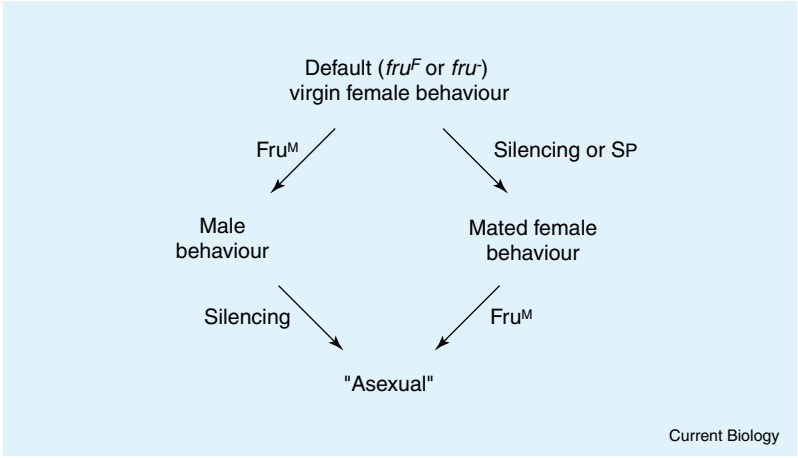

Figure 2. Control of sexual behavior by *fru*^{GAL4} neurons.

Model for the role of *fru*^{GAL4} neurons in *Drosophila* sexual behaviour, based on the results presented in Figure 1 for female behaviours and in [1] for male behaviours. Male mating behaviour is observed in both males and females, provided Fru^{M} is present. Virgin and mated female behaviours are only observed in females (they are not anatomically possible in males). Flies in which the Fru^{M} neurons are both silenced and masculinized perform neither male behaviour, virgin female behaviour, nor mated female behaviour, irrespective of their sex.

(copulation and egg-laying, respectively; Figure 1 experiments 6 and 13, and [1]). Silencing the *fru*^{GAL4} neurons in *fru*^{M} virgin females does not induce egg-laying, as it does in wild-type (*fru*^{F}) females (Figure 1C, experiment 7). Thus, Fru^{M} appears to reconfigure the circuit for male rather than female behaviour in a way that cannot be explained entirely by altered patterns of neuronal activity.

We infer from these data that the distinct reproductive behaviours of males and females are mediated by a common *fru*^{GAL4} neural circuit (Figure 2). Activation of this circuit is required for mating behaviour, which is manifested as male behaviour in *fru*^{M} males and females, but as female behaviour in *fru*^{F} females. In females, the transition from pre-mating to post-mating behaviour is triggered experimentally by silencing these neurons (Figure 1), or naturally by the sex peptide transferred in the male's seminal fluid [5,6]. Sex peptide may therefore promote post-mating behaviours by modulating the activity of the *fru*^{GAL4} circuit in females.

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Positive selection on gene expression in the human brain

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Recent work has shown that the expression levels of genes transcribed in the brains of humans and chimpanzees have changed less than those of genes transcribed in other tissues [1]. However, when gene expression changes are mapped onto the evolutionary lineage in which they occurred, the brain shows more changes than other tissues in the human lineage compared to the chimpanzee lineage [1–3]. There are two possible explanations for this: either positive selection drove more gene expression changes to fixation in the human brain than in the chimpanzee brain, or genes expressed in the brain experienced less purifying selection in humans than in chimpanzees, i.e. gene expression in the human brain is functionally less constrained. The first scenario would be supported if genes that changed their expression in the brain in the human lineage showed more selective sweeps than other genes. Unfortunately, current human genome-wide DNA sequence variation do not allow signatures of selective sweeps to be inferred using frequency-based approaches [4,5]. However, estimates of linkage disequilibrium (LD) — i.e. the extent of non-random association of alleles along chromosomes — are expected to be largely unaffected by frequency ascertainment bias [5], and selective sweeps are expected to increase the amount of LD around a selected gene variant [6–9].

We, thus, analyzed genome-wide LD patterns in three human populations

in conjunction with gene expression in brain, heart, kidney and liver from six humans, five chimpanzees [1], one orang-utan and six rhesus macaques (Supplemental Data). The evolution of human gene expression appears to be largely consistent with the neutral theory of evolution [10]. Furthermore, increased LD caused by selective sweeps is relatively short-lived [8,11] and detectable only for cis-regulatory events. Therefore, any signals of positive selection are expected to be weak. In order to detect signals that increase with the magnitude of expression change, we used progressively increasing cut-offs for expression differences between humans and chimpanzees. Moreover, we enriched genes that changed in the human lineage by using a third species (orang-utan or rhesus macaques) as an outgroup. Finally, we functionally grouped genes based on the gene ontology annotation [12], assuming that genes with similar functions may have experienced similar selection pressures (Supplemental Data).

In the brain, but not in the other three tissues analyzed we find a positive correlation between expression divergence in the human lineage and LD (Figure 1). This correlation increases with the extent of the expression difference and is significantly greater than that observed for 1000 random permutations of LD values in all three populations (p = 0.003 for Africans, p = 0.001 for Europeans, p = 0.001 for Chinese; not corrected for multiple testing in four tissues). However, this is not the case in the other three tissues (p > 0.1; not corrected for multiple testing). Notably, this correlation is not caused by genes expressed exclusively in the brain, as results remain unchanged if the analysis is restricted to genes expressed in all four tissues (data not shown). Furthermore, we do not find a positive correlation between LD and the proportion of expression divergence in the chimpanzee lineage in any of the tissues (data not shown).

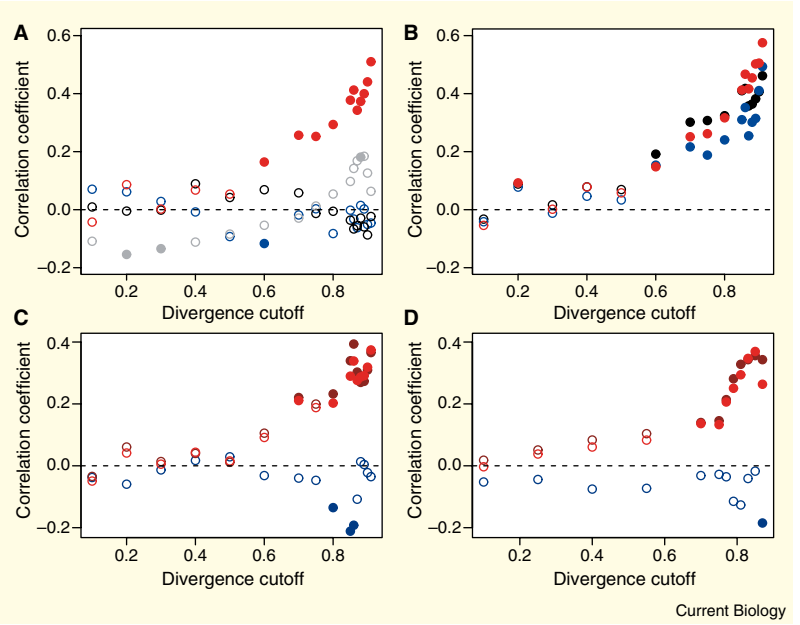

Figure 1. Indication of positive selection on brain gene expression in humans.

Correlation between expression divergence in the human lineage and size of linkage disequilibrium (LD) regions in (A) four different tissues (red: brain; blue: heart; black: kidney; grey: liver); (B) for brain in three human populations (red: Chinese; blue - Europeans; black: Africans); (C) for brain corrected for recombination rate variation across the genome (dark red: LD size with no correction; red: LD size corrected for the recombination rate by partial correlation; dark blue: direct correlation between recombination rate and expression divergence on the human lineage); (D) for brain corrected for recombination rate variation across the genome using independent data for ten humans, one chimpanzee and six macaques (symbols as in (C)). Expression divergence cut-offs are shown as quantiles of the divergence distribution for all genes expressed in a given tissue. Thus, the 0.8 quantile cut-off corresponds to 20% of all expressed genes with the largest expression divergence between humans and chimpanzees in a given tissue. Points represent Spearman rank correlation coefficients for single human populations (B, all measures; C and D, recombination rate measures) or an average of three human populations. The filled circles indicate significant correlations for all included populations at the 1% level (not corrected for multiple testing).

Because recombination rates vary across the genome [13,14], large areas of LD may reflect regions of low recombination rates rather than positive selection. To correct for this, we calculated a partial correlation between expression divergence in humans and LD, after accounting for differences in recombination rates across genomic regions (Supplemental Data). This affected neither the results for the brain (Figure 1C) nor for the other tissues (data not shown). Thus, differences in recombination rates cannot explain most of the positive correlation between expression divergence and the extent of LD. Furthermore, we tested whether a positive correlation is seen using an independent expression dataset for ten humans, six chimpanzees (five were used in the previous study), and six rhesus macaques. Again, we find a positive correlation between expression divergence in the human lineage and the extent of LD in all three human populations, which is independent of differences in recombination rates (Spearman rank correlation test, p < 0.01; Figure 1D).

LD depends on many factors and is not a direct indicator of positive selection. We thus selected, based on function as defined by gene ontology, 22 groups of genes that constitute 5% of the most extreme cases in terms of both expression change in the human lineage and high LD (Supplemental data). We measured positive selection by an excess of rare DNA sequence variants in resequencing projects, where ascertainment of SNPs did not present a problem, as full

sequence data were collected for all individuals. For this purpose, we used the 762 genes in two databases (SeattleSNPs, NIEHS Environmental Genome Project), 402 of which show brain expression in our data set. We find that Tajima's D, a measure of the excess of rare sequence variants, is lower in the 22 gene orthology groups than in the remaining genes expressed in brain ($p = 0.0018$, one-sided Wilcox test). When genes are resampled to yield 10,000 data sets containing the same number of genes taken from the two resequencing databases, as in the observed data set, this observation remains significant ($p = 0.01$).

In summary, our results indicate that the acceleration of gene expression changes in the human lineage is at least partially due to positive selection rather than to a relaxation of functional constraint. It is still possible that these observations are caused by yet unknown factors that do not involve positive selection. However, a non-selective scenario would need to include factors affecting only the brain, and to explain the excess of low-frequency nucleotide variants found in the group of brain-expressed genes with the largest LD and human lineage-specific expression divergence. It should be noted, however, that our results do not necessarily rule out positive selection on gene expression in other tissues. First, the slower rate of expression evolution in brain compared to other tissues [1] may make the assignment of brain expression changes to evolutionary lineages more precise due to fewer recurrent changes. Second, the effects of a selective sweep on LD disappear relatively quickly and can only be detected if associated with cis-regulatory changes. Thus, other tissues may still contain substantial numbers of positively selected changes that either occurred early during human evolution or are mediated by in trans-effects.

Interestingly, the top 5% of gene orthology groups with the highest expression divergence as well as the highest LD contain many genes involved in metabolism (Supplemental data). These genes, most noticeably the ones involved in electron transport and energy pathways, increased significantly in expression in the human versus the chimpanzee brain. It has been argued that amino acid changes in proteins involved in these pathways might have been positively selected for, due to changes in brain size and lifespan [15].

When did positive selection for expression changes in the brain take place? As the increase in LD is seen in human populations on three continents, the selective events are likely to have occurred before these populations separated, less than 100,000 years ago. However, positive selection can be detected for only approximately 200,000 years [5,8,11]. Because we observe correlation between LD and expression changes at the level of functional groups rather than individual genes, some or many of the expression changes selected for in the human lineage may have occurred more than to 200,000 years ago. Consequently, our results do not show that all of the expression changes in brain observed in the human lineage can be explained by events within the last 200,000 years. However, a detectable proportion of these events is recent and potentially associated with the origin of modern humans prior to their spread out of Africa.

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Supplemental data

Supplemental data including Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/16/10/R356/DC1/

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