doi:10.1111/evo.13356

Gene duplication and the evolution of phenotypic diversity in insect societies

Linh M. Chau[1] and Michael A. D. Goodisman[1,2]

[1]*School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332*

[2]*E-mail: michael.goodisman@biology.gatech.edu*

Received March 18, 2017
Accepted August 31, 2017

Gene duplication is an important evolutionary process thought to facilitate the evolution of phenotypic diversity. We investigated if gene duplication was associated with the evolution of phenotypic differences in a highly social insect, the honeybee *Apis mellifera*. We hypothesized that the genetic redundancy provided by gene duplication could promote the evolution of social and sexual phenotypes associated with advanced societies. We found a positive correlation between sociality and rate of gene duplications across the Apoidea, indicating that gene duplication may be associated with sociality. We also discovered that genes showing biased expression between *A. mellifera* alternative phenotypes tended to be found more frequently than expected among duplicated genes than singletons. Moreover, duplicated genes had higher levels of caste-, sex-, behavior-, and tissue-biased expression compared to singletons, as expected if gene duplication facilitated phenotypic differentiation. We also found that duplicated genes were maintained in the *A. mellifera* genome through the processes of conservation, neofunctionalization, and specialization, but not subfunctionalization. Overall, we conclude that gene duplication may have facilitated the evolution of social and sexual phenotypes, as well as tissue differentiation. Thus this study further supports the idea that gene duplication allows species to evolve an increased range of phenotypic diversity.

KEY WORDS: *Apis mellifera*, caste, dimorphism, gene duplication, gene expression, Hymenoptera, sociality.

Individuals within species often belong to distinct phenotypic classes that have different functional roles. These classes (e.g., sexes) may experience contrasting selection pressures on traits associated with their distinct roles. Therefore, alleles favored in one class may be disfavored in the other if different classes share a majority of their genome (Bonduriansky and Chenoweth 2009; Stewart et al. 2010; Pennell and Morrow 2013). Contrasting selection pressures may ultimately displace individuals of both classes from their phenotypic optima. Overall, this "intralocus conflict" represents a fundamentally important process inhibiting adaptation within species (Lande 1980; Rice and Chippindale 2001; Bonduriansky and Chenoweth 2009; Pennell and Morrow 2013; Rice 2013). Problems arising from intralocus conflict can be reduced through mechanisms that decouple the trait genetic correlation between the different phenotypic classes (Lande 1980). This allows each class to express different trait values in reaction to their contrasting selection pressures.

This article corresponds to Harpur, B. A. and N. M. A. Smith. 2017. Digest: Gene duplication and social evolution—Using big, open data to answer big, open questions. Evolution. https://doi.org/10.1111/evo.13390.

Gene duplication has been hypothesized to be a mechanism capable of relieving intralocus conflict (Ellegren and Parsch 2007; Connallon and Clark 2011; Gallach and Betran 2011). After a gene is duplicated, a pair of paralogs are created, each highly similar in sequence and redundant in function (Gu et al. 2003). Such redundancy is thought to release a single paralog from selection after the duplication event, since there is an exact copy retaining its original function (Ohno 1970; Lynch and Conery 2000). Mutation can then alter the function of the focal paralog, which will ultimately determine whether the gene pair is preserved in the genome (Proulx 2012; Cardoso-Moreira et al. 2016).

Gene duplicates are generally thought to undergo one of five possible outcomes within the genome: pseudofunctionalization, conservation, neofunctionalization, subfunctionalization, or specialization (Ohno 1970; Force et al. 1999; Lynch and Conery 2000; He and Zhang 2005; Innan and Kondrashov 2010). Most gene duplicates are expected to undergo pseudofunctionalization, which occurs when one paralog is silenced by mutations and becomes nonfunctional (Lynch and Conery 2000). However, there are circumstances that allow both paralogs to be functional and remain in the genome. Under conservation, the ancestral function

of both paralogs is conserved because there is selective advantage for increased dosage (Ohno 1970). A paralog may also gain novel functions through the process of neofunctionalization (Ohno 1970). Alternatively, the function of the ancestral single copy gene may be divided amongst the two paralogs through subfunctionalization (Force et al. 1999). In this case, both paralogs are subjected to a loss of certain ancestral subfunctions. Thus, to maintain the function of the original, ancestral single-copy ortholog, both paralogs must be preserved (Force et al. 1999). Finally, specialization occurs when neofunctionalization and subfunctionalization work together, creating two copies that are distinct from each other and the ancestral gene (He and Zhang 2005).

Social insects are interesting taxa in which to study the importance of gene duplication in the amelioration of intralocus conflict. These insects, which include the social bees, ants, social wasps, and termites, are among the most dominant organisms on earth (Wilson 1990). The success of social insects arises, in part, from the caste system in which multiple distinct classes of individuals are responsible for completing different tasks within the colony (Wilson 1990).

Hymenopteran insect societies usually consist of three castes: queens, workers, and males. Queens and males are responsible for reproduction and dispersal. Workers perform tasks related to colony growth and maintenance, like brood care and foraging for food. Workers may be further subdivided into behavioral subcastes, such as nurses and foragers (Seeley 1982; Whitfield et al. 2003). The difference in behavior among the castes is often paired with drastic differences in morphology and physiology (Toth et al. 2010; Feldmeyer et al. 2014). Therefore, different castes experience strongly divergent selection pressures.

Importantly, hymenopteran social insect castes share a common genome (though males are haploid and female workers and queens are diploid) (Normark 2003). Thus genetic correlations between the castes can limit the evolution of caste dimorphism in reaction to divergence selection pressures (Gadagkar 1997; Linksvayer and Wade 2005; Kovacs et al. 2010; Hall et al. 2013). Consequently, social insect castes may suffer a variety of intralocus conflicts, which may impede the elaboration of caste differences and limit the evolution of sociality.

The purpose of this study was to investigate if the genetic material provided by gene duplication ameliorated intralocus conflict, facilitating the specialization of social phenotypes within insect societies (Holman 2014). We hypothesized that the evolution of caste specialization was initially constrained by intralocus conflict. We further conjectured that this conflict may have been lessened through the process of gene duplication (Gadagkar 1997). Specifically, duplicated genes may have been co-opted in the development of different castes and thereby allowed the evolution of caste-specific function.

We investigated if gene duplication might be associated with the diversification of castes in the honey bee, *Apis mellifera*. *A. mellifera* societies contain standard queen, worker, and male castes, as well as nurse and forager worker behavioral subcastes. The presence of these alternative phenotypes, and the wealth of data on gene expression differences among castes (Whitfield et al. 2003; Zayed et al. 2012; Cameron et al. 2013; Elsik et al. 2014; Jasper et al. 2015; Ashby et al. 2016), makes *A. mellifera* an ideal system to study the role of duplication in the evolution of alternative phenotypes.

We studied the effects of gene duplication on castes in the honeybee using several approaches. First, we examined the relationship between the level of sociality and gene duplication across the Apoidea to determine if gene duplication was generally associated with the evolution of complex social behavior. Second, we explored differences in biased gene expression between duplicated genes (paralogs) and nonduplicated genes (singletons) within *A. mellifera*. We hypothesized that gene duplication would accelerate the rate of expression divergence between phenotypes by providing new copies of genes that could be co-opted in the development of differential expression. Therefore, we predicted that duplicates would be more likely to be differentially expressed between castes and sexes than singletons (Huminiecki and Wolfe 2004). Third, we examined expression divergence between duplicate pairs. We hypothesized that duplicates gained divergent functions among phenotypes (Connallon and Knowles 2005; Innocenti and Morrow 2010). Therefore, we expected to find divergent expression patterns between duplicated genes. Finally, we examined the evolutionary processes that maintained paralogs in the genome. We predicted that there would be a high proportion of duplicates that were maintained by processes that led to functional diversification like specialization, subfunctionalization, and neofunctionalization. Overall, this study provides new information on the role of gene duplication in the evolution of dimorphism, intralocus conflict, and sociality.

Methods

IDENTIFICATION OF DUPLICATE GENES AND DUPLICATION RATES

We downloaded gene families from OrthoDB v9.1 (Zdobnov et al. 2017). We used custom perl scripts to parse gene duplicates that were duplicated in *A. mellifera* but were single-copy across other Apoidea. We also identified novel duplicates in nine other species in Apoidea, *Apis florea*, *Bombus impatiens*, *Bombus terrestris*, *Eufriesea Mexicana*, *Durfourea novaengliae*, *Habropoda laboriosa*, *Lassioglossum albipes*, *Megachile rotundata*, and *Melipona quadrifasciata*, which vary in level of sociality (Kapheim et al. 2015). We determined species-specific duplication rates by incorporating divergence times from Cardinal and Danforth (2013).

We examined the relationship between species-specific duplication rate and sociality independent of phylogenetic relationship using phylogenetic independent contrasts (PICs) (Felsenstein 1985). PICs for species-specific duplicates per million years and sociality values were generated with the R package Analysis of Phylogenetics and Evolution (APE) (Paradis et al. 2004). This analysis relied on a phylogenetic tree and distances based off of Cardinal and Danforth (2013) and Kapheim et al. (2015): ((Hlab:91, ((Mqua:68, (Bimp:13, Bter:13):55):10, (Emex:62, (Amel:19,Aflo:19):43):16):13):15, Mrot:106):9, (Dnov:85, Lalb:85):30). The species were assigned sociality values from Kapheim et al. (2015): 0 represented solitary species, 1 represented facultative basic eusocial, 2 represented obligate basic eusocial, and 3 represented complex eusocial species. The relationship between the level of sociality and rates of gene duplication per million years were then determined using the Spearman's rank correlation.

GENE EXPRESSION DATA AND ANALYSIS

We investigated patterns of gene expression within *A. mellifera* to understand the relationship between gene expression and gene duplication. We obtained *A. mellifera* RNAseq reads from four different studies that investigated expression differences between *A. mellifera* female castes (queens and workers), sexes (workers and drones), worker behavioral states (nurses and foragers), and worker tissues (Cameron et al. 2013; Jasper et al. 2015; Ashby et al. 2016; Vleurinck et al. 2016). Ashby et al. analyzed expression differences between *A. mellifera* queen, worker, and drone whole body larvae at stage L5 (PRJNA260604). Similarly, Vleurinck et al. assessed caste and sex differences by investigating gene expression in the brains of *A. mellifera* queen, worker, and drone pupae (stages 4–5) (PRJNA193691). In contrast, Cameron et al. studied expression in 60 hour (L3 larval stage) whole body *A. mellifera* queens and workers (PRJNA227348). Finally, Jasper et al. examined gene expression in *A. mellifera* adult worker nurses and foragers across 10 tissues: brain, antennae, midgut, hypopharyngeal gland, malpighian tubule, mandibular gland, muscle, nasonov gland, sting gland, and second thoracic ganglia (PRJNA243651 & PRJNA211831).

All RNAseq data were downloaded from NCBI's sequence read archive. The qualities of the raw RNA-Seq reads were assessed with FastQC v0.11.5 (Andrews 2010). Reads were then trimmed with Trimmomatic v 0.35 (Bolger et al. 2014). Adapter sequences and low-quality bases were trimmed from either side of each read. A sliding window with a minimum quality score of 15 was applied to each read. RSEM 1.3.0 (RNA-Seq by Expectation Maximization) was used to measure expression levels (Li and Dewey 2011). RSEM was used with the Bowtie2 (version 2.2.2) aligner to align reads to the *A. mellifera* reference gene set (Amel OGSv3.2; http://hymenopteragenome.org/beebase/) (Langmead and Salzberg 2012). Expected read count was measured with RSEM with default settings. Bowtie2 within RSEM does not allow for indel, local, and discordant alignments, which may lead to lower alignment rates compared to Bowtie2 itself (Li and Dewey 2011). Also, the use of RSEM allows for the mapping of nonuniquely mapped reads that may have an impact on measuring the expression of duplicate genes. Details of the alignment procedure for each dataset are provided in Table S1.

Each RSEM file was concatenated into single dataset and differential expression of genes was determined with edgeR v 3.16.0 (Robinson et al. 2010). The trimmed mean of M values (TMM) method was used for normalization of gene expression. Pairwise comparisons were made between castes (queens and workers), sexes (drones and workers), and behavioral states (nurses and foragers, brains only) to identify differentially expressed genes. The false discovery rate (FDR) was calculated using the Benjamini–Hochberg correction; an FDR less than or equal to 0.05 was considered significant (Benjamini and Hochberg 1995). Levels of differential expression were calculated as the absolute value of the $\log_2$ fold change between each pair. We calculated tissue expression specificity, τ, per gene across ten tissues (Yanai et al. 2005; Atallah et al. 2013; Jasper et al. 2015). Tau ranges from 0 to 1 with low values indicating that a gene is broadly expressed among tissues and high values indicating that a gene is expressed in few tissues.

We investigated if the frequency of different phenotype-biased genes differed between duplicated genes and singletons. Genes were classed into phenotype-biased gene categories (i.e., phenotypically biased or phenotypically unbiased) based off the FDR cut-off and the magnitude of the expression difference. Genes that had an FDR of 0.05 and fold change equal or greater than two were classified as biased. In contrast, genes that fell outside of these ranges were classified as unbiased. Next, we used a chi-squared test to determine if the proportion of phenotype-biased genes depended on whether the genes were duplicates or singletons. Tests were conducted for caste-biased, sex-biased, and behavior-biased genes. Wilcoxon rank-sum tests were used to compare the levels of expression bias between duplicated genes and singletons.

Each pair of duplicate genes was then categorized based on the pair's joint pattern of expression bias. For example, both copies of a duplicated gene in a queen-worker comparison could show concordant expression, with both genes having the same expression bias (e.g., both queen-biased). Alternatively, the paralogs could show discordant expression patterns with one paralog being more highly expressed in one caste than the other, or one paralog could be caste-biased and the other unbiased. The expected proportions of each paired class were generated by randomly sampling genes 10,000 times from the pool of duplicate pairs to create null distributions of paired genes (Mikhaylova et al. 2008;

Wyman et al. 2012). The mean proportions generated from the null distribution provided the expected proportions of each class (Mikhaylova et al. 2008; Wyman et al. 2012). Chi-squared tests were then used to compare the observed proportions of gene pairs falling into each class to the expected proportions constructed from the randomization approach.

Expression divergence between duplicate gene pairs was calculated for caste-, sex-, behavior-biased expression, and tau. Divergence was calculated as the absolute value of $(x - y)/(x + y)$, with x being the expression measure in one paralog and y being the expression measure in the other. We then used Wilcoxon rank-sum tests to compare the level of expression divergence between duplicates on the same chromosome and different chromosome to determine if the location of duplicate genes in the genome was associated with gene expression divergence between duplicates.

We investigated if there was a correlation in expression bias for paralogs within duplicate pairs. This analysis determined if a paralog that showed highly biased expression in one phenotypic comparison (e.g., queens vs workers) also tended to show highly biased expression in another (e.g., males vs females). We then used the program JMP 11 to perform a multivariate analysis of variance (MANOVA) with the duplicate pair as the independent variable and all measures of phenotype-biased expression (i.e., caste-bias, sex-bias, etc.) as dependent variables. This analysis produced a partial correlation matrix that provided information on whether paralogs tended to show correlations in expression-bias.

IDENTIFYING MODELS OF DUPLICATE GENE MAINTENANCE

We used the methodology of Assis and Bachtrog (2013) to determine the processes that maintained duplicates in *A. mellifera*. Briefly, this method considers the relationships among multiple Euclidean distances between the expression profiles of a single copy ortholog in a closely related species, the expression profiles of both duplicate genes in the focal species, and the combined expression profile of the duplicates. Comparison of these expression distances provides insight into whether conservation, neofunctionalization, subfunctionalization, or specialization maintains the focal duplicate pair in the genome.

We implemented Assis and Bachtrog's approach to identify the evolutionary processes maintaining duplicates in the *A. mellifera* genome (Assis and Bachtrog 2013). We identified genes that were duplicated in *A. mellifera*, but were in single copy in the social bee, *Bombus terrestris*, using custom perl scripts in the OrthoDB v9.1 database (Zdobnov et al. 2017). We used sequence similarity measures from BLAST to classify each *A. mellifera* paralog in a pair as the "D1" or "D2" copy (Assis and Bachtrog 2013; Wang et al. 2016). We used BLASTp to compare each paralog to the single copy ortholog in *B. terrestris,* using the e-value, identity, and alignment length as a measure of sequence similarity. D1 paralogs were those with higher sequence similarity (lower e-value, high identity, and long alignment length) to the *B. terrestris* ortholog whereas the D2 paralogs were those with lower sequence similarity (higher e-value, low identity, and shorter alignment length) to the ortholog. We generated the gene expression profiles for *B. terrestris* queens, workers, and males at adult, larval, and pupal stages using the same methods previously provided for determining expression differences in *A. mellifera* (Harrison et al. 2015). We then determined the processes maintaining duplicates with the R package CDROM (Perry and Assis 2016).

SEQUENCE EVOLUTION OF DUPLICATE GENES

We investigated patterns of sequence divergence of duplicate genes to examine how rates of sequence evolution differed between duplicate pairs. *A. mellifera* (OGSv3.2) duplicates and *B. terrestris* single copy orthologs (NCBI build 1.1) sequences were aligned using MACSE v1.02 (Ranwez et al. 2011). Gene trees were created under the assumption the duplicates were most closely related and the single copy ortholog was used as the outgroup. The codeml package within PAML (v4.7) was used to measure synonymous and nonsynonymous branch-specific substitution rates of the duplicate genes (Yang 2007). All genes with $dS > 3$ were considered to be saturated with mutations and removed from the analysis.

Results

GENE DUPLICATION RATES ACROSS THE APOIDEA

We identified the number of species-specific duplicates across different bee species within Apoidea (Fig. 1). We then determined the rates of species-specific duplication events for each lineage. We found that *A. mellifera* had the highest rate of duplication at 6.1 duplicates per million years. In contrast, bees considered ancestrally solitary, such as *Dufourea novaeangliae* and *Megachile rotundata*, had rates lower than 0.4 duplications per million years. Overall, we observed a significant, positive correlation between the level of sociality and rate of species-specific duplication across the Apoidea ($\rho = 0.6566$, $P = 0.0392$; uncorrected Spearman's correlation) suggesting that gene duplication might be associated with the evolution of sociality in bees. However, when we performed the analysis with the phylogenetic corrected level of sociality and rate of species-specific duplication, the correlation was no longer significant ($\rho = 0.5021$, $P = 0.1684$; phylogenetically corrected Spearman's correlation).

DIFFERENTIAL EXPRESSION BETWEEN DUPLICATES AND SINGLETONS IN A. MELLIFERA

We identified 116 pairs of duplicated genes and 5235 singletons in *A. mellifera*. We then examined the relationship between gene

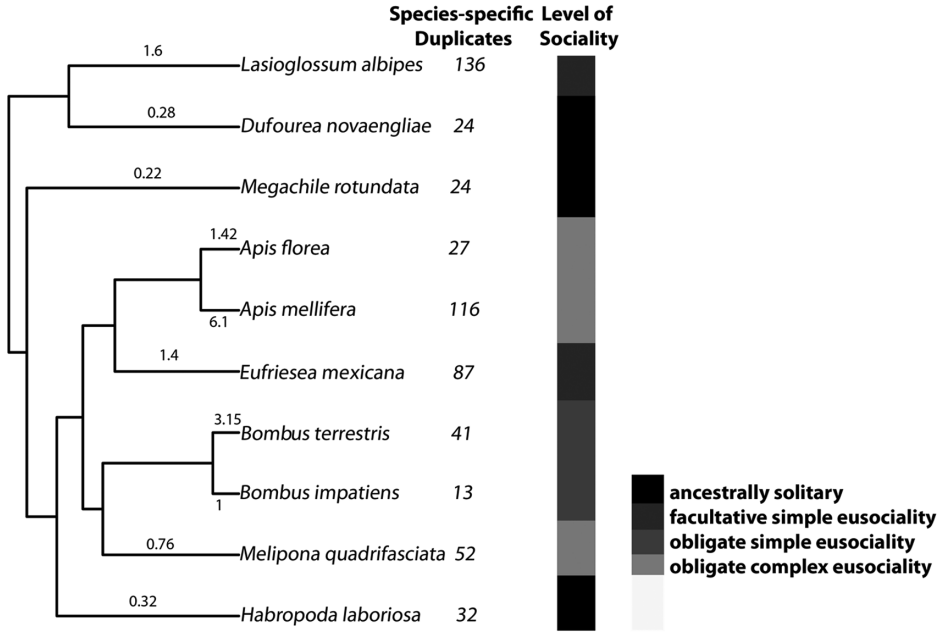

Figure 1. **Species-specific duplications and duplication rates for different bee species varying in level of sociality. Numbers on branches represent species-specific duplication rates in duplicates/MY.**

Table 1. **Observed (and expected) counts of genes differentially expressed across castes for duplicated genes and singletons in three RNAseq datasets comparing queen and worker gene expression differences in *A. mellifera*.**

Dataset	Expression	Duplicates	Singletons	Total
Ashby et al.	Queen-biased	15 (9.89)	243 (248.11)	258
NS	Unbiased	186 (191.43)	4808 (4802.57)	4994
	Worker-biased	3 (2.68)	67 (67.32)	70
	Total	204	5118	5322
Vleurinck et al.	Queen-biased	5 (0.68)	13 (17.32)	18
***	Unbiased	177 (188.74)	4853 (4841.26)	5030
	Worker-biased	18 (10.58)	264 (271.42)	282
	Total	200	5130	5330
Cameron et al.	Queen-biased	3 (1.17)	29 (30.83)	32
*	Unbiased	179 (183.22)	4830 (4825.78)	5009
	Worker-biased	4 (1.61)	40 (42.39)	44
	Total	186	4899	5085

Chi-squared test of independence, NS = not significant, $^{*}P < 0.05$, $^{***}P < 0.001$.

duplication and differential gene expression. First, we compared the proportions of caste-biased genes between duplicates and singletons (Table 1). Since there were a small number of duplicated genes showing biased expression, we performed chi-squared tests by grouping queen- and worker-biased genes into the overall category of "biased" genes. We found that there were significant differences in the percentage of caste-biased (i.e., queen- and worker-biased) genes between duplicated genes and singletons for two out of three datasets analyzed (Ashby et al.: $\chi^2_{df=1} = 2.14$, $P = 0.1435$; Vleurinck et al.: $\chi^2_{df=1} = 12.36$, $P = 0.0004$; Cameron et al.: $\chi^2_{df=1} = 5.24$, $P = 0.0220$, χ^2 test of independence). The patterns among datasets showed some similarities in that duplicated genes tended to show biased expression more often than expected (Table 1).

We next examined the relationship between gene duplication and differential gene expression across the sexes (worker vs drone) (Table 2). We found that the proportion of sex-biased genes differed significantly between duplicates and singletons for both datasets (Ashby et al.: $\chi^2_{df=1} = 30.78$, $P < 0.0001$; Vleurinck et al.: $\chi^2_{df=1} = 12.1$, $P = 0.0005$,

Table 2. Observed (and expected) counts of genes differentially expressed across sexes for duplicated genes and singletons in two RNAseq datasets comparing worker and drone gene expression differences in *A. mellifera*.

Dataset	Expression	Duplicates	Singletons	Total
Ashby et al. ***	Drone biased	25 (8.62)	200 (216.38)	225
	Unbiased	167 (188.25)	4744 (4722.75)	4911
	Worker biased	12 (7.13)	174 (178.87)	186
	Total	204	5118	5322
Vleurinck et al. ***	Drone biased	4 (0.79)	17 (20.21)	21
	Unbiased	191 (197.19)	5064 (5057.81)	5255
	Worker biased	5 (2.03)	49 (51.97)	54
	Total	200	5130	5330

Chi-square test of independence, $^{***}P < 0.001$.

χ^2 test of independence). Both analyses showed a greater frequency of sex-biased, and associated lower frequency of unbiased, genes among the duplicates than the singletons (Table 2).

Next, we compared level of caste-biased expression (as opposed to the number of caste-biased genes) between duplicate genes and singletons (Fig. 2A–C). In this case, we found significant differences in the level of caste-biased expression between duplicates and singletons in all three studies that examined caste differences (Ashby et al.: W = 5.8e + 05, $P = 0.0047$ Vleurinck et al.: W = 6.7e + 05, $P < 0.0001$; Cameron et al.: W = 5.3e + 05, $P < 0.0001$, Wilcoxon rank-sum test). In particular, duplicated genes tended to display significantly higher levels of caste-biased expression. In addition, duplicates had a higher level of sex-biased expression compared to singletons in the two datasets examined (Vleurinck et al.: W = 6.4e + 05, $P < 0.0001$; Ashby et al.: W = 6.4e + 05, $P < 0.0001$, Wilcoxon rank-sum test) (Fig. 2D–E). Duplicates also had a higher level of differential expression in comparisons between nurses and foragers (W = 6.9e + 05, $P < 0.0001$, Wilcoxon rank-sum test) (Fig. 2F). Finally, duplicates displayed a substantially and significantly higher level of tissue-biased expression than singletons (W = 7.0e + 05, $P < 0.0001$, Wilcoxon rank-sum test) (Fig. 2G).

We investigated the correlations of expression-bias within pairs of duplicate genes. Specifically, we were interested in determining if a gene that showed relatively high caste-biased expression, for example, also displayed high levels of sex-biased, behavior-biased, and tissue-biased expression. We first investigated the correlation of caste-biased expression using all genes found in the analyses of Ashby et al., Vleurinck et al., and Cameron et al. We found that the correlations ranged from 0.200 to 0.266 (all pairwise comparisons $P < 0.0001$). Thus there was substantial evidence that genes that showed biased expression in one type of analyses tended to show bias in others.

To determine the prevalence of such correlations within duplicated genes, we considered the partial correlation matrix derived from a MANOVA (Table 3). We found that most of the partial correlations were positive, indicating that there were associations in expression bias for duplicate gene pairs. However, there were two comparisons that resulted in a negative correlation. Nevertheless, as a whole, the partial correlations did indicate that there was a relationship between expression bias for paralogs, revealing that a paralog that showed expression bias in one phenotypic context was likely to show expression bias in another.

GENE EXPRESSION CORRELATION BETWEEN DUPLICATE PAIRS

We compared expression classes of duplicate pairs to determine if the proportion of pairs showing discordant expression between phenotypes differed from random expectations. We found that a majority of the duplicate pairs displayed concordant caste-, sex-, and behavior-biased expression patterns (Table 4). We created a null distribution of pairs to test for the overrepresentation of certain expression pair classes. We did not find significant differences in the observed and expected expression pair classes for castes (Cameron et al.: $\chi^2_{df=1} = 0.06$, $P = 0.807$; Vleurinck et al.: $\chi^2_{df=1} = 2.2$, $P = 0.138$; Ashby et al.: $\chi^2_{df=1} = 0.6$, $P = 0.4396$, χ^2 test) (Table 4). In contrast, when we compared the expression pair classes for sex-biased genes to the null distribution, we saw a significant difference between observed and expected classes for one out of the two datasets (Ashby et al.: $\chi^2_{df=1} = 6.11$, $P = 0.0134$; Vleurinck et al.: $\chi^2_{df=1} = 0.06$, $P = 0.8051$). Overall, however, paired expression classes were generally found at the frequency expected.

We next investigated if expression divergence between paralogs depended on relative location of genes in the genome (Fig. 3). We found that paralogs on different linkage groups had similar levels of expression divergence to those on the same linkage group (Caste: Ashby et al.: W = 947, $P = 0.8761$;

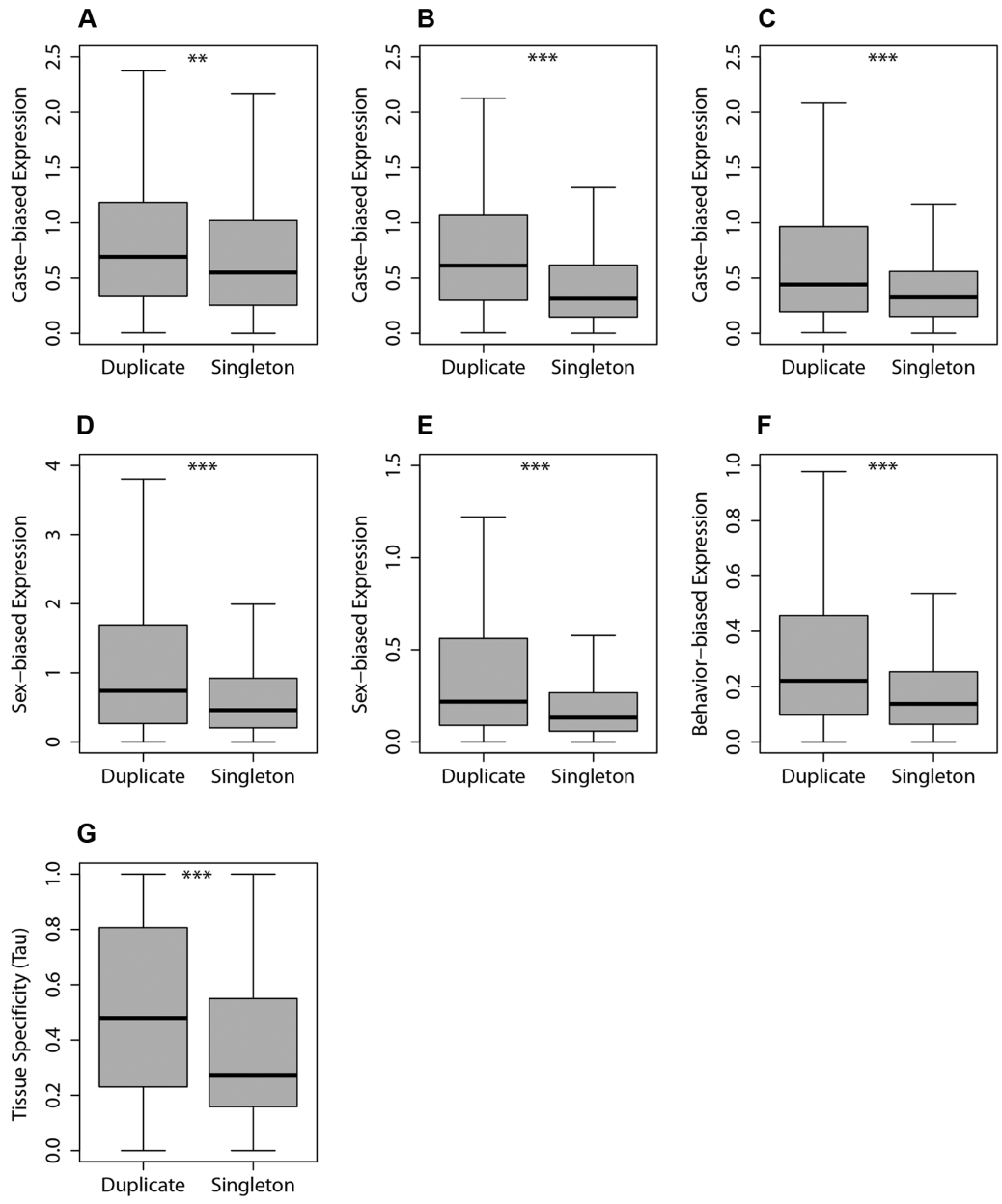

Figure 2. Biased gene expression calculated as the absolute value of the $\log_2$-fold change in expression for duplicated genes and singletons. Caste-biased expression from (A) Ashby et al., (B) Vleurinck et al., and (C) Cameron et al. Sex-biased expression from (D) Ashby et al. and (E) Vleurinck et al. Behavior-bias expression from (F) Jasper et al. (G) Tissue-biased expression (Tau) from Jasper et al. $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 3. Partial correlation matrix between measures of biased expression within duplicate gene pairs.

	A_Caste	J_Tissue	J_Behavior	V_Sex	V_Caste	C_Caste
A_Sex	0.139	0.218	0.083	0.21	0.117	0.156
A_Caste		0.183	0.017	0.029	0.188	–0.127
J_Tissue			0.216	0.204	0.190	–0.089
J_Behavior				0.064	0.159	0.098
V_Sex					0.484	0.091
V_Caste						0.18

A = Ashby et al., V = Vleurinck et al., C = Cameron et al., J = Jasper et al.

Table 4. Observed and expected numbers of pairs of caste-, sex-, and behavior-biased gene expression classes showing correlations of expression classes among duplicate genes.

Phenotype	Dataset	Expression	Observed	Expected
Caste	Ashby	Concordant	79	74.32
		Discordant	11	15.68
		Total	90	90
	Vleurinck	Concordant	76	67.8
		Discordant	10	18.2
		Total	86	86
	Cameron	Concordant	74	72.19
		Discordant	5	6.81
		Total	79	79
Sex	Ashby	Concordant	76	60.83
	*	Discordant	14	29.17
		Total	90	90
	Vleurinck	Concordant	81	79.18
		Discordant	5	6.82
		Total	86	86
Behavior	Jasper	Concordant	98	98.02
		Discordant	2	1.99
		Total	100	100.01

Chi-squared test, $^{*}P < 0.05$.
"Concordant" indicates that the duplicates had the same direction of expression bias (e.g., were both queen-biased) whereas "Discordant" indicates that the duplicate genes showed different patterns of expression bias (e.g., one was queen-biased and the other worker biased).

Vleurinck et al.: W = 894, $P = 0.7921$; Cameron et al.: W = 879, $P = 0.1479$; Sex: Ashby et al.: W = 972, $P = 0.7139$; Vleurinck et al.: W = 927, $P = 0.5766$; Behavior: Jasper et al.: W = 0.5627, $P = 0.5627$, Wilcoxon rank-sum test). There were also no significant differences in the level of tau, which defines tissue-specific expression, between duplicates on the same or different linkage groups (W = 1428, $P = 0.0738$) (Fig. 3G).

CLASSIFICATION OF EVOLUTIONARY PROCESSES MAINTAINING DUPLICATE GENES

We investigated the evolutionary processes maintaining duplicate genes in *A. mellifera* (Assis and Bachtrog 2013, 2015). We found that there were 63 cases of conservation, 28 cases of neofunctionalization (15 of D1 copy, the duplicate with higher sequence similarity to the single copy ortholog, 13 of D2 copy, the duplicate with lower sequence similarity to the single copy ortholog), nine cases of specialization, and no cases of subfunctionalization.

We next investigated evolutionary constraint (dN/dS) and relative expression across alternative phenotypes for genes that arose through conservation. We did not find a significant difference in the level of dN/dS between the duplicate pairs that were subject to conservation (W = 1.3+e03, $P = 0.4227$, Wilcoxon rank-sum test) (Fig. 4A). Since conservation leads to duplicates maintaining similar functions, we expected similar levels of biased gene expression across conserved genes. There was also no significant difference in the level of caste-biased expression ($\chi^2_{df=2} = 4.27$, $P = 0.118$, Kruskal–Wallis test) (Fig. 4E) and sex-biased expression ($\chi^2_{df=2} = 0.34$, $P = 0.8414$) (Fig. 4I) between single copy orthologs and the conserved duplicates.

The level of dN/dS was not significantly different between D1 and D2 for those duplicates maintained through specialization (W = 17, $P = 0.2159$, Wilcoxon rank-sum test) (Fig. 4B). Duplicates that have undergone specialization are expected to have different levels of biased expression for the single copy ortholog and both duplicates. However, we found no difference in the level of caste- ($\chi^2_{df=2} = 3.58$, $P = 0.1671$, Kruskal–Wallis test) or sex-biased gene expression ($\chi^2_{df=2} = 0.79$, $P = 0.6723$) between D1, D2, and single copy orthologs (Fig. 4F and J).

Next, we examined the differences in dN/dS between duplicates that had undergone neofunctionalization. For those duplicates that underwent neofunctionalization of the D1 gene, there was a significantly higher level of dN/dS for the D1 copy (W = 29.5, $P = 0.036$, Wilcoxon rank-sum test) (Fig. 4C). However, this was not the case for those duplicates in which D2 underwent neofunctionalization (W = 57, $P = 0.31$, Wilcoxon rank-sum test) (Fig. 4D). We found no difference in caste-biased expression between the single copy ortholog and both duplicates (D1: $\chi^2_{df=2} = 0.52$, $P = 0.7705$, D2: $\chi^2_{df=2} = 2.31$, $P = 0.3142$, Kruskal–Wallis test) (Fig. 4G and H). There was also no difference in the level of sex-biased expression between genes that underwent neofunctionalization of D1 ($\chi^2_{df=2} = 1.78$, $P = 0.4115$, Kruskal–Wallis test) (Fig. 4K). Though, we saw that D2 had a higher level of sex-biased expression compared the single copy ortholog and D1 copy for those duplicates that underwent neofunctionalization of D2 ($\chi^2_{df=2} = 7.1$, $P = 0.02876$, Kruskal–Wallis test) (Fig. 4L).

Expression patterns of single copy orthologs might limit the evolutionary processes maintaining a duplicate pair in the genome. Therefore, we examined the level of differential expression of single copy orthologs in *B. terrestris* of *A. mellifera* gene duplicates to gain insight into possible constraints on expression evolution of duplicated genes (Fig. 5). We found a significant difference in the level of sex-biased expression between single copy orthologs in *B. terrestris* that have undergone specialization, neofunctionalization, and conservation, with orthologs that underwent neofunctionalization of the D1 copy in *A. mellifera* having the highest level ($\chi^2_{df=3} = 9.18$, $P = 0.027$,

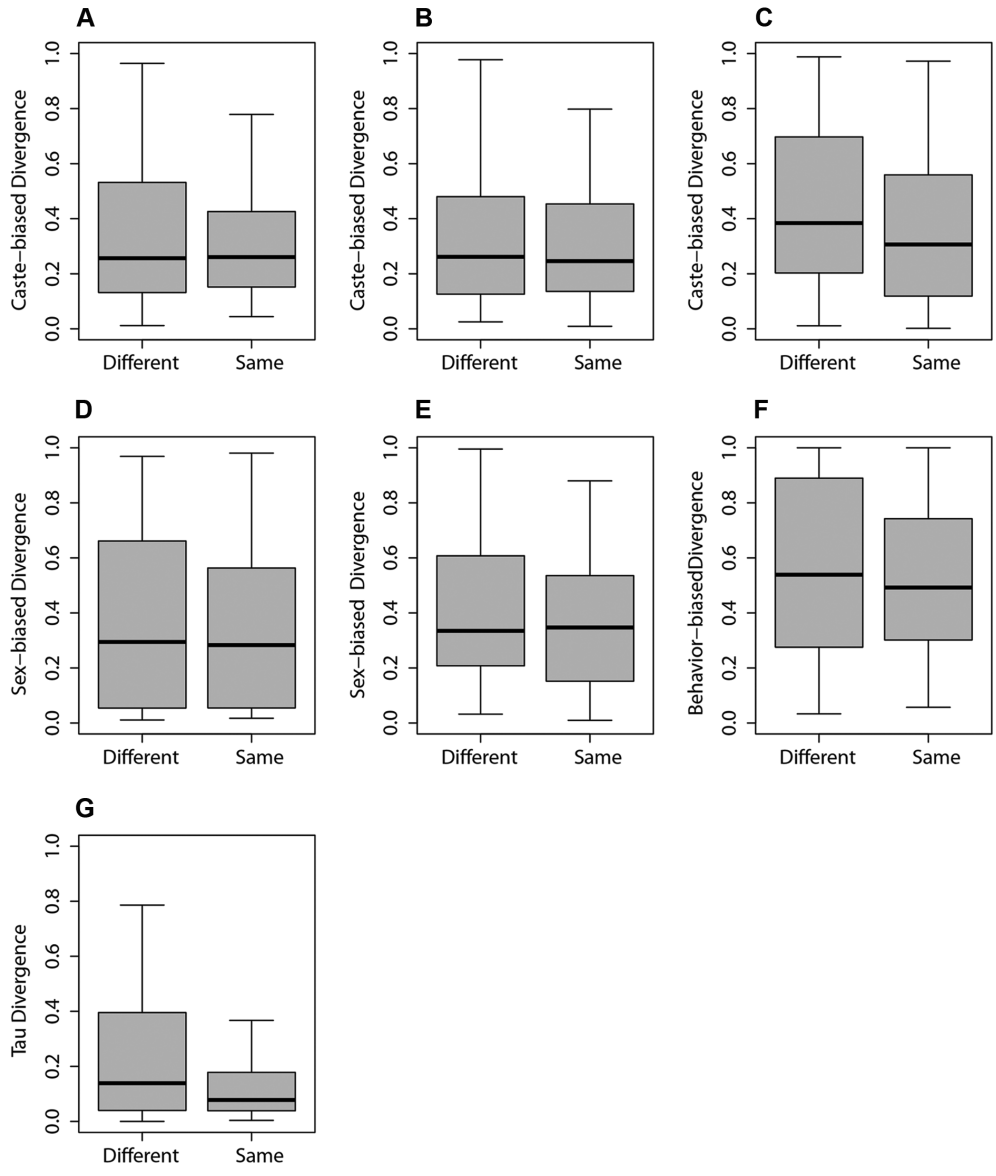

Figure 3. **Divergence in biased gene expression between duplicate pairs on the same or different linkage group. Comparisons between castes from (A) Ashby et al., (B) Vleurinck et al., and (C) Cameron et al., between sexes from (D) Ashby et al. (E) Vleurinck et al., between worker behavioral types from (F) Jasper et al., and among tissues (Tau) from (G) Jasper et al.**

Kruskal–Wallis test) (Fig. 5). However, this trend was not found for genes displaying caste-biased expression ($\chi^2_{df=3} = 6.48$, $P = 0.9039$, Kruskal–Wallis test).

Discussion

Sociality has arisen multiple times in insects. Thus social insects have been the subject of many studies aimed at understanding the genetic changes associated with the evolution of sociality (Woodard et al. 2011; Harpur et al. 2014; Roux et al. 2014; Kapheim et al. 2015). We were interested in the hypothesis that gene duplication had facilitated the evolution of sociality and caste differences in insect societies.

We observed a positive correlation between social complexity and the rate of species-specific gene duplication. This suggests that more highly social bee taxa possess higher rates of gene duplication or lower rates of duplicate gene loss. However, this correlation was not significant with phylogenetic correction. Regardless, the number of species examined in this study was modest and the strength of the correlation was substantial. Therefore, further investigation is needed to determine whether gene duplication rate is correlated with the evolution of sociality.

We hypothesized that gene duplication provided new copies of genes that could be co-opted into the development of social insect phenotypes. We thus expected an enrichment of caste-biased

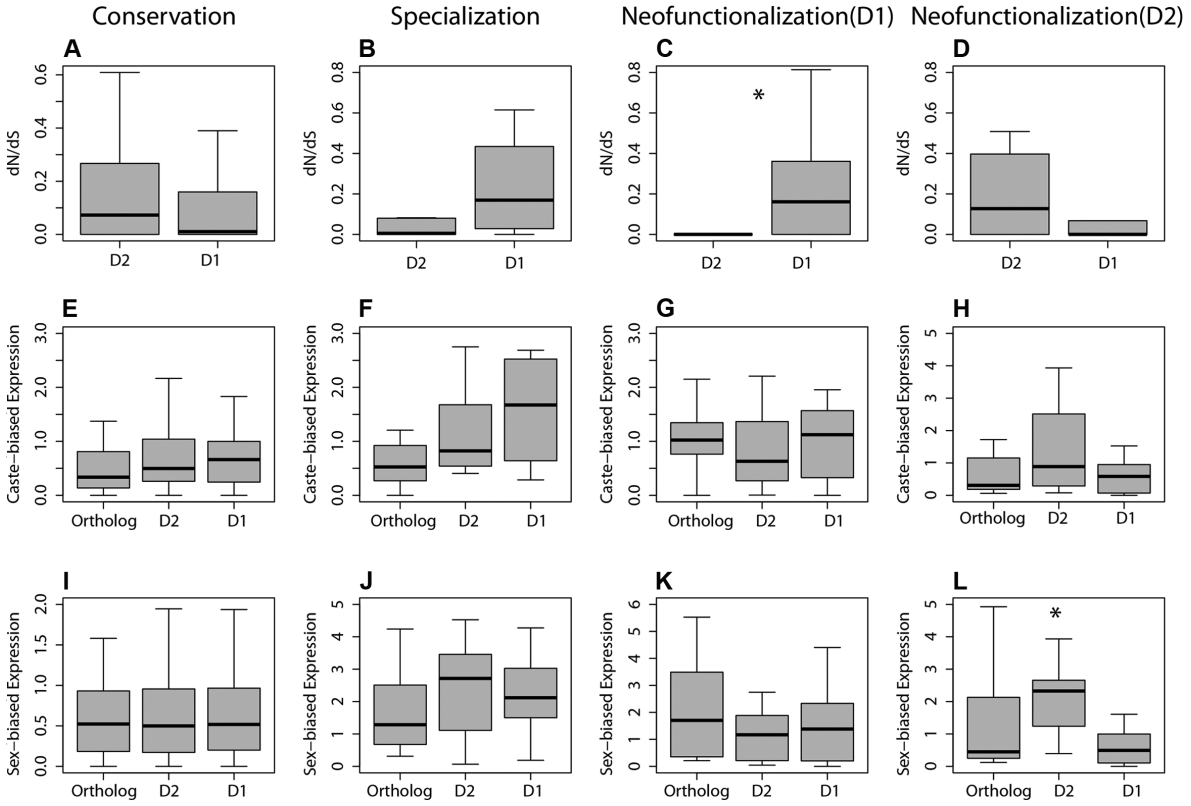

Figure 4. **Comparison of metrics for duplicated genes maintained through conservation, neofunctionalization (D1 copy and D2 copy), and specialization. D1 and D2 are the *A. mellifera* duplicate genes with higher and lower sequence similarity to the single copy *B. terrestris* ortholog, respectively. (A–D) Mean levels of dN/dS for duplicate pairs. (E–H) Caste-biased expression, as measured by absolute value of the $\log_2$ fold change in expression between queens and workers. (I–L) Sex-biased expression, as measured by absolute value of the $\log_2$ fold change in expression between drones and workers.**

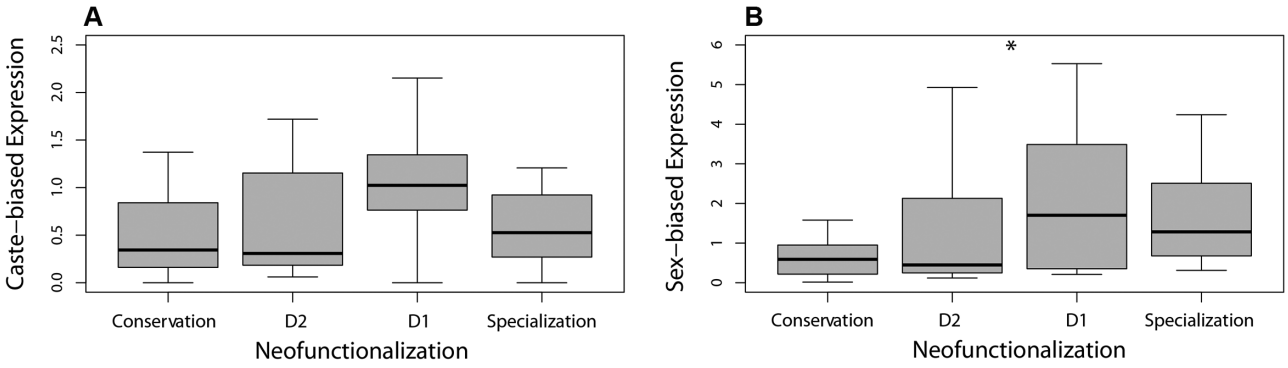

Figure 5. **Comparison of biased expression of single copy orthologs in *B. terrestris* that have been duplicated in *A. mellifera* and been maintained through different evolutionary processes. (A) Levels of caste-biased expression of single copy orthologs in *B. terrestris* ($\chi^2_{df=3} = 6.4817$, $P = 0.09039$, Kruskal–Wallis test). (B) Sex-biased expression between males and workers (female) ($\chi^2_{df=3} = 9.1811$, $P = 0.02698$, Kruskal–Wallis test). $^*P < 0.05$.**

genes in duplicates compared to singletons. We did, in fact, find significantly more caste-biased and sex-biased genes in duplicated genes when compared to singletons (Tables 1 and 2).

Our findings of significant excesses of phenotype-biased genes among duplicates agree with previous studies performed in *D. melanogaster* and *C. elegans* sexes (Cutter and Ward 2005; Wyman et al. 2012). These prior studies found enrichment for duplicates showing phenotype-biased expression. In addition, these investigations uncovered an excess of duplicates with male-biased gene expression, suggesting that gene duplication is frequently involved in the evolution of male-biased traits (Cutter and Ward 2005; Wyman et al. 2012). In our comparison of sex-biased gene

expression between female workers and male drones, we found more drone- and worker-biased duplicates than expected. The datasets we used included individuals from the larval and pupal stages. So it is possible that any strong male effects would not have been identified because we may not have detected the full array of differential gene expression found in adults (Morandin et al. 2015; Ashby et al. 2016; Lockett et al. 2016; Vleurinck et al. 2016).

We expected that duplicates would display a higher level of biased expression compared to singletons. Such a finding would be consistent with the hypothesis that duplicate gene expression can be co-opted into the evolution of different phenotypic forms (Gadagkar 1997; Gallach and Betran 2011). We did find that duplicates tended to have higher levels of caste-, sex-, behavior-, and tissue-biased expression compared to singletons (Fig. 2). Overall, these results agree with past studies that found that duplicates tended to become more specialized in their expression patterns (Freilich et al. 2006; Farré and Albà 2010; Assis and Bachtrog 2013). Similarly, gene families of increasing size have been found to show increasing levels of expression bias (Huminiecki and Wolfe 2004; Tanaka et al. 2015). Our results suggest that gene duplication permits evolution of variation in expression levels and may allow for phenotypic diversification at multiple phenotypic levels.

We further examined the expression patterns of pairs of duplicate genes to determine if they showed concordant expression patterns between different castes, sexes, and worker behavioral types. In general, we did not find significant enrichment of duplicate pairs with concordant expression relative to expectations (Table 4). However, we did see enrichment for duplicates with the similar expression bias for the Ashby et al. dataset when comparing sex-biased expression (Table 4). This excess of duplicate pairs with concordant expression was observed in analysis of *Drosophila* sexes (Wyman et al. 2012). Thus it appears that duplicate gene pairs may maintain similar expression profiles to each other. This might reflect the fact that a new duplicate gene is likely to have maintained its expression profile and function immediately after duplication, and that it takes time for a discordant expression profile to evolve. For example, there is some evidence that duplication of a gene that is already sex-biased may allow the gene's paralog to become even more sex-biased (Wyman et al. 2012). The result that duplicates tend to have higher levels of biased gene expression but tend not to differ in their directional bias may be indicative of this mechanism.

We found that paralogs located on the same chromosome did not necessarily have similar expression patterns compared to paralogs located on different chromosomes (Fig. 3). This result differs from previous studies (Mikhaylova et al. 2008) and suggests that genes on the same chromosome are not necessarily subject to similar regulatory regimes (Ibn-Salem et al. 2016; Lan and Pritchard 2016). Therefore, a new gene duplicate may evolve divergent expression patterns from its parent paralog, even if it is duplicated onto the same chromosome.

We examined the correlation between caste-, sex-, behavior-, and tissue-biased expression for individual duplicate pairs (Table 3). A majority of correlations between these different measures of phenotype-biased expression were positive. This indicates that duplicates that are differentially expressed in one phenotypic context tend to be differentially expressed in other contexts (Hunt et al. 2013). Genes with higher levels of differential expression may be subjected to weakened selective constraint on gene expression compared to genes that are more uniformly expressed among phenotypes (Mank and Ellegren 2009; Hunt et al. 2011; Leichty et al. 2012). Therefore, loci experiencing weak selective constraint may be more likely to be differentially expressed in a variety of contexts (Hunt et al. 2011; Leichty et al. 2012).

Our results suggest that gene duplication may provide genetic material that can be co-opted in the evolution of alternative phenotypes. However, there are other mechanisms that can potentially explain the patterns that we observed. For example, it is possible that ancestral genes that were already differentially expressed between phenotypes were more likely to duplicate because of mutation bias. In addition, copy number variants of differentially expressed genes could be less likely to be under purifying selection, leading to fixation of such genes (Cardoso-Moreira et al. 2016). Or, the genome may be more tolerant of the acquisition of phenotype-biased genes compared to singletons, particularly if phenotypic-biased genes are not essential (Mank and Ellegren 2009). Therefore, biased duplicates may be fixed at a higher rate than biased singletons. Thus there are potentially several molecular evolutionary mechanisms that could lead to the observation of a correlation between phenotype-biased expression and gene duplication.

We investigated the processes that maintained duplicate genes within the *A. mellifera* genome. This analysis used gene expression as a proxy for gene function. However, it is worth noting that genes may diverge in function but not differ in their expression patterns. In addition, comparisons of gene expression patterns across species may be challenging (Montgomery and Mank 2016; Zhang et al. 2007). Thus, the inference of the evolutionary processes maintaining duplicate genes relies on assumptions that may not always be upheld and, therefore, the results of this analysis should be viewed cautiously.

Our analysis suggested that conservation, neofunctionalization, and specialization were the primary evolutionary processes associated with gene duplication in *A. mellifera*. Interestingly, we identified no cases of subfunctionalization. It is notable that prior studies of this type also found that conservation was one of the most common mechanisms maintaining gene duplicates

in mammals and plants (Assis and Bachtrog 2015; Wang et al. 2016). In contrast, neofunctionalization was found to be the most common process maintaining gene duplicates in *Drosophila* (Assis and Bachtrog 2013). The difference between these findings could be due to the differences in effective population size among the studied taxa. Natural selection is less efficient in smaller populations. *Drosophila*, with its large effective population size, may have more neofunctionalized genes maintained by selection. In contrast, natural selection will operate less efficiently in species with smaller effective population size, such as *A. mellifera*, allowing potentially neofunctionalized genes to be fixed less often (Galtier, 2016; Jensen & Bachtrog 2011; Romiguier et al. 2014).

Interestingly, there was a notable lack of subfunctionalization across all studied taxa (Assis and Bachtrog 2015; Lan and Pritchard 2016). This is surprising because it has been suggested that subfunctionalization is an important process in the retention of duplicate genes (Lynch and Conery 2000). Subfunctionalization requires that both duplicates start off with the same function and are in dosage balance. Therefore, subfunctionalization is more likely to occur for large-scale duplications like whole genome duplication events, which maintain the regulatory environments of the focal genes (Casneuf et al. 2006; Fares et al. 2013). The lack of observed subfunctionalization in our analyses could also be due to the datasets used for classification. The analysis classifying duplicates into evolutionary processes was performed using an expression profile across whole-body *A. mellifera* and *B. terrestris* queens, workers, and drones. This may lead to an underestimation of potential expression differences across tissues and time, which may obfuscate some patterns of subfunctionalization (Assis and Bachtrog 2013, 2015).

We examined differences in the level of caste-biased expression of single copy orthologs for duplicate genes maintained by conservation, neofunctionalization, and specialization. Duplicates that underwent conservation tended to arise from single copy orthologs that had lower levels of differential expression (Fig. 5). The low level of differential expression suggests that duplicates that have undergone conservation are more essential and broadly expressed than those that have undergone neofunctionalization and specialization. Genes that are subject to the latter mechanisms generally displayed biased expression among phenotypes, leading to the development of new functions. This suggests that the ancestral function of a pair of duplicates may limit their evolutionary trajectory (Wang et al. 2016). We also examined evolutionary and expression characteristics of duplicates that were maintained through the different evolutionary processes (Assis and Bachtrog 2013, 2015). We found no significant difference in the constraint (dN/dS) between duplicate pairs involved in conservation (Fig. 4A). However, we identified differences in dN/dS between duplicates that underwent neofunctionalization of the D1 copy (Fig. 4C). This is interesting, given that the duplicate that gains the new function, D1, has higher value of dN/dS.

Recently, considerable attention has been paid to the role of novel genes in the evolution of phenotypic diversity in social species (Johnson and Tsutsui 2011; Tautz and Domazet-Lošo 2011; Feldmeyer et al. 2014; Sumner 2014; Jasper et al. 2015). This study provides further insight on the role of new genes, created through the process of gene duplication, in the evolution of insect societies. More highly social bee species may have higher gene duplication rates. Duplicate genes seem to be preferentially co-opted into caste- and sex-specific function. Moreover, duplicated genes are apparently subject to conservation, neofunctionalization, and specialization in *A. mellifera*. Overall, this study adds to the accumulating evidence that gene duplication has played a substantial role in the evolution of complex societies and alternative phenotypes.

AUTHOR CONTRIBUTIONS

L.C. and M.G. conceived of the study. L.C. performed the analyses. L.C. and M.G. wrote the manuscript.

ACKNOWLEDGMENTS

This research was partially supported by the Elizabeth Smithgall Watts and the Bennie H. and Nelson D. Abell Endowment Fund, and the Graduate Assistance in Areas of National Need (GAANN) fellowship.

DATA ARCHIVING

The doi for our data is https://doi.org/10.5061/dryad.9m5h0

LITERATURE CITED

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Ashby, R., S. Forêt, I. Searle, and R. Maleszka. 2016. MicroRNAs in honey bee caste determination. Sci. Rep. 6:18794.

Assis, R., and D. Bachtrog. 2013. Neofunctionalization of young duplicate genes in *Drosophila*. Proc. Natl. Acad. Sci. USA 110:17409–17414.

———. 2015. Rapid divergence and diversification of mammalian duplicate gene functions. BMC Evol. Biol. 15:138.

Atallah, J., D. C. Plachetzki, W. C. Jasper, and B. R. Johnson. 2013. The utility of shallow RNA-Seq for documenting differential gene expression in genes with high and low levels of expression. PLoS One 8:e84160.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. 57:289–300.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Bonduriansky, R., and S. F. Chenoweth. 2009. Intralocus sexual conflict. Trends Ecol. Evol. 24:280–288.

Cameron, R. C., E. J. Duncan, and P. K. Dearden. 2013. Biased gene expression in early honeybee larval development. BMC Genom. 14:903.

Cardinal, S., and B. N. Danforth. 2013. Bees diversified in the age of eudicots. Proc. R. Soc. B 280:20122686.

Cardoso-Moreira, M., J. R. Arguello, S. Gottipati, L. G. Harshman, J. K. Grenier, and A. G. Clark. 2016. Evidence for the fixation of gene duplications by positive selection in *Drosophila*. Genome Res. 26:787–798.

Casneuf, T., S. De Bodt, J. Raes, S. Maere, and Y. Van de Peer. 2006. Nonrandom divergence of gene expression following gene and genome duplications in the flowering plant *Arabidopsis thaliana*. Genome Biol. 7:R13.

Connallon, T., and A. G. Clark. 2011. The resolution of sexual antagonism by gene duplication. Genetics 187:919–937.

Connallon, T., and L. L. Knowles. 2005. Intergenomic conflict revealed by patterns of sex-biased gene expression. Trends Genet. 21:495–499.

Cutter, A. D., and S. Ward. 2005. Sexual and temporal dynamics of molecular evolution in *C. elegans* development. Mol. Biol. Evol. 22:178–188.

Ellegren, H., and J. Parsch. 2007. The evolution of sex-biased genes and sex-biased gene expression. Nat. Rev. Genet. 8:689–698.

Elsik, C., K. Worley, A. Bennett, M. Beye, F. Camara, C. Childers, D. de Graaf, G. Debyser, J. Deng, B. Devreese, et al. 2014. Finding the missing honey bee genes: lessons learned from a genome upgrade. BMC Genom. 15:86.

Fares, M. A., O. M. Keane, C. Toft, L. Carretero-Paulet, and G. W. Jones. 2013. The roles of whole-genome and small-scale duplications in the functional specialization of *Saccharomyces cerevisiae* genes. PLoS Genet. 9: e1003176.

Farré, D., and M. M. Albà. 2010. Heterogeneous patterns of gene-expression diversification in mammalian gene duplicates. Mol. Biol. Evol. 27:325–335.

Feldmeyer, B., D. Elsner, and S. Foitzik. 2014. Gene expression patterns associated with caste and reproductive status in ants: worker-specific genes are more derived than queen-specific ones. Mol. Ecol. 23:151–161.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Force, A., M. Lynch, F. B. Pickett, A. Amores, Y. L. Yan, and J. Postlethwait. 1999. Preservation of duplicate genes by complementary, degenerative mutations. Genetics 151:1531–1545.

Freilich, S., T. Massingham, E. Blanc, L. Goldovsky, and J. M. Thornton. 2006. Relating tissue specialization to the differentiation of expression of singleton and duplicate mouse proteins. Genome Biol. 7:R89.

Gadagkar, R. 1997. The evolution of caste polymorphism in social insects: genetic release followed by diversifying evolution. J. Genet. 76:167–179.

Galtier, N. 2016. Adaptive protein evolution in animals and the effective population size hypothesis. PLOS Genet. 12(1): e1005774.

Gallach, M., and E. Betran. 2011. Intralocus sexual conflict resolved through gene duplication. Trends Ecol. Evol. 26:222–228.

Gu, Z. L., L. M. Steinmetz, X. Gu, C. Scharfe, R. W. Davis, and W. H. Li. 2003. Role of duplicate genes in genetic robustness against null mutations. Nature 421:63–66.

Hall, D. W., S. V. Yi, and M. A. D. Goodisman. 2013. Kin selection, genomics and caste-antagonistic pleiotropy. Biol. Lett. 9 https://doi.org/10.1098/rsbl.2013.0309.

Harpur, B. A., C. F. Kent, D. Molodtsova, J. M. D. Lebon, A. S. Alqarni, A. A. Owayss, and A. Zayed. 2014. Population genomics of the honey bee reveals strong signatures of positive selection on worker traits. Proc. Natl. Acad. Sci. USA 111:2614–2619.

Harrison, M. C., R. L. Hammond, and E. B. Mallon. 2015. Reproductive workers show queenlike gene expression in an intermediately eusocial insect, the buff-tailed bumble bee *Bombus terrestris*. Mol. Ecol. 24:3043–3063.

He, X. L., and J. Z. Zhang. 2005. Rapid subfunctionalization accompanied by prolonged and substantial neofunctionalization in duplicate gene evolution. Genetics 169:1157–1164.

Holman, L. 2014. Caste load and the evolution of reproductive skew. Am. Nat. 183:84–95.

Huminiecki, L., and K. H. Wolfe. 2004. Divergence of spatial gene expression profiles following species-specific gene duplications in human and mouse. Genome Res. 14:1870–1879.

Hunt, B. G., L. Ometto, L. Keller, and M. A. D. Goodisman. 2013. Evolution at two levels in fire ants: the relationship between patterns of gene expression and protein sequence evolution. Mol. Biol. Evol. 30:263–271.

Hunt, B. G., L. Ometto, Y. Wurm, D. Shoemaker, S. V. Yi, L. Keller, and M. A. D. Goodisman. 2011. Relaxed selection is a precursor to the evolution of phenotypic plasticity. Proc. Natl. Acad. Sci. USA 108:15936–15941.

Ibn-Salem, J., E. M. Muro, and M. A. Andrade-Navarro. 2016. Co-regulation of paralog genes in the three-dimensional chromatin architecture. Nucleic Acids Res. 45:81–91.

Innan, H., and F. Kondrashov. 2010. The evolution of gene duplications: classifying and distinguishing between models. Nat. Rev. Genet. 11:97–108.

Innocenti, P., and E. H. Morrow. 2010. The sexually antagonistic genes of *Drosophila melanogaster*. PLoS Biol. 8:e1000335.

Jasper, W. C., T. A. Linksvayer, J. Atallah, D. Friedman, J. C. Chiu, and B. R. Johnson. 2015. Large-scale coding sequence change underlies the evolution of postdevelopmental novelty in honey bees. Mol. Biol. Evol. 32:334–346.

Jensen, J. D., and D. Bachtrog. 2011. Characterizing the influence of effective population size on the rate of adaptation: Gillespie's Darwin domain. Genome Biol. Evol. 3:687–701.

Johnson, B. R., and N. D. Tsutsui. 2011. Taxonomically restricted genes are associated with the evolution of sociality in the honey bee. BMC Genom. 12:164.

Kapheim, K. M., H. Pan, C. Li, S. L. Salzberg, D. Puiu, T. Magoc, H. M. Robertson, M. E. Hudson, A. Venkat, B. J. Fischman, et al. 2015. Genomic signatures of evolutionary transitions from solitary to group living. Science 348:1139–1143.

Kovacs, J. L., E. A. Hoffman, S. M. Marriner, and M. A. D. Goodisman. 2010. Detecting selection on morphological traits in social insect castes: the case of the social wasp *Vespula maculifrons*. Biol. J. Linnean Soc. 101:93–102.

Lan, X., and J. K. Pritchard. 2016. Coregulation of tandem duplicate genes slows evolution of subfunctionalization in mammals. Science 352:1009–1013.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9:U357–U354.

Leichty, A. R., D. W. Pfennig, C. D. Jones, and K. S. Pfennig. 2012. Relaxed genetic constraint is ancestral to the evolution of phenotypic plasticity. Integr. Comp. Biol. 52:16–30.

Li, B., and C. N. Dewey. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323.

Linksvayer, T. A., and M. J. Wade. 2005. The evolutionary origin and elaboration of sociality in the aculeate hymenoptera: maternal effects, sib-social effects, and heterochrony. Q. Rev. Biol. 80:317–336.

Lockett, G. A., E. J. Almond, T. J. Huggins, J. D. Parker, and A. F. G. Bourke. 2016. Gene expression differences in relation to age and social environment in queen and worker bumble bees. Exp. Gerontol. 77: 52–61.

Lynch, M., and J. Conery. 2000. The evolutionary fate and consequences of duplicate genes. Science 290:1151–1155.

Mank, J. E., and H. Ellegren. 2009. Are sex-biased genes more dispensable? Biol. Lett. 5:409–412.

Mikhaylova, L. M., K. Nguyen, and D. I. Nurminsky. 2008. Analysis of the *Drosophila melanogaster* testes transcriptome reveals coordinate regulation of paralogous genes. Genetics 179:305–315.

Morandin, C., K. Dhaygude, J. Paviala, K. Trontti, C. Wheat, and H. Helantera. 2015. Caste-biases in gene expression are specific to developmental stage in the ant *Formica exsecta*. J. Evol. Biol. 28:1705–1718.

Montgomery, S. H., and Mank, J. E. 2016. Inferring regulatory change from gene expression: the confounding effects of tissue scaling. Mol. Ecol. 25:5114–5128.

Normark, B. B. 2003. The evolution of alternative genetic systems in insects. Annu. Rev. Entomol. 48:397–423.

Ohno, S. 1970. Evolution by gene duplication. New York: Springer.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Pennell, T. M., and E. H. Morrow. 2013. Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. Ecol. Evol. 3:1819–1834.

Perry, B. R., and R. Assis. 2016. CDROM: classification of duplicate gene retention mechanisms. BMC Evol. Biol. 16:1–4.

Proulx, S. R. 2012. Multiple routes to subfunctionalization and gene duplicate specialization. Genetics 190:737–751.

Ranwez, V., S. Harispe, F. Delsuc, and E. J. P. Douzery. 2011. MACSE: multiple alignment of coding sequences accounting for frameshifts and stop codons. PLoS ONE 6:e22594.

Rice, W. R. 2013. Nothing in genetics makes sense except in light of genomic conflict. Pp. 217–237 *in* D. J. Futuyma, ed. Annual review of ecology evolution and systematics. Annual Reviews, Palo Alto.

Rice, W. R., and A. K. Chippindale. 2001. Intersexual ontogenetic conflict. J. Evol. Biol. 14:685–693.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. EdgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140.

Roux, J., E. Privman, S. Moretti, J. T. Daub, M. Robinson-Rechavi, and L. Keller. 2014. Patterns of positive selection in seven ant genomes. Mol. Biol. Evol. 31:1661–1685.

Romiguier, J., J. Lourenco, P. Gayral, N. Faivre, L. A. Weinert, S. Ravel, M. Ballenghien, V. Cahais, A. Bernard, E. Loire, et al. 2014. Population genomics of eusocial insects: the costs of a vertebrate-like effective population size. J. Evol. Biol. 27:593–603.

Seeley, T. D. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. Behav. Ecol. Sociobiol. 11:287–293.

Stewart, A. D., A. Pischedda, and W. R. Rice. 2010. Resolving intralocus sexual conflict: genetic mechanisms and time frame. J. Hered. 101:S94–S99.

Sumner, S. 2014. The importance of genomic novelty in social evolution. Mol. Ecol. 23:26–28.

Tanaka, K., Y. Diekmann, A. Hazbun, A. Hijazi, B. Vreede, F. Roch, and É. Sucena. 2015. Multispecies analysis of expression pattern diversification in the recently expanded insect *Ly6* gene family. Mol. Biol. Evol. 32:1730–1747.

Tautz, D., and T. Domazet-Lošo. 2011. The evolutionary origin of orphan genes. Nat. Rev. Genet. 12:692–702.

Toth, A. L., K. Varala, M. T. Henshaw, S. L. Rodriguez-Zas, M. E. Hudson, and G. E. Robinson. 2010. Brain transcriptomic analysis in paper wasps identifies genes associated with behaviour across social insect lineages. Proc. R Soc. B Biol. Sci. 277:2139–2148.

Vleurinck, C., S. Raub, D. Sturgill, B. Oliver, and M. Beye. 2016. Linking genes and brain development of honeybee workers: a whole-transcriptome approach. PLoS ONE 11:e0157980.

Wang, J., F. Tao, N. C. Marowsky, and C. Fan. 2016. Evolutionary fates and dynamic functionalization of young duplicate genes in Arabidopsis genomes. Plant Physiol. 172:427–440.

Whitfield, C. W., A. M. Cziko, and G. E. Robinson. 2003. Gene expression profiles in the brain predict behavior in individual honey bees. Science 302:296–299.

Wilson, E. O. 1990. Success and dominance in ecosystems: the case of the social insects. Ecology Institute Oldendorf/Luhe. Federal Republic of Germany.

Woodard, S. H., B. J. Fischman, A. Venkat, M. E. Hudson, K. Varala, S. A. Cameron, A. G. Clark, and G. E. Robinson. 2011. Genes involved in convergent evolution of eusociality in bees. Proc. Natl. Acad. Sci. USA 108:7472–7477.

Wyman, M. J., A. D. Cutter, and L. Rowe. 2012. Gene duplication in the evolution of sexual dimorphism. Evolution 66:1556–1566.

Yanai, I., H. Benjamin, M. Shmoish, V. Chalifa-Caspi, M. Shklar, R. Ophir, A. Bar-Even, S. Horn-Saban, M. Safran, E. Domany, et al. 2005. Genome-wide midrange transcription profiles reveal expression level relationships in human tissue specification. Bioinformatics 21:650–659.

Yang, Z. H. 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24:1586–1591.

Zayed, A., N. L. Naeger, S. L. Rodriguez-Zas, and G. E. Robinson. 2012. Common and novel transcriptional routes to behavioral maturation in worker and male honey bees. Genes Brain Behav. 11:253–261.

Zhang, Y., D. Sturgill, M. Parisi, S. Kumar, and B. Oliver. 2007. Constraint and turnover in sex-biased gene expression in the genus *Drosophila*. Nature 450:233–237.

Zdobnov, E. M, F. Tegenfeldt, D. Kuznetsov, R. M. Waterhouse, F. A. Simão, P. Ioannidis, M. Seppey, A. Loetscher, and E. V. Kriventseva. 2017. OrthoDB v9.1: cataloging evolutionary and functional annotations for animal, fungal, plant, archaeal, bacterial and viral orthologs. Nucleic Acids Res. 45:D744–D749.

Associate Editor: B. Hollis
Handling Editor: P. Tiffin

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1.