

## ARTICLE PREVIEW

view full access options

## NATURE | ARTICLE

日本語要約

## Evolution of mosquito preference for humans linked to an odorant receptor

Carolyn S. McBride, Felix Baier, Aman B. Omondi, Sarabeth A. Spitzer, Joel Lutomiah, Rosemary Sang, Rickard Ignell & Leslie B. Vosshall

Print

*Nature* **515**, 222–227 (13 November 2014) doi:10.1038/nature13964

Received 09 January 2014 Accepted 13 October 2014 Published online 12 November 2014

## Abstract

Female mosquitoes are major vectors of human disease and the most dangerous are those that preferentially bite humans. A 'domestic' form of the mosquito *Aedes aegypti* has evolved to specialize in biting humans and is the main worldwide vector of dengue, yellow fever, and chikungunya viruses. The domestic form coexists with an ancestral, 'forest' form that prefers to bite non-human animals and is found along the coast of Kenya. We collected the two forms, established laboratory colonies, and document striking divergence in preference for human versus non-human animal odour. We further show that the evolution of preference for human odour in domestic mosquitoes is tightly linked to increases in the expression and ligand-sensitivity of the odorant receptor *AaegOr4*, which we found recognizes a compound present at high levels in human odour. Our results provide a rare example of a gene contributing to behavioural evolution and provide insight into how disease-vectoring mosquitoes came to specialize on humans.

**Subject terms:** Evolutionary genetics Olfactory receptors

## READ THE FULL ARTICLE

Subscribe to  
*Nature* for full  
access:  
**\$199**

Subscribe

ReadCube  
Access\*:  
**\$3.99 rent**  
**\$9.99 buy**

\*printing and sharing  
restrictions apply

Buy/Rent now

Purchase article  
full text and PDF:  
**\$32**

Buy now

Already a subscriber? [Log in now](#) or [Register for online access](#).

## Additional access options:

[Use a document delivery service](#) | [Login via Athens](#) | [Purchase a site license](#) | [Institutional access](#)

## Accession codes

### Primary accessions

GenBank/EMBL/DDBJ	Sequence Read Archive
KF801614	SRP035216
KF801615	
KF801617	
KF801621	

## References

1. Lehane, M. J. *The Biology of Blood-sucking in Insects* (Cambridge Univ.Press, 2005)
2. Christophers, S. R. *Aedes aegypti, the Yellow Fever Mosquito: Its Life History, Bionomics and Structure* Ch. 2 (Cambridge Univ. Press, 1960)
3. Mattingly, P. F. Genetical aspects of the *Aedes aegypti* problem. I: taxonomy and bionomics. *Ann. Trop. Med. Parasitol.* **51**, 392–408 (1957)
4. Brown, J. E. *et al.* Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. *Evolution* **68**, 514–525 (2014)
5. Lumsden, W. H. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952–53. II. general description and epidemiology. *Trans. R. Soc. Trop. Med. Hyg.* **49**, 33–57 (1955)
6. Trpis, M. & Hausermann, W. Demonstration of differential domesticity of *Aedes aegypti* (L) (Diptera, Culicidae) in Africa by mark-release-recapture. *Bull. Entomol. Res.* **65**, 199–208 (1975)
7. Petersen, J. L. *Behavioral Differences in Two Subspecies of Aedes aegypti (L.) (Diptera: Culicidae) in East Africa* PhD thesis, Univ. Notre Dame (1977)
8. Trpis, M. & Hausermann, W. Genetics of house-entering behavior in East African populations of *Aedes aegypti* (L) (Diptera: Culicidae) and its relevance to speciation. *Bull. Entomol. Res.* **68**, 521–532 (1978)
9. Saul, S. H., Novak, R. J. & Ross, Q. E. The role of the preadult stages in the ecological separation of 2 subspecies of *Aedes aegypti*. *Am. Midl. Nat.* **104**, 118–134 (1980)
10. Gouck, H. K. Host preferences of various strains of *Aedes aegypti* and *Aedes simpsoni* as determined by an olfactometer. *Bull. World Health Organ.* **47**, 680–683 (1972)
11. McKenna, R. J. *Attraction of Seven Strains of Aedes aegypti to Man and Guinea-pig in the Laboratory* PhD thesis, Univ. California, Davis (1973)
12. Bhatt, S. *et al.* The global distribution and burden of dengue. *Nature* **496**, 504–507 (2013)
13. Brown, J. E. *et al.* Worldwide patterns of genetic differentiation imply multiple ‘domestications’ of *Aedes aegypti*, a major vector of human diseases. *Proc. R. Soc. B* **278**, 2446–2454 (2011)
14. Mukwaya, L. G. Host preference in *Aedes (Stegomyia)* mosquitoes in Uganda. II. Studies on indoor and outdoor biting and resting behaviour with special reference to *Aedes aegypti* L. *Acta Trop.* **31**, 165–176 (1974)
15. Dekker, T., Geier, M. & Carde, R. Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. *J. Exp. Biol.* **208**, 2963–2972 (2005)
16. Dekker, T., Ibba, I., Siju, K. P., Stensmyr, M. C. & Hansson, B. S. Olfactory shifts parallel superspecialism for toxic fruit in *Drosophila melanogaster* sibling, *D. sechellia*. *Curr. Biol.* **16**, 101–109 (2006)
17. Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T. & Fuyama, Y. Odorant-binding proteins OBP57d and OBP57e affect taste perception and host–plant preference in *Drosophila sechellia*. *PLoS Biol.* **5**, e118 (2007)
18. Linz, J. *et al.* Host plant-driven sensory specialization in *Drosophila erecta*. *Proc. R. Soc. B.* **280**, 20130626 (2013)
19. Rinker, D. C., Zhou, X., Pitts, R. J., Rokas, A. & Zwiebel, L. J. Antennal transcriptome profiles of anopheline mosquitoes reveal human host olfactory specialization in *Anopheles gambiae*. *BMC Genomics* **14**, 749 (2013)

20. Wada-Katsumata, A., Silverman, J. & Schal, C. Changes in taste neurons support the emergence of an adaptive behavior in cockroaches. *Science* **340**, 972–975 (2013)
21. Michelson, R. W., Paran, I. & Kesseli, R. V. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl Acad. Sci. USA* **88**, 9828–9832 (1991)
22. Robertson, H. M., Warr, C. G. & Carlson, J. R. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **100** (Suppl 2), 14537–14542 (2003)
23. Croset, V. *et al.* Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. *PLoS Genet.* **6**, e1001064 (2010)
24. Fan, J., Francis, F., Liu, Y., Chen, J. L. & Cheng, D. F. An overview of odorant-binding protein functions in insect peripheral olfactory reception. *Genet. Mol. Res.* **10**, 3056–3069 (2011)
25. DeGennaro, M. *et al.* *orco* mutant mosquitoes lose strong preference for humans and are not repelled by volatile DEET. *Nature* **498**, 487–491 (2013)
26. Dobritsa, A. A., van der Goes van Naters, W., Warr, C. G., Steinbrecht, R. A. & Carlson, J. R. Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron* **37**, 827–841 (2003)
27. Cork, A. & Park, K. C. Identification of electrophysiologically-active compounds for the malaria mosquito, *Anopheles gambiae*, in human sweat extracts. *Med. Vet. Entomol.* **10**, 269–276 (1996)
28. Labows, J., Preti, G., Hoelzle, E., Leyden, J. & Kligman, A. Analysis of human axillary volatiles: compounds of exogenous origin. *J. Chromatogr.* **163**, 294–299 (1979)
29. Bernier, U. R., Kline, D. L., Barnard, D. R., Schreck, C. E. & Yost, R. A. Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants for the yellow fever mosquito (*Aedes aegypti*). *Anal. Chem.* **72**, 747–756 (2000)
30. Syed, Z. & Leal, W. S. Acute olfactory response of *Culex* mosquitoes to a human- and bird-derived attractant. *Proc. Natl Acad. Sci. USA* **106**, 18803–18808 (2009)
31. Birkett, M. A. *et al.* The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. *Med. Vet. Entomol.* **18**, 313–322 (2004)
32. Jumeau, Z., Gries, R., Unruh, T., Rowland, E. & Gries, G. Identification of the larval aggregation pheromone of codling moth, *Cydia pomonella*. *J. Chem. Ecol.* **31**, 911–924 (2005)
33. Nielsen, B. L. L., Jérôme, N., Saint-Albin, A., Rampin, O. & Maurin, Y. Behavioural response of sexually naïve and experienced male rats to the smell of 6-methyl-5-hepten-2-one and female rat faeces. *Physiol. Behav.* **120**, 150–155 (2013)
34. Stoeffler, M., Maier, T. S., Tolasch, T. & Steidle, J. L. M. Foreign-language skills in rove-beetles? Evidence for chemical mimicry of ant alarm pheromones in myrmecophilous *Pella* beetles (Coleoptera: Staphylinidae). *J. Chem. Ecol.* **33**, 1382–1392 (2007)
35. Rines, H. W., French, R. C. & Daasch, L. W. Nonanal and 6-methyl-5-hepten-2-one: endogenous germination stimulators of uredospores of *Puccinia graminis* var. *tritici* and other rusts. *J. Agric. Food Chem.* **22**, 96–100 (1974)
36. Socaci, S. A. *et al.* Chemometric discrimination of different tomato cultivars based on their volatile fingerprint in relation to lycopene and total phenolics content. *Phytochem. Anal.* **25**, 161–169 (2013)
37. Webster, B. *et al.* Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *J. Chem. Ecol.* **34**, 1153–1161 (2008)
38. Carey, A. F., Wang, G., Su, C. Y., Zwiebel, L. J. & Carlson, J. R. Odorant reception in the malaria mosquito *Anopheles gambiae*. *Nature* **464**, 66–71 (2010)
39. Bohbot, J. *et al.* Molecular characterization of the *Aedes aegypti* odorant receptor gene family. *Insect Mol. Biol.* **16**, 525–537 (2007)
40. Krzywinski, J., Grushko, O. G. & Besansky, N. J. Analysis of the complete mitochondrial DNA from *Anopheles funestus*: an improved dipteran mitochondrial genome annotation and a temporal dimension of mosquito evolution. *Mol. Phylogenet. Evol.* **39**, 417–423 (2006)

41. Pellegrino, M., Steinbach, N., Stensmyr, M. C., Hansson, B. S. & Vosshall, L. B. A natural polymorphism alters odour and DEET sensitivity in an insect odorant receptor. *Nature* **478**, 511–514 (2011)
42. Keller, A., Zhuang, H., Chi, Q., Vosshall, L. B. & Matsunami, H. Genetic variation in a human odorant receptor alters odour perception. *Nature* **449**, 468–472 (2007)
43. Logan, J. G. *et al.* Arm-in-cage testing of natural human-derived mosquito repellents. *Malar. J.* **9**, 239 (2010)
44. Logan, J. G. *et al.* Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. *J. Chem. Ecol.* **34**, 308–322 (2008)
45. Menger, D. J., Van Loon, J. J. A. & Takken, W. Assessing the efficacy of candidate mosquito repellents against the background of an attractive source that mimics a human host. *Med. Vet. Entomol.* <http://dx.doi.org/10.1111/mve.12061> (2014)
46. Greenwood, A. K., Wark, A. R., Yoshida, K. & Peichel, C. L. Genetic and neural modularity underlie the evolution of schooling behavior in threespine sticklebacks. *Curr. Biol.* **23**, 1884–1888 (2013)
47. Weber, J. N., Peterson, B. K. & Hoekstra, H. E. Discrete genetic modules are responsible for complex burrow evolution in *Peromyscus* mice. *Nature* **493**, 402–405 (2013)
48. Martin, A. & Orgogozo, V. The loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. *Evolution* **67**, 1235–1250 (2013)
49. van der Goes van Naters, W. & Carlson, J. R. Insects as chemosensors of humans and crops. *Nature* **444**, 302–307 (2006)
50. Coyne, J. A. & Orr, H. A. *Speciation* Ch. 4 and 5 (Sinauer Associates, 2004)
51. McClelland, G. A. H. A preliminary study of genetics and abdominal colour variations in *Aedes aegypti* (L.). *Ann. Trop. Med. Parasitol.* **54**, 305–320 (1960)
52. McClelland, G. A. H. A worldwide survey of variation in scale pattern of abdominal tergum of *Aedes aegypti* (L.) (Diptera: Culicidae). *Trans. R. Entomolog. Soc.* **126**, 239–259 (1974)
53. Mukwaya, L. Genetic control of feeding preferences in the mosquitoes *Aedes (Stegomyia) simpsoni* and *aegypti*. *Physiol. Entomol.* **2**, 133–145 (1977)
54. Kim, D. *et al.* TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* **14**, R36 (2013)
55. Kent, L. B., Walden, K. K. O. & Robertson, H. M. The Gr family of candidate gustatory and olfactory receptors in the yellow-fever mosquito *Aedes aegypti*. *Chem. Senses* **33**, 79–93 (2008)
56. Zhou, J.-J., He, X., Pickett, J. & Field, L. Identification of odorant-binding proteins of the yellow fever mosquito *Aedes aegypti*: genome annotation and comparative analyses. *Insect Mol. Biol.* **17**, 147–163 (2008)
57. Trapnell, C. *et al.* Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nature Biotechnol.* **31**, 46–53 (2013)
58. Bischof, J., Maeda, R. K., Hediger, M., Karch, F. & Basler, K. An optimized transgenesis system for *Drosophila* using germ-line-specific phiC31 integrases. *Proc. Natl Acad. Sci. USA* **104**, 3312–3317 (2007)
59. Hallem, E. A., Ho, M. & Carlson, J. The molecular basis of odor coding in the *Drosophila* antenna. *Cell* **117**, 965–979 (2004)
60. Harraca, V., Ryne, C., Birgersson, G. & Ignell, R. Smelling your way to food: can bed bugs use our odour? *J. Exp. Biol.* **215**, 623–629 (2012)
61. Stensmyr, M. C., Giordano, E., Balloi, A., Angioy, A. M. & Hansson, B. S. Novel natural ligands for *Drosophila* olfactory receptor neurones. *J. Exp. Biol.* **206**, 715–724 (2003)
62. Huson, D. H. & Bryant, D. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **23**, 254–267 (2006)
63. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990)
64. Krogh, A., Larsson, B., von Heijne, G. & Sonnhammer, E. L. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* **305**, 567–580 (2001)
65. Johns, S. J. TOPO2, transmembrane protein display software. <http://www.sacs.ucsf.edu/TOPO2/>

66. Pellegrino, M., Nakagawa, T. & Vosshall, L. B. Single sensillum recordings in the insects *Drosophila melanogaster* and *Anopheles gambiae*. *J. Vis. Exp.* **36**, 1–5 (2010)
67. Hallem, E. A. & Carlson, J. R. Coding of odors by a receptor repertoire. *Cell* **125**, 143–160 (2006)
68. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nature Methods* **9**, 357–359 (2012)
69. Roberts, A. & Pachter, L. Streaming fragment assignment for real-time analysis of sequencing experiments. *Nature Methods* **10**, 71–73 (2013)

Download references

## Author information

**Present addresses:** Princeton Neuroscience Institute and Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA (C.S.M.); Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138, USA (F.B.); Plant Protection Research Institute, Agricultural Research Council, Private Bag X134, Queenswood 0121, South Africa (A.B.O.); Harvard College, Harvard University, Cambridge, Massachusetts 02138, USA (S.A.S.).

Carolyn S. McBride, Felix Baier, Aman B. Omondi & Sarabeth A. Spitzer

## Affiliations

**Laboratory of Neurogenetics and Behavior, The Rockefeller University, New York, New York 10065, USA**

Carolyn S. McBride, Felix Baier, Sarabeth A. Spitzer & Leslie B. Vosshall

**Howard Hughes Medical Institute, 1230 York Avenue, New York, New York 10065, USA**

Carolyn S. McBride & Leslie B. Vosshall

**Unit of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Box 102, Sundsvägen 14, 230 53 Alnarp, Sweden**

Aman B. Omondi & Rickard Ignell

**Center for Virus Research, Kenya Medical Research Institute, PO Box 54840 – 00200, Off Mbagathi Way, Nairobi, Kenya**

Joel Lutomiah & Rosemary Sang

## Contributions

C.S.M. and L.B.V. conceived the study. C.S.M. participated in the execution and analysis of all aspects of the study. J.L. helped coordinate mosquito collection in Rabai, Kenya under the supervision of R.S. S.A.S. helped design and carry out the morphological assays presented in Fig. 1e–i. F.B. helped clone, analyse, and genotype mosquitoes for the *Or4* alleles presented in Fig. 5a–d, and construct transgenic *Drosophila* lines for use in single sensillum recordings. A.B.O. and R.I. designed, conducted, and analysed the GC–SSR and GC–MS experiments presented in Fig. 4 and carried out pilot experiments comprising dose–response curves and spontaneous activity analysis of alleles A and E, similar to those presented in Fig. 5e–g. C.S.M. and L.B.V. designed all other experiments, interpreted the results, designed the figures, and wrote the paper.

## Competing financial interests

The authors declare no competing financial interests.

## Corresponding author

Correspondence to: Leslie B. Vosshall

Raw RNA-seq data are available for download at the NCBI Sequence Read Archive (accession number SRP035216). Coding sequences of *AaegOr4* alleles are at GenBank (accession numbers KF801614, KF801615 and KF801617–KF801621).

## Extended data figures and tables

## Extended Data Figures

- Extended Data Figure 1: Measuring colour and scaling of adult female *Ae. aegypti* mosquitoes. (410 KB)  
**a**, Representative photograph used to measure scale colour (Fig. 1e, g). Red dots mark the approximate position of 4 points where the colour of dark scales on the scutum was assessed. **b**, Representative photograph used to measure cuticle colour (Fig. 1f, h). Red dots mark the approximate position of 4 points where the colour of bare cuticle on the circular postnotum was assessed. **c**, Representative photographs used to assess the extent of white scaling on the first abdominal tergite (Fig. 1i), outlined with the red rectangle. Each individual is representative of the scaling score shown at the bottom.
- Extended Data Figure 2: Or4 coding sequence variation in human-preferring and guinea-pig-preferring colonies from around the world. (220 KB)  
**a**, Geographical origin of colonies characterized in **b** and **c**. Circle fill colour indicates preference of strains. Circle outline colour indicates origin: Purple, laboratory strain derived from USA; blue, reference genome strain derived from West Africa; orange, Uganda; red, Kenya, green, Thailand. **b**, Host preference assayed in the live host olfactometer. Data for Thailand, K14, K2, K4, K27, K18, K19, and Uganda are reprinted from Fig. 2g. **c**, Frequency of non-synonymous single nucleotide polymorphisms (SNPs) in female antennal RNA-seq reads. SNPs are defined as differences from the A reference allele. SNPs with frequency  $\leq 0.1$  are not shown. Vertical black and red lines indicate SNPs that were present and absent, respectively, in the major alleles subject to functional analysis.
- Extended Data Figure 3: Amino acid differences of major Or4 protein alleles. (108 KB)  
Dots represent amino acid differences with respect to the genome reference, not an inferred ancestor. Red dots indicate differences that are unique to the given allele. Blue dots indicate differences that are shared among multiple alleles. Snake plots are based on the predicted orientation and location of transmembrane domains. Extracellular loops are oriented up and cytoplasmic loops are oriented down. Allele names are indicated to the left of each snake plot.
- Extended Data Figure 4: Evidence that *Or4* is a single copy gene. (61 KB)  
**a**, Histogram showing the number of alleles represented in the Or4-derived PacBio reads obtained for each of 270 parent and F2 hybrid mosquitoes. Alleles were only considered if they received at least 5% of an individual's reads. **b**, Histogram showing the fraction of reads from individual mosquitoes assigned to individual alleles. For all 270 mosquitoes, individual alleles were represented by either very few reads (grey bars, inferred to result from allele or barcode assignment errors or polymerase chain reaction contaminants), approximately half the reads (light blue bars, inferred to represent the two alleles in heterozygotes), or over 98% of all reads (dark blue bars, inferred to represent the single allele carried by homozygotes).
- Extended Data Figure 5: Response of human-preferring mosquitoes to sulcatone-scented guinea-pig odour. (109 KB)  
**a**, Olfactometer apparatus in which 50 mosquitoes per trial were given a choice between guinea-pig odour/CO<sub>2</sub> mix supplemented with solvent on one side and sulcatone 10<sup>-4</sup> on the other side. **b**, Corrected preference for sulcatone vs solvent ports is indicated. Data were corrected for the daily average left-right side bias observed across 2–3 solvent vs solvent tests conducted on each day of testing. An index value of 1 indicates strong preference for the sulcatone side, whereas -1 indicates strong preference for the solvent side. Neither mosquito colony showed a preference significantly different from zero (one-sample *t*-test  $P = 0.76$  for ORL,  $P = 0.11$  for K14). The trials for each colony were performed across 4–8 days ( $n = 40$  for ORL and  $n = 22$  for K14).

## Supplementary information

### Excel files

- Supplementary Table 1 (26 KB)  
This file contains accession numbers and gene names (where known) for differentially expressed genes described in Fig. 3c-g.

© 2014 Macmillan Publishers Limited. All Rights Reserved.

partner of AGORA, HINARI, OARE, INASP, ORCID, CrossRef, COUNTER and COPE