Correspondences

DNA methylation is widespread across social Hymenoptera

Marcus R. Kronforst[1], David C. Gilley[2], Joan E. Strassmann[3] and David C. Queller[3,*]

Genomic imprinting is an epigenetic phenomenon by which the expression of a gene is influenced by the parent from which it is inherited. The evolutionary causes of imprinting are mysterious but it is likely to represent a form of within-genome conflict [1]. For instance, alleles inherited from the father and the mother will be in conflict over treatment of relatives to which they are differently related. In this context, natural selection may favor alleles with effects that differ depending on the allele's parental origin [1,2]. This 'kinship theory of imprinting' has been developed and tested largely in the context of parental provisioning of offspring [1,2]. Given their haplodiploid genetic system and interspecific variation in social traits, the Hymenoptera (ants, bees, and wasps) provide a large variety of novel contexts in which to examine this theory [2]. However, aside from evidence that imprinting determines sex in the parasitic wasp *Nasonia vitripennis* [3], and a QTL that appears to be paternally inherited in the honeybee [4], nothing is known about imprinting in this group of animals. Here we provide evidence that CpG methylation, a hallmark of imprinting, is ubiquitously present in social insects but the proportion of methylated sites varies substantially among species and developmental stages.

Imprinting is mediated by DNA methylation — a heritable, chemical modification of genomic DNA that involves the binding of a methyl group to a nucleotide, often the 5′ carbon of the cytosine pyrimidine ring. In vertebrates, methylated DNA sequences are transcriptionally inactive [5]. In insects, DNA methylation is poorly characterized but it appears to vary widely across species, in terms of both overall amount and genomic location [6]. For instance, *Drosophila melanogaster* shows very little DNA methylation and it is concentrated primarily at CpT and CpA dinucleotides [6]. In contrast, the genome of the moth *Mamestra brassicae* contains, much like vertebrates, approximately 10% 5-methyl-cytosine that is largely concentrated at CpG sites [7]. Recently, a fully functional CpG methylation system was discovered in the honey bee, *Apis mellifera* [8] a finding that could open the door to utilize social insects to study the evolutionary causes and consequences of imprinting. However, the most powerful tests to examine the evolution of genomic imprinting are comparative [2], and it remains unclear whether CpG methylation is widespread across Hymenoptera.

To examine the distribution of DNA methylation across the Hymenoptera, we surveyed a variety of bee, wasp, and ant species using a methylation-sensitive amplified fragment length polymorphism (AFLP) technique [9]. By digesting genomic DNA of each species with two restriction enzymes that have the same cut site (5′-CCGG-3′) but different sensitivities to methylation (*Msp*I and *Hpa*II), we were able to infer the proportion of restriction sites that were methylated in each individual. Our survey included three adult individuals from each of 12 hymenopteran species as well as three *Apis mellifera* individuals from each of three additional developmental stages: young larvae (approximately two days old), old larvae (approximately four days old), and young pupae (approximately 12 days old).

We scored 425 to 878 (mean: 580) restriction sites per group of three individuals (species or developmental stage) and found that each of the surveyed species exhibited evidence of DNA methylation. However, the proportion of methylated sites varied substantially across species. On the low end, we found the bee *Trigona spinipes* with approximately 1% of sites methylated and on the high end was the wasp *Polistes dominulus* with 19% of sites methylated (Figure 1). Methylation also varied across developmental stages in *Apis mellifera*. Approximately 11% of sites were

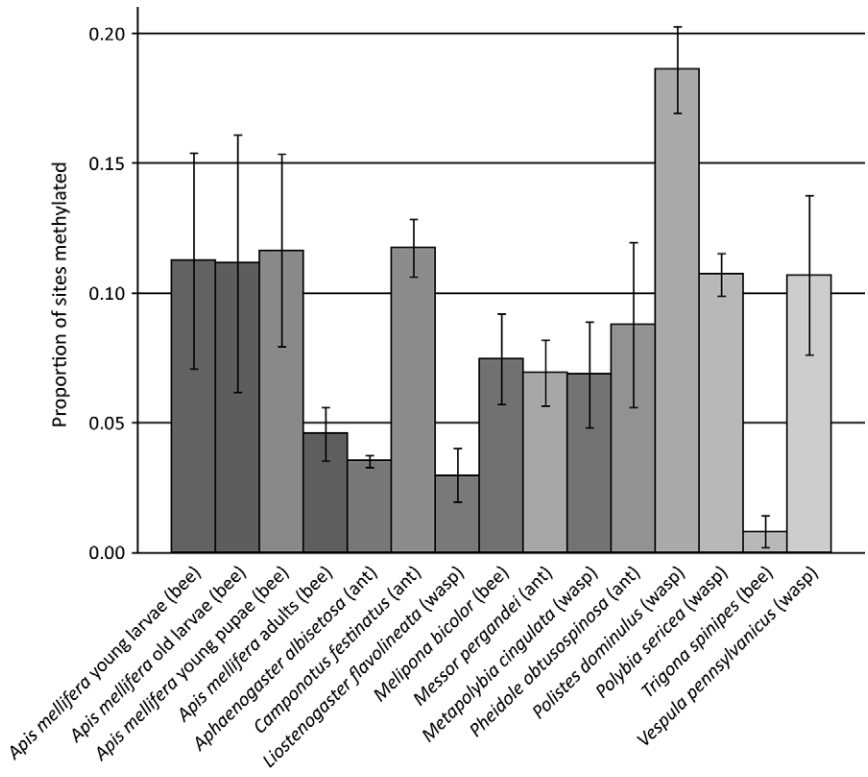

Figure 1. Patterns of DNA methylation across social insects.

For each individual, we calculated the proportion of methylated restriction sites as the number of AFLP fragments exhibiting evidence of methylation or hemimethylation divided by the total number of fragments. We then averaged these across the three individuals in each group (species or developmental stage). Error bars show one standard deviation.

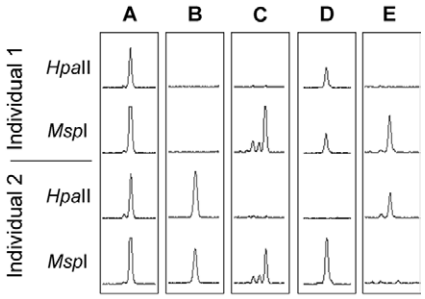

Figure 2. Example of methylation-sensitive AFLP profiles for two *Polistes dominulus* individuals.

(A) A fragment that is present with both restriction enzymes in both individuals, indicating a fixed and unmethylated restriction site. (B) A fragment that is absent in one individual but present with both enzymes in another, indicating a polymorphic and unmethylated restriction site. (C) A fragment that is present with only one restriction enzyme for both individuals, indicating a fixed and methylated restriction site. (D) A fragment that is present with both enzymes in one individual but only present with one enzyme in another individual, indicating a restriction site that is unmethylated in one but methylated in the other. (E) A fragment that is present with one enzyme in one individual but with the other enzyme in another individual, indicating a restriction site that is fully methylated in one but hemimethylated in the other. All markers are pictured at a y-axis scale of 1000 reflectance units.

methylated in young larvae, old larvae, and young pupae of *Apis mellifera* but for adults, this dropped to an average of 4.6%. Intriguingly, we also found evidence for variation between methylation states among individuals of the same species (Figure 2). Across species, 4 to 47 AFLP markers (mean = 20) were present with both restriction enzymes in at least one individual but varied between restriction enzymes in another individual. This is indicative of a restriction site that is not methylated in one individual but methylated in another. Similarly, for a small number of markers (0 to 15 per species, mean: 5) we found that while one individual had a fragment present when digested with *Msp*I and absent when digested with *Hpa*II, another individual showed the opposite pattern with a fragment present with *Hpa*II and absent with *Msp*I. This is indicative of a restriction site that is fully methylated in one individual but hemimethylated in another [10].

Our data reveal that CpG methylation is common in social insects but the overall amount of methylation varies across species and developmental stages. This baseline information sets the stage for a variety of important questions. For instance, does CpG methylation underlie imprinting in social insects? If so, does the variation in methylation we observe among species translate into variation in the extent of genomic imprinting? Finally, and most importantly, do patterns of methylation and imprinting across the social Hymenoptera support the kinship theory of imprinting? If so, this group of insects will provide novel experimental opportunities to study the evolution of genomic conflict.

Supplemental data

Supplemental data including experimental procedures are available at http://www.current-biology.com/cgi/content/full/18/7/R287/DC1

Acknowledgments

We thank Ming Huang for providing *Aphaenogaster albisetosa* and *Messor pergandei* specimens, Barry Sullender and Stefan Cover for assistance identifying ant species, and Amir Karger for assistance with data analysis.

References

1. Haig, D. (2000). The kinship theory of genomic imprinting. Annu. Rev. Ecol. Syst. *31*, 9–32.
2. Queller, D.C. (2003). Theory of genomic imprinting conflict in social insects. BMC Evol. Biol. *3*, 15.
3. Dobson, S.L., and Tanouye, M.A. (1998). Evidence for a genomic imprinting sex determination mechanism in Nasonia vitripennis (Hymenoptera; Chalcidoidea). Genetics *149*, 233–242.
4. Guzman-Novoa, E., Hunt, G.J., Page, R.E., Uribe-Rubio, J.L., Prieto-Merlos, D., and Becerra-Guzman, F. (2005). Paternal effects on the defensive behavior of Honeybees. J. Heredity *96*, 376–380.
5. Bird, A. (2002). DNA methylation patterns and epigenetic memory. Genes Dev. *16*, 6–21.
6. Field, L.M., Lyko, F., Mandrioli, M., and Prantera, G. (2004). DNA methylation in insects. Insect Mol. Biol. *13*, 109–115.
7. Mandrioli, M., and Volpi, N. (2003). The genome of the lepidopteran *Mamestra brassicae* has a vertebrate-like content of methyl-cytosine. Genetica *119*, 187–191.
8. Wang, Y., Jorda, M., Jones, P.L., Maleszka, R., Ling, X., Robertson, H.M., Mizzen, C.A., Peinado, M.A., and Robinson, G.E. (2006). Functional CpG methylation system in a social insect. Science *314*, 645–647.
9. Xu, M., Li, X., and Korban, S.S. (2000). AFLP-based detection of DNA methylation. Plant Mol. Biol. Reptr. *18*, 361–368.
10. Xiong, L.Z., Xu, C.G., Saghai Maroof, M.A., and Zhang, Q. (1999). Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplification polymorphism technique. Mol. Gen. Genet. *261*, 439–446.

[1]FAS Center for Systems Biology, Harvard University, Cambridge, Massachusetts, USA.
[2]Department of Biology, William Paterson University, Wayne, New Jersey, USA.
[3]Department of Ecology and Evolutionary Biology, Rice University, Houston, Texas, USA.
*E-mail: queller@rice.edu

Imitation recognition in great apes

Daniel B.M. Haun[1,2] and Josep Call[1]

Human infants imitate not only to acquire skill, but also as a fundamental part of social interaction [1–3]. They recognise when they are being imitated by showing increased visual attention to imitators (implicit recognition) and by engaging in so-called testing behaviours (explicit recognition). Implicit recognition affords the ability to recognize structural and temporal contingencies between actions across agents, whereas explicit recognition additionally affords the ability to understand the directional impact of one's own actions on others' actions [1–3]. Imitation recognition is thought to foster understanding of social causality, intentionality in others and the formation of a concept of self as different from other [3–5]. Pigtailed macaques (*Macaca nemestrina*) implicitly recognize being imitated [6], but unlike chimpanzees [7], they show no sign of explicit imitation recognition. We investigated imitation recognition in 11 individuals from the four species of non-human great apes. We replicated results previously found with a chimpanzee [7] and, critically, have extended them to the other great ape species. Our results show a general prevalence of

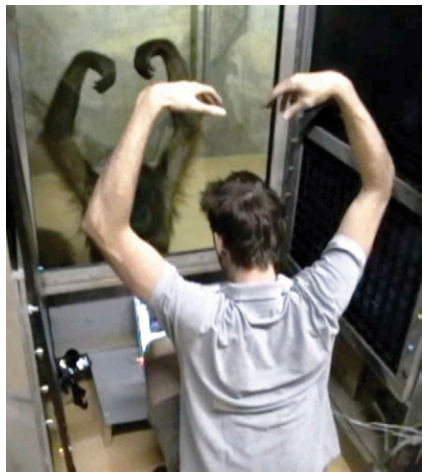

Figure 1. Experimenter and female orangutan (*Pongo pygmaeus*) interacting in the contingent/matching condition showing an example of testing behaviour (testing pose).