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Epigenetics of brain development in workers of the Western honeybee, Apis mellifera*.*

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Worker honeybees change their tasks in the colony with aging from nursing larvae in the hive to foraging in the field. This task transition involves extensive changes of gene expression in the brain and an increase in learning and memory ability. These changes are thought to be regulated by epigenetic mechanisms including DNA methylation. In this study, we compared DNA methylation pattern in brains of nurses and foragers by whole genome bisulfite sequencing to identify differently methylated genes that may be involved in brain development. DNA was extracted from brains of 105 nurses and 195 foragers and subjected to bisulfite treatment. Next generation sequencing were performed using a HiSeq2000 (Illumina), and sequence reads were mapped to the *A. mellifera* genome (Amel 4.5). The *A. mellifera* genome contains 9,498,691 CpG sites, of which more than 80% were covered with at least 5 sequence reads in both nurses and foragers. In these CpG sites, 54,174 sites in nurses and 69,210 sites in foragers were significantly methylated compared with the background level (binomial test with the method of Benjamini and Hochberg, $p<0.05$), suggesting that methylated sites are only approximately 1% of the total CpG sites. Most of the methylated sites were on exons. In comparison between nurses and foragers, 774 CpG sites showed significantly different methylation level: 419 sites were significantly more methylated in nurses than foragers and 355 sites were more methylated in foragers than nurses. More than 85% of these differently methylated sites were placed on genes (exons and introns). We identified 439 genes that include differently methylated CpG sites.