Cell Reports
Article

Frequent Recent Origination of Brain Genes Shaped the Evolution of Foraging Behavior in *Drosophila*

Sidi Chen,[1,5] Maria Spletter,[2,4] Xiaochun Ni,[1,3] Kevin P. White,[1,3] Liqun Luo,[2] and Manyuan Long[1,*]
[1]Department of Ecology and Evolution, The University of Chicago, Chicago, IL 60637, USA
[2]Howard Hughes Medical Institute and Department of Biology, Stanford University, Stanford, CA 94305, USA
[3]Institute for Genomics and Systems Biology, The University of Chicago and Argonne National Laboratory, Chicago, IL 60637, USA
[4]Present address: Laboratory of Muscle Dynamics, Max Planck Institute of Biochemistry, 82152 Martinsried, Germany
[5]Present address: Department of Biology and Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: mlong@uchicago.edu
DOI 10.1016/j.celrep.2011.12.010

SUMMARY

The evolution of the brain and behavior are coupled puzzles. The genetic bases for brain evolution are widely debated, yet whether newly evolved genes impact the evolution of the brain and behavior is vaguely understood. Here, we show that during recent evolution in *Drosophila*, new genes have frequently acquired neuronal expression, particularly in the mushroom bodies. Evolutionary signatures combined with expression profiling showed that natural selection influenced the evolution of young genes expressed in the brain, notably in mushroom bodies. Case analyses showed that two young retrogenes are expressed in the olfactory circuit and facilitate foraging behavior. Comparative behavioral analysis revealed divergence in foraging behavior between species. Our data suggest that during adaptive evolution, new genes gain expression in specific brain structures and evolve new functions in neural circuits, which might contribute to the phenotypic evolution of animal behavior.

INTRODUCTION

Throughout the animal kingdom, the nervous system plays a fundamental role in processing sensory information and forming proper behavioral responses (Beatty, 1995). Originating from a simple protobrain of a single common ancestor, brains have evolved to be the structural and functional center of the nervous system in most metazoan taxa (Shepherd, 1994). There is a large diversity of central nervous systems in both structural organization and functional specialization (Denes et al., 2007). One of the primary functions of the brain, regardless of its level of complexity, is to control the behavior of an animal (Carew, 2000). Behaviors evolve constantly (Brown, 1975; Lowe et al., 2003), and the evolution of behavior is associated with evolution of the brain (Oro, 2004).

Recently, extensive efforts have focused on understanding the genetic basis and molecular mechanisms for brain evolution. Evidence shows that evolutionary changes in the size, shape, structure, and function of the brain are highly correlated with various genetic changes, such as adaptive evolution in protein sequences (Zhang, 2003), regulatory elements (Haygood et al., 2007), and noncoding RNAs (Pollard et al., 2006); changes in gene expression (Cáceres et al., 2003); and occasionally the birth and death of genes (Burki and Kaessmann, 2004; Popesco et al., 2006). However, we know little about how recently evolved genes shape the brain and animal behavior.

New genes are the novel genetic loci, physically distinct and derived segments of DNA that originate in an evolutionary time with newly encoded functional transcripts (Long and Langley, 1993; Long et al., 2003). It is well known that new genes frequently originate during evolution and can acquire functions in different biological processes in various organisms (Kaessmann et al., 2009; Long et al., 2003). However, little is known about young genes that are expressed and potentially function in the brain. We first identified newly evolved genes with neuronal expression in *Drosophila*. Further characterization of their expression patterns revealed significantly enriched expression of new genes in the mushroom bodies (MB). We then detected the signatures of natural selection for the evolution of these genes. Furthermore, we examined the roles of these genes in behavior. We found that animals deficient in two recently originated MB-expressed retrogenes, *Xcpb1* and *Desr,* showed foraging behavior phenotypes.

RESULTS

Identification of Young Brain Genes

To identify recently evolved brain genes in *Drosophila*, we curated genes that originated in the *Drosophila* genus (Table S1) from the literature (Chen et al., 2010; Zhang et al., 2010). Among *D. melanogaster* genes that are younger than 25 million years (Myr), arising after the divergence from *D. pseudoobscura*, we found that 48.8% (161/330) were expressed in the brain, as detected by RT-PCR (Figures 1A and S1, Data Set S1, Extended Experimental Procedures). We refer to those young genes with

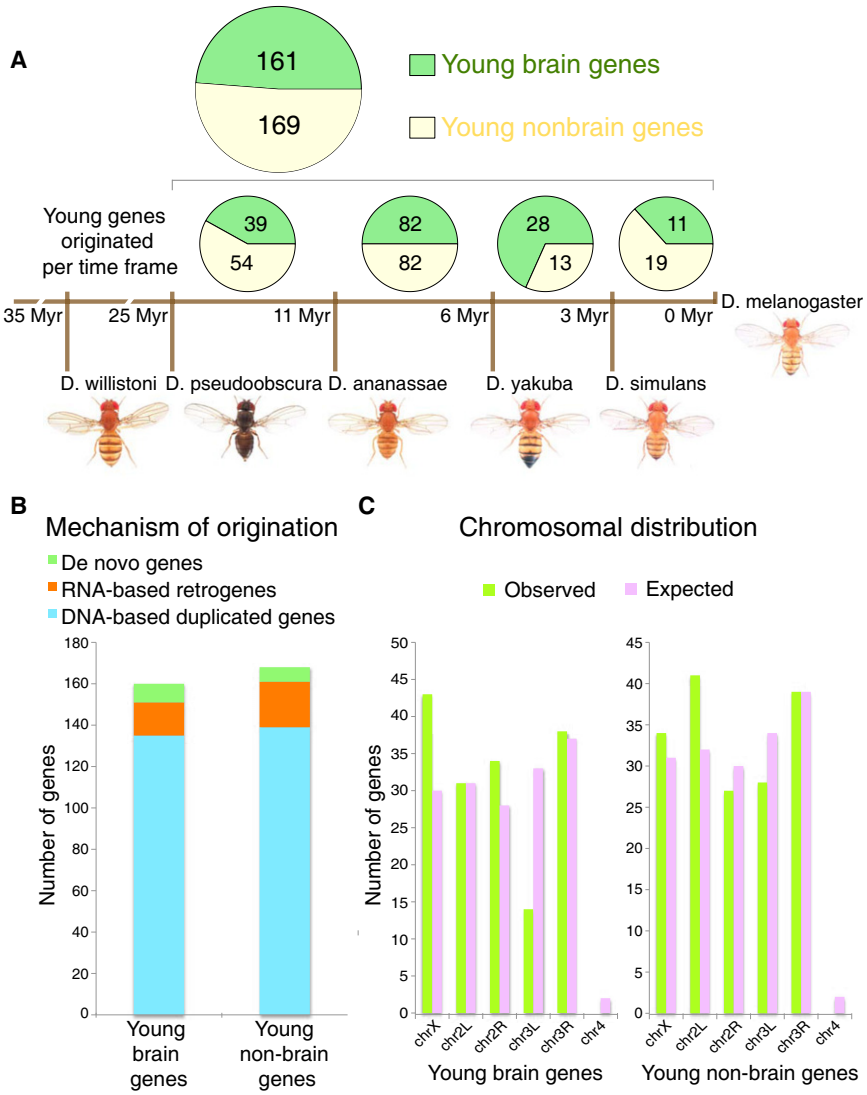

Figure 1. Origin and Evolution of Young Brain Genes in *Drosophila*

(A) Origination and age distribution of young brain genes and young nonbrain genes in *Drosophila*. The phylogenetic tree of representative species is shown in brown solid lines, and split times are labeled above each node as million years (Myr) ago. Pie charts above respective evolutionary branches show numbers of young brain genes and young nonbrain genes that originated on each branch. A nonconstant rate of brain gene origination was observed.

(B) Distribution of young brain genes and young nonbrain genes according to their origination mechanisms: de novo, RNA-based retroposition, and DNA-based duplication.

(C) Chromosomal distribution of young brain genes and young nonbrain genes on major chromosomal arms. Observed and expected values are shown.

(See also: Figure S1)

distinct brain expression as "young brain genes" and other genes as "young nonbrain genes."

We examined the brain gene origination events. Young brain genes can be generated by DNA/RNA-based gene duplication or de novo (Figure 1B). For duplicated genes, 65.2% (105/161) moved to new genomic locations distant from those of their parental genes (Figures S1A and S1B). On the *Drosophila* phylogeny, we observed a burst of brain gene formation between 3 and 6 Myr ago (Figure 1A), a significant enrichment of young brain over young nonbrain genes (Fisher's exact test to compare 28/13 with 132/155, two tailed, $p < 0.01$). Young brain genes are enriched on the X chromosome with a 44% excess over random expectation (χ^2 test, degrees of freedom = 5, $p = 1.5 \times 10^{-3}$), whereas young nonbrain genes are not (Figure 1C), implying that the sex chromosome has gained more brain genes than autosomes recently. Young brain genes encode various protein domains, which are enriched in several biological processes for protein level regulation, such as regulation of kinase activity and phosphorylation, whereas young nonbrain genes are enriched in a unique term proteolysis (Data Set S2).

Young MB Structures Recruited an Excess of Young Brain Gene Expression

We next determined the expression pattern of young brain genes at cellular resolution in the adult brain by using enhancer trap lines, as they often mimic the expression pattern of the genes adjacent to the insertion site of the P-element (Brand and Perrimon, 1993). We obtained 97 *Gal4* enhancer trap lines identified from GETDB (Hayashi et al., 2002) and CBD (Bourbon et al., 2002), representing 35 newly evolved genes. We identified 30 lines that drive clear UAS-mCD8GFP (Lee and Luo, 1999) expression patterns in substructures of the brain, representing 17 genes younger than 25 Myr (Tables S2 and S3, Extended Experimental Procedures). The proportion of genes expressed in the brain identified by enhancer trap (48.6%, or 17/35) agreed with that by RT-PCR (48.8%, or 161/330). Additionally, expression patterns from the few genes with available mRNA in situ hybridization data were consistent with those from the enhancer trap lines (Bourbon et al., 2002; Bousum et al., 2008; Hong and Ganetzky, 1996; Tomancak et al., 2007).

Collectively, young brain genes were expressed in neurons projecting to most major neuropils in the brain of *D. melanogaster* (Figure 2, Table S2). Different genes showed distinct expression patterns in one or more structures. For example, *hog (CG32595),* a ~6- to 11-Myr-old X-linked Forkhead-associated transcription factor known to be involved in neuronal cell migration and differentiation (Bousum et al., 2008), was expressed in all major brain structures we scored (Figure 2, Table S2). In contrast, *CG11825*, a gene that encodes

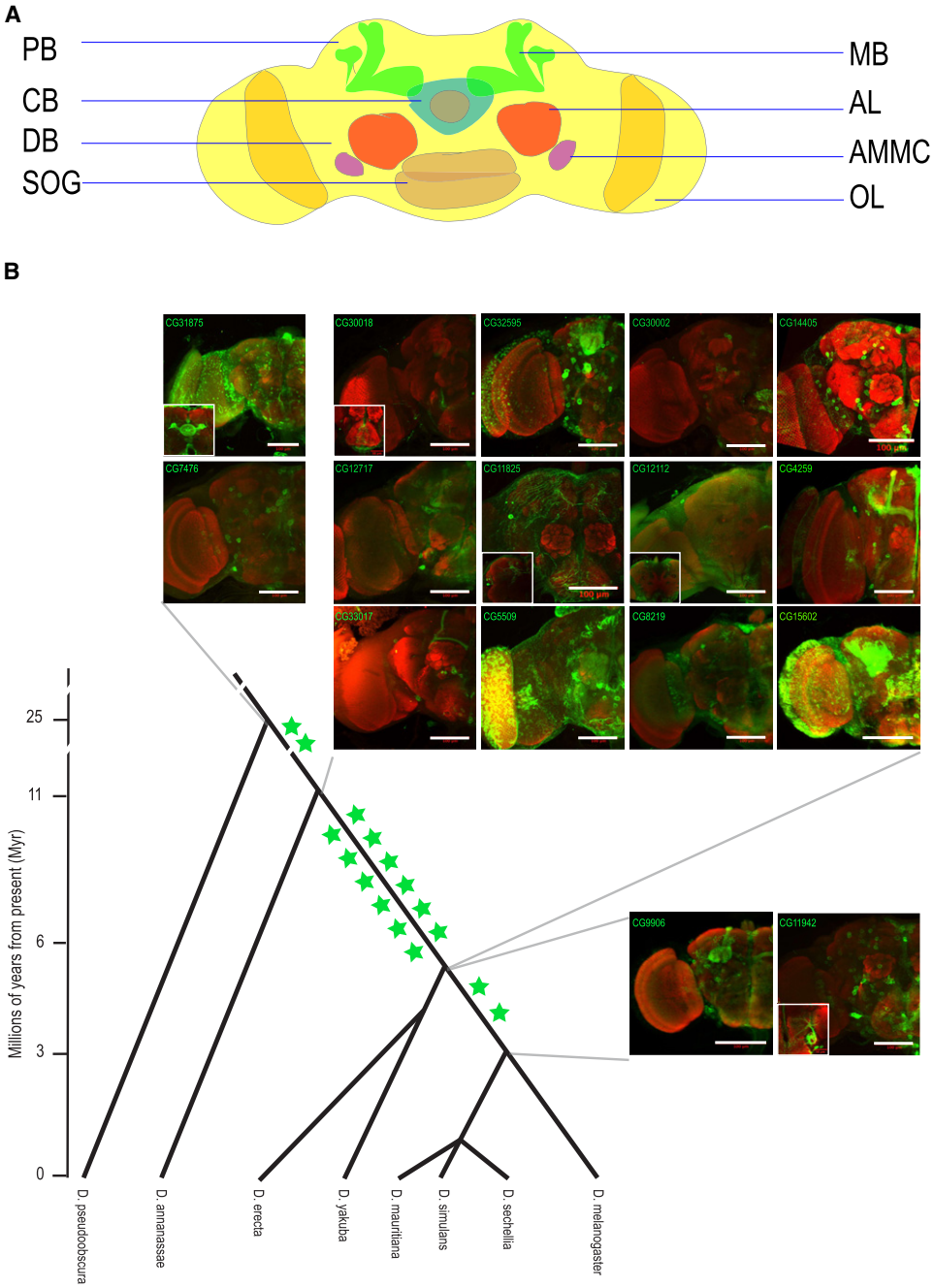

Figure 2. Neuronal Expression Pattern of Young Brain Genes in the *Drosophila* Phylogeny

(A) Schematic representation of the major *Drosophila* brain centers. MB, mushroom body; CB, central body complex; AL, antennal lobe; PB, protocerebrum; DB, deutocerebrum, AMMC, antennal mechanosensory and motor center; SOG, subesophageal ganglia; OL, optic lobe.

(B) Simplified phylogenetic tree showing representative species in the *melanogaster* and *obscura* groups and their divergence time in Myr from present. Green stars denote events of young brain gene origination. Enhancer-trap-based expression patterns in *Drosophila* brain corresponding to representative young brain genes are shown on the right. Magnified insets show the CB (*CG31875*), SOG (*CG30018*), AMMC (*CG11942*), and protocerebra (*CG11825* and *CG12112*). Green, *Gal4*-driven GFP; red, pan-neuropil labeling with nc82. Scale bar represents 100 μm.

(See also: Figure S2)

a putative hypoxia-induced protein, was expressed specifically in a small subset of neurons in the protocerebra and SOG (Figure 2, Table S2). These data demonstrated that recently originated genes acquired stereotypic expression patterns in substructures of the brain, which implies that they acquired neuronal regulation of gene expression.

We next examined how often brain-expressed genes are expressed in the MB. From our set of 35 young genes, of the 17 brain-positive genes, 82% (14/17) are expressed in MBs (Figure S2, Tables S2 and S3). By contrast, from 1934 randomly chosen genes, of the 1231 genes that are expressed in the brain, only 34% (429/1231) are expressed in MBs (E.C. Marin and L.L., unpublished data). An independent enhancer-trap-based study estimated a similar rate of 23% (65/281) for random brain-expressed genes with MB expression (Kelso et al., 2004). While the basal probability of brain expression is similar between young and random genes in the genome, young genes are significantly enriched in the MB (Fisher's exact test, $p = 0.0018$ and $p = 0.022$, respectively). Given that enhancer trap collections represent a relatively random sampling of genomic loci with respect to brain expression (Brand and Perrimon, 1993; Hayashi et al., 2002), these data suggest that the MB is a favored tissue for new genes when they acquire expression in the brain.

The MB consists of three distinct types of neurons, including the α/β, α'/β', and γ neurons (Crittenden et al., 1998; Lee et al., 1999; Tanaka et al., 2008). Interestingly, all of the MB-positive young brain genes are expressed in the α/β neurons, while only four show expression in α/β and γ or α'/β' neurons (Figure S2, Tables S2 and S3). Previous work has shown that the γ lobe is the most ancestral, while the $\alpha\beta$ lobes are derived and the most heterogeneous (Strausfeld and Li, 1999a, Strausfeld and Li, 1999b). The preferential expression of young brain genes in the α/β lobes suggests that the derived substructures may have frequently recruited new genes during recent evolution.

Expression Profiling of the MB Transcriptome

To examine the expression profile of MBs at the genomic level, we profiled the transcriptomes of dissected MBs in parallel with dissected whole brains by RNA sequencing and confirmed the quality of the data set (Figures 3A and S3A–S3D). In brain and MB RNA-seq samples, young brain genes are expressed at higher levels than young nonbrain genes, and young MB genes identified by enhancer trap are expressed significantly higher than young non-MB genes (Figures 3C and 3D, Wilcoxon test, $p < 0.001$ for all comparisons). The correlation between young and parental gene expression is weak (Figure S3E), suggesting that the expression pattern and level of most young genes are not directly inherited from their parental genes (Extended Experimental Procedures). These data provide a genome-wide data set for testing differential gene expression in the brain and MBs.

To estimate the differential expression for each gene between MB and whole brain, we used a generalized linear model framework as previously described (Marioni et al., 2008). A total of 2,940 genes were identified as differentially expressed (multiple testing corrected p value < 0.001), including 58 young genes (< 60 Myr) and 2,272 old genes (> 60 Myr) (Data Set S4). In the old gene group, only half (48.9%, or 1,112/2,272) of the differentially expressed genes are MB-enriched, while in the young gene group, a significantly higher proportion of differentially expressed genes (91.4%, or 53/58) are MB-enriched (Fisher's exact test, two-tailed, $p < 0.0001$, Figures 3B and S3B). This observation was recapitulated with a more stringent young gene data set (Table S1), revealing a stronger pattern: 97.0% (32/33) of young genes are MB-enriched (Figures 3B and S3B, young versus old, $p < 1 \times 10^{-5}$). Similar patterns were not observed in either MB versus testis or brain versus testis differential expression analyses (Data Sets S5 and S6). These data revealed an excess of MB-enriched genes and a paucity of MB-depleted genes in the young gene group, suggesting that differentially expressed young genes tend to be enriched in the MBs.

Natural Selection on Young Brain and Young MB Genes

We examined natural selection on young brain and/or MB genes. Using *Drosophila* polymorphism data (Begun et al., 2007) (DPGP) (Data Set S3), we estimated α (Experimental Procedures); i.e. the proportion of amino acids under positive selection (Eyre-Walker and Keightley, 2009). A higher α is indicative of stronger positive selection. We found that the α of young brain genes (+0.596) is significantly higher than that of young nonbrain genes (0.525), random genes (0.462), or old brain genes (0.464) (Figures 3E and S3F) (Wilcoxon test, $p < 0.00001$ for all comparisons).

Young MB genes identified by enhancer trap have strong positive selection ($\alpha = +0.634$), significantly higher than young non-MB genes (0.344) (Figure 3F, Wilcoxon test, $p = 0.00002$). At the genomic level, MB-enriched genes ($\alpha = +0.534$) showed a stronger signature than MB-depleted genes (0.494) (Figure 3F, Wilcoxon test, $p = 0.00002$). These data suggest that stronger positive Darwinian selection has shaped the evolution of young brain genes and possibly influenced their expression in MBs.

Evolution and Expression Pattern of a Young Brain Gene, *Xcbp1*

Young brain/MB genes may have offered fitness advantages for selection. A young MB gene, *Xcbp1,* showed a strong signal of positive selection (Dn = 195, Ds = 80, Pn = 27, Ps = 23, McDonald and Kreitman test, $p = 0.02$), a starting point for phenotypic examination to test this hypothesis.

Xcbp1 originated roughly 5 Myr ago, after the *D. melanogaster-D. simulans* clade diverged from *D. yakuba* but before the clade split (Figure 4A, Table S2). Its parental gene, *Cnx99a*, encodes a type 1 Calnexin that binds to calcium ions and generally acts as a chaperone to facilitate folding of glycoproteins such as rhodopsins (Rosenbaum et al., 2006; Figure 6A). During its initial retroposition event, *Xcbp1* retained only the CDS portion from its parental gene *Cnx99a* (Figure 4B) (Bai et al., 2007) and integrated into an X-linked cluster of five previously identified neuronal genes (Figure 4B) by nesting into the first intron of *paralytic*, which encodes the major voltage-sensitive sodium channel in *Drosophila* (Hong and Ganetzky, 1996). This genomic region contains multiple binding sites of well-known neuronal transcription factors such as Dichaete, Disconnected, Jumeau, and Senseless (Nègre et al., 2011). *Xcbp1* likely hitchhiked the regulatory elements from this neuronal gene environment and acquired a distinct neuronal expression pattern, as shown previously by in situ hybridization (Hong and Ganetzky, 1996).

To identify neurons that express *Xcbp1*, we utilized the *Gal4* enhancer trap line *pG156* (Bourbon et al., 2002), hereafter called *Xcbp1-Gal4,* which is inserted 1 kb 5′ to the *Xcbp1* transcription start site on the sense strand of *Xcbp1* (Figure 4C). *Xcbp1-Gal4* drives *UAS-mCD8GFP* expression in the peripheral nervous

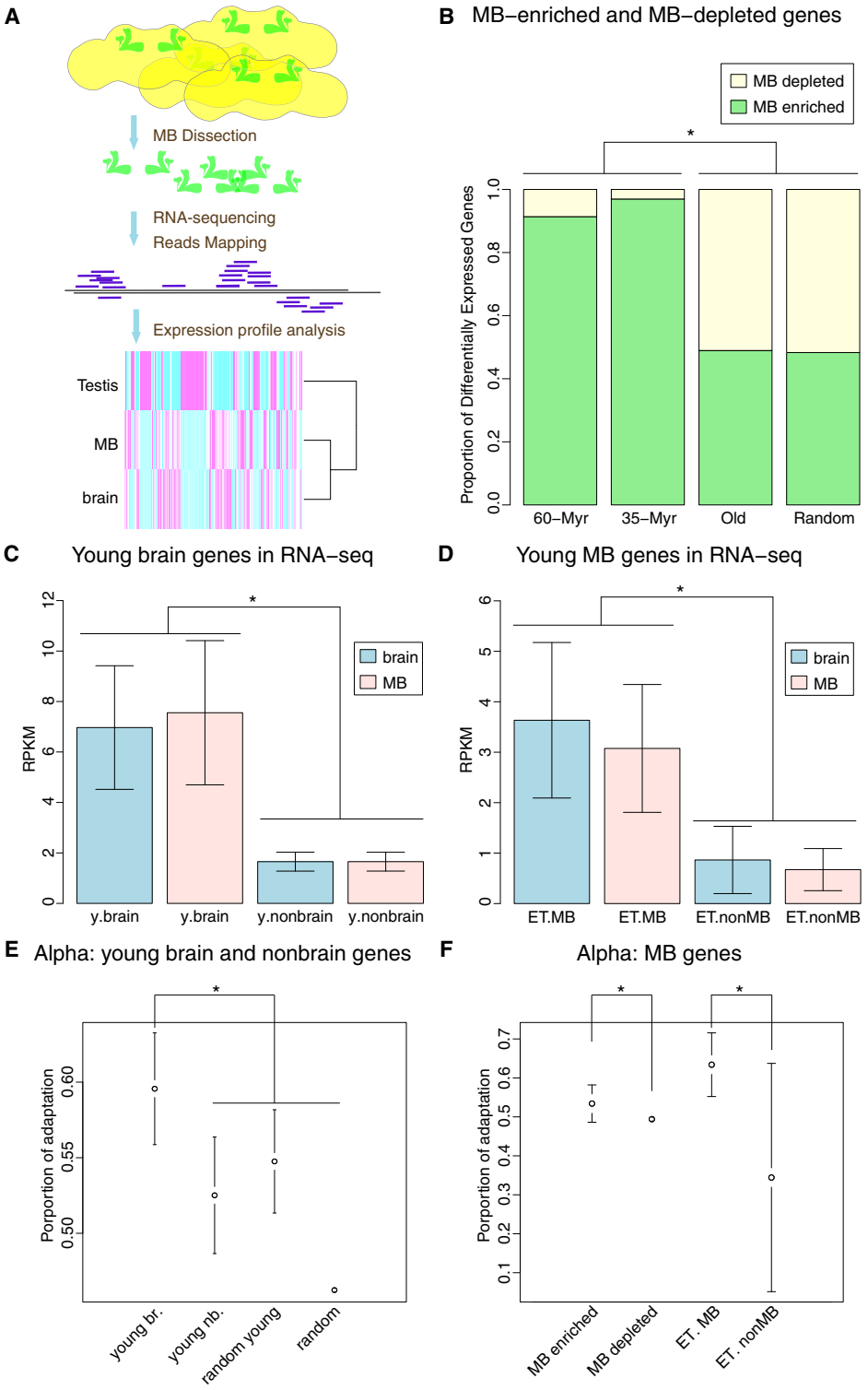

Figure 3. Adaptive Evolution of Young Brain and Young MB Genes

(A) Schematic representation of RNA-seq showing the workflow of experimental (dissection, RNA-sequencing) and analytic procedures, with a heat map of young gene expression in MB, brain, and testis at the bottom.

(B) Statistical summary of MB-enriched and MB-depleted genes in differentially expressed gene sets; 60 Myr, genes younger than 60 million years (n = 447, DE = 58); 35 Myr, genes younger than 35 million years (n = 279, DE = 33); Old, genes older than 60 Myr (n = 9275, DE = 2272); Random, randomly sampled genes from the genome (n = 11820, DE = 2940); DE, number of differentially expressed genes.

system, especially in olfactory receptor neurons (ORNs) in the antennae and maxillary palps (Figure 4D), which are the primary olfactory sensory organs (Stocker, 1994). Furthermore, *Xcbp1-Gal4* is also expressed in α/β MB neurons (Figures 4E and S2), and the MB is a higher brain center known to be involved in olfactory learning and memory (Keene and Waddell, 2007). Using *eyFlp* and *UAS > Stop > mCD8GFP* to limit expression to only ORNs, we verified that *Xcbp1-Gal4*-positive ORNs projected into the antennal lobes, especially to glomeruli VC3m, VC3l, DC4, V, DL5, and DM1 (Figures 4E S4A, and S4B), which are innervated by coeloconic and basiconic ORNs that are likely receptive to food odors (Benton et al., 2009; Hallem et al., 2006). In particular, the DM1 glomerulus has a major role in attraction to food odors (Semmelhack and Wang, 2009), suggesting that *Xcbp1*+ neurons might participate in this process.

Xcbp1 Facilitates Foraging

Xcbp1-Gal4 is primarily expressed in brain centers and sensory organs that are involved in food source detection and odor processing and therefore may have a role in foraging behavior. We designed an assay to measure the foraging ability of *Drosophila* (Figure 5A), and we first tested two wild-type (WT) *D. melanogaster* lines *(Canton-S* and *Oregon-R).* We observed that starved flies efficiently found and immediately started consuming food (Figure 5B, Extended Experimental Procedures, Movie S1). More than half of the WT flies found food within 3 hr, and we observed no significant differences between the WT strains (two-sample Komogorov-Smirnov (KS) test, not significant (NS), Figure 5C). We also quantified foraging performance by calculating an index of foraging speed (FSI) (Figure 5D). These results suggested that WT *D. melanogaster* flies were attracted to the food source in the second compartment in the assay.

We tested whether these *Xcbp1*-positive (*Xcbp1*+) neurons are responsible for foraging behavior. Using a temperature-sensitive dominant-negative *Dynamin* allele, *Shibire^{ts1}* (Kitamoto, 2001), we specifically inactivated synaptic transmission in *Xcbp1-Gal4*+ neurons using *Xcbp1-Gal4*. When *Xcbp1-Gal4 / UAS-Shibire^{ts1}* flies were shifted to restrictive temperature (31°C) before testing to inhibit synaptic transmission in *Xcbp1*+ neurons, there was a sharp reduction in foraging performance as compared to WT flies (Figures S5A and S5B). Both *Xcbp1-Gal4* and *UAS-Shibire^{ts1}* control flies behaved like WT flies at 24°C and 31°C (Figure S5). These data showed that *Xcbp1*+ neurons are necessary for foraging.

We then measured the foraging behavior of *D. melanogaster* when *Xcbp1* was knocked down by RNA interference (RNAi). Constitutive tubulin::*Gal4*-driven *Xcbp1* RNAi (*UAS::ds-Xcbp1*) (Dietzl et al., 2007) reduced *Xcbp1* transcript level sharply compared to controls, with no off-target effect on *Cnx99a* (Figure 5B). Compared to controls, *Xcbp1* RNAi flies (*tubulin::Gal4 > > UAS::ds-Xcbp1*) showed a significant reduction in foraging ability (KS test, $p < 1 \times 10^{-10}$ Figure 5E). This was recapitulated with both *Act5C::Gal4* and *Xcbp1-Gal4* (KS test, $p = 2 \times 10^{-5}$ and $p = 0.0001$, respectively, Figures 5E and 5F). These data indicated that a decrease in *Xcbp1* expression reduces the foraging ability of *D.melanogaster*.

Multiple modalities are important for foraging. We investigated the locomotion and flight abilities of *Xcbp1* RNAi flies with previous methods (Stockinger et al., 2005). Both locomotion and flight were normal for *Xcbp1* RNAi and controls (Figures 5G and 5H). This suggested that neither a locomotion defect nor a flight defect was responsible for the foraging phenotype. Given the prominent expression of *Xcbp1* in the olfactory system, it is possible that defects in olfactory neurons contribute to the foraging deficiency in *Xcbp1-Gal4*-driven *Xcbp1* knockdown (Figure 5E).

Since *Xcbp1-Gal4* is expressed in MBs, which are essential for the olfactory response, we used the pan-MB *Gal4* driver *OK107* to silence *Xcbp1* in the MBs. *OK107*-driven *Xcbp1*-RNAi also led to a reduction in foraging ability equivalent to constitutive or *Xcbp1-Gal4* driven RNAi (Figure 5I), suggesting that *Xcbp1* expression in the MBs is critical for efficient foraging.

Olfaction is essential for foraging (Osborne et al., 2001). The phenotype of *Xcbp1* is consistent with its expression pattern in the olfactory systems for food cue sensing (Carlson, 1996) and olfactory signal processing (Laurent, 2002). *Xcbp1* expression in MB is required for foraging, suggesting that MB processing is critical, though multiple levels might be involved. Together, these data suggest that *Xcbp1* evolved to participate in the neuronal circuit regulating foraging behavior.

Evolution of *Xcbp1* Function

Cnx99a is ancestral and conserved, whereas *Xcbp1* evolved rapidly under positive selection. At origination, *Xcbp1* inherited a calnexin-like structure from its parental gene *Cnx99a* (Figures 6A, S6A, and S6B). Subsequently, *Xcbp1* protein has rapidly evolved 36 amino acid (aa) substitutions, a large de novo 56 aa lysine-glutamate rich insertion in the putative cytosolic

(See also Figure S3B)

(C) Relative gene expression abundance (mean RPKM values from biological replicates) of young brain genes (y.brain) and young nonbrain genes (y.nonbrain) detected by RT-PCR, in brain and MB RNA-seq data sets. Error bars represent mean +/− SEM.

(D) Relative gene expression abundance of young MB genes (ET.MB) and young genes not expressed in the MB (ET.nonMB) detected by enhancer trap, in brain and MB RNA-seq data sets. Error bars represent mean +/− SEM.

(E) Estimation of alpha, the proportion of nonsynonymous substitutions subjected to positive selection, revealed positive selection on young brain genes. Young brain genes (young br.) have significantly higher alpha compared to young nonbrain genes (young nb.), random young genes (random young), or random genes (random), representing the genomic background from random sampling. The "random" error bar is too small to display. Error bars represent the 95% confidence interval.

(F) Estimation of alpha revealed positive selection on MB genes. MB-enriched genes have significantly higher alpha compared to MB-depleted genes; young MB genes (ET.MB) have significantly higher alpha compared to young non-MB genes (ET.nonMB). The "MB-depleted" error bar is too to display.

Stars denote significance in statistical comparison, if applicable. Error bars represent the 95% confidence interval.

(See also: Figure S3)

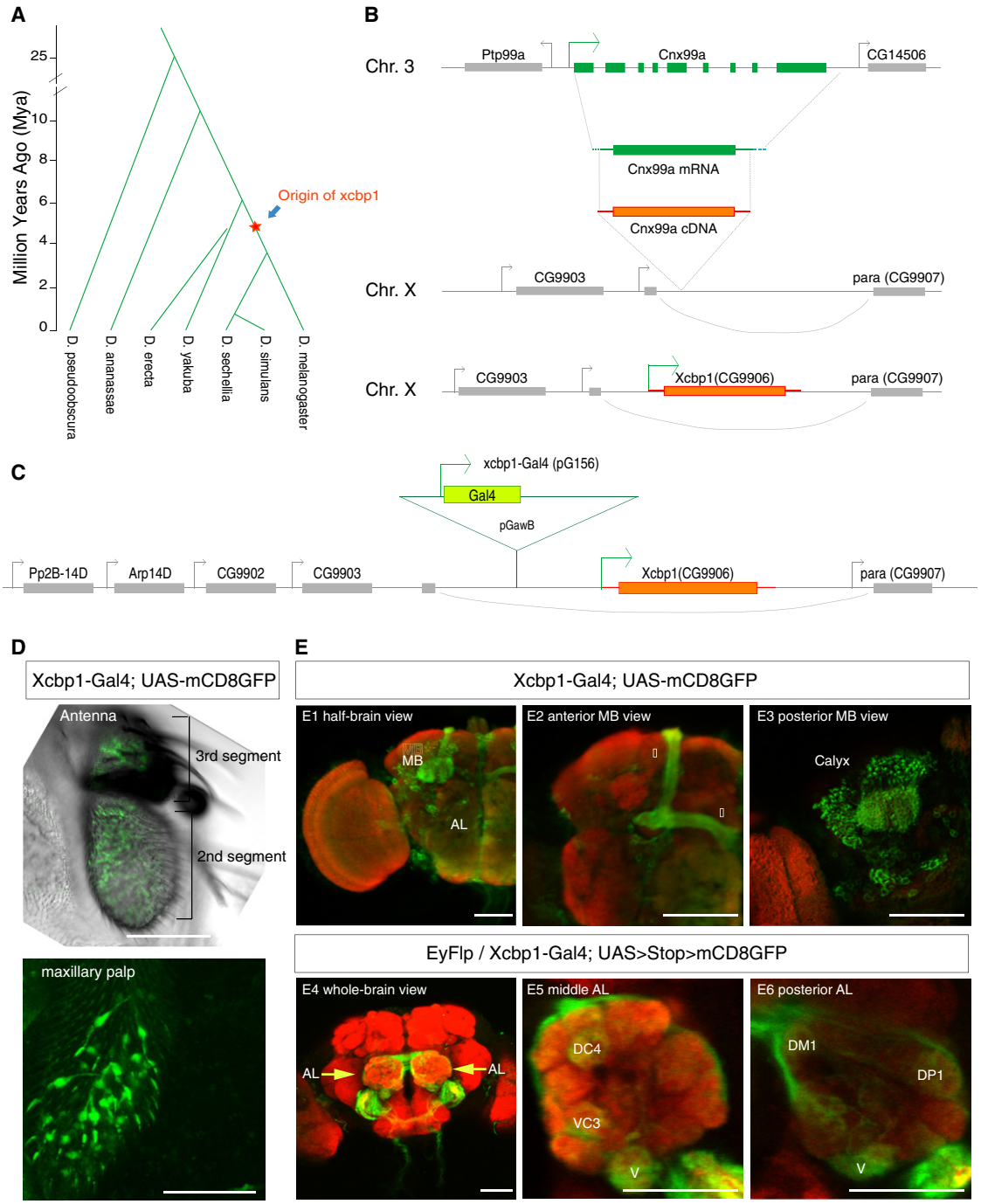

Figure 4. Origin and Expression Pattern of *Xcbp1*

(A) *Drosophila* phylogeny showing the origination of *Xcbp1*.

(B) Scheme illustrating how the CDS of *Cnx99a* (green) retroposed (orange) and inserted into the first intron of *paralytic (para),* generating *Xcbp1* (not drawn to scale).

(C) Illustration of the genomic location and orientation of *Xcbp1-Gal4* (not drawn to scale).

(D) Expression of *Xcbp1-Gal4* in *D. melanogaster* antenna and maxillary palp (GFP, green; scale bars represent 50 μm).

(E) Expression of *Xcbp1-Gal4* in *D. melanogaster* brain (GFP, green; neuropil, red). Top panel: *Xcbp1-Gal4* labeling of MB and AL in a half-brain view, full stack (E1). Magnified anterior (E2) and posterior (E3) views of the MB showing labeling of α and β lobes of the MB and the calyx. Bottom panel: ORN-specific labeling by the intersection of *eyFlp* and *Xcbp1-Gal4* expression. Antennal nerve-specific expression is visible (arrows) in whole-brain view, full stacks (E4). Glomerular labeling of DC4, VC3, V, and DM1 is clearly visible in single planes of the middle (E5) and posterior (E6) layers of the AL. Scale bars represent 50 μm.

(See also: Figure S4)

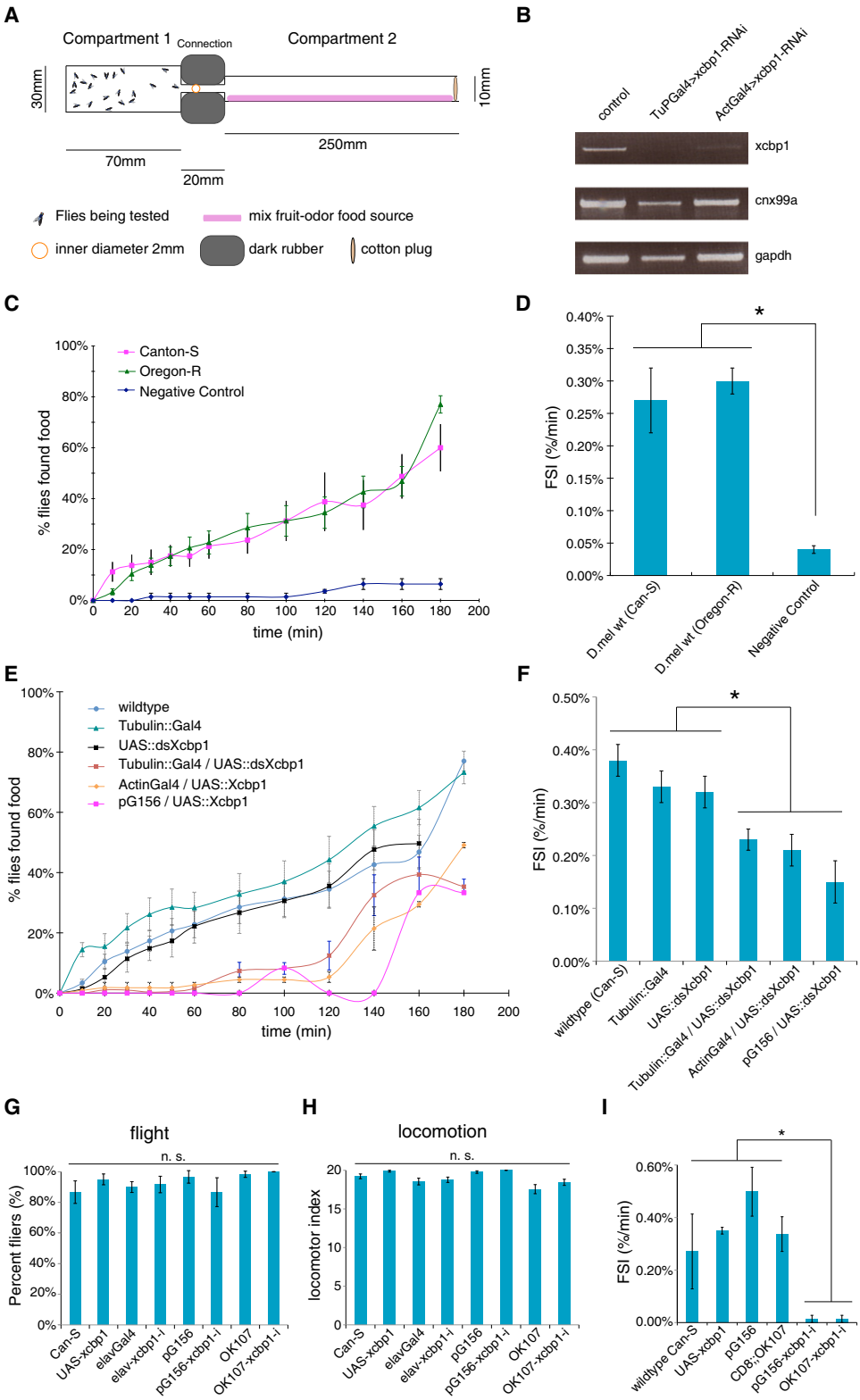

Figure 5. *Xcbp1* Influences Foraging Behavior

(A) Scheme of the foraging assay system design.

(B) Semiquantitative RT-PCR showing the specific and efficient RNAi knockdown of *Xcbp1*. Sample genotypes are shown above and assayed genes on the right.

C-terminal domain, and two small deletions in the putative luminal N-terminal domain (Figures 6A, S6A, and S6B).

Cnx99a has been reported to be important for rhodopsin maturation and photoreceptor survival (Rosenbaum et al., 2006). Consistently, we found that *Cnx99a-RNAi* using eye-specific *GMR-Gal4* caused a disruption in ommatidial structure (Figure 6C). RNAi knockdown of *Cnx99a* by *Act5C-Gal4* caused both shortening and bluntness in notum bristles (Figure 6D), suggesting that *Cnx99a* might also be involved in bristle development. RNAi knockdown of *Xcbp1* in the eye also resulted in a disruption of ommatidial structure (Figure 6C), but knockdown with *Act5C-Gal4* did not result in a bristle defect (Figure 6D). Neuronal knockdown (by *Elav-Gal4*) of *Xcbp1* reduced foraging efficiency, whereas *Cnx99a* knockdown with the same driver did not (Figure 6B). Both *Xcbp1* and *Cna99a* are expressed in the brain; however, whereas *Xcbp1* is enriched in the MB over the brain (LRT, $p = 0.007$), *Cnx99a* is not (LRT, $p = 0.24$) (Data Sets S1–S6). The expression of Cnx99a in the brain is consistent with its function in rhodopsin maturation, while the MB-enriched expression of *Xcbp1* might be related to foraging behavior. In the last five Myr, *Xcbp1* inherited and maintained an ancestral role in eye development and might have acquired a novel role in foraging behavior.

Divergence of Foraging Efficiency and MB Gene Origination

We surveyed the foraging behaviors of several *Drosophila* species with or without *Xcbp1*. Four species within the *D. melanogaster* clade—*D. melanogaster, D. simulans, D. sechellia*, and *D. mauritiana*—possess *Xcbp1* orthologs (Figure 4A). These species showed high FSIs that were not significantly different (Figure 7A). Three outgroup species—*D. yakuba, D. erecta*, and *D. pseudoobscura*—lack an *Xcbp1* ortholog and showed significantly slower foraging speed (test for equality in slopes, $p < 1 \times 10^{-5}$ Figure 7A). These data revealed diversity in the foraging speed of closely related species with the same food cue, possibly because different species have adapted to different cues (Harry et al., 1998).

We used the *OK107-Gal4* line to drive RNAi of other young MB genes in MB neurons and assayed foraging. Out of eight tested, an 11- to 25-Myr-old MB gene, *Drosophila Elm2-Sant retrogene* (*Desr, CG31875*), also showed a significantly reduced FSI when knocked down in the MB (Figures 7B and S7B). *Desr* originated by retroposition from the short splicing isoform of the parental gene *CoREST* (Figures 7C and 7D), which is a transcriptional corepressor essential for nervous system development (Dallman et al., 2004). By retroposition, *Desr* moved into a genomic location near a neuronal gene, *Bib* (Figure 7C). *Desr* also underwent rapid protein evolution compared to its paralogs (Figures 7D). *Desr* recruited novel 5′ and 3′ exons and possibly adjacent enhancers (Figure 7C), acquiring expression in brain structures including ALs and MBs (Figure 7E). These data reveal a second, more ancient event of neuronal gene origination that influenced foraging behavior in *D. melanogaster*. (Figures 7A and 7F). The influences of *Xcbp1* and *Desr* on foraging in other species awaits further study, as different species may have adapted to different foraging cues in unique niches and outgroup species may have a greater foraging ability when tested with food cues most appropriate to their ecology.

DISCUSSION

The evolution of the brain and complex behaviors is an intriguing process. Although most of the basic components of synaptic vesicles and postsynaptic protein complexes are evolutionary conserved (Jiménez and Davletov, 2007), the neuronal roles of clade-specific genes, especially those of recent origin, have rarely been studied. Our data in *D. melanogaster* reveal that new genes with neuronal expression have originated frequently during *Drosophila* evolution. During origination, many young genes either nested in or jumped close to other neuronal genes (Table S2) and might have recruited neuronal enhancers from local genomic environments (Nègre et al., 2011). Indeed, RT-PCR, RNA-seq expression profiling, and enhancer-trap expression verified neuronal expression for many genes in our data set. This finding strengthens enhancer hitchhiking by new genes (Kaessmann et al., 2009). Intriguingly, there is an enrichment of young brain genes on chromosome X compared to either young nonbrain genes or to autosomes, implying a coevolution of the sex chromosome and the brain in terms of new gene origination. Evolutionary analysis suggests that natural selection is actively engaged in the evolution of young brain genes.

We observed a relationship in the evolution of new genes and MB expression at the genomic level. These observations support the idea that young genes rapidly evolved brain expression, and MBs are hotbeds for novel gene expression in the brain. Interestingly, many young MB genes are expressed in the evolutionarily young α/β subtype of MB neurons. The

(C) Foraging curve of WT *D. melanogaster* strains showing the time-dependent increase in the percentage of flies that found food. Flies do not crawl to compartment 2 if the food source is not present (negative control).
(D) FSI measurements of WT *D. melanogaster* strains.
(E) Foraging curve for *Xcbp1* RNAi in *D. melanogaster* with constitutive *Gal4*s and *Xcbp1-Gal4* (*pG156*) or in control (WT, *Gal4* only, UAS only) animals. Genotypes are shown in the inset legend.
(F) FSI measurements of control or *Xcbp1* RNAi knockdown animals. Loss of *Xcbp1* results in a significant decrease in foraging ability. Genotypes are shown at the bottom of each column.
(G-H) *Xcbp1* RNAi knockdown animals show no significant impairment of either flight (G) or locomotion (H) when compared to controls. Genotypes are shown at the bottom of each column.
(I) FSI measurements of control or *Xcbp1* MB RNAi knockdown animals. Loss of *Xcbp1* in MB results in a significant decrease in foraging ability. Genotypes are shown at the bottom of each column.
In barplots, data are represented as mean ± SEM, where error bars denote SEM values; asterisks denote statistical significance ($p < 0.01$ unless otherwise noted) in all pairwise comparisons; n.s., not significant ($p > 0.01$ unless otherwise noted) in all pairwise comparisons.
(See also: Figure S5)

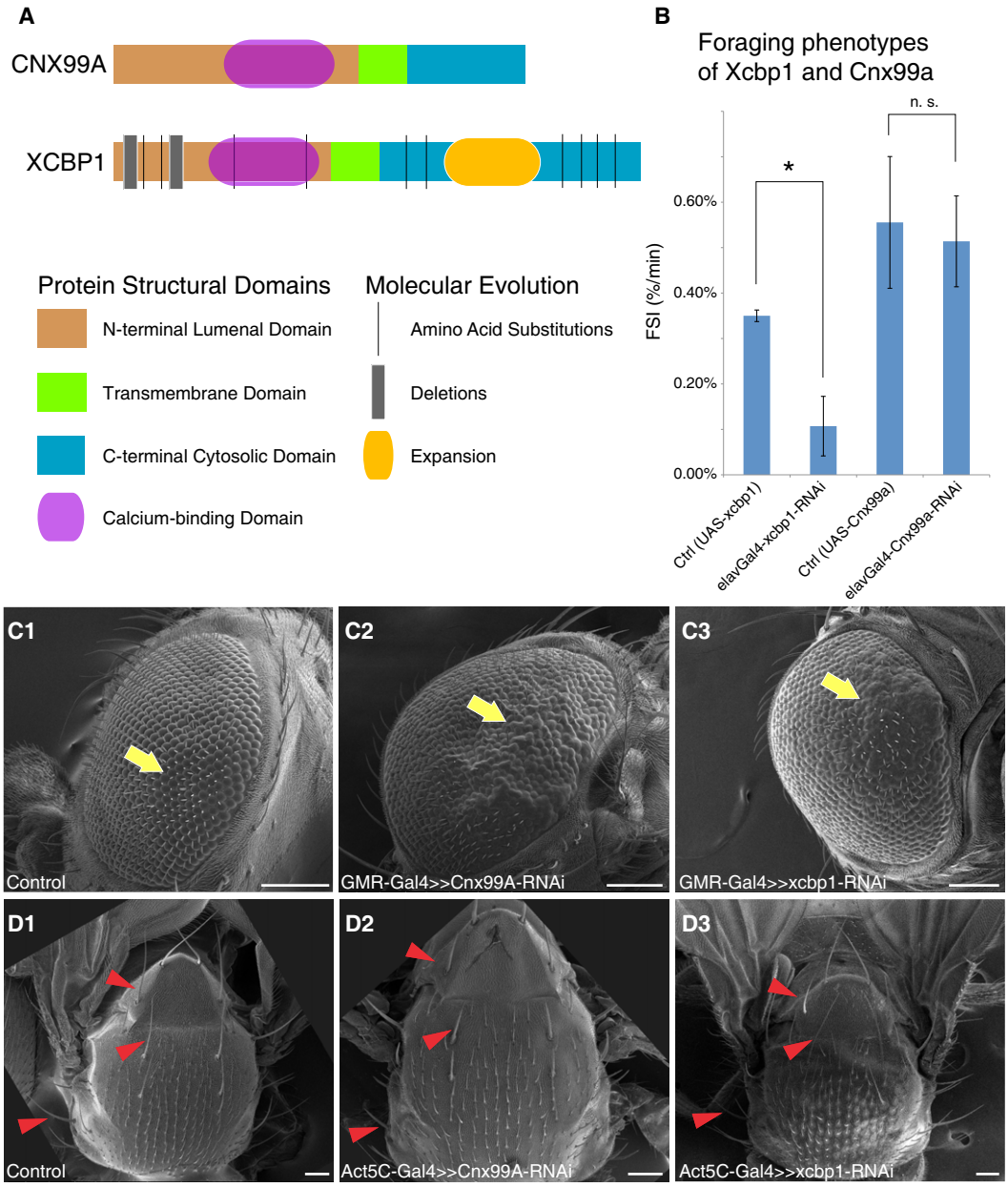

Figure 6. Conservation and Divergence of the *Xcbp1-Cnx99a* Gene Pair

(A) Scheme of the *XCBP1 and CNX99A* proteins. N-terminal domains (NTDs), brown; transmembrane domain (TM), green; C-terminal domains (CTDs), cyan. Amino acid substitutions, deletions, and expansions are noted on the *Xcbp1* diagram.

(B) FSI measurements of control, *Xcbp1* RNAi knockdown, and *Cnx99a* RNAi knockdown animals. Genotypes are shown at the bottom of each column. Data are represented as mean ± SEM. The asterisk denotes statistical significance ($p < 0.01$); n.s., not significant ($p > 0.01$).

(C) SEM images of eye morphology of control animals (C1) or animals with *Cnx99a* (C2) or *Xcbp1* (C3) RNAi knockdown. Yellow arrows point to normal ommatidial structures in control animals and defective or overproliferated ommatidial structures in both *Cnx99a* and *Xcbp1* RNAi animals. Scale bars represent 100 μm.

(D) SEM images of notum morphology of control animals (D1) or animals with *Cnx99a* (D2) or *Xcbp1* (D3) RNAi knockdown. Red arrowheads denote shorter and blunter bristles in *Cnx99a* RNAi that are unaffected in control or *Xcbp1* RNAi flies. Scale bars represent 100 μm.

(See also: Figure S6)

MBs are major control centers for a variety of neuronal functions, such as olfactory information processing, learning, memory, and foraging behavior (Davis, 2001; Krashes et al., 2007; Osborne et al., 2001). *Drosophila* MBs are relatively more complex than those of paleopteran and thysanuran insects (Strausfeld et al., 1998). MBs are thought to have high genetic and synaptic plasticity (de Belle and Heisenberg, 1994; Marin et al., 2002). Such plasticity might enable new genes to be expressed in the

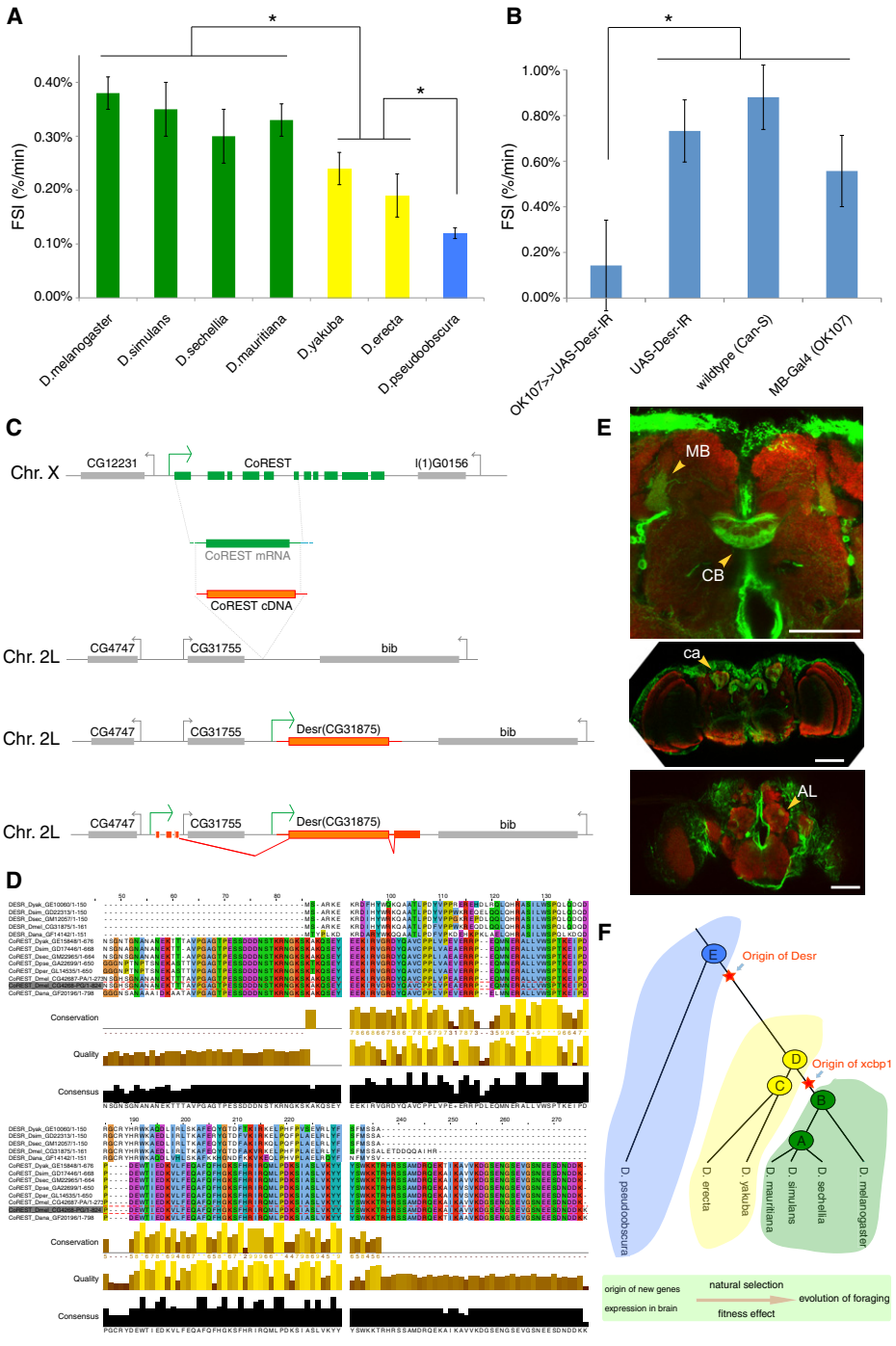

Figure 7. Evolution of Foraging Behavior Driven by New Brain Gene Origination in *Drosophila*

(A) Phylogenetic comparison of FSIs in closely related *Drosophila* species that are color coded according to the phylogeny in (D).

(B) MB-specific knockdown of *Desr* by the *OK107* driver shows a significant decrease in FSI, correlating with the increase in foraging ability observed between nodes E and D (see also D and Figure S4B). Data are represented as mean ± SEM. The asterisk denotes statistical significance (ANOVA, $p < 0.01$); n.s., not significant (ANOVA, $p > 0.01$).

(C) Scheme illustrating how the 11- to 25-Myr-old MB gene *Desr* (orange) retroposed from the X-linked parent *CoREST* (green) into a genomic location on Chr. 2L adjacent to *bib* (gray), and recruited new 5′ and 3′ exons (deep orange). Gene models and distances are not drawn to scale.

(D) Protein sequence alignment of DESR showed homology to the CoREST N-terminal protein, resulting from retroposition of the *CoREST* short isoform. Regional alignment of DESR to its homologs showed evidence for rapid amino acid divergence; DESR arose after the *D. pseudoobscura – D. melanogaster* split and was lost in *D. erecta*. Amino acids color coded by conservation level. Only partial representative sequences are shown for simplicity.

MB and gradually acquire beneficial functions and become integrated as new genetic components.

Changes in the brain have been reported to be associated with rapid protein evolution or *cis*-regulatory mutations. The evolution of language ability is associated with the FOXP2 transcription factor (Konopka et al., 2009). Increased brain complexity in human is thought to be associated with gene expansion (Popesco et al., 2006) and the emergence of noncoding genes (Pollard et al., 2006). The expansions of the soluble ligand gene family in the neural crest are vertebrate innovations (Martinez-Morales et al., 2007). In *Drosophila*, a newborn RNA gene was related to male courtship behavior (Dai et al., 2008). We showed that new genes frequently arose, acquired expression in distinct subsets of neurons, and regulate foraging behavior in *D. melanogaster*. These brain genes encode many different protein domains (Table S2 and Data Set S2), suggesting that the genetic network in the brain may evolve with the origination of new genes that recombine existing protein function with novel neuronal expression.

Animal behaviors are constantly evolving (Brown, 1975; Evans, 1962), and change in existing behavioral genes (Chang et al., 2011; Fitzpatrick et al., 2007; Wheeler et al., 1991) has been suggested as an underlying mechanism. The frequent origin of new genes in the nervous system provides an alternative. The recent origin of both *Xcbp1* and *Desr* contributed to foraging success for *D. melanogaster*. Foraging is critical for animal survival (de Bono and Maricq, 2005; Sokolowski, 1980) because in the natural environment failure to locate and consume food can lead to starvation and death. Foraging success is highly correlated with sensitivity to sensory cues coming from food sources (Asahina et al., 2008); mutations that confer advantages in food cue sensing and/or olfactory processing that might lead to higher foraging success could be favored and quickly get fixed. Foraging behavior is polymorphic (Fitzpatrick et al., 2007) and constantly evolving (Osborne et al., 1997; Sokolowski et al., 1997). *Xcbp1* and *Desr* are such macromutations, although how they interact with previously identified foraging behavior genes and circuits are still open questions (Osborne et al., 2001; Root et al., 2011). We provide two examples of newly evolved brain genes that have facilitated foraging behavior in *D. melanogaster*. Additional components of the foraging circuit and the mechanisms underlying foraging behaviors in other *Drosophila* species await future investigation.

EXPERIMENTAL PROCEDURES

Identification and Expression of Young Brain Genes

Newly evolved genes in the *D. melanogaster* genome were curated from the literature, and their ages were verified with the use of multiple-species genomic sequence alignments. We obtained a larger data set of 886 young genes (< 60 Myr), and a more stringent data set of 566 young genes (< 35 Myr) (Table S1). We designed primers and performed RT-PCR for a set of 330 genes that are younger than 25 Myr (Extended Experimental Procedures, Data Set S1).

Expression Profiling of Brains and MBs

Whole brains and GFP+ MB tissues were dissected from 1- to 7-day-old adult flies from the line *OK107-Gal4 > > UAS-mCD8GFP* with a fluorescence dissecting scope. Total RNA samples were prepared via phenol-chloroform extraction followed by QIAGEN MinElute Kit purification. Single-end RNA sequencing Library preparations and sequencing were performed with the use of the standard Illumina protocols on Solexa (Ilumina). Read mapping and gene expression analysis are described in Extended Experimental Procedures.

Evolutionary Analysis

Evolutionary analyses of new-gene origination were performed as described in Vibranovski et al. (2009) and Zhang et al. (2010). Primary polymorphism data were from DPGP, and we filtered nucleotides with a Phred score < 30 as "N." Polymorphic frequency spectra were analyzed with Polymorphorama (Haddrill et al., 2008). Estimation of α was carried out with DoFE (Eyre-Walker and Keightley, 2009).

Fly Stocks and Crosses

Enhancer trap lines used in this study were ordered from the *Gal4* Enhancer Trap Insertion Database (GETDB) or the Centre de Biologie du Développement (CBD). RNAi lines for *Xcbp1* (GD5597) and *Cnx99a* (GD1335, GD42397) were ordered from the VDRC. UAS::Shibire^{ts1} was provided by Dr. Kitamoto. Other fly stocks used in this paper include *UAS-mCD8GFP, TubP-Gal4, Elav-Gal4, OK107-Gal4*, *eyFlp;UAS-FRT-Stop-FRT-UAS-mCD8GFP* (Lee and Luo, 1999), *Act5C-Gal4* (Flybase), and *GMR-Gal4* (Rebay lab). WT stocks of *D.melanogaster* (Can-S and Oregon-R), *D. simulans* (MD 197), *D. mauritiana* (148 g), *D. sechelia* (Robertson), *D. yakuba* (Tai 6), *D. erecta* (151.4), and *D. pseudoobscura* (MV-25) were obtained from the University of California at San Diego Stock Center.

Immunohistochemistry, Confocal Imaging, and Brain Structure Scoring

Brains were dissected and stained via previously established protocols (Wu and Luo, 2006). Antibodies used include rat-anti-mCD8 (Developmental Studies Hybridoma Bank, DSHB), mouse-anti-nc82 (DSHB), goat-anti-rat-Alexa488 (Invitrogen), and goat-anti-mouse-Cy3 (Invitrogen). Samples were imaged on a Leica SP5 or Zeiss LSM510 confocal microscope. Anatomical structures were identified via methods outlined in Chou et al., 2010; Jefferis et al., 2002; Laissue et al., 1999; Marin et al., 2002.

Foraging Behavior Assay

We measure foraging behavior under standard experimental conditions (see Extended Experimental Procedures) by using a behavioral assay system (Figure 5A) comprised of two transparent compartments linked by a 20 × 2 mm tube: the first 7 × 2.5 × 2.5 cm compartment contains no food, while the second 25 × 1.5 × 1.5 cm compartment contains a yogurt-based food mixed with fruit odors such as strawberry and banana (Hallem and Carlson, 2004a, b, 2006). A cotton plug was inserted into the end of the second compartment to allow air exchange.

Cuticle Preparation and Scanning Electron Microscope Imaging

WT and RNAi F1 adult cuticle samples were air-desiccated, mounted on metal specimen holders with double-stick carbon disks, and coated with 8.0 nm of platinum/paladdium alloy. Samples were imaged with a NanoSEM scanning electron microscope (FEI) with the standard lens at 5 kV.

(E) Expression pattern of *Desr-Gal4* in the brain, particularly in MB axons (top panel) and calyces (ca, middle panel), the CB (top panel), and the AL (bottom panel). (F) *Xcbp1* and *Desr* (red stars) are shown on the *Drosophila* phylogeny. The node for the last common ancestor of the whole group (note E) is colored blue, while common ancestors younger than *Desr* but older than *Xcbp1* (nodes C and D) are labeled yellow, and the common ancestors younger than *Xcbp1* (nodes A and B) are labeled in green. Groups of animals with slow foraging behavior are highlighted in blue, fast foraging in green, and intermediate foraging in yellow. (See also: Figure S7)

ACCESSION NUMBERS

Sequencing data were deposited in the GEO database under accession number GSE33783.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, seven figures, three tables, six data sets, and one movie and can be found with this article online at doi:10.1016/j.celrep.2011.12.010.

LICENSING INFORMATION

ACKNOWLEDGMENTS

We thank Dr. Bourbon, Dr. Tomaru, and Dr. Kitamoto for fly stocks; GETDB and CBD for enhancer trap lines; TRiP and VDRC for RNAi lines; and Dr. Charles Langley and DPGP for population genomics data. We thank Dr. Vytas Bindokas for confocal imaging, Dr. Phillippe Laissue and Dr. Leslie Vosshall for neuroanatomy, Dr. Shujuan Lu for molecular biology, Dr. Wei Du and Xiaoxi Zhuang for fly genetics and assays, and Grace Lee and Bin He for sequence analysis. We thank Dr. Urs Schmidt-Ott, Dr. Margarida Moreira, and Benjamin Krinsky for critically reading the manuscript. We thank all Long and Luo lab members for support and discussions. S.C. was supported by an NSF Doctoral Dissertation Improvement Grant (DEB-1110607). M.S. was supported by an NIH-NRSA fellowship (F31DC008277). M.L. was supported by an NIH grant (R01GM078070-01A1), an NSF CAREER award (MCB0238168), and an NSF grant (MCB 1051826). K.P.W. was supported by an NIH grant (P50GM081892). L.L. was supported by an NIH grant (R01DC005982) and HHMI.

Received: March 7, 2011
Revised: November 7, 2011
Accepted: December 21, 2011
Published online: February 16, 2012

REFERENCES

Asahina, K., Pavlenkovich, V., and Vosshall, L.B. (2008). The survival advantage of olfaction in a competitive environment. Curr. Biol. *18*, 1153–1155.

Bai, Y., Casola, C., Feschotte, C., and Betrán, E. (2007). Comparative genomics reveals a constant rate of origination and convergent acquisition of functional retrogenes in Drosophila. Genome Biol. *8*, R11.

Beatty, J. (1995). Principles of behavioral neuroscience (Madison: Brown & Benchmark Publishers).

Begun, D.J., Holloway, A.K., Stevens, K., Hillier, L.W., Poh, Y.P., Hahn, M.W., Nista, P.M., Jones, C.D., Kern, A.D., Dewey, C.N., et al. (2007). Population genomics: whole-genome analysis of polymorphism and divergence in Drosophila simulans. PLoS Biol. *5*, e310.

Benton, R., Vannice, K.S., Gomez-Diaz, C., and Vosshall, L.B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell *136*, 149–162.

Bourbon, H.M., Gonzy-Treboul, G., Peronnet, F., Alin, M.F., Ardourel, C., Benassayag, C., Cribbs, D., Deutsch, J., Ferrer, P., Haenlin, M., et al. (2002). A P-insertion screen identifying novel X-linked essential genes in Drosophila. Mech. Dev. *110*, 71–83.

Bousum, A., Hogan, J., Price, H., and Kidd, T. (2008). Two studies of Transcriptional Control of Development. Paper presented at: Annual Drosophila Research Conference (San Diego).

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Brown, J.L. (1975). The evolution of behavior, First Edition (New York: Norton).

Burki, F., and Kaessmann, H. (2004). Birth and adaptive evolution of a hominoid gene that supports high neurotransmitter flux. Nat. Genet. *36*, 1061–1063.

Cáceres, M., Lachuer, J., Zapala, M.A., Redmond, J.C., Kudo, L., Geschwind, D.H., Lockhart, D.J., Preuss, T.M., and Barlow, C. (2003). Elevated gene expression levels distinguish human from non-human primate brains. Proc. Natl. Acad. Sci. USA *100*, 13030–13035.

Carew, T.J. (2000). Behavioral neurobiology: the cellular organization of natural behavior (Sunderland, Mass.: Sinauer Associates Publishers).

Carlson, J.R. (1996). Olfaction in Drosophila: from odor to behavior. Trends Genet. *12*, 175–180.

Chang, H.C., Paek, J., and Kim, D.H. (2011). Natural polymorphisms in C. elegans HECW-1 E3 ligase affect pathogen avoidance behaviour. Nature *480*, 525–529.

Chen, S., Zhang, Y.E., and Long, M. (2010). New genes in Drosophila quickly become essential. Science *330*, 1682–1685.

Chou, Y.H., Spletter, M.L., Yaksi, E., Leong, J.C., Wilson, R.I., and Luo, L. (2010). Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe. Nat. Neurosci. *13*, 439–449.

Crittenden, J.R., Skoulakis, E.M., Han, K.A., Kalderon, D., and Davis, R.L. (1998). Tripartite mushroom body architecture revealed by antigenic markers. Learn. Mem. *5*, 38–51.

Dai, H., Chen, Y., Chen, S., Mao, Q., Kennedy, D., Landback, P., Eyre-Walker, A., Du, W., and Long, M. (2008). The evolution of courtship behaviors through the origination of a new gene in Drosophila. Proc. Natl. Acad. Sci. USA *105*, 7478–7483.

Dallman, J.E., Allopenna, J., Bassett, A., Travers, A., and Mandel, G. (2004). A conserved role but different partners for the transcriptional corepressor CoREST in fly and mammalian nervous system formation. J. Neurosci. *24*, 7186–7193.

Davis, R.L. (2001). Mushroom bodies, Ca(2+) oscillations, and the memory gene amnesiac. Neuron *30*, 653–656.

de Belle, J.S., and Heisenberg, M. (1994). Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. Science *263*, 692–695.

de Bono, M., and Maricq, A.V. (2005). Neuronal substrates of complex behaviors in C. elegans. Annu. Rev. Neurosci. *28*, 451–501.

Denes, A.S., Jékely, G., Steinmetz, P.R., Raible, F., Snyman, H., Prud'homme, B., Ferrier, D.E., Balavoine, G., and Arendt, D. (2007). Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. Cell *129*, 277–288.

Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature *448*, 151–156.

Evans, H.E. (1962). The evolution of prey-carrying mechanisms in wasps. Evolution *16*, 468–483.

Eyre-Walker, A., and Keightley, P.D. (2009). Estimating the rate of adaptive molecular evolution in the presence of slightly deleterious mutations and population size change. Mol. Biol. Evol. *26*, 2097–2108.

Fitzpatrick, M.J., Feder, E., Rowe, L., and Sokolowski, M.B. (2007). Maintaining a behaviour polymorphism by frequency-dependent selection on a single gene. Nature *447*, 210–212.

Haddrill, P.R., Bachtrog, D., and Andolfatto, P. (2008). Positive and negative selection on noncoding DNA in Drosophila simulans. Mol. Biol. Evol. *25*, 1825–1834.

Hallem, E.A., and Carlson, J.R. (2004a). The odor coding system of Drosophila. Trends Genet. *20*, 453–459.

Hallem, E.A., and Carlson, J.R. (2004b). The spatial code for odors is changed by conditioning. Neuron *42*, 359–361.

Hallem, E.A., and Carlson, J.R. (2006). Coding of odors by a receptor repertoire. Cell *125*, 143–160.

Hallem, E.A., Dahanukar, A., and Carlson, J.R. (2006). Insect odor and taste receptors. Annu. Rev. Entomol. *51*, 113–135.

Harry, M., Solignac, M., and Lachaise, D. (1998). Molecular evidence for parallel evolution of adaptive syndromes in fig-breeding Lissocephala (Drosophilidae). Mol. Phylogenet. Evol. *9*, 542–551.

Hayashi, S., Ito, K., Sado, Y., Taniguchi, M., Akimoto, A., Takeuchi, H., Aigaki, T., Matsuzaki, F., Nakagoshi, H., Tanimura, T., et al. (2002). GETDB, a database compiling expression patterns and molecular locations of a collection of Gal4 enhancer traps. Genesis *34*, 58–61.

Haygood, R., Fedrigo, O., Hanson, B., Yokoyama, K.D., and Wray, G.A. (2007). Promoter regions of many neural- and nutrition-related genes have experienced positive selection during human evolution. Nat. Genet. *39*, 1140–1144.

Hong, C.S., and Ganetzky, B. (1996). Molecular characterization of neurally expressing genes in the para sodium channel gene cluster of drosophila. Genetics *142*, 879–892.

Jefferis, G.S., Marin, E.C., Watts, R.J., and Luo, L. (2002). Development of neuronal connectivity in Drosophila antennal lobes and mushroom bodies. Curr. Opin. Neurobiol. *12*, 80–86.

Jiménez, J.L., and Davletov, B. (2007). Beta-strand recombination in tricalbin evolution and the origin of synaptotagmin-like C2 domains. Proteins *68*, 770–778.

Kaessmann, H., Vinckenbosch, N., and Long, M. (2009). RNA-based gene duplication: mechanistic and evolutionary insights. Nat. Rev. Genet. *10*, 19–31.

Keene, A.C., and Waddell, S. (2007). Drosophila olfactory memory: single genes to complex neural circuits. Nat. Rev. Neurosci. *8*, 341–354.

Kelso, R.J., Buszczak, M., Quiñones, A.T., Castiblanco, C., Mazzalupo, S., and Cooley, L. (2004). Flytrap, a database documenting a GFP protein-trap insertion screen in Drosophila melanogaster. Nucleic Acids Res. *32* (Database issue), D418–D420.

Kitamoto, T. (2001). Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. J. Neurobiol. *47*, 81–92.

Konopka, G., Bomar, J.M., Winden, K., Coppola, G., Jonsson, Z.O., Gao, F., Peng, S., Preuss, T.M., Wohlschlegel, J.A., and Geschwind, D.H. (2009). Human-specific transcriptional regulation of CNS development genes by FOXP2. Nature *462*, 213–217.

Krashes, M.J., Keene, A.C., Leung, B., Armstrong, J.D., and Waddell, S. (2007). Sequential use of mushroom body neuron subsets during drosophila odor memory processing. Neuron *53*, 103–115.

Laissue, P.P., Reiter, C., Hiesinger, P.R., Halter, S., Fischbach, K.F., and Stocker, R.F. (1999). Three-dimensional reconstruction of the antennal lobe in Drosophila melanogaster. J. Comp. Neurol. *405*, 543–552.

Laurent, G. (2002). Olfactory network dynamics and the coding of multidimensional signals. Nat. Rev. Neurosci. *3*, 884–895.

Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron *22*, 451–461.

Lee, T., Lee, A., and Luo, L. (1999). Development of the Drosophila mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development *126*, 4065–4076.

Long, M., and Langley, C.H. (1993). Natural selection and the origin of jingwei, a chimeric processed functional gene in Drosophila. Science *260*, 91–95.

Long, M., Betrán, E., Thornton, K., and Wang, W. (2003). The origin of new genes: glimpses from the young and old. Nat. Rev. Genet. *4*, 865–875.

Lowe, C.J., Wu, M., Salic, A., Evans, L., Lander, E., Stange-Thomann, N., Gruber, C.E., Gerhart, J., and Kirschner, M. (2003). Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. Cell *113*, 853–865.

Marin, E.C., Jefferis, G.S., Komiyama, T., Zhu, H., and Luo, L. (2002). Representation of the glomerular olfactory map in the Drosophila brain. Cell *109*, 243–255.

Marioni, J.C., Mason, C.E., Mane, S.M., Stephens, M., and Gilad, Y. (2008). RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Res. *18*, 1509–1517.

Martinez-Morales, J.R., Henrich, T., Ramialison, M., and Wittbrodt, J. (2007). New genes in the evolution of the neural crest differentiation program. Genome Biol. *8*, R36.

Nègre, N., Brown, C.D., Ma, L., Bristow, C.A., Miller, S.W., Wagner, U., Kheradpour, P., Eaton, M.L., Loriaux, P., Sealfon, R., et al. (2011). A cis-regulatory map of the Drosophila genome. Nature *471*, 527–531.

Oro, J.J. (2004). Evolution of the brain: from behavior to consciousness in 3.4 billion years. Neurosurgery *54*, 1287–1296.

Osborne, K.A., Robichon, A., Burgess, E., Butland, S., Shaw, R.A., Coulthard, A., Pereira, H.S., Greenspan, R.J., and Sokolowski, M.B. (1997). Natural behavior polymorphism due to a cGMP-dependent protein kinase of Drosophila. Science *277*, 834–836.

Osborne, K.A., de Belle, J.S., and Sokolowski, M.B. (2001). Foraging behaviour in Drosophila larvae: mushroom body ablation. Chem. Senses *26*, 223–230.

Pollard, K.S., Salama, S.R., Lambert, N., Lambot, M.A., Coppens, S., Pedersen, J.S., Katzman, S., King, B., Onodera, C., Siepel, A., et al. (2006). An RNA gene expressed during cortical development evolved rapidly in humans. Nature *443*, 167–172.

Popesco, M.C., Maclaren, E.J., Hopkins, J., Dumas, L., Cox, M., Meltesen, L., McGavran, L., Wyckoff, G.J., and Sikela, J.M. (2006). Human lineage-specific amplification, selection, and neuronal expression of DUF1220 domains. Science *313*, 1304–1307.

Root, C.M., Ko, K.I., Jafari, A., and Wang, J.W. (2011). Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search. Cell *145*, 133–144.

Rosenbaum, E.E., Hardie, R.C., and Colley, N.J. (2006). Calnexin is essential for rhodopsin maturation, Ca2+ regulation, and photoreceptor cell survival. Neuron *49*, 229–241.

Semmelhack, J.L., and Wang, J.W. (2009). Select Drosophila glomeruli mediate innate olfactory attraction and aversion. Nature *459*, 218–223.

Shepherd, G.M. (1994). Neurobiology, Third Edition (New York: Oxford University Press).

Sokolowski, M.B. (1980). Foraging strategies of Drosophila melanogaster: a chromosomal analysis. Behav. Genet. *10*, 291–302.

Sokolowski, M.B., Pereira, H.S., and Hughes, K. (1997). Evolution of foraging behavior in Drosophila by density-dependent selection. Proc. Natl. Acad. Sci. USA *94*, 7373–7377.

Stocker, R.F. (1994). The organization of the chemosensory system in Drosophila melanogaster: a review. Cell Tissue Res. *275*, 3–26.

Stockinger, P., Kvitsiani, D., Rotkopf, S., Tirián, L., and Dickson, B.J. (2005). Neural circuitry that governs Drosophila male courtship behavior. Cell *121*, 795–807.

Strausfeld, N.J., and Li, Y. (1999a). Organization of olfactory and multimodal afferent neurons supplying the calyx and pedunculus of the cockroach mushroom bodies. J. Comp. Neurol. *409*, 603–625.

Strausfeld, N.J., and Li, Y. (1999b). Representation of the calyces in the medial and vertical lobes of cockroach mushroom bodies. J. Comp. Neurol. *409*, 626–646.

Strausfeld, N.J., Hansen, L., Li, Y., Gomez, R.S., and Ito, K. (1998). Evolution, discovery, and interpretations of arthropod mushroom bodies. Learn. Mem. *5*, 11–37.

Tanaka, N.K., Tanimoto, H., and Ito, K. (2008). Neuronal assemblies of the Drosophila mushroom body. J. Comp. Neurol. *508*, 711–755.

Tomancak, P., Berman, B.P., Beaton, A., Weiszmann, R., Kwan, E., Hartenstein, V., Celniker, S.E., and Rubin, G.M. (2007). Global analysis of patterns

of gene expression during Drosophila embryogenesis. Genome Biol. *8*, R145.

Vibranovski, M.D., Zhang, Y., and Long, M. (2009). General gene movement off the X chromosome in the Drosophila genus. Genome Res. *19*, 897–903.

Wheeler, D.A., Kyriacou, C.P., Greenacre, M.L., Yu, Q., Rutila, J.E., Rosbash, M., and Hall, J.C. (1991). Molecular transfer of a species-specific behavior from Drosophila simulans to Drosophila melanogaster. Science *251*, 1082–1085.

Wu, J.S., and Luo, L. (2006). A protocol for dissecting Drosophila melanogaster brains for live imaging or immunostaining. Nat. Protoc. *1*, 2110–2115.

Zhang, J. (2003). Evolution of the human ASPM gene, a major determinant of brain size. Genetics *165*, 2063–2070.

Zhang, Y.E., Vibranovski, M.D., Krinsky, B.H., and Long, M. (2010). Age-dependent chromosomal distribution of male-biased genes in Drosophila. Genome Res. *20*, 1526–1533.