

## Response to Hellenthal et al.:

*To the editor:* In our paper “The Kalash Genetic Isolate: Ancient Divergence, Drift, and Selection,” we reported that the Kalash people of present-day Pakistan experienced no detectable gene flow from their geographic neighbors in Pakistan, or from the other extant western Eurasian populations tested, since their split from a common ancestor.<sup>1</sup> It is probably true that no human population is completely cut off from all others, but populations with limited gene flow to and from the outside, especially when combined with a small effective population size and increased genetic drift, endogamous practices, and a different admixture history from neighboring populations, are commonly described as “isolates.” All these features are observed in the Kalash.

We noted at an early stage of our work that our observations contrasted with the published inference of introgression dated to approximately 910–220 BCE by Hellenthal et al.<sup>2</sup> and contacted some of those authors before submitting our manuscript to explore possible explanations for these differences. We appreciate the time they have spent thinking about these questions and their current input, although unfortunately their suggestion does not resolve the issue. We agree with Hellenthal et al.<sup>3</sup> that we should have discussed the ALDER results presented in Table S4 in more detail in our main text, and present that discussion here. ALDER detected 6-fold more admixture events in the seven other Pakistani populations than in the Kalash. Of the 406 possible surrogate pairings, ALDER detected 14 significant ( $z > |5|$ ) admixture events in the Kalash, of which 85% involved the South Asian Chamar and Kol populations, who have less than 20 individuals each in our dataset (Table S1), and had inconsistent decay rates leading to our low confidence in these results. It has been documented that small sample size in reference populations creates noise in ALDER.<sup>4</sup> The Pathans who are the Kalash’s closest neighbors in north western Pakistan provide an informative contrast: they have a similar Western Eurasian admixture pattern that is significantly ( $z > |5|$ ) supported by 85 surrogate pairings. Instead of writing that ALDER “showed no evidence of gene flow into the Kalash,” we should have written “showed no reliable evidence of gene flow into the Kalash,” and we ask readers to read the sentence in that way. The discrepant conclusions<sup>1,2</sup> thus remain unresolved.

Hellenthal et al.’s comments highlight a topic of more general interest: the limitations of tests that model allele frequency correlation patterns among present-day samples for inferring past events, as also recently demonstrated in a paper on Ethiopian samples.<sup>5</sup> We enthusiastically agree that the analysis of ancient DNA from skeletal remains in north-western Pakistan or Afghanistan should provide further insights into the origins of the Kalash and will probably raise currently-unsuspected lines of inquiry.

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