relationships between stereochemistry and pheromone activity. Some pheromones require a natural mixture of stereoisomers to be active, such as those employed by the ambrosia beetle (*Gnathotrichus sulcatus*), which produces the (R)- and (S)-enantiomers of the aggregation pheromone sulcatol but respond only to a mixture of each [17]. Others are naturally produced in different ratios between individuals, such as the blend of pheromone stereoisomers produced by male Asian elephants that change with maturity and elicit stronger responses when mixed at 1:1 ratios [18]. Still others are only active as one stereoisomer, inhibited by stereoisomers, or even elicit stronger responses as unnatural stereoisomers [19].

The relationship between stereochemistry and pheromone activity is also apparent in fish. The pheromones of the masu salmon and the sea lamprey are known to bind only with a specific stereochemistry, where L- but not D-kynurenine is attractive to male Masu salmon [3] and the 5α- but not 5β-3keto-petromyzonol sulfate elicits strong olfactory responses in sea lamprey [20]. In Mozambique tilapia, Keller-Costa *et al.* [4] have shown that a mixture of the two steroid stereoisomers increases release of 17,20β-P. The 20 α-PG and 20 β-PG stereoisomers appear to be detected by the same receptor mechanisms, and the higher binding affinity and release rate of 20 β-PG may be evidence for higher bioactivity. These results lead to questions on how the 20 α-PG:20 β-PG ratio in mixtures and individual stereoisomers affect the pheromone function, which can now be examined efficiently in the Mozambique tilapia model. Indeed, the identification of the 20 α-PG and 20 β-PG stereoisomers as natural mixtures in the tilapia pheromone sets a foundation for future studies on the importance of ratios and stereoisomers in fish pheromone systems.

In addition to mediating female mate choice, male-released urinary pheromones convey social status to other males [11]. Dominant males increase urination frequency during aggressive displays with subordinate males. In contrast, subordinate males do not increase urination frequency when confronted by dominant males. Dominant male urine stifles aggressive behavior in other dominant males, but subordinate male urine increases aggressive behavior in dominant males (unpublished data). Whether the same 20 α-PG and 20 β-PG stereoisomers mediate aggressive male–male interactions is yet to be determined. Regardless, the urine inspired social structures of Mozambique tilapia are sure to inspire further studies that will advance our understanding of pheromone communication.

References

1. Wyatt, T.D. (2014). Pheromones and Animal Behavior: Chemical Signals and Signature Mixes, Second Edition (Cambridge University Press).
2. Stacey, N.E. (2003). Hormones, pheromones and reproductive behavior. Fish Physiol. Biochem. *28*, 229–235.
3. Yambe, H., Kitamura, S., Kamio, M., Yamada, M., Matsunaga, S., Fusetani, N., and Yamazaki, F. (2006). L-Kynurenine, an amino acid identified as a sex pheromone in the urine of ovulated female masu salmon. Proc. Natl. Acad. Sci. USA *103*, 15370–15374.
4. Keller-Costa, T., Hubbard, P.C., Paetz, C., Nakamura, Y., da Silva, J.P., Rato, A., Barata, E.N., Schneider, B., and Canario, A.V.M. (2014). Identity of a tilapia phenomone released by dominant males that primes females for reproduction. Curr. Biol. *24*, 2130–2135.
5. Bruton, M.N., and Boltt, R.E. (1975). Aspects of the biology of *Tilapia mossambica* Peters (Pisces: Cichlidae) in a natural freshwater lake (Lake Sibaya, South Africa). J. Fish Biol. *7*, 423–445.
6. Kirkpatrick, M., and Ryan, M.J. (1991). The evolution of mating preferences and the paradox of the lek. Nature *350*, 33–38.
7. Oliveira, R.F., and Almada, V.C. (1996). Dominance hierarchies and social structure in captive groups of the Mozambique tilapia *Oreochromis mossambicus* (Teleostei Cichlidae). Ethol. Ecol. Evol. *8*, 39–55.
8. Oliveira, R.F., Almada, V.C., and Canario, A.V. (1996). Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*. Horm. Behav. *30*, 2–12.
9. Neil, E.H. (1966). Observations on the behavior of *Tilapia mossambica* (Pisces, Cichlidae) in Hawaiian ponds. Copeia *1*, 50–56.
10. Oliveira, R.F., and Almada, V.C. (1998). Mating tactics and male-male courtship in the lek-breeding cichlid *Oreochromis mossambicus*. J. Fish Biol. *52*, 1115–1129.
11. Barata, E.N., Fine, J.M., Hubbard, P.C., Almeida, O.G., Frade, P., Sorensen, P.W., and Canario, A.V. (2008). A sterol-like odorant in the urine of Mozambique tilapia males likely signals social dominance to females. J. Chem. Ecol. *34*, 438–449.
12. Keller-Costa, T., Lopes, O.S., Lima, M., Hubbard, P.C., Iacovella, A., Canário, A.V.M., Almeida, O., and Barata, E.N. (2012). Muscular hypertrophy of urinary bladders in dominant tilapia facilitates the control of aggression through urinary signals. Behaviour *149*, 953–975.
13. Barata, E., Hubbard, P., Almeida, O., Miranda, A., and Canario, A. (2007). Male urine signals social rank in the Mozambique tilapia (*Oreochromis mossambicus*). BMC Biol. *5*, 54.
14. Huertas, M., Almeida, O.G., Canário, A.V.M., and Hubbard, P.C. (2014). Tilapia male urinary pheromone stimulates female reproductive axis. Gen. Comp. Endocrinol. *196*, 106–111.
15. Karnofsky, E., Atema, J., and Elgin, R. (1989). Field observations of social behavior, shelter use, and foraging in the lobster, *Hamarus americanus*. Biol. Bull. *185*, 321–322.
16. Atema, J. (1985). Chemoreception in the sea. Adaptation of cheomreceptors and behavior to aquatic stimulus conditions. Soc. Exp. Biol. Symp. *37*, 387–442.
17. Borden, J.H., Chong, L., McLean, J.A., Slessor, K.N., and Mori, K. (1976). Gnathotrichus sulcatus: Synergistic response to enantiomers of the aggregation pheromone sulcatol. Science *192*, 894–896.
18. Greenwood, D.R., Comeskey, D., Hunt, M.B., and Rasmussen, L.E.L. (2005). Chemical communication: chirality in elephant pheromones. Nature *438*, 1097–1098.
19. Mori, K. (2007). Significance of chirality in pheromone science. Bioorg. Med. Chem. *15*, 7505–7523.
20. Burns, A.C., Sorensen, P.W., and Hoye, T.R. (2011). Synthesis and olfactory activity of unnatural, sulfated 5β-bile acid derivatives in the sea lamprey (*Petromyzon marinus*). Steroids *76*, 291–300.

Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48848, USA.
*E-mail: liweim@msu.edu

http://dx.doi.org/10.1016/j.cub.2014.08.001

Human Evolution: Genomic Gifts from Archaic Hominins

The dispersal of humans throughout the world was accompanied by adaptations to local environments. New research shows that a previously identified haplotype of the *EPAS1* gene, which allows Tibetans to live at high altitude, was inherited from archaic hominin ancestors.

Benjamin Vernot and Joshua M. Akey*

Anatomically modern humans arose in East Africa approximately 150 thousand years ago (kya) [1], and roughly 60 kya began an odyssey that resulted in the peopling of nearly all habitable regions of the world [2]. The varied climates and pathogens our ancestors encountered as they dispersed into new environments were

Figure 1. High-altitude landscape.

A view of the Brahmaputra river from the Ganden Monastery in Tibet, which is at an altitude of approximately 4,300 m. (Photo: Antoine Taveneaux/Wikimedia Commons.)

powerful agents of selective change, resulting in adaptation to regional niches. The signatures of local adaptation still remain in our genomes today, and a large number of genomic scans for traces of selection have been performed, typically identifying haplotypes that have risen to high frequency in a short amount of time [3]. A striking example of recent, strong and geographically restricted selection that has emerged from such analyses is a particular haplotype in the *EPAS1* gene [4], which confers adaptation to high altitude in Tibetan populations (Figure 1). *EPAS1* encodes a transcription factor that is expressed when oxygen levels are low, and the adaptive haplotype is associated with lower hemoglobin levels, possibly compensating for increases in hemoglobin in response to high altitude, a primary symptom of mountain sickness [5]. Although *EPAS1* has unquestionably been a substrate of selection in Tibetans, the origin of the adaptive haplotype has been ambiguous. In a new study, Huerta-Sánchez *et al.* [6] now show that the adaptive haplotype of *EPAS1* did not arise in modern humans, but in an archaic hominin population, and only recently entered the modern human gene pool through hybridization between these archaic hominins and modern humans.

To unravel the evolutionary history of the *EPAS1* gene, Huerta-Sánchez *et al.* [6] resequenced a 129 kilobase region in 40 Tibetans and 40 Han Chinese individuals. Consistent with previous studies [4], they found that many *EPAS1* variants exhibited substantial allele frequency differences between Tibetan and non-Tibetan populations, as would be expected for a gene influenced by geographically restricted selection. The peak of differentiation was concentrated in a 32.7 kb region that spans six exons and is present in 86% of the sequenced Tibetans, but is nearly absent in the geographically diverse sample of human individuals in the 1000 Genomes Project [7]. This genomic region is the most likely location of the specific allele (or alleles) conferring high-altitude adaptation. Additionally, the adaptive *EPAS1* haplotype exhibits very high levels of sequence divergence from non-adaptive *EPAS1* haplotypes in Tibetans. Through simulations, the authors show that these levels of intra- and inter-population divergence at the adaptive *EPAS1* haplotype are unlikely to be explained by classic models of selection acting on either *de novo* or standing variation.

Given the extreme divergence of the adaptive *EPAS1* haplotype, the authors hypothesized that it might have been inherited from an archaic hominin ancestor. The number of mutations that accumulate between DNA sequences (and thus the amount of divergence) is a function of the amount of time that has elapsed since they shared a common ancestor. The hundreds of thousands of years of separation between archaic hominins and modern humans would be sufficient to account for the high divergence of the adaptive *EPAS1* haplotype found in Tibetans compared to *EPAS1* haplotypes

carried by other modern humans. To date, two archaic hominin individuals have been sequenced — one Neanderthal and one Denisovan. These individuals diverged from modern humans ~550 kya, and from each other ~380 kya [8]. Strikingly, of the 20 most differentiated variants on the adaptive *EPAS1* haplotype, 15 match the high-coverage Denisovan genome, whereas only 4 match the high-coverage Neanderthal genome, suggesting that the haplotype may have been inherited from an archaic hominin more closely related to the sequenced Denisovan individual. Additionally, the adaptive Tibetan haplotype is far more similar to the Denisovan sequence than to other modern human *EPAS1* haplotypes. The authors performed two formal tests for introgression, both of which were statistically significant, further strengthening the case that the adaptive *EPAS1* haplotype originally arose in an archaic hominin group, and only recently was introduced into modern humans through mating.

This study adds to a growing body of evidence that sequences inherited from archaic hominins have helped modern humans adapt to new environmental challenges as they dispersed out of Africa [9–12]. For example, two recent studies [11,12] found that 20–30% of the Neanderthal genome still persists in modern humans. Although most Neanderthal sequences exist at low frequency in contemporary human populations, in a handful of regions, the Neanderthal haplotype is too common to be accounted for by neutral evolutionary forces. These regions are therefore likely to be additional examples of adaptive introgression — sequences conferring an advantage in modern humans and inherited from archaic hominin ancestors. Strikingly, both studies [11,12] found an enrichment of putative adaptive introgression from Neanderthals in genes involved in keratinocyte biology, including *BNC2*, which influences pigmentation levels in Europeans [13], and *POU2F3*, which mediates keratinocyte proliferation and differentiation [14]. Adaptive introgression of archaic haplotypes has been postulated for several additional genes involved in immune function, such as *STAT2* [9] and regions of the HLA [10]. More generally, adaptive introgression has been observed in many other animal species [15], for example introgression of a warfarin resistance allele from the Algerian mouse to the house mouse [16]. Thus, hybridization can be an efficient mechanism for adaptation, allowing alleles to be acquired whose fitness effects have already been screened by evolution.

Despite the fascinating insights of Huerta-Sánchez *et al.* [6], the adaptive *EPAS1* haplotype found in Tibetans may have further stories to tell. Perhaps most intriguing is the question of which ancestral group this haplotype originated in. Although the adaptive *EPAS1* haplotype shows more sequence similarity with Denisovans than it does with Neanderthals, it is not a direct match, differing at twelve nucleotides over the ~33 kb haplotype. In addition, significant Denisovan admixture has so far only been found in individuals of Melanesian ancestry [17], although very low levels of admixture may have occurred elsewhere [8]. It is therefore possible that the *EPAS1* haplotype was inherited from an unknown archaic hominin group. While provocative, signatures of introgression from unknown hominins have been found in contemporary African populations [18,19], suggesting additional groups may have overlapped in time and space with modern humans. However, sequence data are only available from a single Denisovan and a small number of Neanderthals, and it remains possible that the adaptive *EPAS1* haplotype was inherited from an archaic individual belonging to one of these populations whose sequence differs from the reference archaic genomes.

Furthermore, it is not clear if the high frequency *EPAS1* haplotype was adaptive in the archaic population, and if so, whether the advantage was related to high altitude or a different phenotype. It is also possible that the adaptive *EPAS1* variant of modern Tibetans arose on the introgressed haplotype only in the last few thousand years, long after the haplotype was introduced into modern humans. It will be difficult, if not impossible, to address these questions without identifying the specific causal variant (or variants) conferring high-altitude adaptation in Tibetans. If the causal difference was present in both Neanderthals and Denisovans, it seems unlikely that the same selective pressures were acting in these populations as in Tibetans, perhaps suggesting that the haplotype was either neutral or had pleiotropic effects in the originating archaic population.

More generally, the work of Huerta-Sánchez *et al.* [6] is a powerful reminder that our genomes are a mosaic patchwork of differing ancestries, shaped by admixture that occurred throughout our species' existence. Although current genetic data suggest that hybridization between modern humans and archaic hominins was limited [11,12], its consequences were not, bestowing upon our ancestors the genetic resources to help adapt to challenging new environments.

References

1. White, T.D., Asfaw, B., DeGusta, D., Gilbert, H., Richards, G.D., Suwa, G., and Clark Howell, F. (2003). Pleistocene Homo sapiens from Middle Awash, Ethiopia. Nature *423*, 742–747.
2. Mellars, P. (2006). Going east: new genetic and archaeological perspectives on the modern human colonization of Eurasia. Science *313*, 796–800.
3. Akey, J.M. (2009). Constructing genomic maps of positive selection in humans: Where do we go from here? Genome Res. *19*, 711–722.
4. Yi, X., Liang, Y., Huerta-Sanchez, E., Jin, X., Cuo, Z.X.P., Pool, J.E., Xu, X., Jiang, H., Vinckenbosch, N., Korneliussen, T.S., *et al*. (2010). Sequencing of 50 human exomes reveals adaptation to high altitude. Science *329*, 75–78.
5. Beall, C.M., Cavalleri, G.L., Deng, L., Elston, R.C., Gao, Y., Knight, J., Li, C., Li, J.C., Liang, Y., McCormack, M., *et al*. (2010). Natural selection on EPAS1 (HIF2α) associated with low hemoglobin concentration in Tibetan highlanders. Proc. Natl. Acad. Sci. USA *107*, 11459–11464.
6. Huerta-Sánchez, E., Jin, X., Asan, Bianba, Z., Peter, B.M., Vinckenbosch, N., Liang, Y., Yi, X., He, M., Somel, M., *et al*. (2014). Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. Nature *512*, 194–197.
7. Consortium, T. 1000 G. P. (2012). An integrated map of genetic variation from 1,092 human genomes. Nature *491*, 56–65.
8. Prüfer, K., Racimo, F., Patterson, N., Jay, F., Sankararaman, S., Sawyer, S., Heinze, A., Renaud, G., Sudmant, P.H., de Filippo, C., *et al*. (2014). The complete genome sequence of a Neanderthal from the Altai Mountains. Nature *505*, 43–49.
9. Mendez, F.L., Watkins, J.C., and Hammer, M.F. (2012). A haplotype at STAT2 introgressed from neanderthals and serves as a candidate of positive selection in Papua New Guinea. Am. J. Hum. Genet. *91*, 265–274.
10. Abi-Rached, L., Jobin, M.J., Kulkarni, S., McWhinnie, A., Dalva, K., Gragert, L., Babrzadeh, F., Gharizadeh, B., Luo, M., Plummer, F.A., *et al*. (2011). The shaping of modern human immune systems by multiregional admixture with archaic humans. Science *334*, 89–94.
11. Vernot, B., and Akey, J.M. (2014). Resurrecting surviving Neandertal lineages from modern human genomes. Science *343*, 1017–1021.
12. Sankararaman, S., Mallick, S., Dannemann, M., Prüfer, K., Kelso, J., Pääbo, S., Patterson, N., and Reich, D. (2014). The genomic landscape of Neanderthal ancestry in present-day humans. Nature *507*, 354–357.

13. Jacobs, L.C., Wollstein, A., Lao, O., Hofman, A., Klaver, C.C., Uitterlinden, A.G., Nijsten, T., Kayser, M., and Liu, F. (2013). Comprehensive candidate gene study highlights UGT1A and BNC2 as new genes determining continuous skin color variation in Europeans. Hum. Genet. *132*, 147–158.
14. Takemoto, H., Tamai, K., Akasaka, E., Rokunohe, D., Takiyoshi, N., Umegaki, N., Nakajima, K., Aizu, T., Kaneko, T., Nakano, H., *et al*. (2010). Relation between the expression levels of the POU transcription factors Skn-1a and Skn-1n and keratinocyte differentiation. J. Dermatol. Sci. *60*, 203–205.
15. Hedrick, P.W. (2013). Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol. Ecol. *22*, 4606–4618.
16. Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F.-R., Shih, C.-H., Nachman, M.W., and Kohn, M.H. (2011). Adaptive introgression of anticoagulant rodent poison resistance by hybridization between Old World mice. Curr. Biol. *21*, 1296–1301.
17. Reich, D., Green, R.E., Kircher, M., Krause, J., Patterson, N., Durand, E.Y., Viola, B., Briggs, A.W., Stenzel, U., Johnson, P.L.F., *et al*. (2010). Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature *468*, 1053–1060.
18. Hammer, M.F., Woerner, A.E., Mendez, F.L., Watkins, J.C., and Wall, J.D. (2011). Genetic evidence for archaic admixture in Africa. Proc. Natl. Acad. Sci. USA *108*, 15123–15128.
19. Lachance, J., Vernot, B., Elbers, C.C., Ferwerda, B., Froment, A., Bodo, J.-M., Lema, G., Fu, W., Nyambo, T.B., Rebbeck, T.R., *et al*. (2012). Evolutionary history and adaptation from high-coverage whole-genome sequences of diverse African hunter-gatherers. Cell *150*, 457–469.

Department of Genome Sciences, University of Washington, 3720 15th Ave NE, Box 355065, Seattle WA 98195-5065, USA.
*E-mail: akeyj@uw.edu

http://dx.doi.org/10.1016/j.cub.2014.07.079