## Bonds smooth conflicts

Humans tend to respond differently between each other to rewards being dished out unfairly in different social groups, yet little is known about the potential for such variation in other species. A new study has examined responses to such inequity in several groups of chimpanzees (*Pan troglodytes*). The study, by Sarah Brosnan, Hillary Schiff and Frans de Waal, at the Yerkes National Primate Center at Emory University, Atlanta, in the Proceedings of the Royal Society series B (published online), demonstrates that chimpanzees show a response to inequity that depends to considerable extent on the social context that the rewards are given.

The researchers used an experimental procedure whereby animals were given a token, in this case a piece of white PVC pipe, which they then had to give back to the researcher to receive a food reward. Food rewards were placed in identical buckets on the floor in front of the chimpanzees so that all animals in the experiment could see what was in the buckets, but none knew what reward they would receive until they had successfully returned the token.

The rewards were chosen on the basis of previous preference tests in which grapes were most highly prized by the animals and cucumber and celery, the least sought.

The researchers found that the animals were averse to any inequity but only in subjects that lived in pairs or in a relatively newly established social group. In a far older group, with a tightly knit social structure characterised by intense integration and social reciprocity, inequity caused barely a ripple from the animals disappointingly receiving a cucumber or celery reward.

This finding may reflect human responses in close relationships, and inequity may be tolerated more as apes develop the mutual dependencies and bonds that serve a wide range of benefits derived from sociality. "If so, tolerance of inequity may increase with social closeness between partners, such as friends and family, in a wide variety of species, a hypothesis that deserves further testing in humans and non-human primates," the authors say.

Such tolerance may not marry with the experience of some humans, but its apparent existence in our closest cousins is intriguing.

**Finders keepers:** a new study has shown that closer social ties help chimpanzees play down inequities in food rewards (Photo: Oxford Scientific Films).

**Correspondence**

# Functional conservation of an insect odorant receptor gene across 250 million years of evolution

**Walton D. Jones[1], Thuy-Ai T. Nguyen[2], Brian Kloss[2], Kevin J. Lee[2], and Leslie B. Vosshall[1]**

Pest insects have a profound negative impact on agriculture and human health. Significant global losses of crops, stored agricultural products, timber and livestock can be attributed to damage and destruction by insects [1]. Blood-feeding insects such as mosquitoes, flies and ticks transmit many of humanity's most devastating infectious diseases. Insect-borne diseases account for more than one million annual fatalities, and insect-associated illnesses surpass 300 million annual reported cases [2]. The medical and economic impact of these animals can be ascribed in part to the sensitivity and selectivity of their olfactory systems, essential for location of their preferred plant and animal hosts.

In the fruit fly *Drosophila melanogaster*, the sense of smell is mediated by a family of 62 odorant receptor (OR) proteins, 61 of which are individually expressed in small non-overlapping sub-populations of olfactory sensory neurons (OSNs) of the antenna and maxillary palps [3]. A comparison of the OR repertoire of fruit fly with that of the mosquito *Anopheles gambiae* reveals dramatic sequence divergence. In each insect there are remarkable species-specific expansions of distinct OR gene subfamilies. The mosquito genome contains 27 ORs that have no corresponding gene in the fruit fly; similarly, the fruit fly genome contains 18 ORs with no

corresponding mosquito genes [4]. It has been suggested that the cognate ligands of these species-specific subfamilies of ORs could be the odorants that define host preference — fruit volatiles for *Drosophila* and human odors for *Anopheles* [4].

Among the 62 *Drosophila* OR genes, there is a single, atypical receptor, *Or83b*, that is co-expressed with the conventional ORs in nearly all olfactory neurons. Recent genetic studies in *Drosophila* have shown that *Or83b* has an important general function in olfaction, and is essential for localizing conventional OR proteins to the sensory dendrite. The absence of ORs in the OSN dendrites, the site of interaction with airborne odorants, eliminates odor-evoked potentials and severely attenuates olfactory-associated behaviors in *Or83b* mutant flies [5].

*Or83b* also stands apart from other conventional members of the OR gene family in that clear orthologs have been described in the *Anopheles* genome (*AgOR7* [4,6]) and in moth and beetle [7]. Unlike conventional ORs, whose inter-species sequence diversity may reflect the distinct food preferences and ecological niches used by insects, the remarkable sequence conservation of *Or83b* suggests a unique and essential function in insect olfaction that has been conserved through insect evolution. To test this notion, we asked whether *Or83b* orthologs from diverse insect species can functionally substitute for *Drosophila Or83b*. We focused on three major insect pests: the medfly, a citrus pest; the corn earworm moth, which damages corn and tobacco; and the malaria mosquito.

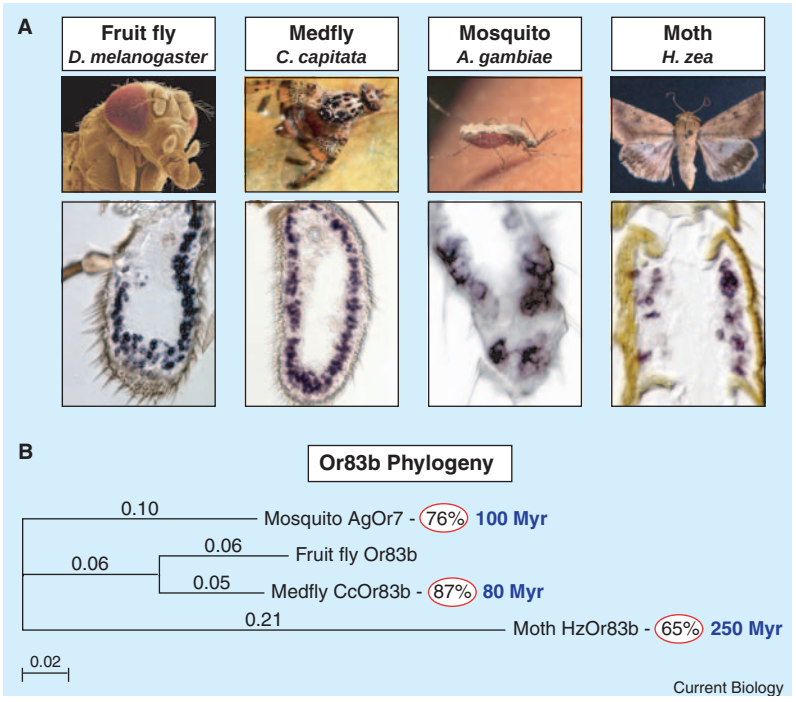


Figure 1. *Or83b* orthologs in diverse insect species.

(A) Broad expression of *Or83b* orthologs in antennal olfactory neurons of four divergent species as visualized by RNA *in situ* hybridization of antennal sections (see Supplemental Methods on-line). Insect photo credits: fruit fly (Juergen Berger, Max Planck Institute, Tuebingen, Germany); medfly (USDA); mosquito (Ekisei Sonoda); moth (John L. Capinera, University of Florida). (B) Best-fit *Or83b* phylogenetic tree generated using the neighbor-joining algorithm in MacVector. Values are uncorrected ('*p*') distance, with scale indicated at the lower left. Percent amino acid identities of *Or83b* orthologs compared to *Drosophila Or83b* are circled in red to the right of each species. Phylogenetic distance of each species to *Drosophila* (in million years, Myr) is indicated in blue text. Insect orders are: fruit fly, medfly, and mosquito, Diptera; moth, Lepidoptera. Full-length cDNA sequences have been deposited in Genbank: *Anopheles gambiae*, AY843205; *Ceratitis capitata*, AY843206; *Helicoverpa zea*, AY843204.

Orthologs of *Or83b* were cloned by cDNA library screening and RT-PCR (see Supplemental Methods online). RNA *in situ* hybridization shows widespread OSN-specific expression of each orthologous gene (Figure 1A). Analysis of predicted protein coding sequences from each insect shows 65–87% amino acid identity (Supplementary Figure 1). Phylogenetic analysis of these proteins is consistent with the inferred phylogeny of their corresponding species (Figure 1B).

We next examined whether these *Or83b* orthologs can functionally complement the defects in OR localization and odor-evoked potentials characteristic of *Or83b* mutant flies, using the GAL4–UAS system to express the orthologs in OSNs that normally express *Or83b* [8]. Transgenic expression of *Drosophila Or83b* and its orthologs from medfly, mosquito and moth in $Or83b^{-/-}$ flies rescues the mutant phenotype, alleviating the block in Or22a/b trafficking in sensory dendrites (Figure 2A) and rescuing odor-evoked electrophysiological defects (Figure 2B,C). We made multiple independent transgenic lines for each construct and saw variability in the degree of rescue. The consistently smaller EAG responses of the mosquito *AgOr7* rescue may be explained by position effects, sequence divergence or both; we favor position effects as the explanation because the more divergent moth ortholog is indistinguishable from *Drosophila Or83b*.

These data show functional conservation of a single OR gene across about 250 million years of evolution since the *Lepidopteran* and *Dipteran* lineages diverged. The strong selective pressure on *Or83b* orthologs clearly demonstrates the critical importance of this gene for insect olfaction. This conservation of function may reflect a requirement for *Or83b* to interact with other conserved cellular machinery. The results further suggest that *Or83b* might be an 'Achilles heel' for insects; rational design of novel insect control strategies targeted against *Or83b* orthologs might be

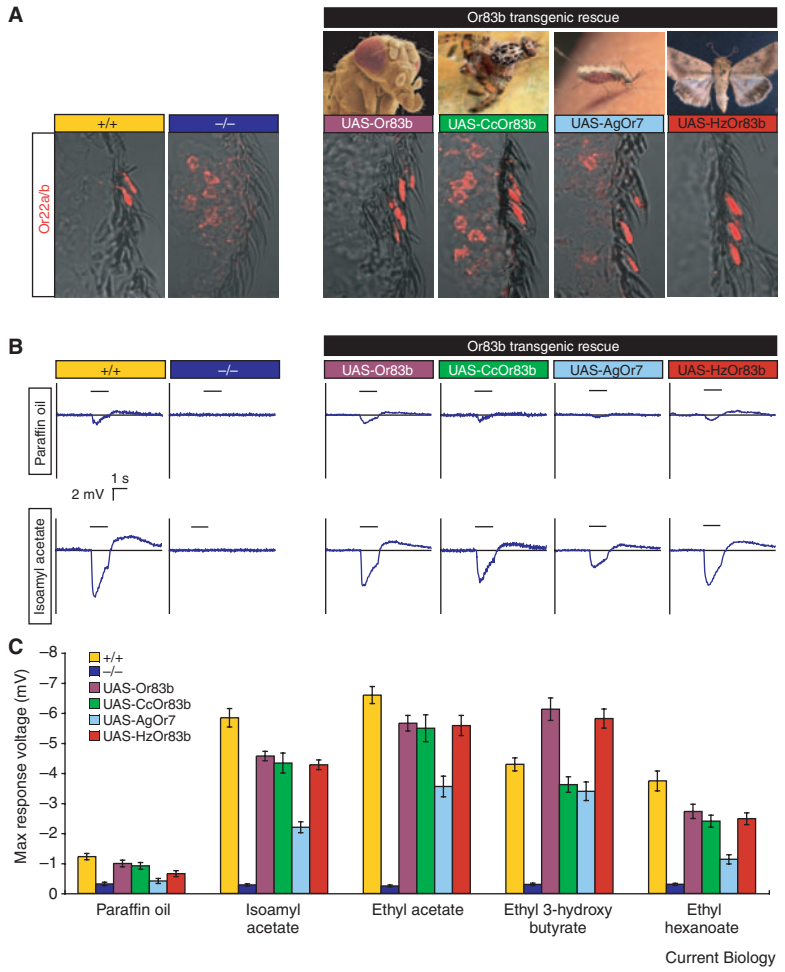


Figure 2. Transgenic rescue of *Or83b* orthologs restores function to *Or83b*$^{-/-}$ flies.

(A) Left panels: normal Or22a/b dendritic localization observed in +/+ (wild-type Berlin) flies is abolished in *Or83b*$^{-/-}$ (*Or83b*-GAL4; *Or83b*$^{2}$/*Or83b*$^{3}$) flies. Right panels: transgenic rescue with *Drosophila Or83b* and with the medfly (UAS-*CcOr83b*), mosquito (UAS-*AgOr7*), and moth (UAS-*HzOr83b*) *Or83b* orthologs restores Or22a/b localization in the OSN dendrites. Genotypes: *Or83b*-GAL4/UAS-species; *Or83b*$^{2}$/*Or83b*$^{3}$. Immunostaining performed as described [5]. (B) Electroantennograms (EAGs) measure robust odor-evoked activity in wild-type antennae that is abolished in *Or83b* mutants. *Or83b* orthologs from medfly, mosquito, and moth rescue this odor-evoked activity. Representative plots for isoamyl acetate and the solvent control (paraffin oil) are shown. Odorants (Sigma-Aldrich) were diluted 1:100 in paraffin oil (Fluka) and were of the highest purity available. Genotypes as in (A). EAGs performed as described [5]. To verify consistent contact with the antenna, all *Or83b* mutant antennae were tested with carbon dioxide before and after application of the other odorants. $CO_2$-responsive neurons are *Or83b*-independent and produce robust potentials (data not shown). (C) Summary of EAG data plotted as mean ± SEM of the peak response voltage, $n$ = 9 or 10 antennae per genotype. Responses of each transgenic rescue to the four odorants tested are significantly different from *Or83b*$^{-/-}$ mutant responses to the same odorants in pairwise comparisons ($P$<0.001; two-tailed t test).

effective in blocking host-seeking behavior in diverse insect pests.

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*Conflict of interest statement*: L.B.V. is an equity holder in Sentigen Holding Corporation and T.T.N., B.K. and K.J.L. are current or former employees of Sentigen Biosciences Inc., a wholly-owned subsidiary of Sentigen Holding Corporation. The intellectual property rights of *Or83b* have been licenced by Sentigen for the purposes of developing novel insect control agents.

## Supplemental data

Supplemental data comparing Or83b ortholog sequences are available at http://www.current-biology.com/cgi/content/full/15/4/Rxx/DC1/

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# Supplemental data: Functional Conservation of an Insect Odorant Receptor Gene Family Member Across 250 Million Years of Evolution

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## Supplemental Methods Online

### Cloning of *Helicoverpa zea* HzOr83b cDNA

An *H. zea* antennal cDNA library constructed in lambdaZAP was screened with *D. melanogaster Or83b* cDNA at low stringency by prehybridizing at 42°C in 5X SSCP (10X Denhardt's, 25% formamide, 0.1% SDS, 0.25 mg/ml salmon sperm DNA). Filters were hybridized with a $^{32}$P-labeled probe in same buffer overnight at 42°C. Filters were washed at low stringency (2x30 min in 2X SSC/0.1% SDS at 25°C; 2x30 min in 0.5X SSC/0.1% SDS at 42°C). Plasmids containing positively hybridizing inserts were obtained by in vivo excision and sequenced.

### Cloning of *Anopheles gambiae* AgOr7 cDNA

The *D. melanogaster Or83b* cDNA sequence was used to search a collection of random *A. gambiae* genomic DNA sequences (compiled by Genoscope and the Institut Pasteur, France) by BLAST and two sequence fragments with significant similarity to the query sequence were identified. These *A. gambiae* sequences were used to design oligonucleotide primers to amplify a portion of the *A. gambiae AgOr83b gene* (since renamed *AgOr7*) gene by PCR of mosquito genomic DNA. The resulting 3Kb PCR product was used to screen an *A. gambiae* genomic DNA library. Several genomic clones were isolated and sequenced and the exon/intron structure of the *AgOr7* gene was predicted using GENSCAN. Oligonucleotide primers flanking the predicted *AgOr7* ORF were synthesized and used to amplify the mosquito *AgOr7* cDNA by RT-PCR of *A. gambiae* adult head mRNA. PCR products were purified, cloned into pGEM-T Easy, and sequenced.

### Cloning of *Ceratitis capitata* CcOr83b cDNA

The *D. melanogaster Or83b* cDNA was used to screen a *C. capitata* genomic DNA library at low stringency as described above. Several clones were isolated and sequenced and the exon/intron structure of the *CcOr83b* gene was predicted using GENSCAN. Oligonucleotide primers flanking the predicted *CcOr83b* open reading frame were synthesized and used to amplify the *CcOr83b* cDNA by RT-PCR from *C. capitata* adult antennal mRNA. PCR products were purified, cloned into pGEM-T Easy, and sequenced.

### RNA *in situ* hybridization

Antennal tissues were embedded and frozen sections obtained. RNA in situ hybridization was carried out as described, except detergents were omitted and all sample manipulations after sectioning were carried out horizontally without cover slips [1]

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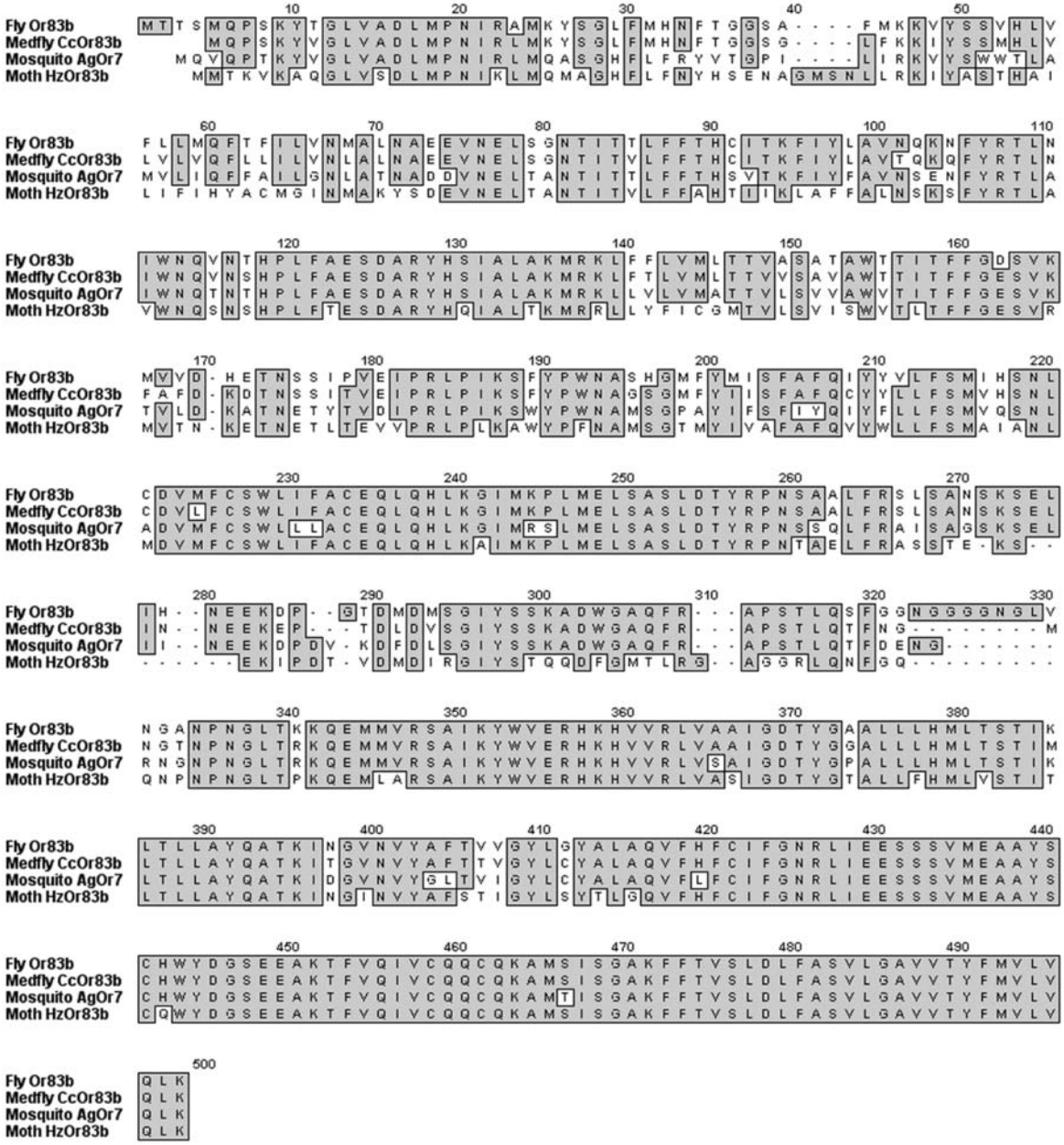


Supplemental Figure 1

Sequence Alignment of Or83b orthologs. Proteins predicted from cDNA sequences were aligned using the Clustal W algorithm (MacVector). Identical amino acids are indicated with gray shading.