

information and events, and which have been also proposed as a mechanism for trace conditioning in humans and mammals<sup>6</sup>. Whether such a conscious recollection exists in insects is unknown but the previous components, ellipsoid-body-based working memory and dopamine-dependent attentional processes<sup>17,18</sup>, have been characterized in flies. Their underlying networks may provide, therefore, modulatory signals inducing the selective changes reported for both CS and US processing by Grover *et al.*<sup>7</sup>. Which specific neural pathways are placed 'on top' of ellipsoid-body ring neurons and PPM3 dopaminergic neurons to modify their activity selectively for trace conditioning remains to be determined.

Grover *et al.*<sup>7</sup> underlined the fact that mushroom bodies were neither involved in delay nor in trace conditioning, thus questioning their role as a "site for general-purpose conditioning". Although this statement is strictly correct, it should not surprise that mushroom bodies were dispensable for the conditioning protocol employed in their work. Mushroom bodies in adult *Drosophila* receive consistent visual input but are predominantly olfactory<sup>19</sup>, so their involvement in visual conditioning should not be necessarily expected. On the contrary, the fact that short-term memory traces of visual patterns similar to those employed by Grover *et al.*<sup>7</sup> were localized in the fan-shaped body<sup>20</sup>, another region of the central complex, underlines the importance of this region for experience-dependent visual plasticity. In olfactory delay and trace conditioning, the opposite result could be predicted, namely an involvement of mushroom bodies and a dispensability of central-complex structures. This hypothesis awaits appropriate testing.

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

#### REFERENCES

- Pavlov, I.P. (1927). *Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex* (Oxford University Press).
- Droege, P., Weiss, D.J., Schwob, N., and Braithwaite, V. (2021). Trace conditioning as a test for animal consciousness: a new approach. *Anim. Cogn.* **24**, 1299–1304.
- Kim, J.J., and Thompson, R.F. (1997). Cerebellar circuits and synaptic mechanisms involved in classical eyeblink conditioning. *Trends Neurosci.* **20**, 177–181.
- Cheng, D.T., Disterhoft, J.F., Power, J.M., Ellis, D.A., and Desmond, J.E. (2008). Neural substrates underlying human delay and trace eyeblink conditioning. *Proc. Natl. Acad. Sci. USA* **105**, 8108–8113.
- Clark, R.E., Manns, J.R., and Squire, L.R. (2002). Classical conditioning, awareness, and brain systems. *Trends Cogn. Sci.* **6**, 524–531.
- Connor, D.A., and Gould, T.J. (2016). The role of working memory and declarative memory in trace conditioning. *Neurobiol. Learn. Mem.* **134**, 193–209.
- Grover, D., Chen, J.Y., Xie, J., Li, J., Changeux, J.P., and Greenspan, R.J. (2022). Differential mechanisms underlie trace and delay conditioning in *Drosophila*. *Nature* **603**, 302–308.
- Szyszkka, P., Demmler, C., Oemisch, M., Sommer, L., Biergans, S., Birnbach, B., Silbering, A.F., and Galizia, C.G. (2011). Mind the gap: olfactory trace conditioning in honeybees. *J. Neurosci.* **31**, 7229–7239.
- Ito, I., Ong, R.C.-y., Raman, B., and Stopfer, M. (2008). Sparse odor representation and olfactory learning. *Nat. Neurosci.* **11**, 1177–1184.
- Dylla, K.V., Raiser, G., Galizia, C.G., and Szyszkka, P. (2017). Trace conditioning in *Drosophila* induces associative plasticity in mushroom body Kenyon cells and dopaminergic neurons. *Front. Neural Circuits* **11**, 42.
- Gallili, D.S., Ludke, A., Galizia, C.G., Szyszkka, P., and Tanimoto, H. (2011). Olfactory trace conditioning in *Drosophila*. *J. Neurosci.* **31**, 7240–7248.
- Birch, J. (2022). The search for invertebrate consciousness. *Nous* **56**, 133–153.
- Brembs, B., and Heisenberg, M. (2000). The operant and the classical in conditioned orientation of *Drosophila melanogaster* at the flight simulator. *Learn. Mem.* **7**, 104–115.
- Honkanen, A., Adden, A., da Silva Freitas, J., and Heinze, S. (2019). The insect central complex and the neural basis of navigational strategies. *J. Exp. Biol.* **222**, jeb188854.
- García-Hoz, V. (2014). Signalization and stimulus-substitution in Pavlov's theory of conditioning. *Span. J. Psychol.* **6**, 168–176.
- Baddeley, A.D. (1986). *Working Memory* (Oxford University Press).
- Koenig, S., Wolf, R., and Heisenberg, M. (2016). Visual attention in flies — Dopamine in the mushroom bodies mediates the after-effect of cueing. *PLoS One* **11**, e0161412.
- van Swinderen, B. (2011). Attention in *Drosophila*. *Int. Rev. Neurobiol.* **99**, 51–85.
- Li, F., Lindsey, J.W., Marin, E.C., Otto, N., Dreher, M., Dempsey, G., Stark, I., Bates, A.S., Pleijzier, M.W., Schlegel, P., *et al.* (2020). The connectome of the adult *Drosophila* mushroom body provides insights into function. *eLife* **9**, e62576.
- Liu, G., Seiler, H., Wen, A., Zars, T., Ito, K., Wolf, R., Heisenberg, M., and Liu, L. (2006). Distinct memory traces for two visual features in the *Drosophila* brain. *Nature* **439**, 551–556.

## Paleogenomics: The demographic past of prehistoric Europeans

Jeremy Choin<sup>1,2</sup> and Lluís Quintana-Murci<sup>1,2,\*</sup>

<sup>1</sup>Institut Pasteur, Université Paris Cité, CNRS UMR2000, Unit of Human Evolutionary Genetics, F-75015 Paris, France

<sup>2</sup>Chair of Human Genomics and Evolution, Collège de France, F-75005 Paris, France

\*Correspondence: quintana@pasteur.fr

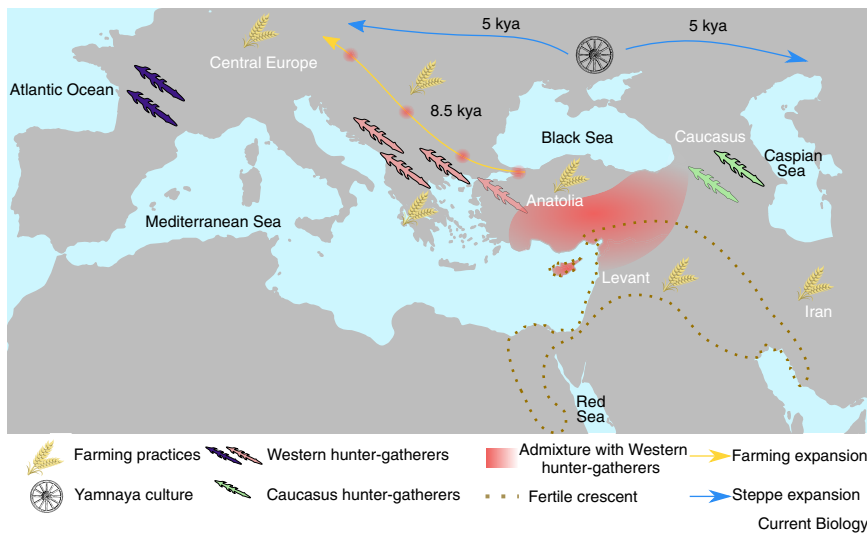
<https://doi.org/10.1016/j.cub.2022.04.081>

Ancient DNA provides answers to long-standing debates about past human history. New work using demographic modeling on ancient genomes documents the nature and timing of the demographic processes — population size changes, divergences and admixture — that took place in prehistoric Europe.

The era of ancient DNA studies, focusing on DNA extracted from fossil remains, began in 1984 with the publication of the

sequence of a mitochondrial DNA segment from the quagga, a South African zebra that became extinct in the





**Figure 1. The paleogenomic history of Europe.**

Ancient genomics studies have shown that present-day Europeans are the result of admixture between three main ancestry components associated with Mesolithic hunter-gatherers, Anatolian Neolithic farmers, and pastoralist populations from the Pontic-Caspian region. The first inhabitants of Europe were groups of hunter-gatherers that were present in the region by at least 40,000 years ago. These populations differentiated into separate ancestry groups from 25,000 years ago onward, during the Last Glacial Maximum (LGM). The ancestors of Anatolian/Aegean/European early farmers admixed twice with Western hunter-gatherers: first, around 14,000 years ago likely in Anatolia and/or the Northern Levant, and a second time between 13,000 and 9,000 years ago, after their divergence from Caucasus foragers (13,000–9,000 years ago). Anatolian early farmers then expanded around 8,500 years ago to central Europe where they brought farming practices. During this dispersal, early farmers admixed with already established groups of Western hunter-gatherers, bringing a new genetic ancestry to the European continent. Lastly, starting around 5,000 years ago, groups from the Pontic-Caspian steppes and associated with the Yamnaya culture migrated into Europe. This massive expansion of pastoralist communities changed again the genetic makeup of Holocene Europeans and likely introduced proto-European languages in the region.

late 19th century<sup>1</sup>. However, it was not until midway through the first decade of this century that the study of ancient DNA entered its golden age and paleogenomics was founded as a discipline<sup>2</sup>. Over the last decade, we have witnessed results that would have been science fiction just a few years ago, such as genome-wide data from mammoths over one million years old<sup>3</sup>. Likewise, the sequencing of Neanderthal and Denisovan genomes has shown that contemporary populations of non-African ancestry can carry up to 6% of ‘archaic’ genetic material<sup>4</sup>, and that the presence of such archaic segments in the genomes of modern humans can facilitate adaptation to environmental cues, including pathogens<sup>4–6</sup>. Paleogenomics has also shed light on how humans have biologically adapted to varying environmental pressures through different epochs, and on the precise spatiotemporal details of the peopling of the planet<sup>2</sup>. With an increasing amount of ancient genome data available, it now

becomes possible to paint an ever more fine-grained picture of human prehistory. In this vein, a new study by Nina Marchi and colleagues<sup>7</sup> provides a picture of the demographic processes — population size changes, split times and admixture — that took place in prehistoric Europe.

For several reasons, Europe is the best-studied region in paleogenomics. We now know that present-day Europeans are the result of several admixture events between three main ancestry components associated with Mesolithic hunter-gatherers, Anatolian Neolithic farmers and pastoralist ‘steppe’ populations from the Pontic-Caspian region<sup>8</sup> (Figure 1). Modern humans were present in Europe, in the form of small groups of hunter-gatherers, at least 40,000 years ago. Around 8,500 years ago during the Neolithic era, a new ancestry that was highly differentiated from that of Mesolithic hunter-gatherers expanded throughout Europe starting from the southeast. This expansion, which was related to the emergence of

farming in the fertile crescent, involved the replacement of local hunter-gatherers in some cases, and extensive admixture between the two groups in others. The next major change in the European human genomic landscape came with the large-scale migration, beginning about 5,000 years ago, of communities from the Eurasian steppe. These communities were associated with the Yamnaya cultural complex and probably introduced proto-European languages into Europe. However, despite the considerable knowledge generated by paleogenomic studies of west Eurasians<sup>2,8–12</sup>, the nature and timing of the demographic processes underlying the emergence of these ancestries, and of early Neolithic farmers in particular, have remained elusive.

This is precisely the question that Marchi and colleagues<sup>7</sup> have addressed in their new study, in which they report 15 new high-quality ancient genomes from early Holocene hunter-gatherers and farmers, from a temporal and geographic transect extending from the Near East to the Rhine basin. This study is innovative in its methodology. By combining this new dataset with available genomes from ancient and modern west Eurasians, the authors have jointly estimated demographic parameters, such as divergence times between populations, changes in effective population size over time and gene flow between groups, using a maximum likelihood framework and coalescent theory. Specifically, Marchi and colleagues<sup>7</sup> have inferred parameters characterizing the demographic past of hunter-gatherers and early farmers from Europe, Anatolia and Iran, by comparing alternative ‘continent–island’ models. In this approach, each island represents a sampled population and each continent represents a large metapopulation composed of multiple unsampled and smaller populations interconnected through exchanges of migrants.

In the context of pre-Neolithic history, Marchi and colleagues<sup>7</sup> report that the best-supported demographic scenario indicates an initial splitting of Western hunter-gatherers from the ancestors of Iranian early farmers and Anatolian/Aegean/European early farmers around 25,000 years ago, during the Last Glacial Maximum, followed by the divergence of the ancestors of the two groups of early

farmers around 16,000 years ago. These estimates are at odds with previous models, from which earlier divergence times were inferred, assuming an absence of fluctuations in population size over time and of gene flow between groups<sup>10,13</sup>. The detailed demographic modeling by Marchi and colleagues<sup>7</sup> also revealed that the ancestors of European hunter-gatherer populations experienced a drastic bottleneck during the glacial maximum, but their effective population sizes were actually larger than those of early farmer populations, which may have been smaller due to their sedentary nature. Thus, contrary to previous interpretations<sup>9</sup>, this work suggests that the low genetic diversity of European hunter-gatherers may have been due to the severe bottlenecks experienced by their ancestors during the last ice age. This interpretation is particularly relevant for Western European foragers, who experienced an additional bottleneck after their divergence from the ancestors of European/Anatolian and Iranian early farmers.

The demographic modeling of Marchi and colleagues<sup>7</sup> also further refines our knowledge of the extent of admixture between ancestral groups. Previous studies have shown that the hunter-gatherers of the Caucasus were genetically closer to Iranian early farmers than to Anatolian/European early farmers; this observation was interpreted as indicating a lack of genetic continuity between Caucasus hunter-gatherers and Anatolian/European farmers<sup>11</sup>. However, the demographic model that best fits the data in this new study indicates that these two groups are related and descended from a common ancestral population that admixed with European hunter-gatherers around 14,200 years ago. Furthermore, Marchi and colleagues<sup>7</sup> found that the ancestors of Anatolian/European early farmers admixed a second time with Western European hunter-gatherers, shortly after the divergence of these farmers from Caucasus hunter-gatherers. This separation occurred between 13,000 and 9,000 years ago and was accompanied by strong founder effects associated with range expansions during the Allerød interstadial period, during which climatic conditions became more favorable. So why do Caucasus hunter-gatherers appear to be genetically

closer to Iranian early farmers than to European/Anatolian early farmers in analyses based on descriptive statistics or simpler demographic models? Based on simulations of genomic data under the most likely demographic scenario, Marchi and colleagues<sup>7</sup> suggest that the strong genetic drift experienced by Anatolian/European early farmers masked the footprints of their admixture with European hunter-gatherers, thereby increasing their differentiation from Caucasus hunter-gatherers. This analysis highlights the need for demographic modeling to elucidate finer events in populations that have experienced strong genetic drift.

Lastly, Marchi and colleagues<sup>7</sup> revisit the long-standing question of how agricultural practices spread across Europe during the Neolithic period, by comparing a model of stepwise expansion from the Aegean region to central Europe with a model involving long-distance migrations. The most supported scenario indicates a westward expansion of early farmers from the Aegean region around 8,000 years ago, followed by migrations towards Serbia, the Balkans and the Danube corridor, with Austria and Southern Germany ultimately reached around 7,500 years ago. According to this scenario, early farmers would have admixed with established communities of Western European hunter-gatherers, consistent with previous findings<sup>12,14</sup>. The demographic modeling of early farmer populations confirms that the Neolithization of Central Europe from the Aegean region involved actual movements of people (Figure 1), rather than the simple cultural transmission of new technology brought from the fertile crescent, in support of the ‘demic diffusion model’.

Overall, the study by Marchi and colleagues<sup>7</sup> demonstrates the advantages of the demographic modeling of ancient genomes for revealing more subtle aspects of past human history, which may be missed when using either simpler demographic models that do not consider ancient gene flow and population size changes, or descriptive and summary statistics only. Despite such advances in our understanding of the history of ancient Europe, much remains unknown about the demographic past of many other human populations worldwide,

partly due to the poor preservation of fossil remains in some areas of the world and, more generally, the bias of genomic studies towards individuals and populations of European ancestry. However, technological improvements that enable retrieval of DNA from poorly preserved fossils from Europe and other regions of the world are anticipated soon. For example, ancient DNA data for populations from Southeast Asia and the Pacific remain extremely scarce<sup>15,16</sup>. Yet, these populations display the highest levels of combined Neanderthal/Denisovan ancestry worldwide<sup>17,18</sup>, and paleogenomic studies could shed light on the complex peopling history of this region and on the admixture of our species with probably different, highly-structured groups of Denisovans<sup>18–20</sup> or even with other hominins. It is to be hoped that the next decade of paleogenomics research will yield extraordinary insight into, and probably some surprises about, the demographic, adaptive and cultural past of our species.

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

#### REFERENCES

- Higuchi, R., Bowman, B., Freiberger, M., Ryder, O.A., and Wilson, A.C. (1984). DNA sequences from the quagga, an extinct member of the horse family. *Nature* 312, 282–284.
- Skoglund, P., and Mathieson, I. (2018). Ancient genomics of modern humans: The first decade. *Annu. Rev. Genomics Hum. Genet.* 19, 381–404.
- van der Valk, T., Pecnerova, P., Diez-Del-Molino, D., Bergstrom, A., Oppenheimer, J., Hartmann, S., Xenikoudakis, G., Thomas, J.A., Dehasque, M., Saglican, E., et al. (2021). Million-year-old DNA sheds light on the genomic history of mammoths. *Nature* 591, 265–269.
- Dannemann, M., and Racimo, F. (2018). Something old, something borrowed: admixture and adaptation in human evolution. *Curr. Opin. Genet. Dev.* 53, 1–8.
- Quach, H., Rotival, M., Pothlichet, J., Loh, Y.E., Dannemann, M., Zidane, N., Laval, G., Patin, E., Harmant, C., Lopez, M., et al. (2016). Genetic adaptation and Neanderthal admixture shaped the immune system of human populations. *Cell* 167, 643–656.e617.
- Enard, D., and Petrov, D.A. (2018). Evidence that RNA viruses drove adaptive introgression between Neanderthals and modern humans. *Cell* 175, 360–371.e3.

7. Marchi, N., Winkelback, L., Schulz, I., Brami, M., Hofmanova, Z., Blocher, J., Reyna-Blanco, C.S., Diekmann, Y., Thiéry, A., Kapopoulou, A., *et al.* (2022). The genomic origins of the world's first farmers. *Cell*, <https://doi.org/10.1016/j.cell.2022.04.008>.
8. Lazaridis, I., Patterson, N., Mitnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P.H., Schraiber, J.G., Castellano, S., Lipson, M., *et al.* (2014). Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* 513, 409–413.
9. Gunther, T., Malmstrom, H., Svensson, E.M., Omrak, A., Sanchez-Quinto, F., Kilinc, G.M., Krzewinska, M., Eriksson, G., Fraser, M., Edlund, H., *et al.* (2018). Population genomics of Mesolithic Scandinavia: Investigating early postglacial migration routes and high-latitude adaptation. *PLoS Biol.* 16, e2003703.
10. Jones, E.R., Gonzalez-Forbes, G., Connell, S., Siska, V., Eriksson, A., Martiniano, R., McLaughlin, R.L., Gallego Llorente, M., Cassidy, L.M., Gamba, C., *et al.* (2015). Upper Palaeolithic genomes reveal deep roots of modern Eurasians. *Nat. Commun.* 6, 8912.
11. Lazaridis, I., Nadel, D., Rollefson, G., Merrett, D.C., Rohland, N., Mallick, S., Fernandes, D., Novak, M., Gamarra, B., Sirak, K., *et al.* (2016). Genomic insights into the origin of farming in the ancient Near East. *Nature* 536, 419–424.
12. Lipson, M., Szecsenyi-Nagy, A., Mallick, S., Posa, A., Stegmar, B., Keerl, V., Rohland, N., Stewardson, K., Ferry, M., Michel, M., *et al.* (2017). Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature* 551, 368–372.
13. Broushaki, F., Thomas, M.G., Link, V., Lopez, S., van Dorp, L., Kirsanow, K., Hofmanova, Z., Diekmann, Y., Cassidy, L.M., Diez-Del-Molino, D., *et al.* (2016). Early Neolithic genomes from the eastern Fertile Crescent. *Science* 353, 499–503.
14. Nikitin, A.G., Stadler, P., Kotova, N., Teschler-Nicola, M., Price, T.D., Hoover, J., Kennett, D.J., Lazaridis, I., Rohland, N., Lipson, M., and Reich, D. (2019). Interactions between earliest Linearbandkeramik farmers and central European hunter gatherers at the dawn of European Neolithization. *Sci. Rep.* 9, 19544.
15. Lipson, M., Spriggs, M., Valentin, F., Bedford, S., Shing, R., Zinger, W., Buckley, H., Petchey, F., Matanik, R., Cheronet, O., *et al.* (2020). Three phases of ancient migration shaped the ancestry of human populations in Vanuatu. *Curr. Biol.* 30, 4846–4856.e6.
16. Posth, C., Nagele, K., Colleran, H., Valentin, F., Bedford, S., Kami, K.W., Shing, R., Buckley, H., Kinaston, R., Walworth, M., *et al.* (2018). Language continuity despite population replacement in Remote Oceania. *Nat. Ecol. Evol.* 2, 731–740.
17. Qin, P., and Stoneking, M. (2015). Denisovan ancestry in East Eurasian and Native American populations. *Mol. Biol. Evol.* 32, 2665–2674.
18. Choin, J., Mendoza-Revilla, J., Arauna, L.R., Cuadros-Espinoza, S., Cassar, O., Larena, M., Ko, A.M., Harmant, C., Laurent, R., Verdu, P., *et al.* (2021). Genomic insights into population history and biological adaptation in Oceania. *Nature* 592, 583–589.
19. Jacobs, G.S., Hudjashov, G., Saag, L., Kusuma, P., Darusallam, C.C., Lawson, D.J., Mondal, M., Pagani, L., Ricaut, F.X., Stoneking, M., *et al.* (2019). Multiple deeply divergent Denisovan ancestries in Papuans. *Cell* 177, 1010–1021.e32.
20. Browning, S.R., Browning, B.L., Zhou, Y., Tucci, S., and Akey, J.M. (2018). Analysis of human sequence data reveals two pulses of archaic Denisovan admixture. *Cell* 173, 53–61.e9.