

Genome composition, caste, and molecular evolution in eusocial insects

Kent et al. (1) recently investigated genome evolution in the honey bee and proposed “strong links between recombination, biased gene conversion, GC-content evolution, and worker behavior.” However, we feel these connections may be more nuanced than suggested.

Kent et al. (1) illustrated dramatically higher guanine and cytosine (GC) content for worker-biased genes than for queen-biased and drone-biased genes (Fig. 2B of ref. 1). These results were based on combining two studies that separately investigated queen-worker and drone-worker expression differences.

However, we found that a direct comparison of queen and worker gene expression showed only small differences in third codon position GC content (Fig. 1A). Thus, the exceptionally high GC-content profile noted by Kent et al. (1) arises in worker-biased genes only when defined relative to drone-biased genes (Fig. 1B). Moreover, the correlation between GC content and the ratio of queen-to-worker gene expression is actually not significant (Spearman’s $\rho = -0.014$, $P = 0.4444$; $n = 2,864$ genes), whereas the correlation between GC content and the absolute value of this ratio is highly significant (Spearman’s $\rho = 0.148$, $P < 0.0001$; $n = 2,864$ genes). This suggests that queen- and worker-biased genes both display high GC content relative to unbiased genes in the honey bee (Fig. 1A).

To provide greater perspective to the relationship between eusociality and GC content, we analyzed data from two ants (2). We found that GC content for worker-biased genes was significantly higher than for queen-biased genes in each species, consistent with Kent et al.’s hypothesis (1) (Fig. 2A and B). However, differences of a similar (Fig. 2C) or larger (Fig. 2D) magnitude were observed between egg-biased and larval-biased genes. Overall, these analyses suggest that differences in GC content are not uniquely associated with queen-worker differences but may be a more general property of genes showing biased expression among phenotypes (3).

Kent et al. (1) further investigated sequence divergence for 23 honey bee genes, suggesting that “higher recombination rates increase the evolutionary rate of genes associated with worker behavior.” To support this assertion, Kent et al. (1) showed that GC content, reported to be high in worker-biased genes, was positively correlated with both recombination rate and evolutionary rate. However, in contrast to the hypothesis that worker genes tend to evolve rapidly, an analysis of 1,511 honey bee genes reported that (i) worker-biased genes evolved at lower rates than queen-biased genes and (ii) GC content was negatively correlated with the rate of protein evolution (4).

To investigate this discrepancy further, we assessed the 13 genes present in both datasets (1, 4) and observed a positive, albeit nonsignificant, correlation between GC content and the rate of protein evolution as measured by Hunt et al. (4) (Spearman’s $\rho = 0.154$, $P = 0.6158$). This relative agreement between datasets raises the possibility that the discrepancy between published results (1, 4) did not arise due to differences in evolutionary time scale or methodology, but perhaps due to differences in the number of loci investigated. This prospect suggests that more loci are needed to resolve whether accelerated protein evolution became coupled with high GC content and worker behavior following the advent of eusociality.

ACKNOWLEDGMENTS. This work was supported by US National Science Foundation Grant DEB-0640690.

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Author contributions: B.G.H., K.M.G., and M.A.D.G. designed research; B.G.H. and K.M.G. performed research; B.G.H. and K.M.G. analyzed data; and B.G.H., K.M.G., and M.A.D.G. wrote the paper.

The authors declare no conflict of interest.

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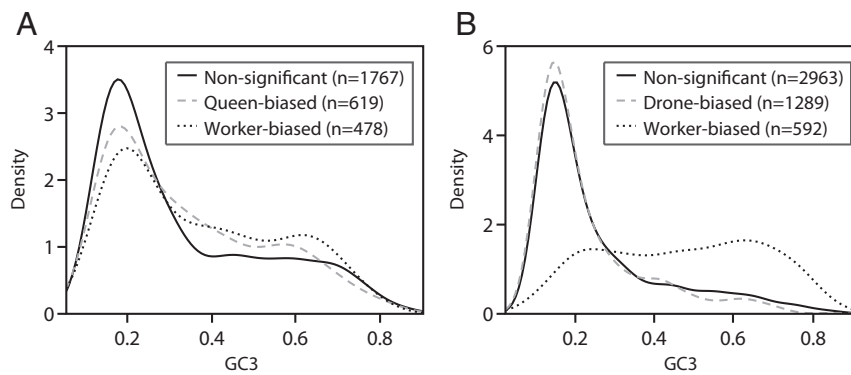


Fig. 1. GC content is weakly associated with eusocial specialization in the honey bee *Apis mellifera*. (A) Comparison of third codon position GC content (GC3) based on gene expression differences between brains of queens and sterile workers. According to Wilcoxon rank-sum tests, worker-biased genes differ significantly in GC3 from nonsignificantly biased genes ($P < 0.0001$) and queen-biased genes ($P = 0.0218$). (B) Comparison of GC3 based on gene expression differences between brains of drones and workers. Worker-biased genes exhibit dramatic, significant differences in GC3 compared with nonsignificantly biased genes ($P < 0.0001$) and drone-biased genes ($P < 0.0001$), but differences of this magnitude are not inherent to the female caste specialization that underpins eusocial behavior shown in A.

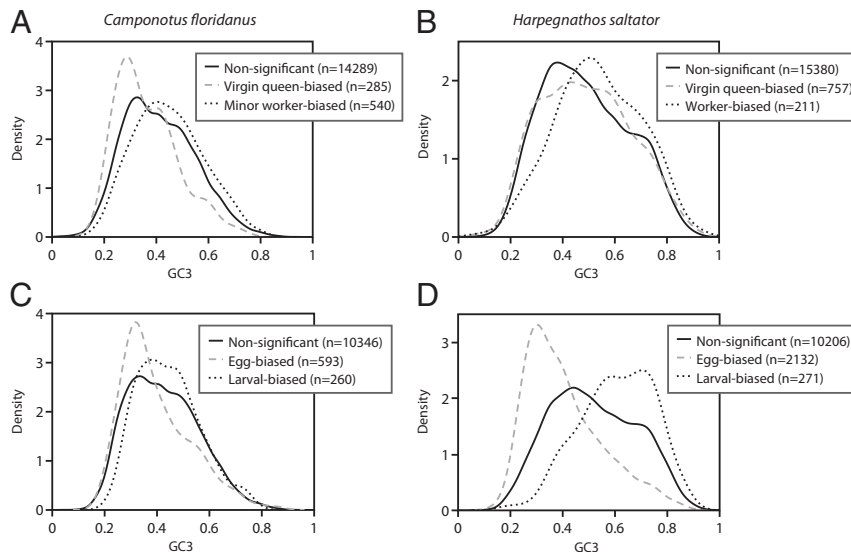


Fig. 2. GC content differs according to caste- and developmentally biased gene expression in two ant species. A comparison of third codon position GC content (GC3) based on gene expression differences between bodies of virgin queens and workers from *Camponotus floridanus* and *Harpegnathos saltator* is made. According to Wilcoxon rank-sum tests, *C. floridanus* worker-biased genes differ significantly in GC3 from nonsignificantly biased genes ($P < 0.0001$) and queen-biased genes ($P < 0.0001$) (A), and *H. saltator* worker-biased genes differ significantly in GC3 from nonsignificantly biased genes ($P < 0.0001$) and queen-biased genes ($P = 0.0006$) (B). *C. floridanus* larval-biased genes differ significantly in GC3 from nonsignificantly biased genes ($P < 0.0102$) and from egg-biased genes ($P < 0.0001$) (C), and *H. saltator* larval-biased genes differ significantly in GC3 from nonsignificantly biased genes ($P < 0.0001$) and egg-biased genes ($P < 0.0001$) (D). To approximate pairwise differential expression calls from these unreplicated RNA-seq data, we used NOISeq (5) to assign significantly biased categories based on simulated replication.