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innervate two of the lateral transverse (LT) muscles and that they can be colabeled with antibodies against Fasciclin 2 (Fas2) (Fig. 4H), demonstrating these to be motorneurons. LT muscles are innervated by Bar-H1$^+$ motorneurons (fig. S8A), so we used *Bar-H1*-Gal4 as a second driver to demonstrate that appropriate Ubx levels in these cells are required for normal SR behavior (fig. S8B), establishing the SRN cells as the LT-MNs.

We have therefore shown that miRNA-dependent *Hox* gene repression within a distinct group of motorneurons (SRN/LT-MNs) is required for the control of a specific locomotor behavior in the early *Drosophila* larva. Our finding that *Hox* gene posttranscriptional regulation is involved in SR control suggests that other RNA-based regulatory processes affecting *Hox* gene expression might also impinge on specific neural outputs; we are currently investigating this possibility, with special regard to the roles of the *Hox* genes in the specification of neural lineages with axial-specific architectures, and systematically testing the roles of other miRNAs on behavior.

That we could not detect any obvious neuroanatomical changes in miRNA mutant embryos suggests that these are either very subtle or that the role of miRNA regulation may be primarily behavioral, in the sense of affecting the performance of a correctly wired neural system, rather than developmental, contributing to the development of the network (*26*). Given that *miR-iab4/iab8* is involved in adult ovary innervation (*16*), it seems that miRNAs—much like ordinary protein-coding genes—can be involved in several distinct roles within the organism.

The results of this study contribute to the understanding of how complex innate behaviors are represented in the genetic program. Our data lead us to propose that other miRNAs might also be involved in the control of behavior in *Drosophila* and other species.

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SUPPLEMENTARY MATERIALS

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Figs. S1 to S11
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Movies S1 to S7

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HUMAN EVOLUTION

Ancient Ethiopian genome reveals extensive Eurasian admixture in Eastern Africa

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Characterizing genetic diversity in Africa is a crucial step for most analyses reconstructing the evolutionary history of anatomically modern humans. However, historic migrations from Eurasia into Africa have affected many contemporary populations, confounding inferences. Here, we present a 12.5× coverage ancient genome of an Ethiopian male ("Mota") who lived approximately 4500 years ago. We use this genome to demonstrate that the Eurasian backflow into Africa came from a population closely related to Early Neolithic farmers, who had colonized Europe 4000 years earlier.

The ability to sequence ancient genomes has revolutionized our understanding of human evolution. However, genetic analyses of ancient material have focused on individuals from temperate and Arctic regions, where ancient DNA is preserved over longer time frames (*1*). Africa has so far failed to yield skeletal remains with much ancient DNA, with the exception of a few poorly preserved specimens from which only mitochondrial DNA could be extracted (*2*). This is particularly unfortunate, as African genetic diversity is crucial to most analyses reconstructing the evolutionary history of anatomically modern humans, by providing the baseline against which other events are defined. In the absence of ancient DNA, geneticists rely on contemporary African populations, but a number of historic events, in particular a genetic backflow from West Eurasia into Eastern Africa (*3*, *4*), act as confounding factors.

Here, we present an ancient human genome from Africa and use it to disentangle the effects of recent population movement into Africa. By sampling the petrous bone (*5*), we sequenced the genome of a male from Mota Cave (herein referred to as "Mota") in the southern Ethiopian highlands, with a mean coverage of 12.5× (*6*). Contamination was estimated to be between 0.29 and 1.26% (*6*). Mota's remains were dated to ~4500 years ago [direct calibrated radiocarbon date (*6*)] and thus predate both the Bantu expansion (*7*) and, more importantly, the 3000-year-old West Eurasian backflow, which has left strong genetic signatures in the whole of Eastern and, to a lesser extent, Southern Africa (*3*, *4*).

We compared Mota to contemporary human populations (*6*). Both principal component analysis (PCA) (Fig. 1A) and outgroup f_3 analysis using Ju|'hoansi (Khoisan) from Southern Africa as the outgroup (Fig. 1, B and C) place this ancient

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individual close to contemporary Ethiopian populations, and more specifically to the Ari, a group of Omotic speakers from southern Ethiopia, to the west of the highland region where Mota lived. Our ancient genome confirms the view that the divergence of this language family results from the relative isolation of its speakers (*8*), and indicates population continuity over the last ~4500 years in this region of Eastern Africa.

The age of Mota means that he should predate the West Eurasian backflow, which has been dated to ~3000 years ago (*3*, *4*). We formally tested this proposition by using an f_4 ratio estimating the West Eurasian component (*6*), following the approach adopted by Pickrell *et al.* (*3*). As expected, we failed to find any West Eurasian component in Mota (table S5), thus providing support for previous dating of that event (*3*, *4*).

Given that Mota predates the backflow, we searched for its most likely source by modeling the Ari, the contemporary population closest to our ancient genome, as a mixture of Mota and another West Eurasian population (*6*). We investigated both contemporary sources (*3*) and other Eurasian ancient genomes (*5*, *9*). In this analysis, contemporary Sardinians and the early Neolithic LBK (Stuttgart) genome stand out (Fig. 2A). Previous analyses have shown Sardinians to be the closest modern representatives of early Neolithic farmers (*10*, *11*), implying that the backflow came from the same genetic source that fueled the Neolithic expansion into Europe from the Near East/Anatolia, before recent historic events changed the genetic makeup of populations living in that region. An analysis with haplotype sharing also identified a connection between contemporary Ethiopians and Anatolia (*4*, *12*). Interestingly, archaeological evidence dates the arrival of Near Eastern domesticates (such as wheat, barley, and lentils) to the same time period (~3000 years ago) (*13*, *14*), suggesting that the direct descendants of the farmers that earlier brought agriculture into Europe may have also played a role in the development of new forms of food production in the Horn of Africa.

Using Mota as an unadmixed African reference and the early farmer LBK as the source of the West Eurasian component, it is possible to reassess the magnitude and geographic extent of historical migrations, avoiding the complications of using admixed contemporary populations (*6*). We estimated a substantially higher Eurasian backflow admixture than previously detected (*3*), with an additional 4 to 7% of the genome of most African populations tracing back to a Eurasian source. Moreover, we detected a much broader geographical impact of the backflow, going all the way to West and Southern Africa (Fig. 2B). Even though the West Eurasian component in these regions is smaller than in Eastern Africa, it is still sizable, with Yoruba and Mbuti, who are often used as African reference populations (*15*, *16*), showing 7% and 6%, respectively, of their genomes to be of Eurasian origin (table S5).

Since Mota predates recent demographic events, his genome can act as an ideal African reference

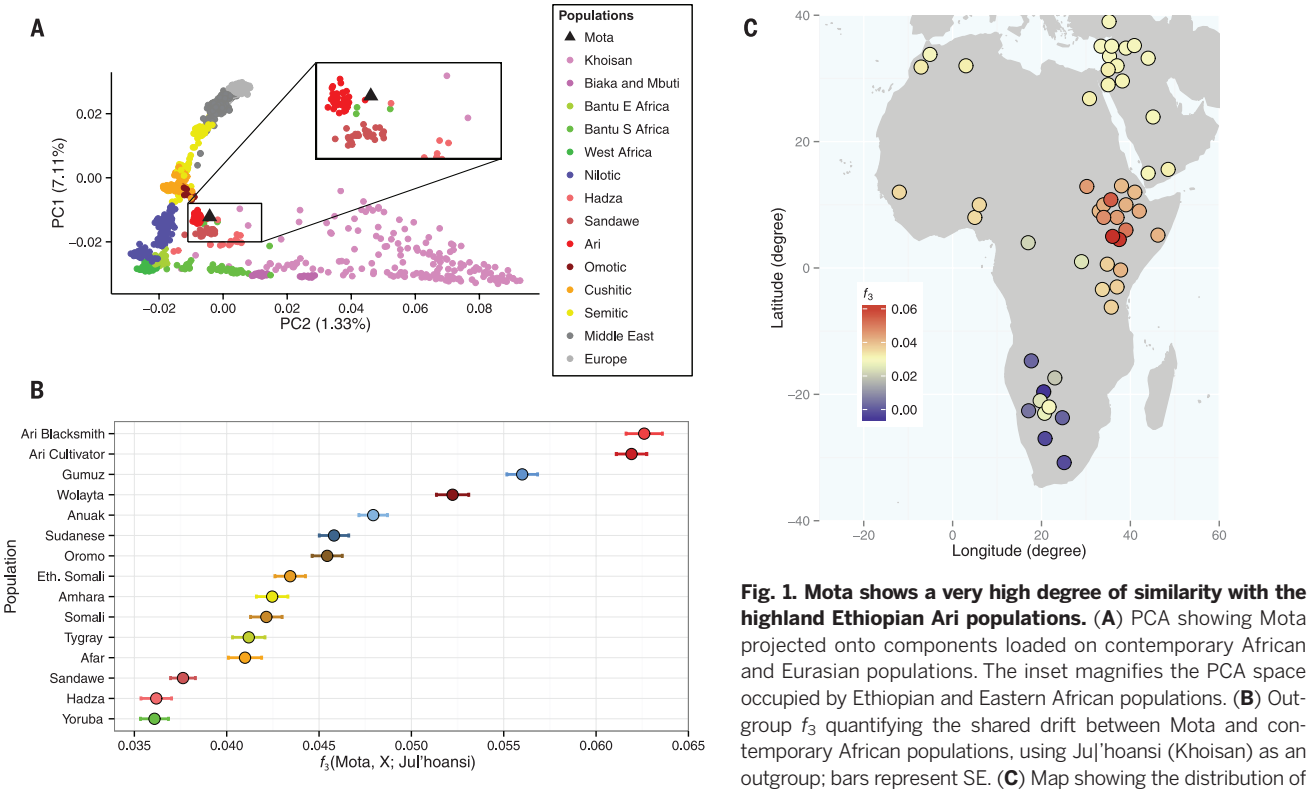

Fig. 1. Mota shows a very high degree of similarity with the highland Ethiopian Ari populations. (**A**) PCA showing Mota projected onto components loaded on contemporary African and Eurasian populations. The inset magnifies the PCA space occupied by Ethiopian and Eastern African populations. (**B**) Outgroup f_3 quantifying the shared drift between Mota and contemporary African populations, using Ju|'hoansi (Khoisan) as an outgroup; bars represent SE. (**C**) Map showing the distribution of outgroup f_3 values across the African continent. In (A) and (B), populations speaking Nilo-Saharan languages are marked with blue shades, Omotic speakers with red, Cushitic with orange, Semitic with yellow, and Bantu with green. Mota is denoted by a black symbol.

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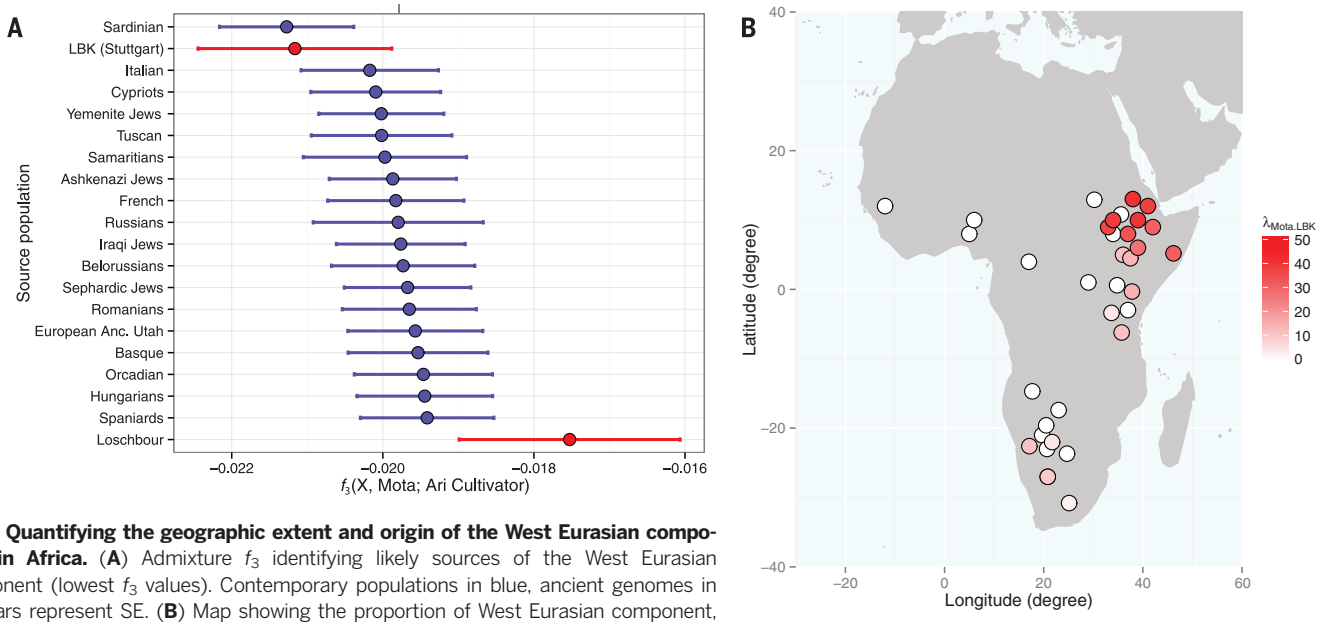

Fig. 2. Quantifying the geographic extent and origin of the West Eurasian component in Africa. (**A**) Admixture f_3 identifying likely sources of the West Eurasian component (lowest f_3 values). Contemporary populations in blue, ancient genomes in red; bars represent SE. (**B**) Map showing the proportion of West Eurasian component, $\lambda_{Mota,LBK}$, across the African continent.

to understand episodes during the out-of-Africa expansion. We used him as the African reference to quantify Neandertal introgression in a number of contemporary genomes (*6*). Both Yoruba and Mbuti, which are routinely used as African references for this type of analysis (*15*, *16*), show a marginally closer affinity with Neandertal than Mota on the basis of *D* statistics, and an f_4 ratio analysis detected a small Neandertal component in these genomes at around 0.2 to 0.7%—greater than previously suggested (*16*) and consistent with our estimates of the magnitude of their Western Eurasian ancestry (*6*). Although the magnitude of Neandertal ancestry in these contemporary African populations is not enough to change conclusions qualitatively (estimates of Neandertal ancestry in French and Han only increased marginally when tested with Mota as a reference), it should be accounted for when looking for specific introgressed haplotypes (*17*) or searching for unknown ancient hominins who might have hybridized with African populations (*18*).

We also investigated the Mota genome for a number of phenotypes of interest (*6*). As expected, Mota lacked any of the derived alleles found in Eurasian populations for eye and skin color, suggesting that he had brown eyes and dark skin. Mota lacked any of the currently known alleles that confer lactose tolerance, which may have implications concerning when pastoralism appeared in southwestern Ethiopia. In addition, Mota did possess all three selected alleles that recently have been shown to play a role in the adaptation to altitude in contemporary highland Ethiopian populations (*19*). The presence of these mutations supports our conclusion that Mota is the descendant of highland dwellers, who have lived in this environment long enough to accumulate adaptations to the altitude (*20*, *21*).

Until now, it has been necessary to use contemporary African populations as the baseline against which events during the worldwide expansion of anatomically modern humans are defined (*16*, *22*–*24*). By obtaining an ancient whole genome from this continent, we have shown that having an unadmixed reference that predates the large number of recent historical migrations can greatly improve our inference. This result stresses the importance of obtaining unadmixed baseline data to reconstruct demographic events, and the limitations of analyses that are solely based on contemporary populations. Even older African genomes will thus be needed to investigate key demographic events that predate Mota, such as earlier instances of backflows into Africa (*25*).

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SUPPLEMENTARY MATERIALS

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Supplementary Text
Figs. S1 to S8
Tables S1 to S14
References (*26*–*74*)

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ERRATUM

Erratum for the Report "Ancient Ethiopian genome reveals extensive Eurasian admixture in Eastern Africa" (previously titled "Ancient Ethiopian genome reveals extensive Eurasian admixture throughout the African continent") by M. Gallego Llorente, E. R. Jones, A. Eriksson, V. Siska, K. W. Arthur, J. W. Arthur, M. C. Curtis, J. T. Stock, M. Coltorti, P. Pieruccini, S. Stretton, F. Brock, T. Higham, Y. Park, M. Hofreiter, D. G. Bradley, J. Bhak, R. Pinhasi, A. Manica

In the Report "Ancient Ethiopian genome reveals extensive Eurasian admixture in Eastern Africa," the results were affected by a bioinformatics error. A script necessary to convert the input produced by samtools v0.1.19 to be compatible with PLINK was not run when merging the ancient genome, Mota, with the contemporary populations SNP panel, leading to homozygote positions to the human reference genome being dropped as missing data (the analysis of admixture with Neandertals and Denisovans was not affected). When those positions were included, 255,922 SNP out of 256,540 from the contemporary reference panel could be called in Mota. These changes are reflected in the corrected Fig. 2B, fig. S6, and table S5. Tables S6 and S7 have been removed from the corrected Supplementary Material, because there is no detectable Western Eurasian component in Yoruba and Mbuti. The conclusion of a migration into East Africa from Western Eurasia, and more precisely from a source genetically close to the early Neolithic farmers, is not affected. However, the geographic extent of the genetic impact of this migration was overestimated: The Western Eurasian backflow mostly affected East Africa and only a few Sub-Saharan populations; the Yoruba and Mbuti do not show higher levels of Western Eurasian ancestry compared to Mota. Hence, the title and abstract of the published paper did not accurately represent the geographical extent of the admixture, and both have been corrected accordingly. The authors acknowledge Pontus Skoglund and David Reich for detecting these problems.

Science

Ancient Ethiopian genome reveals extensive Eurasian admixture in Eastern Africa

M. Gallego Llorente, E. R. Jones, A. Eriksson, V. Siska, K. W. Arthur, J. W. Arthur, M. C. Curtis, J. T. Stock, M. Coltorti, P. Pieruccini, S. Stretton, F. Brock, T. Higham, Y. Park, M. Hofreiter, D. G. Bradley, J. Bhak, R. Pinhasi and A. Manica

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Ancient African helps to explain the present

Tracing the migrations of anatomically modern humans has been complicated by human movements both out of and into Africa, especially in relatively recent history. Gallego Llorente *et al.* sequenced an Ethiopian individual, "Mota," who lived approximately 4500 years ago, predating one such wave of individuals into Africa from Eurasia. The genetic information from Mota suggests that present-day Sardinians were the likely source of the Eurasian backflow. Furthermore, 4 to 7% of most African genomes, including Yoruba and Mbuti Pygmies, originated from this Eurasian gene flow.

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