

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 *MspI* and absent when digested with *HpaII*, another individual showed the opposite pattern with a fragment present with *HpaII* and absent with *MspI*. 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>

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References

- Haig, D. (2000). The kinship theory of genomic imprinting. *Annu. Rev. Ecol. Syst.* 31, 9–32.
- Queller, D.C. (2003). Theory of genomic imprinting conflict in social insects. *BMC Evol. Biol.* 3, 15.
- 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.
- Guzman-Novoa, E., Hunt, G.J., Page, R.E., Uribe-Rubio, J.L., Prieto-Merios, D., and Becerra-Guzman, F. (2005). Paternal effects on the defensive behavior of Honeybees. *J. Heredity* 96, 376–380.
- Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev.* 16, 6–21.
- Field, L.M., Lyko, F., Mandrioli, M., and Pranter, G. (2004). DNA methylation in insects. *Insect Mol. Biol.* 13, 109–115.
- 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.
- 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.
- Xu, M., Li, X., and Korban, S.S. (2000). AFLP-based detection of DNA methylation. *Plant Mol. Biol. Repr.* 18, 361–368.
- 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.

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Imitation recognition in great apes

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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).

imitation recognition in all great apes and thereby demonstrate important differences between great apes and monkeys in their understanding of contingent social interactions.

In phase 1 of the study, the experimenter (E) interacted with non-human great apes (henceforth apes) in four different conditions manipulating the structural and temporal contingency between the apes' and his actions. Testing behaviours are defined as actions, performed to test the contingent relationship between two interacting individuals [1–3]. In human children, these include either deliberately odd behaviours to test form contingencies or odd or repetitive sequences of behaviours to test timing contingencies [2,3,5]. Based on previous work [7], therefore, we coded testing behaviour in apes as behavioural patterns which were enacted while facing E, if they fell outside of the described repertoire of the species (for example, testing poses; see Figure 1), or were combined in non-standard sequences (testing sequences) or uncharacteristically long bouts of repetitions (behaviour repetitions). Out of 46 coded bouts of testing behaviour, four were testing poses, zero testing sequences and 42 behaviour repetitions (for detailed Supplemental Experimental Procedures, see the Supplemental Data available on-line with this issue).

Apes demonstrated more testing behaviours in conditions in which: E acted synchronous to their actions, compared to a two second delay; E repeated the apes' action, compared to responding with a different action; and E repeated the apes' action at the same time, hence matching timing and form of his actions to the apes' actions (Figure 2A). Apes never displayed testing sequences.

Based on previous work [7], we administered an additional test (phase 2) to assess whether apes responded to the imitative nature of E's actions or to some behavioural peculiarity unconsciously exhibited by E. In this case, E either imitated the ape, or, observing the videotapes of phase 1, replicated all actions as the ape had exhibited them eight weeks earlier. Apes demonstrated more testing behaviours when E imitated their current rather than their past actions (Figure 2B). Of a total of 26

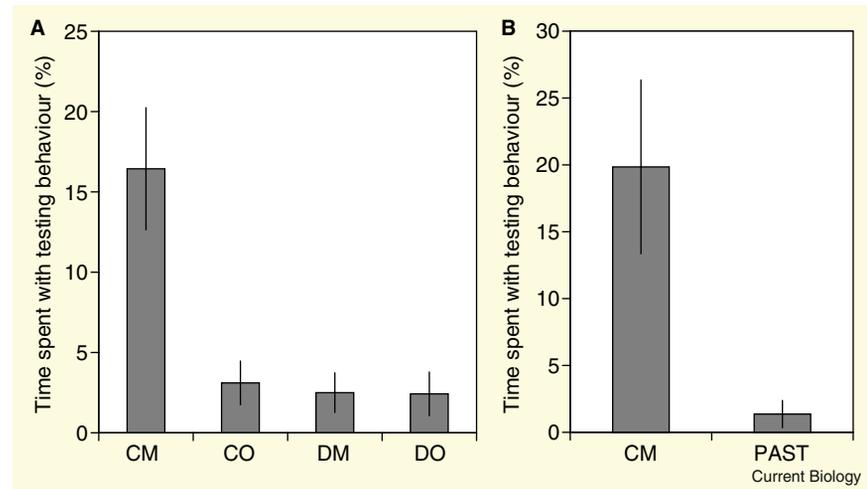


Figure 2. Imitation recognition in great apes.

(A) Mean percentage (+SE) of total time (four trials x 30 seconds) apes spent engaged in testing behaviour in each of the four conditions of phase 1. Contingent/matching (CM), the experimenter (E) replicated all actions as the ape exhibited them; contingent/other (CO), as the ape performed an action, E performed a different action synchronously; delayed/matching (DM), E replicated all actions two seconds after the ape exhibited them; delayed/other (DO), two seconds after the ape performed an action, E performed a different action (CM: M = 16.49, SE = 3.82; CO: M = 3.15, SE = 1.40; DM: M = 2.50, SE = 1.24; DO: M = 2.47, SE = 1.40). A mixed ANOVA with the within-subject factors contingency (contingent/delayed) and match (matching/other) and species as a between-subject factor revealed significant effects of contingency ($F_{(1,7)} = 11.45$; $p = 0.012$; partial $\eta^2 = .62$), match ($F_{(1,7)} = 8.94$; $p = 0.020$; partial $\eta^2 = 0.46$) and contingency x match ($F_{(1,7)} = 8.60$; $p = 0.022$; partial $\eta^2 = 0.55$). There were no significant differences between species and no other interactions. In the CM condition 9 of 11 animals exhibited testing behaviour at least once. In the CO, DM and DO conditions, only 5/11, 3/11 and 4/11 animals showed testing behaviour at least once. (B) Mean percentage (+SE) of total time (four trials x 30 seconds) apes spent engaged in testing behaviour in each of the two conditions of phase 2. CM (see Figure 2A); PAST: E observed the videotapes of the CM condition of phase 1, and replicated all actions as the apes (and E) had exhibited them eight weeks earlier (CM: M = 19.85, SE = 6.50; PAST: M = 1.36, SE = 1.02). A repeated-measures ANOVA with the within-subject factor condition (CM/PAST) revealed a significant main effects of condition ($F_{(1,7)} = 7.74$; $p = 0.019$; partial $\eta^2 = 0.44$). In the CM condition 7 of 11 animals exhibited testing behaviour at least once, while in the PAST condition only 2/11 animals showed any testing behaviour at all.

coded bouts of testing behaviour, five were testing poses, zero were testing sequences and 21 behaviour repetitions. These results replicated those of phase 1 and showed that testing behaviour was indeed caused by the imitative quality of the interaction and not by inadvertently peculiar behaviour of E.

We found that, in response to being imitated, apes reliably displayed behaviours more closely aligned to the behaviours observed in human infants than to those observed in monkeys. The observed behaviours are interpreted as testing the structural and temporal contingencies of an interaction. Apes displayed odd behaviours testing form contingencies and repetitive sequences of behaviours testing timing contingencies. Unlike children, however, apes never exhibited testing sequences. Our data replicated previous results with a chimpanzee [7]

and are consistent with the hypotheses that apes have the ability to explicitly recognize the contingency between actions in a social interaction and the directional impact of their own actions on others' actions.

Our data reveal an important difference between great apes and monkeys in understanding contingent social interactions. Further, the absence of testing sequences in our ape sample contrasts starkly with their abundance in human children [1–3,5], suggesting a potential species difference within the great apes. Taking a phylogenetic perspective [8,9], we infer that the competences for contingent social interactions increased around the time of the common ancestor of great apes, approximately 15 million years ago, and possibly again in the hominid line. The increased social competence in great apes relative to monkeys fits with

theories arguing that dispersed social systems, common in great ape but not monkey societies, caused selection pressures favouring advanced social cognitive abilities [10].

Supplemental data

Supplemental data are available at <http://www.current-biology.com/cgi/content/full/18/7/R288/DC1>

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References

1. Nadel, J. (2002). Imitation and imitation recognition: functional use in preverbal infants and nonverbal children with autism. In *The Imitative Mind: Development, Evolution and Brain Bases*, A.N. Meltzoff and W. Prinz, eds. (Cambridge: Cambridge University Press), pp. 63–73.
2. Asendorpf, J.B., Warkentin, V., and Baudonniere, P.-M. (1996). Self-awareness and other-awareness 2: mirror self-recognition, social contingency awareness, and synchronic imitation. *Dev. Psych.* 32, 313–321.
3. Meltzoff, A.N. (1990). Foundations for developing a concept of self: the role of imitation in relating self to other and the value of social mirroring, social modelling, and the self practice in infancy. In *The Self in Transition: Infancy to Childhood*, D. Cicchetti and M. Beeghly, eds. (Chicago: University of Chicago Press), pp. 139–164.
4. Schütz-Bosbach, S., and Prinz, W. (2007). Perceptual resonance: action-induced modulation of perception. *Trends Cogn. Sci.* 11, 349–355.
5. Agnetta, B., and Rochat, P. (2004). Imitative games by 9-, 14-, and 18-month-old infants. *Infancy* 6, 1–36.
6. Paukner, A., Anderson, J.R., Borelli, E., Visalberghi, E., and Ferrari, P.F. (2005). Macaques (*Macaca nemestrina*) recognize when they are being imitated. *Biol. Lett.* 1, 219–222.
7. Nielsen, M., Collier-Baker, E., Davis, J.M., and Suddendorf, T. (2005). Imitation recognition in a captive chimpanzee (*Pan troglodytes*). *Anim. Cogn.* 8, 31–36.
8. Haun, D.B.M., Call, J., Janzen, G., and Levinson, S.C. (2006). Evolutionary psychology of spatial representations in the hominidae. *Curr. Biol.* 16, 1736–1740.
9. Haun, D.B.M., Rapold, C.J., Call, J., Janzen, G., and Levinson, S.C. (2006). Cognitive cladistics and cultural override in Hominid spatial cognition. *Proc. Natl. Acad. Sci. USA* 103, 17568–17573.
10. Barrett, L., Henzi, P., and Dunbar, R. (2003). Primate cognition: from ‘what know?’ to ‘what if?’ *Trends Cogn. Sci.* 7, 494–497.

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Use-induced motor plasticity affects the processing of abstract and concrete language

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Traditional analyses of language [1] emphasize an arbitrary correspondence between linguistic symbols and their extensions in the world, but recent behavioral and neurophysiological [2,3] studies have demonstrated a processing link between a symbol and its extension: that is, comprehension of language about concrete events relies in part on a simulation process that calls on neural systems used in perceiving and acting on those extensions. It is an open question, however, whether this simulation process is necessary for abstract language understanding [4,5]. Here we report how, using a new technique based on use-induced neural plasticity [6], we have obtained evidence for a causal link between the motor system and the comprehension of both concrete and abstract language.

Participants were required to move, one at a time, 600 beans from a wide-mouthed container to a target — a narrow-mouthed container, an arm’s length away. The movement direction was either toward or away from the body, as determined by the location of the target. After moving all the beans, participants read nonsense and sensible sentences describing transfer of concrete objects or abstract information toward or away from themselves (see Table S1 in the Supplemental Data for examples). On measuring the time required to judge the sentences as sensible, we observed an interaction between the direction of previous bean movement and the direction of described transfer (toward or away) for both the concrete and abstract sentences. That is, modifying the motor system affected processes used in the comprehension of both concrete and abstract language.

In the first experiment, after moving the beans (for approximately 20 minutes), participants read (for approximately 10 minutes) sensible

and nonsense sentences. The grammatical objects named concrete items or abstract information, and the sentences described transfer of the objects either toward the reader, away from the reader, or no transfer. There was a significant interaction such that participants were slower to make the sensible judgment, by pressing a key on a keyboard with the right index finger, when sentences described transfer in a direction that matched the direction of previous bean practice ($p = 0.02$; abstract alone, $p = 0.04$, see Figure 1 and details in Supplemental Data).

Repeated production or perception of a word results in a temporary loss of meaning termed semantic satiation [7]. If participants are covertly saying “toward” or “away” while performing the bean task, then later sentence comprehension might suffer because of the satiation phenomenon. We tested this alternative in experiment 2 by replacing the bean task with 600 trials of judging if a stimulus was a word (“toward” or “away”) or an anagram of that word. Then, participants judged the same sentences as in experiment 1. The absence of any interactions ($ps > 0.35$) between stimulus word and sentence direction indicates that the interaction found in experiment 1 is not due to the sort of verbal repetition that produces semantic satiation.

The concrete sentences were changed in experiment 3 so that they did not describe any movement. Instead, they described events that were near or far from the grammatical subject, and manipulated the grammatical person of the sentence subject (see Table S1). We replicated the interaction for abstract sentences describing transfer (although at $p = 0.06$), but now in the absence of concrete sentences describing transfer that might have biased a motoric interpretation of the abstract sentences. Furthermore, failure to find the critical interaction for the concrete sentences demonstrates that, in the absence of language about transfer, differences in location ($p = 0.68$) or grammatical subject ($p = 0.11$ in the wrong direction) do not contribute to the effect.

How abstract are the motor system controllers that the bean task changes? In experiment 4, participants moved the beans with the right arm and hand, but half