Wasp Gene Expression Supports an Evolutionary Link Between Maternal Behavior and Eusociality

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Atlantic are available, except a recent record from the Irish margin by Peck *et al*. (*23*). The authors recorded identical $\delta^{18}O$ *N. pachyderma* (s) and *G. bulloides* values during the LGM that were attributed to a continuous discharge of meltwater from the British Ice Sheet and year round mixing that homogenized the upper waters.

Several processes can create variability in the $\delta^{18}O$ of foraminifera. Seasonal variability was interpreted by Ganssen and Kroon (*19*) to explain why *G. bulloides* $\delta^{18}O$ was more positive than *G. inflata* $\delta^{18}O$ in the modern North Atlantic at 57°N, which was attributed to a later seasonal period of *G. bulloides* production further south. The uniform $\delta^{18}O$ of the foraminifera during HEs would require improbable ecological changes in preferred depth-habitat zones or in seasonal behavior if these values were not the result of uniform upper–water-mass conditions. Upwelling of ^{18}O-depleted water produced by brine-rejection at higher latitudes might affect the $\delta^{18}O$ of deeper-dwelling foraminifera; however, it is difficult to imagine it influencing $\delta^{18}O$ values in all three taxa. Without a decrease in temperature by ~2.5°C, it is impossible to lower the salinity by 0.8 per mil (‰) (*24*) (and by extension to lower $\delta^{18}O$ by 0.5‰) while remaining along the same isopycnal surface, which by itself would correspond to a 0.5‰ increase in the $\delta^{18}O$ of calcite. Because these effects cancel each other out, such a mechanism is inadequate to explain the anomalously low $\delta^{18}O$ values in all three planktonic foraminifera (Fig. 3B).

Intensified vertical mixing and deepening of the mixed layer during HEs is the mechanism remaining to explain the data. Atmospheric conditions directly influence the mixed layer through turbulence, and wind driven Langmuir circulation could be the prime driver of the turbulence (*25*, *26*). As a result, the upper ocean often becomes well mixed to depths as great as 600 m (*27*). Our $\delta^{18}O$ data from planktonic foraminifera that live at different depth ranges illustrate the extent of this process, suggesting that during the times of HEs the near-surface waters were homogenized by stronger mixing.

During the last glacial cycle, large ice sheets in the Northern Hemisphere and steeper meridional temperature gradients in the atmosphere must have reorganized atmospheric circulation. As a result, winter sea-ice cover extended further south, and glacial winds were stronger and more zonal (*28*, *29*). These winds would have intensified the vertical mixing and turbulence in the upper water masses. It is counterintuitive to visualize such a mechanism during HEs when the glacial North Atlantic was flooded with meltwater resulting in stronger stratification. However, our $\delta^{18}O$ data demonstrate that homogenization of upper water masses did occur, suggesting that this mechanism functioned at the core site. Additional evidence of this mechanism comes from the measurements of Ca^{+2} and Na^{+} ions derived from sea salt and continental dust in the Greenland ice core (*30*). Both Ca^{+2} and Na^{+} ions in the ice core show rapid increases from their ambient concentration during stadials. Abrupt, manyfold increases of these chemical species suggest that storminess during the glacial period caused stronger vertical mixing at the atmosphere/ocean boundary at the subpolar and subtropical fronts.

The question of why weaker homogenization of near-surface waters occurred during other D/O cycles not associated with HEs could be raised, because Greenland ice core data show similar patterns of glaciochemical species. One possibility is that unfavorable composition or insufficient volumes of meltwater were available to perturb the near-surface waters during these D/O ice-rafting cycles as the icebergs originated from the smaller ice sheets. Hence, even though the glacial climate was windier and stormier, the near-surface waters continued to be stratified.

References and Notes

1. H. Heinrich, *Quat. Res.* **29**, 143 (1988).
2. G. C. Bond *et al*., *Nature* **365**, 143 (1993).
3. H. Rashid, R. Hesse, D. J. W. Piper, *Paleoceanography* **18**, 1077 (2003).
4. R. B. Alley, D. R. MacAyeal, *Paleoceanography* **9**, 503 (1994).
5. C. Huber *et al*., *Earth Planet. Sci. Lett.* **243**, 504 (2006).
6. S. Manabe, R. J. Stouffer, *Nature* **378**, 165 (1995).
7. D. Rind *et al*., *J. Geophys. Res.* **106**, 27335 (2001).
8. J. Flückiger, R. Knutti, J. W. C. White, *Paleoceanography* **21**, 1204 (2006).
9. E. A. Boyle, L. Keigwin, *Earth Planet. Sci. Lett.* **76**, 135 (1985/86).
10. W. F. Ruddiman, *Geol. Soc. Am. Bull.* **88**, 1813 (1977).
11. L. Keigwin, S. Lehman, *Paleoceanography* **9**, 185 (1994).
12. M. Stuiver, P. J. Reimer, *Radiocarbon* **35**, 215 (1993).
13. R. G. Fairbanks *et al*., *Quat. Sci. Rev.* **24**, 1781 (2005).
14. H. Rashid, R. Hesse, D. J. W. Piper, *Earth Planet. Sci. Lett.* **208**, 319 (2003).
15. H. Rashid, E. Grosjean, *Paleoceanography* **21**, 1240 (2006).
16. J. J. Ottens, *Oceanol. Acta* **14**, 123 (1991).
17. W. G. Deuser, *J. Foraminifer. Res.* **17**, 14 (1987).
18. R. G. Fairbanks, P. H. Wiebe, A. W. Bé, *Science* **207**, 61 (1980).
19. G. Ganssen, D. Kroon, *J. Geol. Soc.* **157**, 693 (2000).
20. S. Mulitza, A. Dürkoop, W. Hale, G. Wefer, H. S. Niebler, *Geology* **25**, 335 (1997).
21. J. R. Luyten, A. J. Pedlosky, H. Stommel, *J. Phys. Oceanogr.* **13**, 192 (1983).
22. L. Labeyrie *et al*., *AGU Monogr.* **112**, 77 (1999).
23. V. Peck *et al*., *Earth Planet. Sci. Lett.* **243**, 476 (2006).
24. G. L. Pickard, W. J. Emery, *Descriptive Physical Oceanography* (Pergamon, Oxford, ed. 5, 1990).
25. S. K. Gulev, B. Barnier, H. Knochel, J.-M. Molines, M. Cottet, *J. Clim.* **16**, 3085 (2003).
26. K. Hanawa, T. Suga, in *Ocean-Atmosphere Interactions*, Y. Toba, Ed. (Kluwer Academic, Tokyo, 2003), pp. 63–109.
27. M. K. Robinson *et al*., *Atlas of the North Atlantic-Indian Ocean Monthly Mean Temperatures and Mean Salinities of the Surface Layer* (U.S. Naval Oceanogr. Office Reference Publication 18, Washington, DC, 1978).
28. M. Sarnthein, U. Pflaumann, M. Weinelt, *Paleoceanography* **18**, 771 (2003).
29. H. Gildor, E. Tzipperman, *Philos. Trans. R. Soc. London Ser. A* **361**, 1935 (2003).
30. P. A. Mayewski *et al*., *Science* **263**, 1747 (1994).

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Wasp Gene Expression Supports an Evolutionary Link Between Maternal Behavior and Eusociality

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The presence of workers that forgo reproduction and care for their siblings is a defining feature of eusociality and a major challenge for evolutionary theory. It has been proposed that worker behavior evolved from maternal care behavior. We explored this idea by studying gene expression in the primitively eusocial wasp *Polistes metricus*. Because little genomic information existed for this species, we used 454 sequencing to generate 391,157 brain complementary DNA reads, resulting in robust hits to 3017 genes from the honey bee genome, from which we identified and assayed orthologs of 32 honey bee behaviorally related genes. Wasp brain gene expression in workers was more similar to that in foundresses, which show maternal care, than to that in queens and gynes, which do not. Insulin-related genes were among the differentially regulated genes, suggesting that the evolution of eusociality involved major nutritional and reproductive pathways.

A major challenge in biology is to understand the evolution of animal society in molecular terms. Eusociality is the most extreme form of cooperation, typified by individuals that care for siblings rather than reproduce themselves, i.e., "workers." The evolution

of eusociality has been ascribed to kin or colony-level selection (*1*, *2*), but these explanations do not specify mechanistic routes.

It has long been suggested (*3*–*5*) that sibling care by hymenopteran (ant, bee, wasp) workers evolved from maternal care, which involves provisioning brood by foraging for food and then feeding them. According to this idea, two principal behaviors exhibited by solitary Hymenoptera, reproduction (egg-laying) and maternal care (brood provisioning), became uncoupled during the early stages of social evolution (*6*), and these behaviors eventually occurred in separate castes, queens and workers, respectively (*7*). Linksvayer and Wade (*8*) added a molecular dimension to this idea by predicting that sibling care and maternal care behaviors should be regulated by similar patterns of gene expression.

We used *Polistes* paper wasps to test Linksvayer and Wade's idea. *Polistes* are primitively eusocial, which means that although individuals specialize as either workers or reproductive individuals, these two castes are less distinct than in advanced eusocial species. In *Polistes*, both workers and reproductives display provisioning behavior, but at different points in the life of a colony. Advanced eusocial insects, by contrast, have morphologically distinct queen and worker castes, and in some species, such as the honey bee, queens no longer exhibit any maternal care, which precludes comparing the molecular basis of sibling and maternal care. Primitively eusocial insects like *Polistes* afford the opportunity to explore the molecular basis of maternal and worker behavior within a single species.

We measured brain gene expression in 87 individuals from four distinct behavioral groups of females from naturally occurring colonies of the temperate species *Polistes metricus* (Fig. 1A). Foundresses are females that establish new colonies in the spring, often as solitary individuals. Foundresses exhibit both reproductive (egg-laying) and maternal (foraging and brood-feeding) behavior. After rearing a first generation of female brood that develop into workers, successful foundresses become queens and cease caring for brood. Workers take over provisioning the brood—their siblings—by foraging for food and then feeding them; workers show little, if any, reproductive behavior. By contrast, queens focus exclusively on reproductive behavior. Gynes are reared late in the season; they engage in no reproductive or maternal care behavior (*9*). After successfully mating, gynes overwinter and then become foundresses (*10*). We hypothesized that brain gene expression patterns in *P. metricus* workers and foundresses should be most similar to each other from among these four groups, because they both show brood provisioning behavior despite their different reproductive status. Alternatively, if brain gene expression more closely reflects reproductive behavior, expression in foundresses and queens should be most similar to each other.

Social behavior is a complex and polygenic trait, so an appropriate test of the idea that maternal and worker behavior share a common molecular basis requires analysis of multiple genes in different pathways. But *Polistes* wasps, though venerable models for studies of social evolution (*11*, *12*), have until recently lacked genomic sequence information (*13*). To provide a ready source of test genes for quantitative reverse transcription–polymerase chain reaction analysis, we used 454 sequencing to obtain 45 megabases (Mb) in 391,157 cDNA sequence fragments from the *P. metricus* brain transcriptome (*14*). We were interested to see whether this low-cost, high-throughput sequencing method would be successful for this purpose, despite short se-

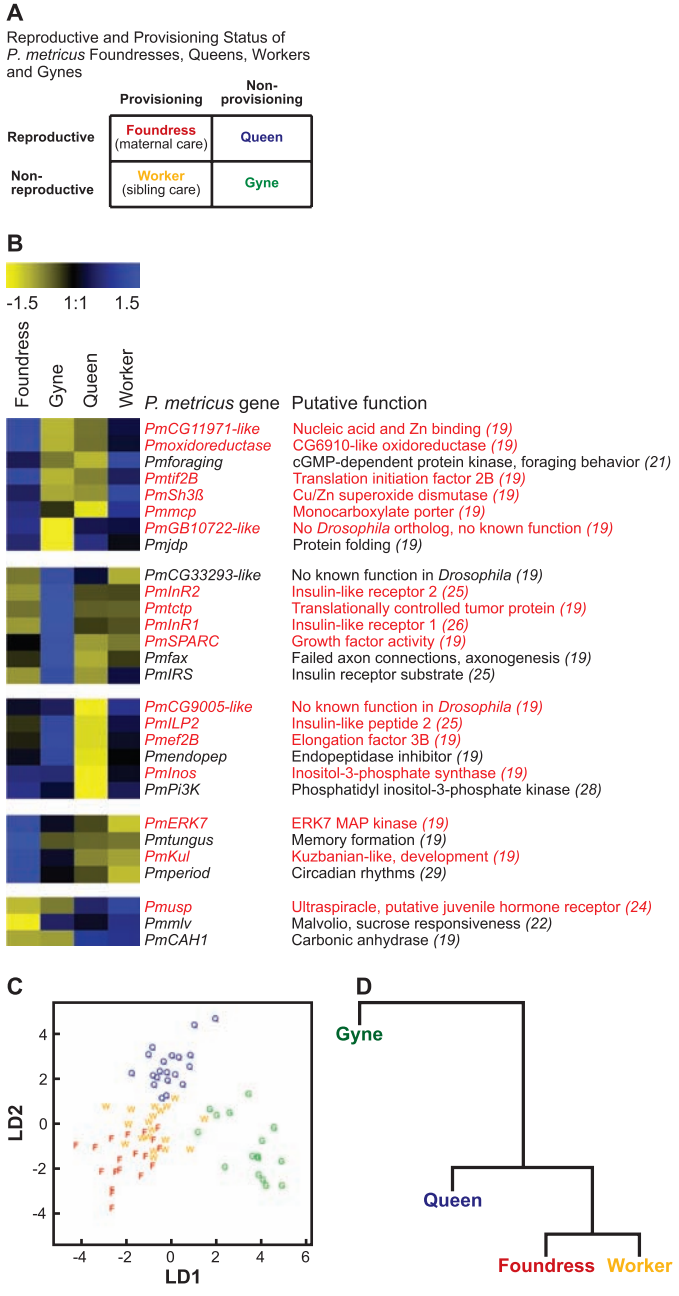

Fig. 1. *P. metricus* wasp brain gene expression analysis tests the prediction that maternal and worker (eusocial) behavior share a common molecular basis. (**A**) Similarities and differences in reproductive and brood provisioning status for the four behavioral groups analyzed in this study: foundresses (n = 22), gynes (n = 20), queens (n = 23), and workers (n = 22). Each individual wasp (total of 87) was assigned to a behavioral group on the basis of physiological measurements (*14*). (**B** to **D**) Results for 28 genes selected for their known involvement in worker (honey bee) behavior. (**B**) Heatmap of mean expression values by group and a summary of analysis of variance (ANOVA) results for each gene. Genes were clustered by K-means clustering (*37*); those in red showed significant differences (ANOVA, $P < 0.05$; table S1) between the behavioral groups. *P. metricus* gene names were assigned on the basis of orthology to honey bee genes (reference in parentheses); putative functions were assigned on the basis of similarity to *Drosophila melanogaster* genes. (C) Results of linear discriminant analysis show that foundress and worker brain profiles are more similar to each other than to the other groups. (D) Results of hierarchical clustering show the same result (based on group mean expression value for each gene). Four genes (*PmVg*, *Pmg5sd*, *PmGlyP*, and *PmRfaBp*) were excluded from these analyses because they showed high levels of expression in tissue adjacent to the brain (fig. S2); results for all three analyses were similar with and without these four genes (fig. S3).

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quence read lengths (average of 120 bp) and an estimated 100- to 150-million-year divergence time between *P. metricus* and the honey bee, *Apis mellifera* (*15*), the most closely related species with a sequenced genome to use as reference (*16*).

We generated a map of the honey bee genome combined with known transcripts and their relative abundance in the combined bee expressed sequence tag (EST) data sets (*16*–*18*). *P. metricus* transcript fragments predicted to encode proteins orthologous to those encoded by *A. mellifera* genes were plotted on the map according to the number of fragments identified for a particular locus; matches were found for 39% of all honey bee mRNAs. The relative abundance of *P. metricus* sequence fragments corresponded well with the abundance of *A. mellifera* ESTs for the respective loci (Fig. 2). The combined *P. metricus*–*A. mellifera* transcriptome data set was then used to select the genes for this study.

Prior information allowed us to focus on genes implicated in honey bee foraging and provisioning behavior, rather than a set of randomly chosen genes that might be less informative. We selected 32 genes (Fig. 1B and table S1) from the *P. metricus* EST set that are orthologs of *A. mellifera* genes known to be associated in some way with worker bee behavior, based on results from studies with microarrays (22 genes) (*19*, *20*) and candidate genes (10 genes) (*21*–*29*). Twenty-two of the genes have been shown by microarray analysis to be both differentially expressed in the brains of honey bees engaged in foraging or feeding brood [on the list of the "top 100" genes most consistently associated with bee foraging behavior (*19*)] and regulated by juvenile hormone (*20*), which also causes worker bee foraging behavior (*30*). Five candidate genes are differentially expressed in honey bees engaged in foraging or feeding brood (*21*–*24*, *29*), three of which also have been shown to play causal roles in the regulation of worker bee foraging behavior (*21*, *22*, *31*). Five additional candidate genes involved in insulin signaling were selected because this pathway is implicated in honey bee queen-worker caste determination (*25*, *26*, *32*) and worker foraging behavior (*27*, *28*). Patterns of gene expression in *P. metricus* were not used as criteria for gene selection.

There was a robust association between individual wasp brain gene expression and naturally occurring behavioral differences among the wasp groups. Leave-one-out cross-validation analysis (*19*) resulted in 68, 69, 70, and 47% correct assignments to the foundress, gyne, queen, and worker groups, respectively. For the less conservative resubstitution method (*33*), the results were 89, 100, 100, and 95%. The predictions from both classification methods were significantly better than random (Chi-square tests, $P < 0.0001$, 25% expected). This honey bee–derived gene set thus demonstrates extensive brain regulation across the four wasp groups, making it an informative set to explore the molecular relationship between maternal and worker behavior in *P. metricus*.

Sixty-two percent of the genes in the gene set were differentially regulated in *P. metricus* as a function of reproductive or provisioning behavior (Fig. 1B and table S1). Multivariate analysis of variance showed that brain gene expression varied significantly with reproduction ($F = 3.28$, $P = 0.0002$) and provisioning ($F = 4.76$, $P < 0.0001$), with a significant provisioning × reproduction interaction ($F = 2.48$, $P = 0.002$). Three out of the five insulin-related genes showed significant associations with provisioning and/or reproductive behavior, consistent with known nutritional effects on behavior and physiology in honey bees and other social insects (*34*).

Three statistical analyses demonstrated that brain gene expression for worker wasps was more similar to that of maternal females (foundresses) than to that of females not showing maternal care (queens and gynes). First, K-means clustering (Fig. 1B and fig. S1) revealed five clusters of coexpressed genes. The first cluster contained genes ($n = 8$) that showed coexpression in foundresses and workers compared to queens and gynes. The second cluster of genes ($n = 7$) was mainly characterized by upregulation in gynes, and the third ($n = 6$) by down-regulation in queens, but in both of these clusters, foundresses and workers also showed patterns of expression that were similar to each other (Fig. 1B and fig. S1).

The second statistical analysis, linear discriminant (LD) analysis, also showed similarities between foundress and worker brain gene expression (Fig. 1C). A plot of LD1 versus LD2 (which accounted for 90% of the variation in brain gene expression across all four groups) revealed group-specific expression patterns, but foundresses and workers showed the greatest overlap. This is consistent with the poorer performance of classification methods (described above) for those two groups; overlap in gene expression patterns made them difficult to distinguish from each other. Gynes, which engage in neither reproductive nor provisioning behavior, were the most distinct group. The third statistical analysis, hierarchical clustering by group, supported the patterns found in the other two analyses—brain gene expression of workers and foundresses was most similar, and that of gynes was most divergent (Fig. 1D).

There are marked temporal changes in brain gene expression as females shift from foundress to queen status, i.e., from maternal to reproductive behavior. These findings demonstrate heterochronic expression of genes associated with maternal behavior, a form of plasticity that is considered to be necessary for the evolution of worker behavior (*8*). They also reflect the apparent modularity of egg-laying and brood provisioning behavior and their underlying regulatory networks; this type of modularity also is thought to be important in the evolution of novel traits (*35*).

We used the honey bee genome, together with "next-generation" sequencing technology, to rapidly bring genomics to the relatively closely

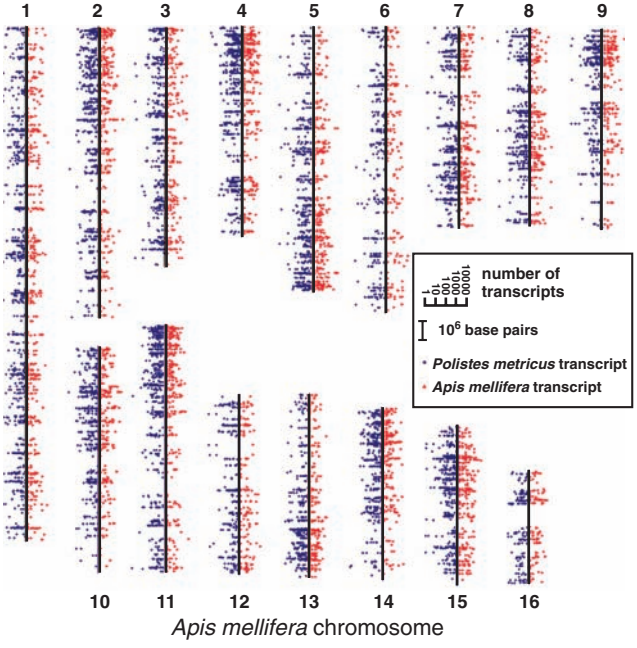

Fig. 2. A representation of *P. metricus* brain transcripts overlaid on a honey bee genome template (*16*) shows wide coverage and similar transcript abundance for *P. metricus* relative to known honey bee transcripts. *P. metricus* brain cDNA sequence fragments were matched as predicted proteins to *A. mellifera* transcripts with experimental support (known cDNA or EST sequences). *A. mellifera* transcripts (red points, right of axis) and their closest *P. metricus* orthologs from our survey (blue points, left of axis) were then mapped to the corresponding genomic locus in the *A. mellifera* genome. The vertical lines represent *A. mellifera* chromosomes 1 to 16. The distance of each point from the midline is proportional to the logarithm of the abundance of the mRNA (the number of sequences for each *P. metricus* or *A. mellifera* transcript corresponding to the *A. mellifera* gene at that locus) (*16*–*18*). *P. metricus* orthologs were obtained for a total of 3017 *A. mellifera* transcripts. The *P. metricus* transcriptome data contained putative orthologs for 39% of known *A. mellifera* mRNAs. An additional 252,556 transcript sequence fragments obtained from *P. metricus* did not have a clearly orthologous transcript in *A. mellifera*.

related wasp *P. metricus*; this is an early example of the utility of 454 sequencing for transcriptomics (*36*). Our results demonstrate that it is possible to use species that have had their genomes sequenced as "hubs" to efficiently generate genomic resources for clusters of related species that might each be especially well suited to address particular evolutionary problems. This "hub and spokes" approach should enable genomics to be deployed for a broader range of species than is currently being done, until whole-genome sequencing of eukaryote genomes becomes routine.

References and Notes

1. E. O. Wilson, B. Hölldobler, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13367 (2005).
2. L. Lehmann, L. Keller, *J. Evol. Biol.* **19**, 1365 (2006).
3. W. M. Wheeler, *The Social Insects: Their Origin and Evolution* (Harcourt, Brace, New York, 1928).
4. H. E. Evans, M. J. West-Eberhard, *The Wasps* (Univ. of Michigan Press, Ann Arbor, MI, 1970).
5. J. H. Hunt, *Evolution* **53**, 225 (1999).
6. M. J. West-Eberhard, in *Natural History and Evolution of Paper-Wasps*, S. Turillazzi, M. J. West-Eberhard, Eds. (Oxford Univ. Press, New York, 1996), pp. 290–317.
7. E. O. Wilson, *The Insect Societies* (Belknap, Cambridge, MA, 1971).
8. T. A. Linksvayer, M. J. Wade, *Q. Rev. Biol.* **80**, 317 (2005).
9. M. J. West-Eberhard, *Misc. Publ. Mus. Zool. Univ. Mich.* **140**, 1 (1969).
10. J. H. Hunt, *The Evolution of Social Wasps* (Oxford Univ. Press, New York, 2007).
11. S. Turillazzi, M. J. West-Eberhard, Eds., *Natural History and Evolution of Paper-Wasps* (Oxford Univ. Press, New York, 1996).
12. H. K. Reeve, in *The Social Biology of Wasps*, K. G. Ross, R. W. Matthews, Eds. (Cornell Univ. Press, Ithaca, NY, 1991).
13. S. Sumner, J. J. M. Pereboom, W. C. Jordan, *Proc. R. Soc. London B Biol. Sci.* **273**, 19 (2006).
14. Materials and methods are available as supporting material on *Science* Online.
15. B. N. Danforth, S. G. Brady, S. D. Sipes, A. Pearson, *Syst. Biol.* **53**, 309 (2004).
16. Honey Bee Genome Sequencing Consortium, *Nature* **443**, 931 (2006).
17. F. M. F. Nunes *et al*., *BMC Genomics* **5**, 84 (2004).
18. C. W. Whitfield *et al*., *Genome Res.* **12**, 555 (2002).
19. C. W. Whitfield, A. M. Cziko, G. E. Robinson, *Science* **302**, 296 (2003).
20. C. W. Whitfield *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16068 (2006).
21. Y. Ben-Shahar, A. Robichon, M. B. Sokolowski, G. E. Robinson, *Science* **296**, 741 (2002).
22. Y. Ben-Shahar, N. L. Dudek, G. E. Robinson, *J. Exp. Biol.* **207**, 3281 (2004).
23. G. V. Amdam, K. Norberg, M. K. Fondrk, R. E. Page, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 11350 (2004).
24. A. R. Barchuk, R. Maleszka, Z. L. P. Simoes, *Insect Mol. Biol.* **13**, 459 (2004).
25. D. E. Wheeler, N. Buck, J. D. Evans, *Insect Mol. Biol.* **15**, 597 (2006).
26. M. Corona *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 7128 (2007).
27. S. A. Ament, R. A. Verlarde, G. E. Robinson, *Society for Neuroscience Itinerary Planner and Abstract Viewer*, "Neuropeptide Y signaling and nutritionally mediated social behavior in the honey bee" (2006).
28. G. J. Hunt *et al*., *Naturwissenschaften* **94**, 247 (2007).
29. G. Bloch, D. P. Toma, G. E. Robinson, *J. Biol. Rhythms* **16**, 444 (2001).
30. G. Bloch, D. E. Wheeler, G. E. Robinson, in *Hormones, Brain and Behavior*, D. W. Pfaff, A. Arnold, A. Etgen, S. Fahrbach, R. Rubin, Eds. (Elsevier Science, St. Louis, MO, 2002), vol. 3, pp. 195–235.
31. M. Nelson, K. Ihle, M. K. Fondrk, R. E. Page, G. V. Amdam, *PLoS Biol.* **5**, e62 (2007).
32. A. Patel *et al*., *PLoS One* **2**, e509 (2007).
33. W. N. Venables, B. D. Ripley, *Modern Applied Statistics with S* (Springer, New York, ed. 4, 2002).
34. A. L. Toth, S. Kantarovich, A. F. Meisel, G. E. Robinson, *J. Exp. Biol.* **208**, 4641 (2005).
35. M. J. West-Eberhard, *Developmental Plasticity and Evolution* (Oxford Univ. Press, New York, 2003).
36. M. E. Hudson, *Mol. Ecol. Notes*, 10.1111/j.1471-8286.2007.02019.x (2007).
37. A. Sturn, J. Quackenbush, Z. Trajanoski, *Bioinformatics* **18**, 207 (2002).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S3
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JMJD6 Is a Histone Arginine Demethylase

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Arginine methylation occurs on a number of proteins involved in a variety of cellular functions. Histone tails are known to be mono- and dimethylated on multiple arginine residues where they influence chromatin remodeling and gene expression. To date, no enzyme has been shown to reverse these regulatory modifications. We demonstrate that the Jumonji domain–containing 6 protein (JMJD6) is a JmjC-containing iron- and 2-oxoglutarate–dependent dioxygenase that demethylates histone H3 at arginine 2 (H3R2) and histone H4 at arginine 3 (H4R3) in both biochemical and cell-based assays. These findings may help explain the many developmental defects observed in the JMJD6$^{-/-}$ knockout mice.

Iron- and 2-oxoglutarate–dependent dioxygenases have been shown to oxidize a variety of substrates including metabolites, nucleic acids, and proteins (*1*). A candidate dioxygenase, JMJD6, shares extensive sequence and predicted structural homology with an asparaginyl hydroxylase (*2*, *3*) as well as the JmjC domains found in several histone lysine demethylases (fig. S1A) (*4*–*8*). Given the predicted conservation of structural elements and key residues (*9*–*11*), it is likely that JMJD6 retains an analogous catalytic activity. Here we report in vitro and in vivo data that clearly indicate that JMJD6 functions as an arginine demethylase.

To test whether JMJD6 demethylates the N-terminal tails of histone H3 or H4, we incubated bulk histones with JMJD6 in the presence of Fe(II), 2-oxoglutarate, and ascorbate (*12*). Antibodies specific for various methylated sites on histones H3 and H4 were used to assess demethylation. Although no lysine demethylation was observed, a substantial reduction in H3R2me2 and H4R3me2 was observed in the presence of JMJD6 compared with buffer alone (Fig. 1A). These effects were site-specific as no changes in dimethylarginine were seen at positions H3R17 or H3R26. Previously, no enzyme had been shown to reverse regulatory arginine methylation, although deiminases can convert methylarginine to citrulline via demethylimination (*13*, *14*). However, the requisite chemistry is analogous to that demonstrated for demethylation of alkylated nitrogens by other dioxygenases (fig. S1C).

To investigate the preference for the substrate methylation state, we used antibodies specific for either mono- or dimethylated (symmetric) H4R3 (Fig. 1B). The recombinant JMJD6 was able to demethylate H4R3me2 when either heterogeneous bulk histones or synthetic peptides encompassing the N-terminal 30 residues of histone H4 were used as substrates (Fig. 1C). To a lesser extent, JMJD6 could also demethylate H4R3me1-containing substrates (Fig. 1C). Mutation of the residues predicted to mediate Fe(II) binding (mut JMJD6) prevented demethylation (Fig. 1C).

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