this element in other animal genomes. We used the *Famar1* sequence[42] as query to perform online blastn similarity searches (all default options, including "-task megablast") on a total of 8,180 animal genomes belonging to 11 insect orders as well as to Annelida, Chelicerata, Chiroptera, Cnidaria, Myriapoda, Nematoda, Platyhelminthes and Teleostei. We found full length copies showing >79% of nucleotide identity to this element in a total of 37 species. We aligned up to ten copies from each genome the most similar to *Famar1* using Muscle.[105] We then reconstructed a maximum-likelihood phylogeny of these copies using IqTree after nucleotide model detection using ModelFinder. Node support was quantified using ultrafast bootstrap as implemented in IqTree.

Gene family evolution

We used CAFE v. 5[47] to model and infer gene family evolution. We conducted CAFE5 using an error model on the 19,011 orthgroups generated by OrthoFinder and using the time-calibrated phylogenetic tree of the 42 dipteran and hymenopteran species. The analysis showed that *B. coeca* has gained 439 while losing 1,517 protein-coding genes in agreement with the low gene content of this species. To gain further functional insights on the ecological or biological relevance of the evolving genes, we grouped OrthoFinder orthogroups into functional gene groups using the customized perl script OG2GG.pl (https://github.com/AmirYassinLab/OG2GG) leveraging the proximity of *B. coeca* to *D. melanogaster*. The script assigns each *D. melanogaster* ortholog to its largest functional gene groups in Flybase[48] ("gene_group_data_fb_2023_02.tsv") and then assigns each orthogroup to the largest gene group of its constituent genes. *D. melanogaster* has 13,545 protein-coding genes that were clustered into 10,497 orthogroups. However, 8,202 *D. melanogaster* genes are assigned to at least one of 10,670 functional gene groups in the FlyBase database, of which some concern RNA genes that, by definition, are not analyzed by OrthoFinder. Because of the hierarchical nature of the functional gene groups annotation in FlyBase as well as to the pleiotropy of certain genes, each *D. melanogaster* gene was assigned to its largest group, *i.e.* the group with biggest number of genes. Consequently, 5,733 protein-coding genes were assigned to an orthogroup and a gene group. Because some orthogroups can have multiple genes with some assigned to different gene groups, each orthogroup was assigned to the largest gene group of its constituent genes. Orthogroups were then clustered according to their assigned functional groups, e.g., the odorant receptors family contained 16 orthgroups (and 60 *D.*

melanogaster genes). Because some of the genes found in the orthogroups based on their sequence similarity have no functional annotation in Flybase, the total number of *D. melanogaster* protein-coding genes to be grouped into gene groups was 7,820 genes (and 6,317 protein-coding genes for *B. coeca*).

CAFE5 was then run on the gene groups' gene counts using four different birth rate models in an increasing order (lambda = 1, 2, 3 and 4) and the error model to correct for possible assembly and annotation errors. For each model we run four iterations. The likelihood of only the two simplest models, *i.e.* one- and two-lambda models, converged across the four iterations. Likelihood ratio test using the lr.test function of the extRemes R package[106] showed that the two-lambda better fit our data. This model imposed a different rate for only *B. coeca* compared to the rest of the tree and it was chosen for four-iterations of subsequent analyses using the estimated error rate. Multiple testing corrections were conducted using the False Discovery Rate (FDR) analysis of the FDRestimation[108] package implemented in R.

Chemosensory superfamilies evolution

To curate *B. coeca* gustatory receptors (GR) and odorant receptors (OR) genes, we queried *D. melanogaster* GRs and ORs protein sequences on *B. coeca*, *L. varia* and *P. variegata* assemblies using Exonerate ver. 2.2[107] with option –maxintron 2000 and -p pam250. The output, along with the assembly, were fed to InsectOR[54] with option 7tm_7 and 7tm_6 activated for GR and OR analyses, respectively. From the output files, we extracted 300-500 amino acids-long complete sequences with 7tm_7 or 7tm_6 motif detected and with start codon present and no internal stop codon, *i.e.* pseudogenes excluded. Protein sequences were aligned using MAFFT and a maximum-likelihood phylogenetic tree for each family using IqTREE 2 with the same options as the phylogenomic analysis. The literature was reviewed to classify GRs into bitter, sweet, and CO_2 categories[109] and identify volatile ligands eliciting the strongest response in odorant neurons in *D. melanogaster*.[60] Because CAFE5 inferred ancestral counts for orthogroups with significant deviation only, we estimated and visualized ancestral counts for each orthogroups of these two families using FastAnc command in the R package Phytools.

QUANTIFICATION AND STATISTICAL ANALYSIS

Genome size and protein-coding gene content analyses

We compared the estimates of genome size and protein-coding gene content of *B. coeca* to the distributions of these values for the 10 drosophilid genomes (data given in Table S1) using one-sample Student's *t* test and after testing for normality using Shapiro-Wilk test as implemented in R.

Likelihood Ratio Test (LTR) comparison of CAFE5 models

We compared the likelihood of the two simplest CAFE5 models, *i.e.* one- and two-lambda models which were the only ones to converge across the four iterations, using the lr.test function of the extRemes R package.[106] For each model, the likelihood estimates were averaged across the four iterations.

False Discovery Rate (FDR) estimation of the* p-*values of the best CAFE5 model

The two-lambda model had the best likelihood and was therefore subsequently run for four iterations. *p*-values inferred for each gene group in the branch leading to *B. coeca* in the iteration with the best likelihood were extracted, and multiple testing corrections were conducted using the False Discovery Rate (FDR) analysis of the FDRestimation[108] package implemented in R.

List of supplementary materials

Table S1. List of all genome assemblies and annotations downloaded or generated in this study with data on genome size, gene content and BUSCO scores.

References

1. Cini, A., Sumner, S., and Cervo, R. (2019). Inquiline social parasites as tools to unlock the secrets of insect sociality. Philosophical Transactions of the Royal Society B: Biological Sciences *374*, 20180193. 10.1098/rstb.2018.0193.
2. Rabeling, C., Schultz, T.R., Pierce, N.E., and Bacci, M. (2014). A Social Parasite Evolved Reproductive Isolation from Its Fungus-Growing Ant Host in Sympatry. Current Biology *24*, 2047–2052. 10.1016/j.cub.2014.07.048.
3. Borowiec, M.L., Cover, S.P., and Rabeling, C. (2021). The evolution of social parasitism in Formica ants revealed by a global phylogeny. Proceedings of the National Academy of Sciences *118*, e2026029118. 10.1073/pnas.2026029118.
4. Dejean, A., Orivel, J., Azémar, F., Hérault, B., and Corbara, B. (2016). A cuckoo-like parasitic moth leads African weaver ant colonies to their ruin. Sci Rep *6*, 23778. 10.1038/srep23778.
5. Maruyama, M., and Parker, J. (2017). Deep-Time Convergence in Rove Beetle Symbionts of Army Ants. Current Biology *27*, 920–926. 10.1016/j.cub.2017.02.030.
6. Skaife, S.H. (1922). On *Braula Coeca* , Nitzsch, a Dipterous parasite of the honey bee. Transactions of the Royal Society of South Africa *10*, 41–48. 10.1080/00359192209519263.
7. Imms, A.D. (1942). On Braula coeca Nitsch and its affinities. Parasitology *34*, 88–

100. 10.1017/S0031182000016012.
8. Bayless, K.M., Trautwein, M.D., Meusemann, K., Shin, S., Petersen, M., Donath, A., Podsiadlowski, L., Mayer, C., Niehuis, O., Peters, R.S., et al. (2021). Beyond Drosophila: resolving the rapid radiation of schizophoran flies with phylotranscriptomics. BMC Biol *19*, 23. 10.1186/s12915-020-00944-8.
9. Winkler, I.S., Kirk-Spriggs, A.H., Bayless, K.M., Soghigian, J., Meier, R., Pape, T., Yeates, D.K., Carvalho, A.B., Copeland, R.S., and Wiegmann, B.M. (2022). Phylogenetic resolution of the fly superfamily Ephydroidea–Molecular systematics of the enigmatic and diverse relatives of Drosophilidae. PLOS ONE *17*, e0274292. 10.1371/journal.pone.0274292.
10. Avalos, J., Rosero, H., Maldonado, G., and Reynaldi, F.J. (2019). Honey bee louse (Braula schmitzi) as a honey bee virus vector? Journal of Apicultural Research *58*, 427–429. 10.1080/00218839.2019.1565726.
11. de Réaumur, R.-A.F. (1740). Mémoires pour servir à l'histoire des insectes... Vol. 5 (de l'Imprimerie royale).
12. Nitzsch, C. (1818). Die Familien und Gattungen der Thierinsekten (insecta epizoica); als Prodromus einer Naturgeschichte derselben. In.
13. Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., and Zdobnov, E.M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics *31*, 3210–3212. 10.1093/bioinformatics/btv351.
14. Lewin, H.A., Robinson, G.E., Kress, W.J., Baker, W.J., Coddington, J., Crandall, K.A., Durbin, R., Edwards, S.V., Forest, F., Gilbert, M.T.P., et al. (2018). Earth BioGenome Project: Sequencing life for the future of life. Proceedings of the National Academy of Sciences *115*, 4325–4333. 10.1073/pnas.1720115115.
15. Rhie, A., Walenz, B.P., Koren, S., and Phillippy, A.M. (2020). Merqury: reference-free quality, completeness, and phasing assessment for genome assemblies. Genome Biology *21*, 245. 10.1186/s13059-020-02134-9.
16. Koren, S., Phillippy, A.M., Simpson, J.T., Loman, N.J., and Loose, M. (2019). Reply to 'Errors in long-read assemblies can critically affect protein prediction.' Nat Biotechnol *37*, 127–128. 10.1038/s41587-018-0005-y.
17. Yassin, A. (2013). Phylogenetic classification of the Drosophilidae Rondani (Diptera): the role of morphology in the postgenomic era. Systematic Entomology *38*, 349–364.
18. Rondani, C. (1856). Dipterologiae Italicae prodromus: Genera Italica Ordinis Dipterorum (Stocchi).
19. Egger, S. (1853). Himmlische Waffenrüstung für die Jugend bestehend aus den heiligen Sakramenten der Busse, des Altars und der Firmung: Ein praktischer Unterricht (Schmid).
20. Throckmorton, L. (1975). The phylogeny, ecology, and geography of *Drosophila*. In.
21. Keiper, J.B., Walton, W.E., and Foote, B.A. (2002). Biology and ecology of higher Diptera from freshwater wetlands. Annu. Rev. Entomol. *47*, 207–232. 10.1146/annurev.ento.47.091201.145159.
22. Pollock, J.N. (2002). Observations on the biology and anatomy of Curtonotidae (Diptera: Schizophora). Journal of Natural History *36*, 1725–1745. 10.1080/00222930110061869.
23. Markow, T.A., and O'Grady, P.M. (2005). Evolutionary genetics of reproductive behavior in *Drosophila*: connecting the dots. Annu. Rev. Genet. *39*, 263–291. 10.1146/annurev.genet.39.073003.112454.
24. Ashburner, M. (1981). Entomophagous and other bizarre *Drosophilidae*. In.
25. Duchêne, D., Duchêne, S., and Ho, S.Y.W. (2014). Tree imbalance causes a bias in phylogenetic estimation of evolutionary timescales using heterochronous sequences. Molecular Ecology Resources *15*, 785–794. 10.1111/1755-0998.12352.

26. Shell, W.A., Steffen, M.A., Pare, H.K., Seetharam, A.S., Severin, A.J., Toth, A.L., and Rehan, S.M. (2021). Sociality sculpts similar patterns of molecular evolution in two independently evolved lineages of eusocial bees. Commun Biol *4*, 253. 10.1038/s42003-021-01770-6.
27. Noll, F.B. (2002). Behavioral phylogeny of corbiculate Apidae (Hymenoptera; Apinae), with special reference to social behavior. Cladistics *18*, 137–153. 10.1111/j.1096-0031.2002.tb00146.x.
28. Galang, K.C., Croft, J.R., Thompson, G.J., and Percival-Smith, A. (2019). Analysis of the Drosophila melanogaster anti-ovarian response to honey bee queen mandibular pheromone. Insect Molecular Biology *28*, 99–111. 10.1111/imb.12531.
29. Grimaldi, D., and Underwood, B.A. (1986). Megabraula, a new genus for two new species of Braulidae (Diptera), and a discussion of braulid evolution. System Entomol *11*, 427–438. 10.1111/j.1365-3113.1986.tb00534.x.
30. Dobson, J.R. (1999). A “bee-louse" *Braula schmitzi* örösi-pál (Diptera: Braulidae) new to the British Isles, and the status of *Braula* spp. in England and Wales.
31. Kelley, J.L., Peyton, J.T., Fiston-Lavier, A.-S., Teets, N.M., Yee, M.-C., Johnston, J.S., Bustamante, C.D., Lee, R.E., and Denlinger, D.L. (2014). Compact genome of the Antarctic midge is likely an adaptation to an extreme environment. Nat Commun *5*, 4611. 10.1038/ncomms5611.
32. Kim, B.Y., Wang, J.R., Miller, D.E., Barmina, O., Delaney, E., Thompson, A., Comeault, A.A., Peede, D., D’Agostino, E.R., Pelaez, J., et al. (2021). Highly contiguous assemblies of 101 drosophilid genomes. eLife *10*, e66405. 10.7554/eLife.66405.
33. Cantarel, B.L., Korf, I., Robb, S.M.C., Parra, G., Ross, E., Moore, B., Holt, C., Alvarado, A.S., and Yandell, M. (2008). MAKER: An easy-to-use annotation pipeline designed for emerging model organism genomes. Genome Res. *18*, 188–196. 10.1101/gr.6743907.
34. Korf, I. (2004). Gene finding in novel genomes. BMC Bioinformatics *5*, 59. 10.1186/1471-2105-5-59.
35. König, S., Romoth, L.W., Gerischer, L., and Stanke, M. (2016). Simultaneous gene finding in multiple genomes. Bioinformatics *32*, 3388–3395. 10.1093/bioinformatics/btw494.
36. van Breugel, F., and Dickinson, M.H. (2017). Superhydrophobic diving flies (*Ephydra hians*) and the hypersaline waters of Mono Lake. Proceedings of the National Academy of Sciences *114*, 13483–13488. 10.1073/pnas.1714874114.
37. Flynn, J.M., Hubley, R., Goubert, C., Rosen, J., Clark, A.G., Feschotte, C., and Smit, A.F. (2020). RepeatModeler2 for automated genomic discovery of transposable element families. Proceedings of the National Academy of Sciences *117*, 9451–9457. 10.1073/pnas.1921046117.
38. Smit, A.F.A., Hubley, R., and Green, P. (2015). RepeatMasker Open-4.0. 2013–2015 http://www.repeatmasker.org/.
39. Mérel, V., Boulesteix, M., Fablet, M., and Vieira, C. (2020). Transposable elements in *Drosophila*. Mobile DNA *11*, 23. 10.1186/s13100-020-00213-z.
40. Gilbert, C., Peccoud, J., and Cordaux, R. (2021). Transposable elements and the evolution of insects. Annu. Rev. Entomol. *66*, 355–372. 10.1146/annurev-ento-070720-074650.
41. Venner, S., Miele, V., Terzian, C., Biémont, C., Daubin, V., Feschotte, C., and Pontier, D. (2017). Ecological networks to unravel the routes to horizontal transposon transfers. PLOS Biology *15*, e2001536. 10.1371/journal.pbio.2001536.
42. Barry, E.G., Witherspoon, D.J., and Lampe, D.J. (2004). A Bacterial Genetic Screen Identifies Functional Coding Sequences of the Insect *mariner* Transposable Element *Famar1* Amplified From the Genome of the Earwig, *Forficula auricularia*. Genetics *166*, 823–833.

10.1534/genetics.166.2.823.
43. Carr, S.M. (2023). Multiple mitogenomes indicate things fall apart with Out of Africa or Asia hypotheses for the phylogeographic evolution of honey bees (*Apis mellifera*). Sci Rep *13*, 9386. 10.1038/s41598-023-35937-4.
44. Gilbert, C., Schaack, S., Pace II, J.K., Brindley, P.J., and Feschotte, C. (2010). A role for host–parasite interactions in the horizontal transfer of transposons across phyla. Nature *464*, 1347–1350. 10.1038/nature08939.
45. Gilbert, C., and Maumus, F. (2022). Multiple Horizontal Acquisitions of Plant Genes in the Whitefly Bemisia tabaci. Genome Biology and Evolution *14*, evac141. 10.1093/gbe/evac141.
46. Emms, D.M., and Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol *20*, 1–14. 10.1186/s13059-019-1832-y.
47. Mendes, F.K., Vanderpool, D., Fulton, B., and Hahn, M.W. (2020). CAFE 5 models variation in evolutionary rates among gene families. Bioinformatics *36*, 5516–5518. 10.1093/bioinformatics/btaa1022.
48. Thurmond, J., Goodman, J.L., Strelets, V.B., Attrill, H., Gramates, L.S., Marygold, S.J., Matthews, B.B., Millburn, G., Antonazzo, G., Trovisco, V., et al. (2019). FlyBase 2.0: the next generation. Nucleic Acids Research *47*, D759–D765. 10.1093/nar/gky1003.
49. Evans, J.D., Aronstein, K., Chen, Y.P., and Hetru, C. (2006). Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology.
50. Berenbaum, M.R., and Johnson, R.M. (2015). Xenobiotic detoxification pathways in honey bees. Current Opinion in Insect Science *10*, 51–58. 10.1016/j.cois.2015.03.005.
51. Chan, Q.W.T., Chan, M.Y., Logan, M., Fang, Y., Higo, H., and Foster, L.J. (2013). Honey bee protein atlas at organ-level resolution. Genome Res. *23*, 1951–1960. 10.1101/gr.155994.113.
52. Winston, M.L. (1987). The Biology of the Honey Bee (Harvard University Press).
53. Weiss, L.A., Dahanukar, A., Kwon, J.Y., Banerjee, D., and Carlson, J.R. (2011). The Molecular and Cellular Basis of Bitter Taste in Drosophila. Neuron *69*, 258–272. 10.1016/j.neuron.2011.01.001.
54. Karpe, S.D., Tiwari, V., and Ramanathan, S. (2021). InsectOR—Webserver for sensitive identification of insect olfactory receptor genes from non-model genomes. PLoS ONE *16*, e0245324. 10.1371/journal.pone.0245324.
55. Sadd, B.M., Barribeau, S.M., Bloch, G., de Graaf, D.C., Dearden, P., Elsik, C.G., Gadau, J., Grimmelikhuijzen, C.J., Hasselmann, M., Lozier, J.D., et al. (2015). The genomes of two key bumblebee species with primitive eusocial organization. Genome Biol *16*, 1–32. 10.1186/s13059-015-0623-3.
56. Robertson, H.M., and Wanner, K.W. (2006). The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. Genome Res. *16*, 1395–1403. 10.1101/gr.5057506.
57. Ni, L. (2021). The structure and function of ionotropic receptors in *Drosophila*. Front. Mol. Neurosci. *13*, 638839. 10.3389/fnmol.2020.638839.
58. Park, D., Jung, J.W., Choi, B.-S., Jayakodi, M., Lee, J., Lim, J., Yu, Y., Choi, Y.-S., Lee, M.-L., Park, Y., et al. (2015). Uncovering the novel characteristics of Asian honey bee, *Apis cerana*, by whole genome sequencing. BMC Genomics *16*, 1. 10.1186/1471-2164-16-1.
59. Karpe, S.D., Jain, R., Brockmann, A., and Sowdhamini, R. (2016). Identification of Complete Repertoire of Apis florea Odorant Receptors Reveals Complex Orthologous Relationships with Apis mellifera. Genome Biology and Evolution *8*, 2879–2895. 10.1093/gbe/evw202.
60. Münch, D., and Galizia, C.G. (2016). DoOR 2.0 - Comprehensive mapping of *Drosophila melanogaster* odorant responses. Sci Rep *6*, 21841. 10.1038/srep21841.

855 61. Wang, Z., and Tan, K. (2019). Honey Bee Alarm Pheromone Mediates
856 Communication in Plant–Pollinator–Predator Interactions. Insects *10*, 366.
857 10.3390/insects10100366.
858 62. Machado, A.M., Miguel, M.G., Vilas-Boas, M., and Figueiredo, A.C. (2020). Honey
859 Volatiles as a Fingerprint for Botanical Origin—A Review on their Occurrence on Monofloral
860 Honeys. Molecules *25*, 374. 10.3390/molecules25020374.
861 63. Starowicz, M., Hanus, P., Lamparski, G., and Sawicki, T. (2021). Characterizing the
862 Volatile and Sensory Profiles, and Sugar Content of Beeswax, Beebread, Bee Pollen, and
863 Honey. Molecules *26*, 3410. 10.3390/molecules26113410.
864 64. Noël, A., Dumas, C., Rottier, E., Beslay, D., Costagliola, G., Ginies, C., Nicolè, F.,
865 Rau, A., Conte, Y.L., and Mondet, F. (2023). Detailed chemical analysis of honey bee *(Apis*
866 *mellifera)* worker brood volatile profile from egg to emergence. PLOS ONE *18*, e0282120.
867 10.1371/journal.pone.0282120.
868 65. Collins, A.M., and Blum, M.S. (1983). Alarm responses caused by newly identified
869 compounds derived from the honeybee sting. J Chem Ecol *9*, 57–65. 10.1007/BF00987770.
870 66. Torto, B., Boucias, D.G., Arbogast, R.T., Tumlinson, J.H., and Teal, P.E.A. (2007).
871 Multitrophic interaction facilitates parasite–host relationship between an invasive beetle and
872 the honey bee. Proceedings of the National Academy of Sciences *104*, 8374–8378.
873 10.1073/pnas.0702813104.
874 67. Van Oystaeyen, A., Oliveira, R.C., Holman, L., van Zweden, J.S., Romero, C., Oi,
875 C.A., d'Ettorre, P., Khalesi, M., Billen, J., Wäckers, F., et al. (2014). Conserved Class of
876 Queen Pheromones Stops Social Insect Workers from Reproducing. Science *343*, 287–290.
877 10.1126/science.1244899.
878 68. Camiletti, A.L., Percival-Smith, A., Croft, J.R., and Thompson, G.J. (2016). A novel
879 screen for genes associated with pheromone-induced sterility. Sci Rep *6*, 36041.
880 10.1038/srep36041.
881 69. Stensmyr, M.C., Dweck, H.K.M., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz,
882 J., Grabe, V., Steck, K., Lavista-Llanos, S., et al. (2012). A Conserved Dedicated Olfactory
883 Circuit for Detecting Harmful Microbes in Drosophila. Cell *151*, 1345–1357.
884 10.1016/j.cell.2012.09.046.
885 70. Senthilan, P.R., and Helfrich-Förster, C. (2016). Rhodopsin 7–The unusual Rhodopsin
886 in Drosophila. PeerJ *4*, e2427. 10.7717/peerj.2427.
887 71. Zanini, D., Giraldo, D., Warren, B., Katana, R., Andrés, M., Reddy, S., Pauls, S.,
888 Schwedhelm-Domeyer, N., Geurten, B.R.H., and Göpfert, M.C. (2018). Proprioceptive Opsin
889 Functions in Drosophila Larval Locomotion. Neuron *98*, 67-74.e4.
890 10.1016/j.neuron.2018.02.028.
891 72. Leung, N.Y., and Montell, C. (2017). Unconventional Roles of Opsins. Annu. Rev.
892 Cell Dev. Biol. *33*, 241–264. 10.1146/annurev-cellbio-100616-060432.
893 73. Mishra, A.K., Fritsch, C., Voutev, R., Mann, R.S., and Sprecher, S.G. (2021).
894 Homothorax controls a binary Rhodopsin switch in Drosophila ocelli. PLOS Genetics *17*,
895 e1009460. 10.1371/journal.pgen.1009460.
896 74. Partha, R., Chauhan, B.K., Ferreira, Z., Robinson, J.D., Lathrop, K., Nischal, K.K.,
897 Chikina, M., and Clark, N.L. (2017). Subterranean mammals show convergent regression in
898 ocular genes and enhancers, along with adaptation to tunneling. eLife *6*, e25884.
899 10.7554/eLife.25884.
900 75. Policarpo, M., Fumey, J., Lafargeas, P., Naquin, D., Thermes, C., Naville, M.,
901 Dechaud, C., Volff, J.-N., Cabau, C., Klopp, C., et al. (2021). Contrasting Gene Decay in
902 Subterranean Vertebrates: Insights from Cavefishes and Fossorial Mammals. Molecular
903 Biology and Evolution *38*, 589–605. 10.1093/molbev/msaa249.
904 76. Büscher, T.H., Petersen, D.S., Bijma, N.N., Bäumler, F., Pirk, C.W.W., Büsse, S.,

Heepe, L., and Gorb, S.N. (2022). The exceptional attachment ability of the ectoparasitic bee louse Braula coeca (Diptera, Braulidae) on the honeybee. Physiological Entomology *47*, 83–95. 10.1111/phen.12378.

77. Roff, D.A. (1990). The Evolution of Flightlessness in Insects. Ecological Monographs *60*, 389–421. 10.2307/1943013.

78. Miller, D.E., Staber, C., Zeitlinger, J., and Hawley, R.S. (2018). Highly Contiguous Genome Assemblies of 15 Drosophila Species Generated Using Nanopore Sequencing. G3: Genes, Genomes, Genetics *8*, 3131–3141. 10.1534/g3.118.200160.

79. Lu, H., Giordano, F., and Ning, Z. (2016). Oxford Nanopore MinION Sequencing and Genome Assembly. Genomics, Proteomics & Bioinformatics *14*, 265–279. 10.1016/j.gpb.2016.05.004.

80. Leger, A., and Leonardi, T. (2019). pycoQC, interactive quality control for Oxford Nanopore Sequencing. JOSS *4*, 1236. 10.21105/joss.01236.

81. Zimin, A.V., Puiu, D., Luo, M.-C., Zhu, T., Koren, S., Marçais, G., Yorke, J.A., Dvořák, J., and Salzberg, S.L. (2017). Hybrid assembly of the large and highly repetitive genome of Aegilops tauschii, a progenitor of bread wheat, with the MaSuRCA mega-reads algorithm. Genome Res. *27*, 787–792. 10.1101/gr.213405.116.

82. Kokot, M., Długosz, M., and Deorowicz, S. (2017). KMC 3: counting and manipulating k-mer statistics. Bioinformatics *33*, 2759–2761. 10.1093/bioinformatics/btx304.

83. Ranallo-Benavidez, T.R., Jaron, K.S., and Schatz, M.C. (2020). GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. Nat Commun *11*, 1432. 10.1038/s41467-020-14998-3.

84. Laetsch, D.R., and Blaxter, M.L. (2017). BlobTools: Interrogation of genome assemblies. Preprint at F1000Research, 10.12688/f1000research.12232.1 10.12688/f1000research.12232.1.

85. Buchfink, B., Xie, C., and Huson, D.H. (2015). Fast and sensitive protein alignment using DIAMOND. Nat Methods *12*, 59–60. 10.1038/nmeth.3176.

86. Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics *34*, 3094–3100. 10.1093/bioinformatics/bty191.

87. Muller, H., Ogereau, D., Da Lage, J.-L., Capdevielle, C., Pollet, N., Fortuna, T., Jeannette, R., Kaiser, L., and Gilbert, C. (2021). Draft nuclear genome and complete mitogenome of the Mediterranean corn borer, Sesamia nonagrioides, a major pest of maize. G3 Genes|Genomes|Genetics *11*, jkab155. 10.1093/g3journal/jkab155.

88. Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. (2009). BLAST+: architecture and applications. BMC Bioinformatics *10*, 421. 10.1186/1471-2105-10-421.

89. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al. (2011). Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. Nat Biotechnol *29*, 644–652. 10.1038/nbt.1883.

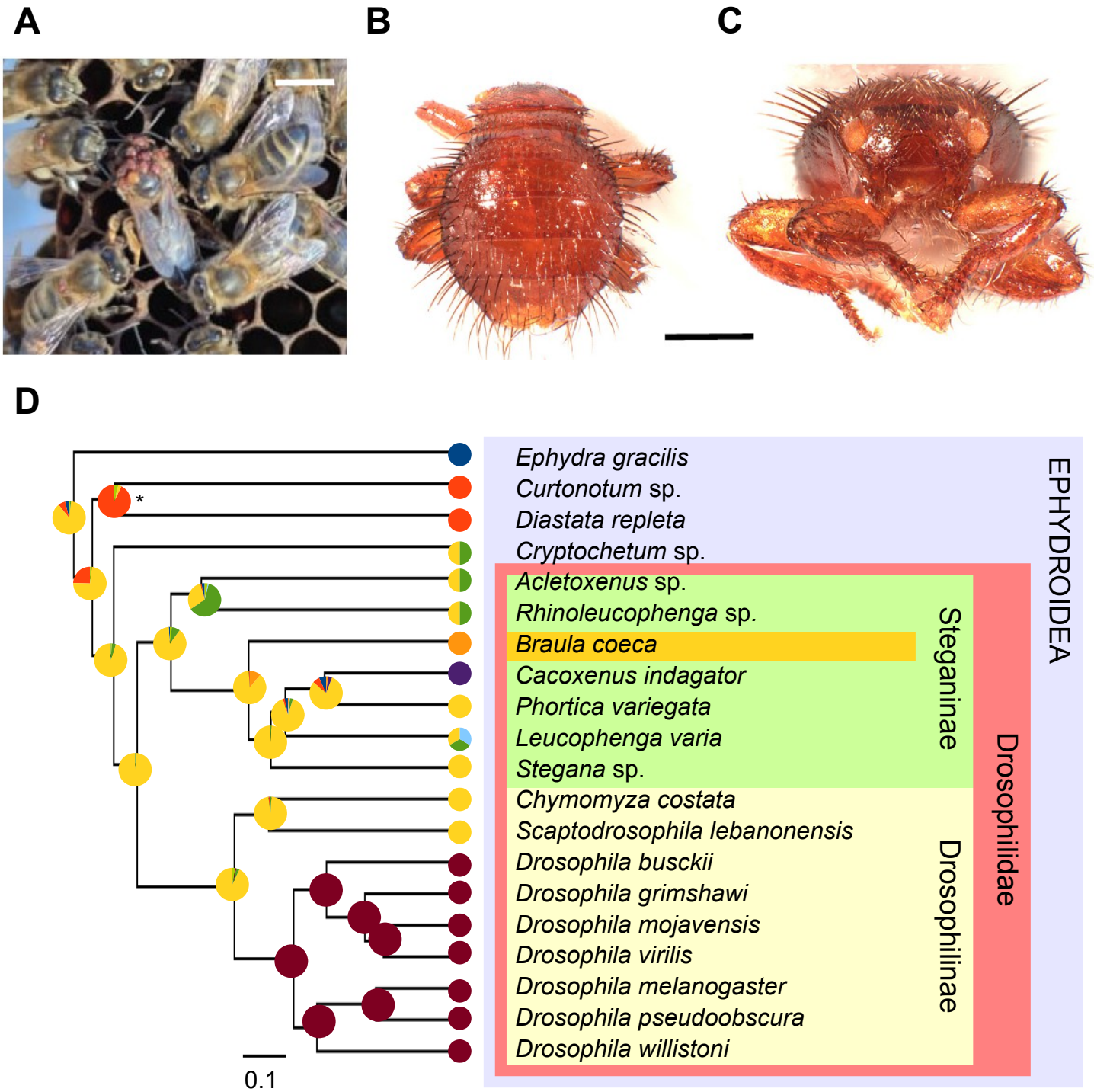
90. Boekel, J., Chilton, J.M., Cooke, I.R., Horvatovich, P.L., Jagtap, P.D., Käll, L., Lehtiö, J., Lukasse, P., Moerland, P.D., and Griffin, T.J. (2015). Multi-omic data analysis using Galaxy. Nat Biotechnol *33*, 137–139. 10.1038/nbt.3134.

91. Hiltemann, S., Rasche, H., Gladman, S., Hotz, H.-R., Larivière, D., Blankenberg, D., Jagtap, P.D., Wollmann, T., Bretaudeau, A., Goué, N., et al. (2023). Galaxy Training: A powerful framework for teaching! PLOS Computational Biology *19*, e1010752. 10.1371/journal.pcbi.1010752.

92. Katoh, K., and Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution *30*, 772–780. 10.1093/molbev/mst010.

93. Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., and Lanfear, R. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution *37*, 1530–1534. 10.1093/molbev/msaa015.
94. Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., and Vinh, L.S. (2018). UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution *35*, 518–522. 10.1093/molbev/msx281.
95. Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., and Jermiin, L.S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods *14*, 587–589. 10.1038/nmeth.4285.
96. Okada, T. (1962). Bleeding sap preference of the Drosophilid flies. Japanese journal of applied entomology and zoology *6*, 216–229. 10.1303/jjaez.6.216.
97. Pagel, M., and Meade, A. Bayesian analysis of correlated evolution of discrete characters by reversible-jump markov chain monte carlo.
98. Yang, Z. (2007). PAML 4: phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution *24*, 1586–1591. 10.1093/molbev/msm088.
99. Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware, J., Flouri, T., Beutel, R.G., et al. (2014). Phylogenomics resolves the timing and pattern of insect evolution. Science *346*, 763–767. 10.1126/science.1257570.
100. Suvorov, A., Kim, B.Y., Wang, J., Armstrong, E.E., Peede, D., D'Agostino, E.R.R., Price, D.K., Waddell, P.J., Lang, M., Courtier-Orgogozo, V., et al. (2022). Widespread introgression across a phylogeny of 155 *Drosophila* genomes. Current Biology *32*, 111-123.e5. 10.1016/j.cub.2021.10.052.
101. Revell, L.J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution *3*, 217–223. 10.1111/j.2041-210X.2011.00169.x.
102. Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. PeerJ *4*, e2584. 10.7717/peerj.2584.
103. Lampe, D.J., Witherspoon, D.J., Soto-Adames, F.N., and Robertson, H.M. (2003). Recent horizontal transfer of mellifera subfamily mariner transposons into insect lineages representing four different orders shows that selection acts only during horizontal transfer. Mol Biol Evol *20*, 554–562. 10.1093/molbev/msg069.
104. Zhang, H.-H., Peccoud, J., Xu, M.-R.-X., Zhang, X.-G., and Gilbert, C. (2020). Horizontal transfer and evolution of transposable elements in vertebrates. Nat Commun *11*, 1362. 10.1038/s41467-020-15149-4.
105. Edgar, R.C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics *5*, 113. 10.1186/1471-2105-5-113.
106. Gilleland, E., and Katz, R.W. (2016). extRemes 2.0: An Extreme Value Analysis Package in R. Journal of Statistical Software *72*, 1–39. 10.18637/jss.v072.i08.
107. Slater, G.S.C., and Birney, E. (2005). Automated generation of heuristics for biological sequence comparison. BMC Bioinformatics *6*, 31. 10.1186/1471-2105-6-31.
108. Murray, M.H., and Blume, J.D. (2021). FDRestimation: Flexible False Discovery Rate Computation in R. F1000Res *10*, 441. 10.12688/f1000research.52999.2.
109. Montell, C. (2009). A taste of the *Drosophila* gustatory receptors. Current Opinion in Neurobiology *19*, 345–353. 10.1016/j.conb.2009.07.001.

Figure 1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B
C
D
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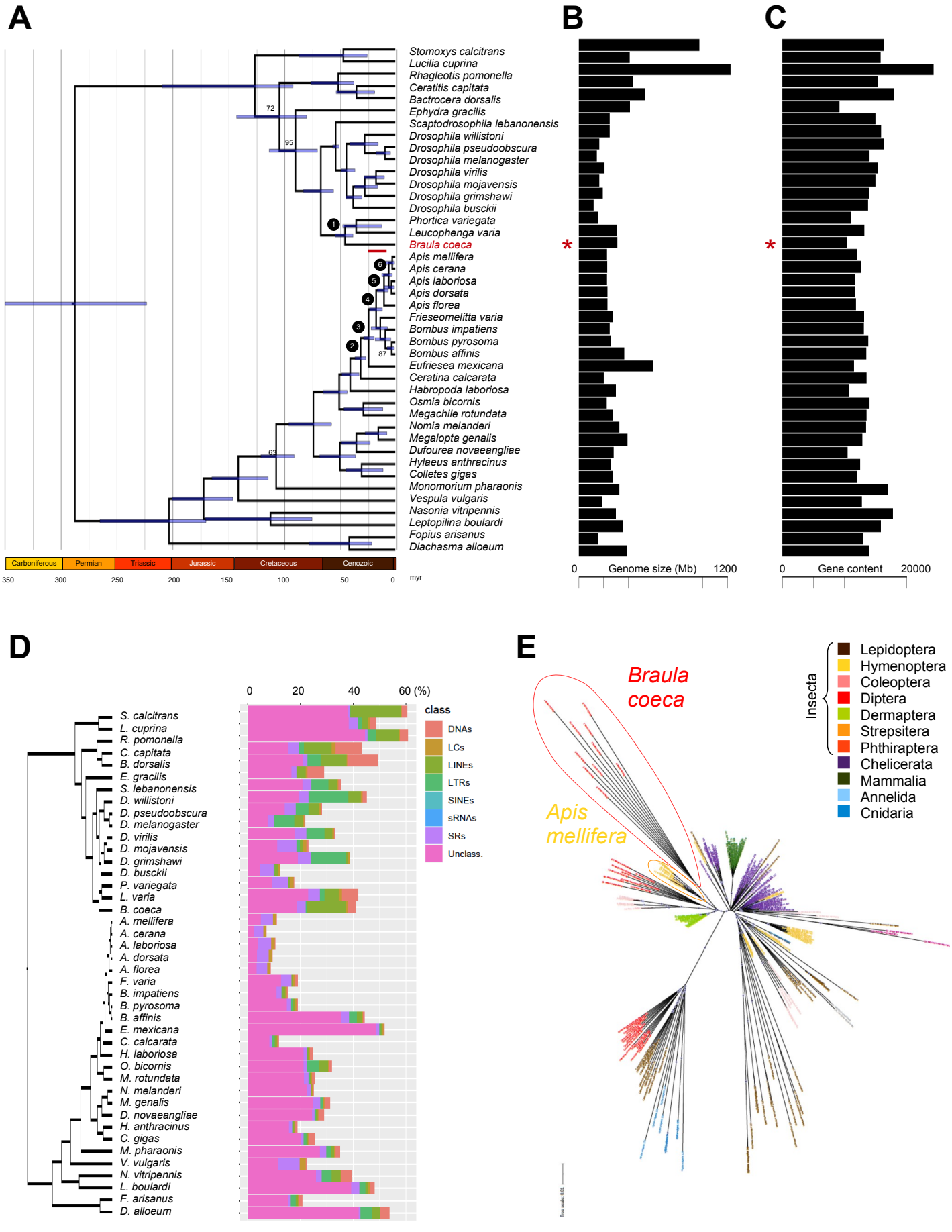
Diastata repleta
Cryptochetum sp.
Acletoxenus sp.
Rhinoleucophenga sp.
Braula coeca
Cacoxenus indagator
Phortica variegata
Leucophenga varia
Stegana sp.
Chymomyza costata
Scaptodrosophila lebanonensis
Drosophila busckii
Drosophila grimshawi
Drosophila mojavensis
Drosophila virilis
Drosophila melanogaster
Drosophila pseudoobscura
Drosophila willistoni
Steganinae
Drosophilinae
Drosophilidae
EPHYDROIDEA
Predominant niche
Algae + marsh plants
Dung
Plant sap
Scale insects
Fruits
Fungi
Solitary bee inquilinism
Social bee inquilinism

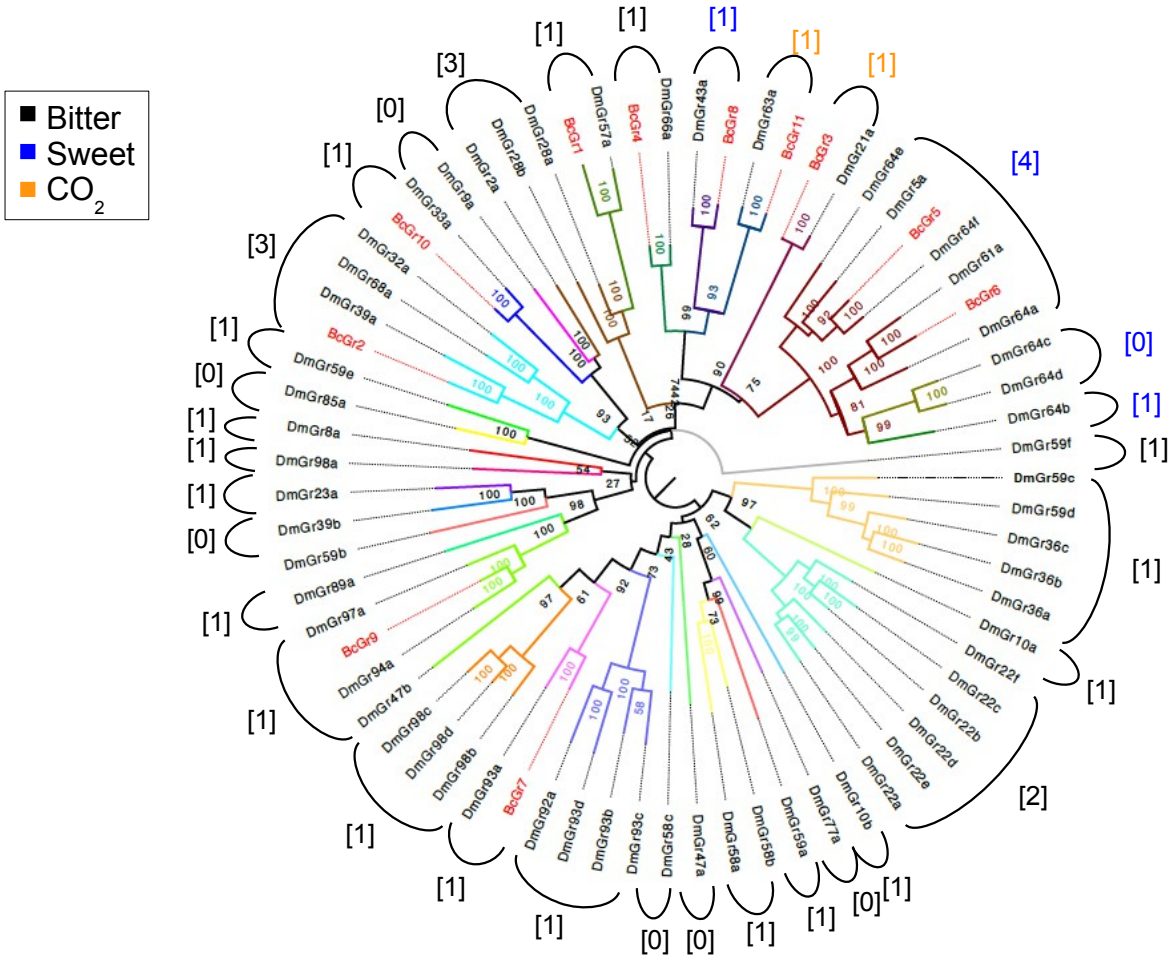
Figure 2


A
B
C
Stomoxys calcitrans
Lucilia cuprina
Rhagleotis pomonella
Ceratitis capitata
Bactrocera dorsalis
Ephydra gracilis
Scaptodrosophila lebanonensis
Drosophila willistoni
Drosophila pseudoobscura
Drosophila melanogaster
Drosophila virilis
Drosophila mojavensis
Drosophila grimshawi
Drosophila busckii
Phortica variegata
Leucophenga varia
Braula coeca
Apis mellifera
Apis cerana
Apis laboriosa
Apis dorsata
Apis florea
Frieseomelitta varia
Bombus impatiens
Bombus pyrosoma
Bombus affinis
Eufriesea mexicana
Ceratina calcarata
Habropoda laboriosa
Osmia bicornis
Megachile rotundata
Nomia melanderi
Megalopta genalis
Dufourea novaeangliae
Hylaeus anthracinus
Colletes gigas
Monomorium pharaonis
Vespula vulgaris
Nasonia vitripennis
Leptopilina boulardi
Fopius arisanus
Diachasma alloeum
72
95
87
63
Carboniferous
Permian
Triassic
Jurassic
Cretaceous
Cenozoic
350
300
250
200
150
100
50
0
myr
0
Genome size (Mb)
1200
0
Gene content
20000

D
E
S. calcitrans
L. cuprina
R. pomonella
C. capitata
B. dorsalis
E. gracilis
S. lebanonensis
D. willistoni
D. pseudoobscura
D. melanogaster
D. virilis
D. mojavensis
D. grimshawi
D. busckii
P. variegata
L. varia
B. coeca
A. mellifera
A. cerana
A. laboriosa
A. dorsata
A. florea
F. varia
B. impatiens
B. pyrosoma
B. affinis
E. mexicana
C. calcarata
H. laboriosa
O. bicornis
M. rotundata
N. melanderi
M. genalis
D. novaeangliae
H. anthracinus
C. gigas
M. pharaonis
V. vulgaris
N. vitripennis
L. boulardi
F. arisanus
D. alloeum
0
20
40
60 (%)
class
DNAs
LCs
LINEs
LTRs
SINEs
sRNAs
SRs
Unclass.
Braula coeca
Apis mellifera
Insecta
Lepidoptera
Hymenoptera
Coleoptera
Diptera
Dermaptera
Strepsitera
Phthiraptera
Chelicerata
Mammalia
Annelida
Cnidaria

Figure 3

A


Bitter
Sweet
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DmGr59b
DmGr89a
DmGr97a
DmGr94a
DmGr47b
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DmGr56c
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DmGr66a
DmGr57a
BcGr1
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BcGr9
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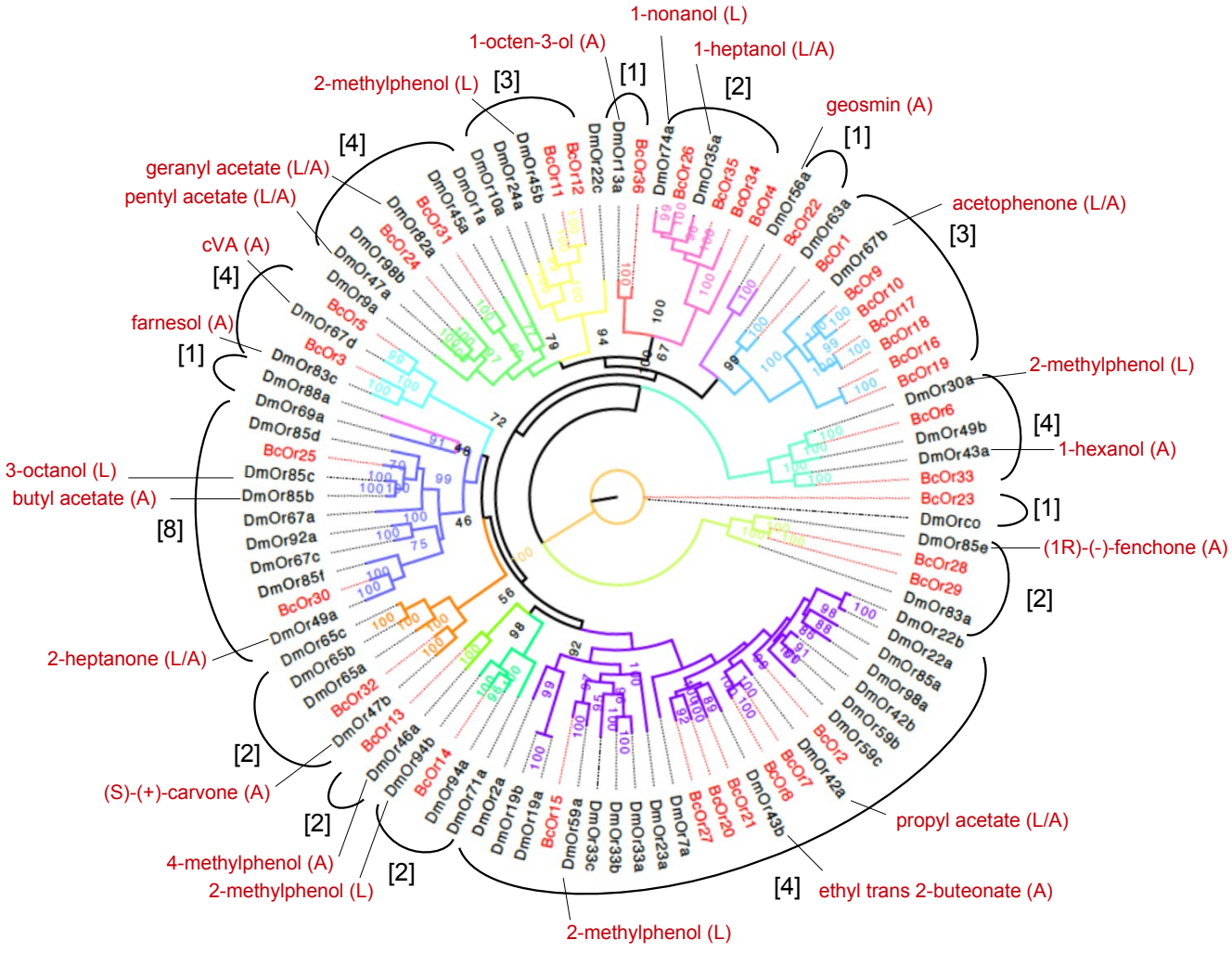
BcGr11
BcGr13

B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1-octen-3-ol (A)
1-heptanol (L/A)
2-methylphenol (L)
geosmin (A)
geranyl acetate (L/A)
pentyl acetate (L/A)
acetophenone (L/A)
cVA (A)
farnesol (A)
2-methylphenol (L)
1-hexanol (A)
3-octanol (L)
butyl acetate (A)
(1R)-(-)-fenchone (A)
2-heptanone (L/A)
propyl acetate (L/A)
(S)-(+)-carvone (A)
4-methylphenol (A)
2-methylphenol (L)
ethyl trans 2-buteonate (A)
2-methylphenol (L)
DmOr45b
DmOr24a
DmOr10a
DmOr1a
DmOr45a
DmOr82a
DmOr98b
DmOr47a
DmOr9a
DmOr67d
DmOr83c
DmOr88a
DmOr69a
DmOr85d
DmOr85c
DmOr85b
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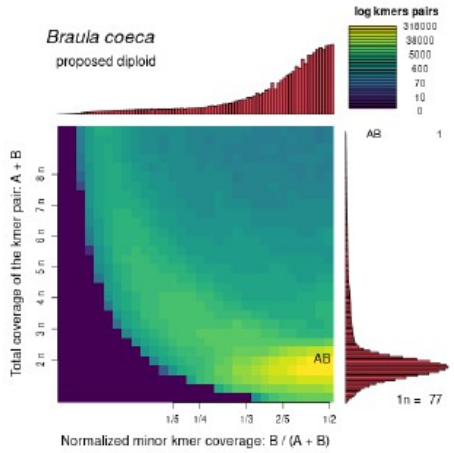
DmOr49b
DmOr6
DmOr30a
DmOr16
DmOr19
DmOr18
DmOr17
DmOr10
DmOr9
DmOr1
DmOr67b
DmOr63a
DmOr56a
DmOr35a
DmOr74a
DmOr13a
DmOr22c
BcOr11
BcOr12
BcOr36
BcOr26
BcOr35
BcOr34
BcOr4
BcOr22
BcOr31
BcOr24
BcOr5
BcOr3
BcOr25
BcOr30
BcOr32
BcOr13
BcOr14
BcOr15
BcOr21
BcOr20
BcOr27
BcOr8
BcOr7
BcOr2
BcOr28
BcOr29

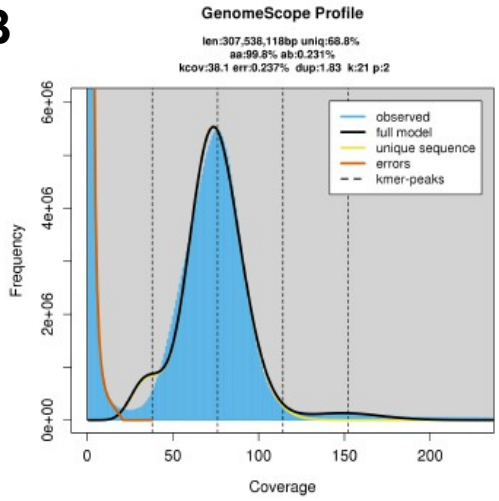
Table 1 – **Rapidly evolving gene groups in *Braula coeca* show parallel reduction with bees. See also Figure S3.** Gene groups were defined according to *D. melanogaster* genes clustered with orthologous sequences from 42 dipteran and hymenopteran genomes by OrthoFinder. Putative functions of each group are given following FlyBase definitions and references therein. Evolutionary rate was estimated by CAFE5, with *p*-values corrected for multiple testing using False Discovery Rate (FDR) analysis.

Gene group	Function	Evolution in *Braula*		Evolution in bees
		Change	FDR *p*-value	
GUSTATORY RECEPTORS	Chemosensory	Reduced	1.7×10^{-4}	Reduced in bees
DIVERGENT IONOTROPIC RECEPTORS	Chemosensory	Reduced	1.7×10^{-4}	Reduced in Hymenoptera
S1A NON-PEPTIDASE HOMOLOGS	Immunity, morphogenesis	Reduced	1.9×10^{-4}	Reduced in bees (particularly in *A. mellifera*)
CYTOCHROME P450 - CYP3 CLAN	Detoxification	Reduced	9.8×10^{-4}	Reduced in *Apis* (particularly in *A. mellifera*)
ODORANT RECEPTORS	Chemosensory	Reduced	9.9×10^{-4}	Reduced in the Apidae
ODORANT BINDING PROTEINS	Chemosensory	Reduced	0.0012	Reduced in bees
CYTOSOLIC GLUTATHIONE S-TRANSFERASES	Detoxification	Reduced	0.0034	Reduced in *Apis*
ECDYSTEROID KINASE-LIKE	Detoxification	Reduced	0.0034	Reduced in bees
C-TYPE LECTIN-LIKE	Immunity	Reduced	0.0072	Reduced in Hymenoptera
SLC22 FAMILY OF ORGANIC IONS TRANSPORTERS	Development, detoxification	Reduced	0.0084	Reduced in *Apis*
SLC2 FAMILY OF HEXOSE SUGAR TRANSPORTERS	Metabolism	Reduced	0.0144	
S1A SERINE PROTEASES - CHYMOTRYPSIN-LIKE	Metabolism, immunity, morphogenesis	Reduced	0.0144	Reduced in bees
CHITIN BINDING DOMAIN-CONTAINING PROTEINS	Morphogenesis, immunity	Reduced	0.0233	Reduced in bees
CARBOXYLESTERASES	Detoxification	Reduced	0.0450	Reduced in longue-tongued bees (but not in *A. mellifera*)
S1A SERINE PROTEASES - TRYPSIN-LIKE	Metabolism, immunity, morphogenesis	Reduced	0.0450	Reduced in bees
CYTOCHROME P450 - CYP4 CLAN	Detoxification	Reduced	0.0478	Reduced in bees
DORSAL GROUP	Morphogenesis, immunity	Reduced	0.0478	Reduced in Hymenoptera

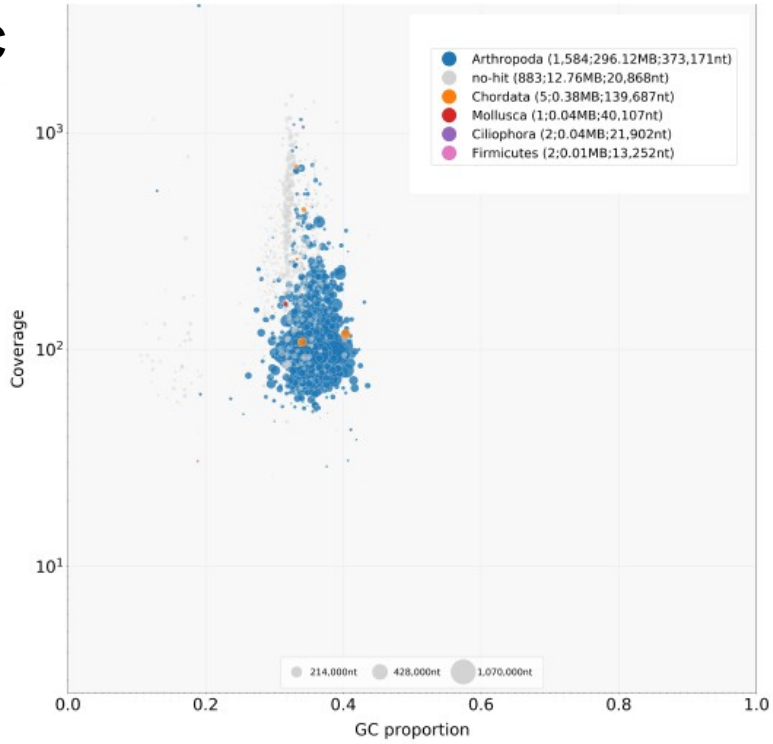
A


Braula coeca
proposed diploid
log kmers pairs
Total coverage of the kmer pair: A + B
Normalized minor kmer coverage: B / (A + B)
AB
1n = 77

B


GenomeScope Profile
len:307,538,118bp uniq:68.8%
aa:99.8% ab:0.231%
kcov:38.1 err:0.237% dup:1.83 k:21 p:2
observed
full model
unique sequence
errors
kmer-peaks
Frequency
Coverage

C


Arthropoda (1,584;296.12MB;373,171nt)
no-hit (883;12.76MB;20,868nt)
Chordata (5;0.38MB;139,687nt)
Mollusca (1;0.04MB;40,107nt)
Ciliophora (2;0.04MB;21,902nt)
Firmicutes (2;0.01MB;13,252nt)
Coverage
GC proportion

D

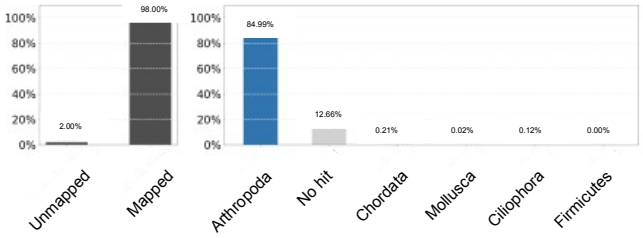

2.00%
98.00%
84.99%
12.66%
0.21%
0.02%
0.12%
0.00%
Unmapped
Mapped
Arthropoda
No hit
Chordata
Mollusca
Ciliophora
Firmicutes

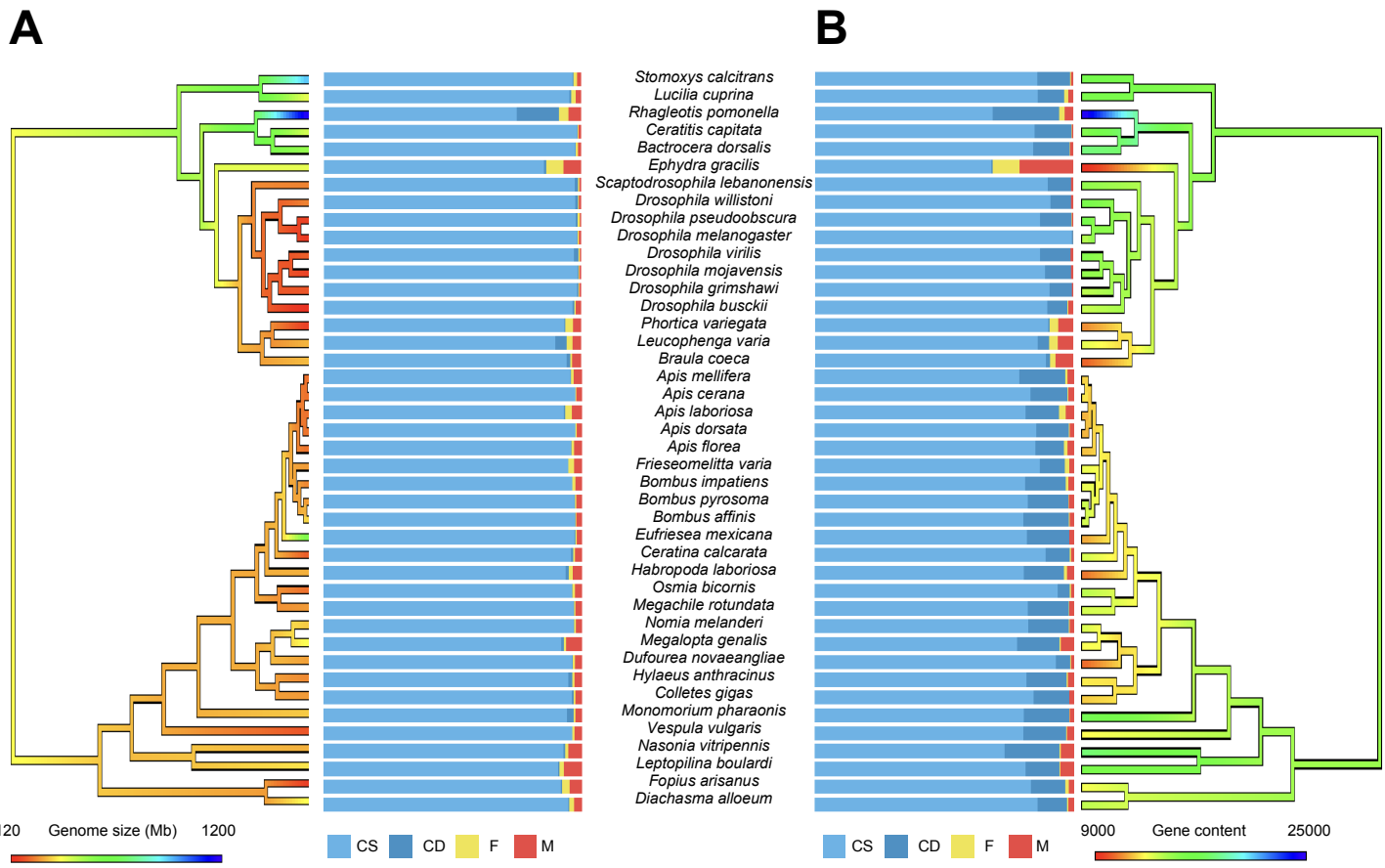
Figure S1. Features of *Braula coeca* genome assembly, Related to STAR Methods.

(A) Smudgeplot comparing the sum of heterozygous kmer pair coverages (A+B) to their relative coverage of the minor kmer (B/A+B). The hottest smudge corresponds to expected diploid kmer pairs (AB).

(B) Kmer profile spectrum estimating the length of the genome at 307,538,118 bp generated by GenomeScope2.

(C) Square-binned blob plot showing the distribution of assembly scaffolds on GC proportion and coverage axes. Squares within each bin are colored according to taxonomic annotation and scaled according to total span.

(D) ReadCovPlots generated by Blobtools visualising the proportion of reads of *B. coeca* that are unmapped or mapped and showing the percentage of mapped reads by taxonomic group, as barcharts.


A
B
Stomoxys calcitrans
Lucilia cuprina
Rhagleotis pomonella
Ceratitis capitata
Bactrocera dorsalis
Ephydra gracilis
Scaptodrosophila lebanonensis
Drosophila willistoni
Drosophila pseudoobscura
Drosophila melanogaster
Drosophila virilis
Drosophila mojavensis
Drosophila grimshawi
Drosophila busckii
Phortica variegata
Leucophenga varia
Braula coeca
Apis mellifera
Apis cerana
Apis laboriosa
Apis dorsata
Apis florea
Frieseomelitta varia
Bombus impatiens
Bombus pyrosoma
Bombus affinis
Eufriesea mexicana
Ceratina calcarata
Habropoda laboriosa
Osmia bicornis
Megachile rotundata
Nomia melanderi
Megalopta genalis
Dufourea novaeangliae
Hylaeus anthracinus
Colletes gigas
Monomorium pharaonis
Vespula vulgaris
Nasonia vitripennis
Leptopilina boulardi
Fopius arisanus
Diachasma alloeum
120
Genome size (Mb)
1200
CS
CD
F
M
CS
CD
F
M
9000
Gene content
25000

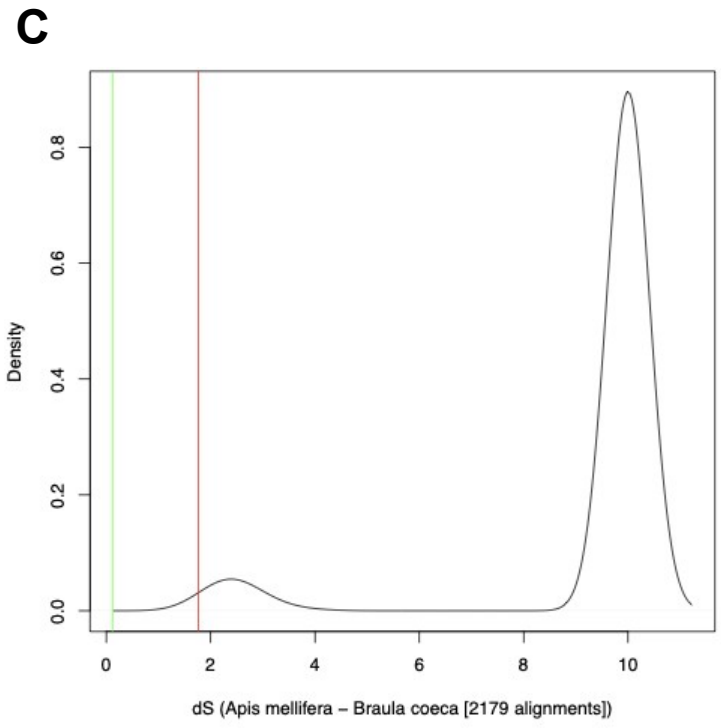

C
Density
0.0
0.2
0.4
0.6
0.8
0
2
4
6
8
10
dS (Apis mellifera – Braula coeca [2179 alignments])

Figure S2. Genome size, gene content and completeness of assembly and annotation of 42 dipteran and hymenopteran genomes and evidence for a horizontal transposon transfer between *B. coeca* and *A. mellifera*, Related to Figure 2.

(A) Genome-size evolution and genome assembly completeness inferred by BUSCO across 42 dipteran and hymenopteran species.

(B) Gene content evolution and genome annotation completeness inferred by BUSCO across 42 dipteran and hymenopteran species.

Branch colors in A and B reflect inferred ancestral reconstructions using fastanc in phytools. CS = complete and single-copy BUSCOs; CD = complete and duplicated BUSCOs; F = fragmented BUSCOs; M = missing BUSCOs.

(C) Horizontal transposon transfer between *Braula coeca* and *Apis mellifera*.

Comparison of Famar1-like transposon synonymous distance (dS) and orthologous gene synonymous distances between *Braula coeca* and *Apis mellifera*. The red line indicates the 0.5% quantile (=1.76) of the distribution of orthologous gene dS calculated over 2,179 codon alignments. The distribution is bimodal, with genes having highly saturated dS values showing a peak centered on 9.99 and highly conserved genes showing less saturated dS values showing another peak around 2.5. The *Famar1*-like dS (green line, = 0.12) was calculated over the transposase open reading frame of one copy of the element extracted from the *A. mellifera* genome and another copy from the *B. coeca* genome.

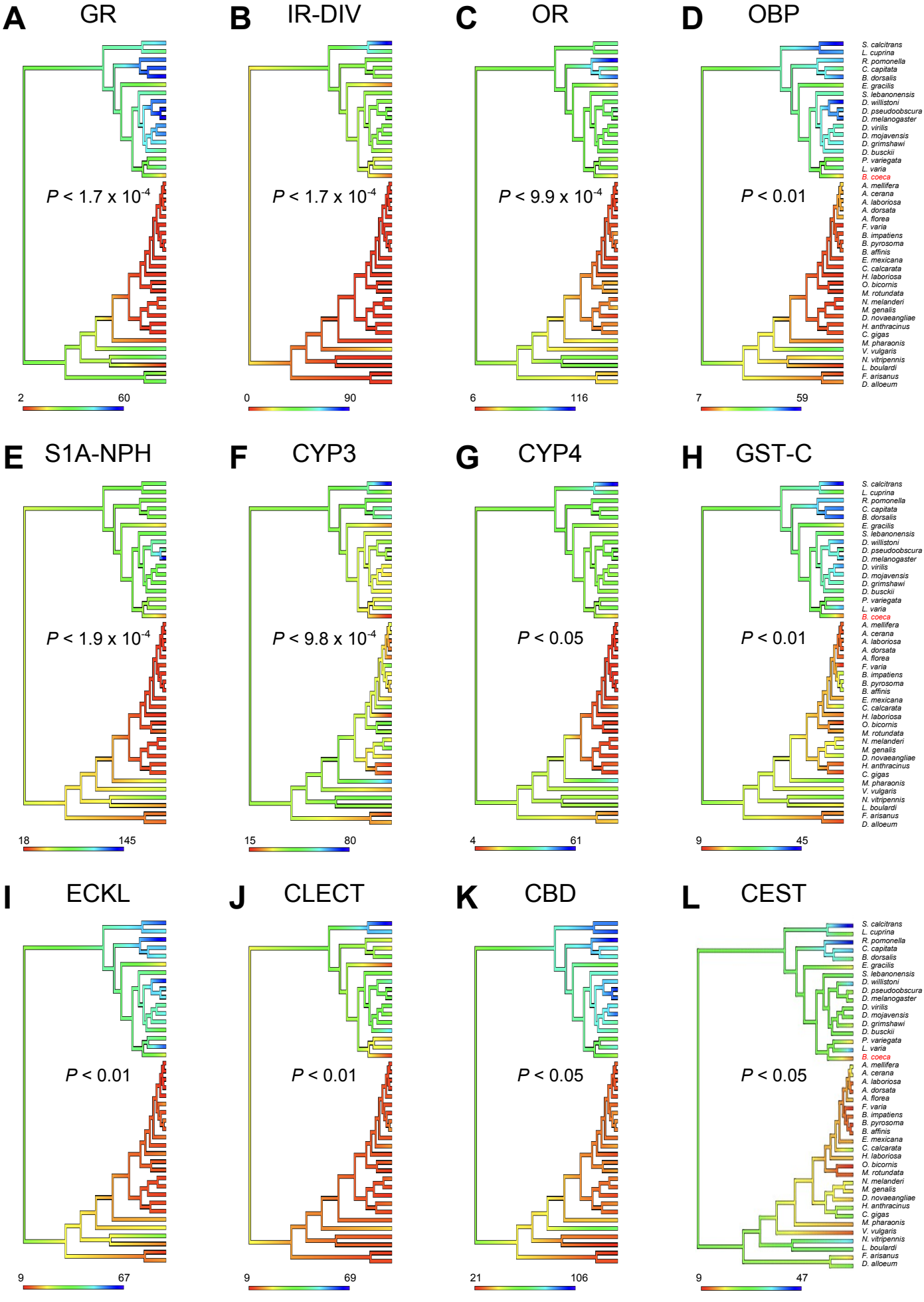

A
GR
P < 1.7 x 10-4
2
60
B
IR-DIV
P < 1.7 x 10-4
0
90
C
OR
P < 9.9 x 10-4
6
116
D
OBP
P < 0.01
7
59
S. calcitrans
L. cuprina
R. pomonella
C. capitata
B. dorsalis
E. gracilis
S. lebanonensis
D. willistoni
D. pseudoobscura
D. melanogaster
D. virilis
D. mojavensis
D. grimshawi
D. busckii
P. variegata
L. varia
B. coeca
A. mellifera
A. cerana
A. laboriosa
A. dorsata
A. florea
F. varia
B. impatiens
B. pyrosoma
B. affinis
E. mexicana
C. calcarata
H. laboriosa
O. bicornis
M. rotundata
N. melanderi
M. genalis
D. novaeangliae
H. anthracinus
C. gigas
M. pharaonis
V. vulgaris
N. vitripennis
L. boulardi
F. arisanus
D. alloeum
E
S1A-NPH
P < 1.9 x 10-4
18
145
F
CYP3
P < 9.8 x 10-4
15
80
G
CYP4
P < 0.05
4
61
H
GST-C
P < 0.01
9
45
I
ECKL
P < 0.01
9
67
J
CLECT
P < 0.01
9
69
K
CBD
P < 0.05
21
106
L
CEST
P < 0.05
9
47

Figure S3. Ancestral gene count reconstructions of 12 gene groups significantly evolving in *Braula coeca* after correction for multiple testing, Related to Table 1. For each panel, the species tree from Figure 2 is given with branch colors reflecting inferred ancestral family size reconstruction using fastanc in phytools.

(A) GR = Gustatory Receptors

(B) IR-DIV = Divergent Ionotropic Receptors

(C) OR = Odorant Receptors

(D) OBP = Odorant Binding Proteins

(E) S1A-NPH = S1a Non-Peptidase Homologs

(F) CYP3 = Cytochrome P450 - CYP3 Clan

(G) CYP4 = Cytochrome P450 – CYP4 Clan

(H) GST-C = Cytosolic Glutathione S-Transferases

(I) ECKL = Ecdysteroid Kinase-Like

(J) CLECT = C-Type Lectin-Like

(K) CBD = Chitin Biding Domain-Containing Proteins

(L) CEST = Carboxylesterases

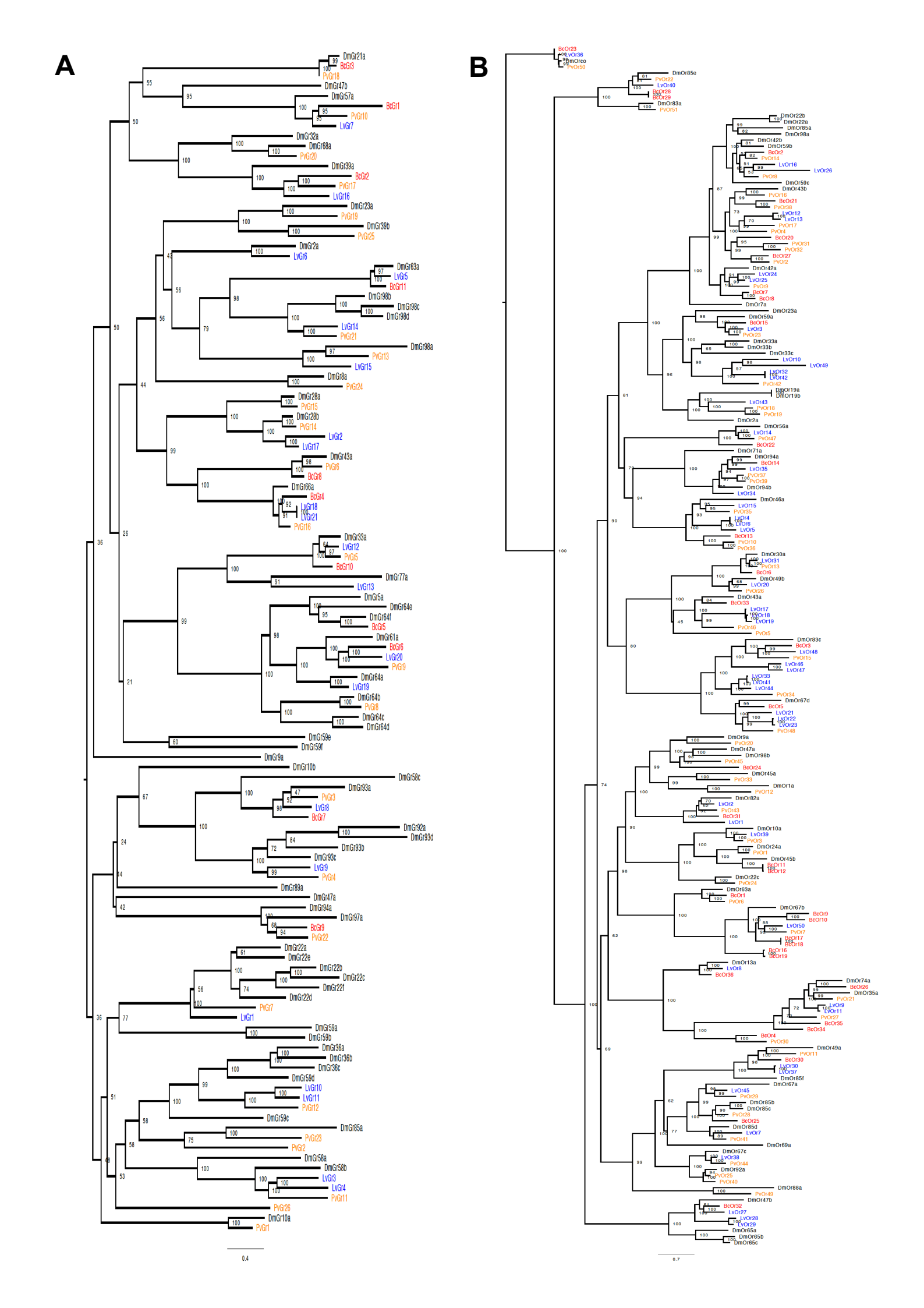

A
DmGr21a
BcGr3
PvGr18
DmGr47b
DmGr57a
BcGr1
PvGr10
LvGr7
DmGr32a
DmGr68a
PvGr20
DmGr39a
BcGr2
PvGr17
LvGr16
DmGr23a
PvGr19
DmGr39b
PvGr25
DmGr2a
LvGr6
DmGr63a
LvGr5
BcGr11
DmGr98b
DmGr98c
DmGr98d
LvGr14
PvGr21
DmGr98a
PvGr13
LvGr15
DmGr8a
PvGr24
DmGr28a
PvGr15
DmGr28b
PvGr14
LvGr2
LvGr17
DmGr43a
PvGr6
BcGr8
DmGr66a
BcGr4
LvGr18
LvGr21
PvGr16
DmGr33a
LvGr12
PvGr5
BcGr10
DmGr77a
LvGr13
DmGr5a
DmGr64e
DmGr64f
BcGr5
DmGr61a
BcGr6
LvGr20
PvGr9
DmGr64a
LvGr19
DmGr64b
PvGr8
DmGr64c
DmGr64d
DmGr59e
DmGr59f
DmGr9a
DmGr10b
DmGr58c
DmGr93a
PvGr3
LvGr8
BcGr7
DmGr92a
DmGr93d
DmGr93b
DmGr93c
LvGr9
PvGr4
DmGr89a
DmGr47a
DmGr94a
DmGr97a
BcGr9
PvGr22
DmGr22a
DmGr22e
DmGr22b
DmGr22c
DmGr22f
DmGr22d
PvGr7
LvGr1
DmGr59a
DmGr59b
DmGr36a
DmGr36b
DmGr36c
DmGr59d
LvGr10
LvGr11
PvGr12
DmGr59c
DmGr85a
PvGr23
PvGr2
DmGr58a
DmGr58b
LvGr3
LvGr4
PvGr11
PvGr26
DmGr10a
PvGr1
0.4
B
BcOr23
LvOr36
DmOrco
PvOr50
DmOr85e
PvOr22
LvOr40
BcOr28
BcOr29
DmOr83a
PvOr51
DmOr22b
DmOr22a
DmOr85a
DmOr98a
DmOr42b
DmOr59b
BcOr2
PvOr14
LvOr16
LvOr26
PvOr8
DmOr59c
DmOr43b
PvOr16
BcOr21
PvOr38
LvOr12
LvOr13
PvOr17
PvOr4
BcOr20
PvOr31
PvOr32
BcOr27
PvOr2
DmOr42a
LvOr24
LvOr25
PvOr9
BcOr7
BcOr8
DmOr7a
DmOr23a
DmOr59a
BcOr15
LvOr3
PvOr23
DmOr33a
DmOr33b
DmOr33c
LvOr10
LvOr49
LvOr32
LvOr42
PvOr42
DmOr19a
DmOr19b
LvOr43
PvOr18
PvOr19
DmOr2a
DmOr56a
LvOr14
PvOr47
BcOr22
DmOr71a
DmOr94a
BcOr14
LvOr35
PvOr37
PvOr39
DmOr94b
LvOr34
DmOr46a
LvOr15
PvOr35
LvOr4
LvOr6
LvOr5
BcOr13
PvOr10
PvOr36
DmOr30a
LvOr31
PvOr13
BcOr6
DmOr49b
LvOr20
PvOr26
DmOr43a
BcOr33
LvOr17
LvOr18
LvOr19
PvOr46
PvOr5
DmOr83c
BcOr3
LvOr48
PvOr15
LvOr46
LvOr47
LvOr33
LvOr41
LvOr44
PvOr34
DmOr67d
BcOr5
LvOr21
LvOr22
LvOr23
PvOr48
DmOr9a
PvOr30
DmOr47a
DmOr98b
PvOr45
BcOr24
DmOr45a
PvOr33
DmOr1a
PvOr12
DmOr82a
LvOr2
PvOr43
BcOr31
LvOr1
DmOr10a
LvOr39
PvOr3
DmOr24a
PvOr1
DmOr45b
BcOr11
BcOr12
DmOr22c
PvOr24
DmOr63a
BcOr1
PvOr6
DmOr67b
BcOr9
BcOr10
LvOr50
PvOr7
BcOr17
BcOr18
BcOr16
BcOr19
DmOr13a
LvOr8
BcOr36
DmOr74a
BcOr26
DmOr35a
PvOr21
LvOr9
LvOr11
PvOr27
BcOr35
BcOr34
BcOr4
PvOr30
DmOr49a
PvOr11
BcOr30
LvOr30
LvOr37
DmOr85f
DmOr67a
LvOr45
PvOr29
DmOr85b
DmOr85c
PvOr28
BcOr25
DmOr85d
LvOr7
PvOr41
DmOr69a
DmOr67c
LvOr38
PvOr44
DmOr92a
PvOr25
PvOr40
DmOr88a
PvOr49
DmOr47b
BcOr32
LvOr27
LvOr28
LvOr29
DmOr65a
DmOr65b
DmOr65c
0.7

Figure S4. Maximum-likelihood phylogeny of gustatory receptors (GRs) and odorant receptors (ORs), Related to Figure 3.

(A) Maximum-likelihood phylogeny of GRs from *D. melanogaster*, *B. coeca*, *L. varia* and *P. variegata*.

(B) Maximum-likelihood phylogeny of ORs from *D. melanogaster*, *B. coeca*, *L. varia* and *P. variegata*.