## A 'Denisovan' genetic history of recent human evolution

João C. Teixeira<sup>1,2\*</sup> & Alan Cooper<sup>1,2</sup>

<sup>1</sup>Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Australia <sup>2</sup>Australian Research Council Centre of Excellence for Australian Biodiversity and Heritage

\*Corresponding author: joao.teixeira@adelaide.edu.au

As anatomically modern humans (AMHs) migrated out of Africa and around the rest of the world, they met and interbred with multiple extinct hominid species<sup>1</sup>. The traces of genetic input from these past interbreeding events, recorded in the genomes of modern populations, have created a powerful record of recent human migrations. The first of these events occurred between Neandertals, and a small group of AMH shortly after they left Africa, somewhere in western Eurasia around 55-50 ka<sup>2</sup>, and left a genomic signal of about 2% Neandertal DNA that was subsequently spread across the rest of the world. The timing of this interbreeding event has been precisely calculated using the size of introgressed Neandertal DNA fragments in the genome of an early (45-43 ka) AMH specimen from western Siberia<sup>2,3</sup>. Subsequent additional Neandertal interbreeding events left much smaller localised genomic signals in different parts of Eurasia, presumably due to the much larger size of AMH populations involved<sup>4</sup>.

In contrast to the Neandertals, the interbreeding events with other extinct hominid groups – such as the Denisovans, the east Eurasian sister group of Neandertals – remain poorly understood, but are potentially far more complex. Our knowledge of the Denisovans remains intimately tied to genetic data retrieved from *Denisova* cave in the Altai mountains, in southern Siberia, which contains the only currently known fossil record of this group<sup>5,6</sup> (Fig. 1). A complete Denisovan genome was reconstructed from DNA surviving in a distal phalanx from the cave<sup>7</sup>, while a Denisovan-Neandertal hybrid was detected from bone fragments<sup>8</sup>. A recent morphological description of three hominid crania uncovered in Xuchang, central China<sup>9</sup>, has led to speculations about the likely presence of Denisovans in China, while the extensive diversity of fossil forms in South East Asia (SEA) raises the potential of yet more species<sup>5</sup>. As a result, the Denisovans and relatives remain some of the more elusive members of the recent human family.

The distribution of Denisovan ancestry in present-day human populations is markedly uneven<sup>10</sup>, and surprisingly is mostly found in geographical regions east of Wallace's Line, one of the world's most notable geographic barriers for faunal dispersion, thousands of kilometers away from Siberia<sup>6</sup>. Early studies reported that the highest genomic proportions of Denisovan ancestry are found in Aboriginal Australians and New Guinea Papuan Highlanders (Australo-Papuans) at ~3-6%, along with Oceanian populations with high Papuan ancestry. These are followed by huntergatherer populations in the Philippines (about half of that observed in Australo-Papuans), while trace amounts (<1%) of Denisovan signals have been reported in the genomes of many South and East Asian groups<sup>11</sup> (Fig. 1).

The nature and amount of introgressed hominid DNA provides a unique means to infer multiple aspects of the history of AMH movement throughout Eurasia. Recent studies have inferred the existence of at least two pulses of hominid 'Denisovan-like' introgression into AMH from genetically very distinct groups<sup>12,13</sup>. The source of the first genetic pulse is so distantly related to Denisovans it is potentially a new group, equidistant to both Neandertal and Denisovans<sup>13</sup> and

which we term here EH1 (extinct hominid 1) (Fig 1). Extensive demographic modelling indicates the divergent EH1 group interbred with the ancestor of all Asian and Australo-Papuan populations, before each of the latter split from one another, resulting in around 2.6-3.4% EH1 ancestry across these populations (Fig 1). This signal, albeit diluted, can still be detected in modern Indian, Andamanese, and East Asian populations<sup>13</sup>. A second phase of admixture with a Denisovan group closely related to the sequenced Altai Denisovan took place with the ancestors of Australo-Papuans and related groups after they split from other Asian populations, resulting in an additional ~1.6% genomic contribution<sup>13</sup> (red 2, Fig 1C). Modern Island South East Asia (ISEA) populations such as the Philippines and Flores have Denisovan genomic content roughly proportional to their Australo-Papuan shared ancestry<sup>10</sup>, suggesting they are derived from this same ancestral event (Fig. 1B).

The isolation of Australo-Papuan populations in Sahul (the continent of New Guinea-Australia-Tasmania) around 50 ka<sup>3</sup> preserved their EH1 and Denisovan genomic content from dilution by admixture with later AMH populations lacking these signals, such as Holocene farming groups, which are likely responsible for the low hominid genomic signals seen in modern AMH populations across Asia and ISEA<sup>14</sup> (Fig 1C). However, the lower than expected amounts of Denisovan ancestry in long-isolated SEA hunter-gatherer groups, such as the Andaman Islanders and Malaysian Jehai<sup>10,15</sup>, potentially indicates an early phase of admixture with non-Denisovan containing AMH populations. While some genetic studies have also reported a unique Denisovanlike introgression signal in East Asian populations<sup>12</sup>, the finding of the ancestral EH1 genomic contribution potentially explains both this, and the slightly higher Neandertal genomic ancestry described in these populations<sup>13</sup>. Either way, it seems safe to assume that the first introgression event, with EH1, occurred somewhere in South Asia, prior to the split and geographic spread of the different populations across South and East Asia, ISEA and Oceania. We depict this scenario in Figure 1.

The markedly higher amount of Denisovan genomic content observed in populations across Wallace's Line (Fig. 1), including many hunter-gatherer groups, correlates with the extent of their shared ancestry with the ancestral Australo-Papuan populations who first moved through the area<sup>10,11</sup>. Intriguingly however, relatively higher Denisovan proportions are detected in Philippines hunter-gatherer groups<sup>10,15</sup>, apparently recording a further independent introgression with Denisovans, following their separation from the ancestors of Australo-Papuan populations<sup>15</sup> (as depicted in Fig 1B). This raises the distinct possibility that the introgression events recorded in the Philippines populations took place east of Wallace's Line, and if so, that perhaps multiple populations of Denisovans were present on other islands in the area. An early hominid presence on the Philippines is supported by the finding of a 70 ka fossil hominid finger bone in Callao Cave<sup>16</sup>, well before AMH arrival in the area<sup>3</sup>, while butchered rhino bones indicate hominid presence as early as 700 ka<sup>17</sup>.

The idea that multiple hominid populations may have been present east of Wallace's Line is further supported by recent genetic analyses of the modern, very short-statured population living near Liang Bua cave on Flores, where fossils of the diminutive hominid *H. floresiensis* were discovered<sup>18</sup>. While no genetic signals consistent with *H. floresiensis* (or *H. erectus*) were detected in the genomes of the modern population around Liang Bua, the study detected an enigmatic 'unknown' genomic signature compatible with introgression from a hominid source as divergent

from modern humans as Neandertals and Denisovans<sup>18</sup>. Importantly, this signal was only detected on Flores and not elsewhere in ISEA/Melanesia, demonstrating that it is not the widespread initial EH1 genomic signal (Fig 1), or the Denisovan genomic component observed in the Australo-Papuan and Philippines populations<sup>18</sup>. This appears to point to an introgression event with a further extinct hominid (EH2), which given the location on Flores, implies that it had probably crossed Wallace's Line. This model raises important questions about whether *H. floresiensis* is in fact closely related to *H. erectus*, or instead might actually represent EH2, or even another taxon within this group. Further work is clearly required to determine what EH2 might represent (Fig. 1B).

Further paleontological and genetic research is required to resolve the many outstanding issues outlined here, but the current genetic evidence suggests that as they first moved through the area, modern humans interbred with one hominid population in south Asia, and at least two more in ISEA. Of these hominids, only Denisovans and Neandertals are currently known. Islands in SEA were only separated from mainland southeast Asia by narrow marine gaps during low sea levels in glacial periods, and the fluctuating extent and nature of land connections during glacial cycles likely played a key role in the original dispersal and subsequent isolation of early hominid groups in the area.

We present a parsimonious scenario of the current data in Fig. 1, although the precise location of the introgression events currently remains unknown. For example, the Denisovan introgression with the ancestor of the Australo-Papuans, Philippines, and Flores populations would appear to be on the Sunda shelf, prior to the divergence of these groups (Fig 1C), so we have tentatively placed it near Borneo due to recent studies suggesting the settlement of Sahul occurred via a route that transited through Borneo and Sulawesi before arriving in Sahul in what is now West Papua<sup>19</sup> (Fig. 1B). Alternatively, it is possible that the Denisovan introgression might also have taken place east of Wallace's Line, for example on Sulawesi, where archaeological evidence suggests hominids were present by at least 120 ka<sup>3,5,20</sup>. Importantly however, Sulawesi marks the eastern most point where introgression is plausible and there remain no clear signs that archaic hominid migrations extended as far as the Sahul continent itself.

## References

- 1. Skoglund, P. & Mathieson, I. Ancient human genomics: The first decade. *Ann Rev Genomics Human Genetics* **19**, 381-404 (2018).
- 2. Fu, Q. *et al.* Genome sequence of a 45,000-year-old modern human from western Siberia. *Nature* **514**, 445-449 (2014).
- 3. O'Connell, J. F. *et al.* When did *Homo sapiens* first reach Southeast Asia and Sahul? *PNAS* 115, 8482-8490 (2018).
- 4. Villanea, F.A. & Schraiber, J. G. Multiple episodes of interbreeding between Neanderthal and modern humans. *Nature Ecol Evol* **3**, 39-44 (2019).
- 5. Kaifu, Y. Archaic hominin populations in Asia before the arrival of modern humans: Their phylogeny and implications for the "Southern Denisovans". *Current Anthropology* **58**, S418-S433 (2017).
- 6. Cooper, A. & Stringer, C. B. Did the Denisovans cross Wallace's line? Science 342, 321-323 (2013).
- 7. Meyer, M. *et al.* A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222–226 (2012).
- 8. Slon, V. *et al.* The genome of the offspring of a Neanderthal mother and a Denisovan father. *Nature* **561**,113-116 (2018).
- 9. Li, Z. I. et al. Late Pleistocene archaic human crania from Xuchang, China. Science 355, 969-972 (2017).
- 10. Reich, D. *et al.* Denisova admixture and first modern human dispersals into Southeast Asia and Oceania. *Am J Hum Genet* **89**, 516-528 (2011).
- 11. Qin, P. & Stoneking, M. Denisovan ancestry in East Eurasian and Native American populations. *Mol Biol Evol* **32**, 2665-2674 (2015).
- 12. Browning, S. R. *et al.* Analysis of Human sequence data reveals two pulses of archaic Denisovan admixture. *Cell* **173**, 1-9 (2018).
- 13. Mondal, M., Bertranpetit, J. & Lao, O. Approximate Bayesian Computation with deep learning supports a third archaic introgression in Asia and Oceania. *Nat Comm* **10**, 246 (2019).
- 14. Lipson, M. *et al.* Ancient genomes document multiple waves of migration in Southeast Asian prehistory. *Science* **361**, 92-95 (2018).
- 15. Jinam, T. A. *et al.* Discerning the origins of the Negritos, first Sundaland people: deep divergence and archaic admixture. *Genome Biology and Evolution* **9**, 2013-2022 (2017).
- 16. Mijares, A. S. *et al.* New evidence for a 67,000-year-old human presence at Callao Cave, Luzon, Philippines. *Journal of Human Evolution* **59**, 123-132 (2010).
- 17. Ingicco, T. *et al.* Earliest known hominin activity in the Philippines by 709 thousand years ago. *Nature* **557**, 233-237 (2018).
- 18. Tucci, S. *et al.* Evolutionary history and adaptation of a human pygmy population of Flores Island, Indonesia. *Science* **361**, 511-516 (2018).
- 19. Kealy, S. *et al.* Least-cost pathway models indicate northern human dispersal from Sunda to Sahul. *Journal of Human Evolution* **125**, 59-70 (2018).
- 20. van den Bergh, G. D. et al. Earliest hominin occupation of Sulawesi, Indonesia. Nature 529, 208-211 (2016).

## Peer Preprints



Fig 1A. Proposed route of AMH movement out of Africa, around 60-50ka<sup>1,3</sup>, following areas of savannah-like habitat reconstructed from BIOME4 CO2 climate models (http://www.bridge.bris.ac.uk/resources/simulations/). Around 55-50ka, a small founding AMH population met and interbred with Neandertals somewhere in western Eurasia (blue circle N), resulting in a Neandertal genomic signal of around 2% that was subsequently distributed globally outside of Africa<sup>1,3</sup>. Sometime after the first event, the AMH population split, with one branch leading to the ancestors of Europeans, and the other to the common ancestor of South and East Asians, Australo-Papuans, and related populations. Modelling of genetic data<sup>13</sup> suggests that as the latter moved across south Asia, it experienced an initial introgression event (purple circle 1) with a unknown hominid (Extinct hominid 1; EH1), that was roughly equidistant to Denisovans and Neandertals. This resulting genomic signal (estimated to have originally been 2.6-3.4% <sup>13</sup>) is detected in groups as geographically distant as Andaman Islanders and Aboriginal Australians.

Fig 1B. Recent hominid introgression events in South East Asia: Phylogenetic tree showing approximate relationships and date estimates for the various extinct hominids, and modern AMH populations. Five inferred hominid introgression events are shown, starting with the Neandertal around 55-50 ka (Blue N), followed by EH1 (above, purple 1) which we have positioned in NE India. The ancestor of Australo-Papuans, Philippines and other ISEA groups is then inferred to have experienced admixture with Denisovans (red 2), with a second subsequent phase (red 3) also detected in Philippines hunter-gatherer populations. Lastly, the genomes of modern short-statured AMH populations on Flores record input from yet another extinct hominid (EH2), seemingly different from EH1<sup>18</sup>. The phylogenetic relationships amongst the extinct hominids remains unclear, but appear to be of roughly similar genetic divergence, occurring around 600 ka. The geographic location of introgression events 2-4 are not precisely known, and have been inferred parsimoniously. The timing of the above events is constrained by the initial Neandertal introgression at 55-50ka<sup>1,3</sup>, and the colonization of Australia at 50 ka<sup>3</sup>.

Fig 1C. Yellow and red arrows indicate the inferred route of AMH movement through Island South East Asia around 50ka<sup>19</sup> (shown with lowered sea levels), following reconstructed areas of savannah-like habitat as above. Modern hunter gatherer populations with genetic data are shown with red labels, and farming populations in black. The estimated genomic content of EH1 (purple) and Denisovan (red) in modern populations is shown in pie charts, relative to Australo-Papuans, with all populations containing large amounts of Denisovan genomic content found east of Wallace's Line. Independent introgression events with Denisovan groups are inferred for both the common ancestor of Australo-Papuan, Philippines and ISEA populations (red, circle 2), and separately for the Philippines (red, circle 3). Genomic data from modern populations on Flores record introgression with a further unknown extinct hominid (EH2), roughly equally related to Neandertals and Denisovans (brown, circle 4). The precise location of all the introgression events currently remains unknown. Pie charts with black borders have estimated hominid proportions.