

1     Testing an alternative explanation for relatively greater base-sharing  
2                   between Neanderthals and non-African humans.  
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14    **Abstract**

15  
16    **Most accept that non-African humans share ~2% of their genome with Neanderthals**  
17    **(1) and that inter-breeding occurred between several archaic lineages (2-4). However,**  
18    **most evidence assumes that mutation rate is constant. It has been suggested that**  
19    **heterozygosity is mutagenic (5-8). If so, an alternative explanation of the data becomes**  
20    **possible. Instead of non-Africans sharing relatively more bases with Neanderthals due**  
21    **to interbreeding, Africans could appear unexpectedly divergent due to their mutation**  
22    **rate not having been lowered when diversity was lost during the out of Africa**  
23    **bottleneck. I therefore tested a series of predictions aimed at distinguishing mutation**  
24    **slowdown from inter-breeding. Predictions from mutation slowdown are generally**  
25    **better supported. For example, the signal used to infer inter-breeding remains even**  
26    **when Neanderthal sequences are excluded. I conclude that, while some inter-breeding**  
27    **probably did occur, an appreciable component of the signal seems better explained by**  
28    **mutation slowdown.**  
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## 31 Introduction

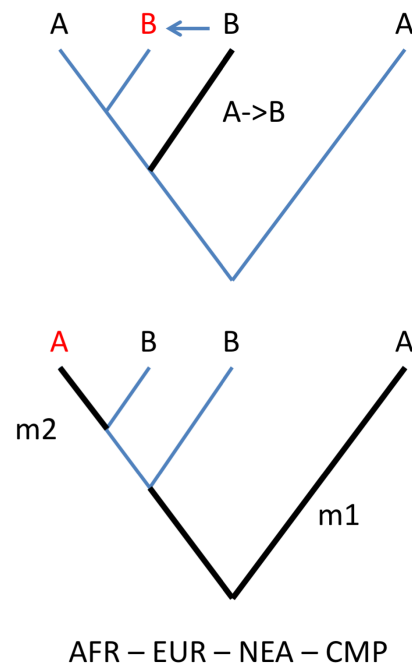
32  
33 The draft Neanderthal genome revealed more shared nucleotide bases with modern non-  
34 African humans compared with Africans (1). This asymmetric base-sharing was used to  
35 argue that Neanderthals inter-bred with the ancestors of modern non-Africans, leaving a  
36 modern genetic legacy of ~2%. Subsequent studies have reinforced this model, with the  
37 discovery of skeletons with recent hybrid origin (9), an estimated 5% legacy from Denisovans  
38 in Oceania (10-12) and a decrease in the inferred size of introgressed blocks over time (13,  
39 14), consistent with recombination breaking down introgressed blocks.

40  
41 Inter-breeding with Neanderthals was originally inferred from four-way DNA sequence  
42 alignments comprising two humans, a Neanderthal and a chimpanzee (the 'ABBA-BABA' test  
43 (15-17)) (1, 16). Focus centres on bases where the Neanderthal and chimpanzee differ, with  
44 each matching one of the two humans (states 'ABBA' and 'BABA'). Under a null model, with  
45 no introgression and constant mutation rate, ABBA and BABA sites should be equally  
46 frequent. In practice, when the two humans comprise one African and one non-African, the  
47 counts of ABBA and BABA are significantly asymmetrical (1). Such asymmetry, usually  
48 expressed as Patterson's D (18), is most obviously explained by introgression of Neanderthal  
49 DNA into the non-African.

50  
51 Subsequent studies have developed the ABBA-BABA into derivative statistics such as  $D_{\text{enhanced}}$   
52 (10) and  $f_4$ -ratios (18). Despite these developments, the underlying principles remain largely  
53 unchanged. Ultimately, all methods attempt to quantify excess base-sharing between an  
54 archaic genome and one modern genome relative to a control, usually a second modern  
55 human. Clusters of shared bases have been interpreted as introgressed haplotypes [12, 19].  
56 However, many aspects of the mutation process remain poorly understood, including the  
57 lability and strength of mutation hotspots (19), the tendency of mutations to cluster on the  
58 same chromatid (20-22), and the mechanism and strength of the correlation between  
59 mutation rate and recombination rate (23-25). Consequently, distinguishing unexpected  
60 clusters of related mutations from genuine non-human fragments is not trivial.

61  
62 D statistics assume explicitly that mutation rate is constant (18), or at least does not vary  
63 enough to distort the expectation of symmetric base-sharing. If this assumption holds, then  
64 inter-breeding with archaic hominins offers the only viable mechanism capable of generating  
65 asymmetrical base-sharing and significant deviations from zero can be used to help  
66 understand patterns human migration (26) and selection (27). However, if the assumption of  
67 mutation rate constancy is relaxed, an alternative explanation becomes possible, based on the  
68 genome-wide mutation rate being higher among humans who stayed in Africa compared with  
69 those who migrated out to colonise the rest of the world (**Figure 1**). This alternative model  
70 can be seen as the converse of the inter-breeding model: instead of non-Africans being  
71 unexpectedly *similar* to Neanderthals, Africans would be seen as being unexpectedly  
72 *dissimilar*.

73



74

75 **Figure 1. Alternative hypotheses to explain excess base sharing with Neanderthals.** Each  
 76 tree depicts a mechanism by which an “ABBA” pattern is generated, wherein a European human  
 77 (EUR) shares a base with the Neanderthal (NEA) while, at the same site, an African human  
 78 (AFR) shares a base with the chimpanzee, *Pan troglodytes* (CMP). Both trees assume only two  
 79 states, ‘A’ and ‘B’, with CMP = ‘A’. Heavy black lines indicate lineages on which mutations  
 80 occurred and key bases appear in red. Top panel: a single mutation creates a new ‘B’ allele in  
 81 NEA alone which then enters EUR by inter-breeding. Bottom panel: a first mutation m1 creates  
 82 a “BBBA” pattern then a back-mutation m2 then recreates an ‘A’ allele in AFR.

83

84 Mutation rate variation occurs within many groups of organisms, including higher primates  
 85 (28) though the mechanisms remains largely unclear (29, 30). Among human populations  
 86 there are reports both of differences in the mutation process (31, 32) and of variation in  
 87 mutation rate, though the latter evidence is inconsistent, one study reporting a higher rate in  
 88 Africans (8) and a second finding a lower rate (33) (see also discussion below). One possible  
 89 mechanism capable of driving variation in mutation rate is heterozygote instability (HI) (5,  
 90 34), whereby gene conversion events that target heterozygous sites in heteroduplex DNA  
 91 formed during synapsis (35) provide extra opportunities for mutations (34). Under HI, the  
 92 loss of heterozygosity as humans migrated out of Africa (36, 37) would have reduced the  
 93 mutation rate in non-Africans (8). Although not yet widely accepted, the HI hypothesis  
 94 enjoys support from microsatellite data (5, 38, 39), from the correlation between  
 95 heterozygosity lost by humans out of Africa and excess mutation rate in Africa (8) and, more  
 96 recently, from direct mutation counting in parents and their progeny (7). HI is also consistent  
 97 with patterns of SNP clustering in humans (20).

98

99 Here I attempt to address the question of whether any of the observed asymmetrical base-  
 100 sharing can be attributed to mutation slowdown rather than inter-breeding with archaic  
 101 hominins. To do this I construct a series of tests designed to yield opposing predictions under  
 102 the two models, finding, for the most part, that mutation slowdown is better supported.  
 103 Given that the processes associated with mutation slowdown are poorly understood, I do not

104 attempt to explain the large body of recent observations relating to archaic hybridisation but  
105 hope that my observations will stimulate future research in this area.

106

## 107 Results

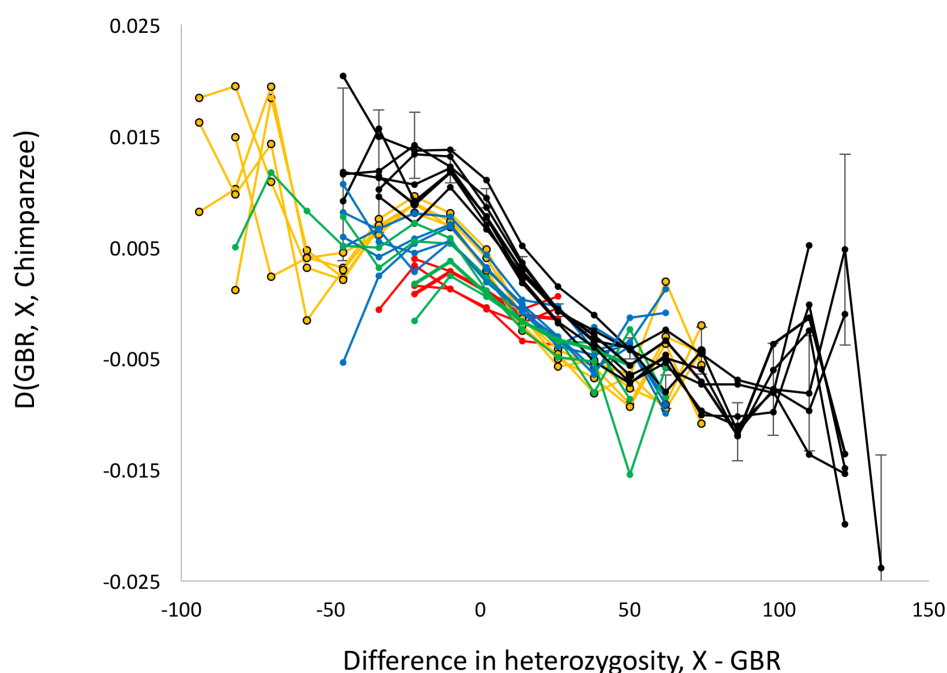
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### 109 ***Mutation rate variation in modern humans and heterozygosity***

110

111 A key requirement of the mutation slowdown hypothesis is that, since humans migrated out  
112 of Africa, the mutation rate inside Africa has been appreciably higher than outside. A three  
113 taxon D,  $D(\text{Europe, Africa, chimpanzee})$ , has given conflicting results. The first study, based on  
114 Complete Genomics data, found a higher mutation rate in Africa (8), while a second used the  
115 Simons Genome Diversity panel to show a higher rate outside (33). Repeating the analysis  
116 using data from the 1000 genomes project (40), I found a lower rate in Africa, in agreement  
117 with Mallick et al. (33). Although it is unclear why my first analysis gave conflicting results,  
118 the key quantity in the context of ABBA-BABA is the relative mutation rate in Africans and  
119 non-Africans *since* humans left Africa, and this is not necessarily reflected when all variants  
120 are considered.

121



122

123

124 **Figure 2. Relationship between heterozygosity difference and relative mutation rate**  
125 ***within the genome.*** Data presented are for all pairwise population comparisons including GBR  
126 and a second population, X, colour-coded: Africa, black; Europe, red; South Asia, blue; East Asia,  
127 yellow; America, green. To construct this plot, the autosomal genome was divided into 1Mb  
128 blocks and each one used to measure the relative mutation rate, expressed as  $D(\text{GBR, X,}$   
129  $\text{chimpanzee})$  and heterozygosity difference, expressed as the difference in expected number of  
130 heterozygous sites. Data were then binned by heterozygosity difference to generate average D  
131 values and data for bins with >10 values plotted. For clarity, example error bars, 1 standard  
132 error of the mean, are only included for one African population. D is calculated as  $(\text{BAA} -$   
133  $\text{ABA})/(\text{BAA} + \text{ABA})$  such that +ve D values indicate a higher mutation rate in GBR relative to X.  
134

135 Since population bottlenecks impact greatly on the allele frequency spectrum, it is not valid  
136 simply to focus on alleles rare enough mostly to have arisen since humans left Africa.  
137 However, the large loss of heterozygosity non-Africans suffered was modulated across the  
138 genome by selection (36, 37, 41). Consequently, if HI operates, then changes in  
139 heterozygosity will drive parallel changes in D, creating a correlation between heterozygosity  
140 and D. Specifically, excess mutation rate in Africa should be greatest in regions of the genome  
141 where excess heterozygosity in Africa is greatest. On the other hand, since D is not impacted  
142 by demography (8, 33), if HI does not operate then a correlation cannot exist. In practice,  
143 heterozygosity difference and D are correlated in a way predicted by HI (illustrated for all  
144 pairwise population comparisons involving GBR, **Figure 2**). The complexity of the profiles yet  
145 strong overall similarity of shape between regions argues for a common mechanism.

146  
147 I next tested more directly whether recent mutations occur preferentially in regions of  
148 elevated heterozygosity. Even though mutation hotspots are known to evolve rapidly (42),  
149 they should be similar in strength and locations among human populations drawn from the  
150 same geographic region. Nonetheless, drift will lead to variation in heterozygosity between  
151 populations and across the genome. If HI operates, new mutations should be more frequent in  
152 population / genomic location combinations that by chance carry higher heterozygosity.  
153 Detecting genuine de novo mutations requires deep sequencing of many parent-offspring  
154 trios. However, variants occurring just twice in the 1000 genomes data (= 'doubletons') are  
155 usually less than 500 generations old (43), so their distribution can conveniently be used as a  
156 surrogate measure of where mutations are currently most likely to occur.

157  
158 I identified all doubletons in the 1000 genome data where both copies occurred in the same  
159 population. For each doubleton I calculated heterozygosity within 1kb either side, both in the  
160 population where the doubleton occurred, *HetMut*, and as an average for the same window  
161 across each of the other populations from the same geographic region, *HetOther*. In 24 of the  
162 26 1000g populations the average difference between *HetMut* and *HetOther* is highly  
163 significantly positive (**Table 1**). The two exceptions are Finland and Peru, both populations  
164 that have the lowest heterozygosity in their region. After correcting for differences in  
165 genome-wide heterozygosity, the all populations show similar, highly significant, positive  
166 differences (mean = 0.022 +/- 0.004 s.d., range = 0.018 to 0.038). Thus, recent variants do  
167 seem to occur preferentially in genomic regions where heterozygosity is higher.

### 168 169 ***Are there sufficient numbers of back-mutations?***

170 The mutation slowdown hypothesis requires large numbers of back-mutations. Until now,  
171 back-mutation have been assumed to be too rare to be important (1, 18), as the following toy  
172 example illustrates. If the mutation rate is  $10^{-8}$  per base per generation and 2000 generations  
173 have elapsed since 'out of Africa', the 8,156,936 BBBA states reported by Green et al. ((1),  
174 Supplementary Materials p. 138) would yield only 163 back-mutations. Even if all these  
175 back-mutations occur in Africa, the number is still 50 fold fewer than the observed ABBA-  
176 BABA excess of around 8,000. However, this calculation is naïve because real mutations are  
177 strongly clustered (21, 22). Since the probability of a back-mutation scales with mutation  
178 rate squared, back-mutations are disproportionately likely in mutation hotspots, and likelier  
179 still if HI involves the active 'correction' of heterozygous sites by gene conversion (19, 35, 44).

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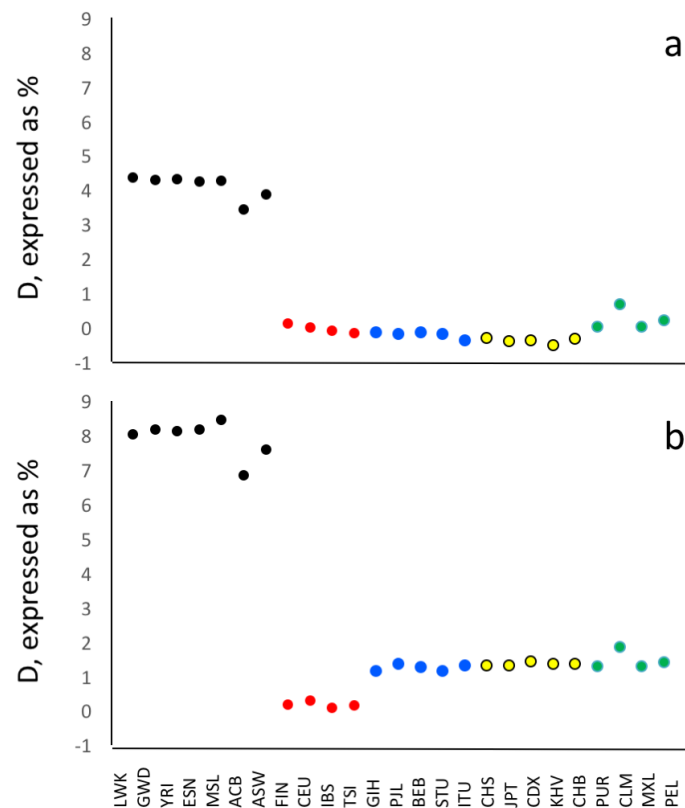
181 Although the true rate of back-mutations is difficult to estimate, several lines of evidence  
182 suggest it may be of the order required by the mutation slowdown hypothesis. Thus, Green et  
183 al. list just over 6000 instances each of BCBA and CBBA ((1), SOM p. 138). Both these base  
184 conformations require two independent mutations at the same site, at least one of which  
185 must be a transversion. Since transitions outnumber transversions by around two to one,  
186 despite representing only half the possible changes, the relative probability of a transition to  
187 a transversion is around four (45), implying around 60,000 ABBA and BABA due to back-  
188 mutation in the Green et al. dataset and 240,000 genomewide (Green et al. generate  
189 alignments for about one quarter of the genome). Even this number is likely to be an under-  
190 estimate. First, sites with three plus states are probably under-reported, particularly in lower  
191 coverage genomes. Second, site-specific and sequence-context considerations (45-47) may  
192 cause some sites to mutate mainly by transitions while others would favour transversions.  
193 Third, if heterozygous sites are recognised and 'repaired' by gene conversion as they are in  
194 yeast (35), this would provide a directed mechanism that actively promotes back-mutations.  
195

### 196 *Consequence of Excluding Neanderthal sequences*

197 Mutation slowdown and inter-breeding give opposing predictions concerning the  
198 dependence of D on the Neanderthal genome. In the inter-breeding model, D will show  
199 complete dependence on inclusion of Neanderthal sequences, because Neanderthal bases  
200 dictate which sites are informative. Conversely, under the mutation slowdown model,  
201 asymmetrical ABBA BABA counts are endogenous to modern humans so different outgroups  
202 will give similar results. To test this prediction I used GBR as a reference population and  
203 compared  $D(X, GBR, Neanderthal, Chimpanzee)$  with  $D(X, GBR, AA, Chimpanzee)$ , where X is  
204 one of 25 non-GBR 1000g populations and 'AA' is the ancestral human allele inferred by  
205 1000g. To prevent any possible spill-over signal, all informative Neanderthal sites  
206 contributing to  $D(X, GBR, Neanderthal, chimpanzee)$  were excluded from the calculation of  
207  $D(X, GBR, AA, chimpanzee)$ . The resulting patterns are highly similar and correlated with each  
208 other (**Figures 3a, 3b**), suggesting a common cause.

209

210 Interestingly, D actually strengthens when all Neanderthal information is removed. This  
211 strengthening might be expected. When the ancestral allele is used as a form of outgroup, the  
212 time-depth of the hominin trio is shallower than when the Neanderthal is used.  
213 Consequently, the asymmetrically distributed ABBA-BABA counts will make up a relatively  
214 larger proportion of all ABBA-BABA states. The fact that larger Ds result when Neanderthal  
215 information is excluded indicates that, in this analysis, Neanderthal introgression cannot be  
216 the dominant mechanism driving positive D. This point is emphasised by the fact that  
217 replacing Neanderthal and chimpanzee with various combinations of higher primates (gorilla  
218 and orangutan) also generate significant D (data not shown).  
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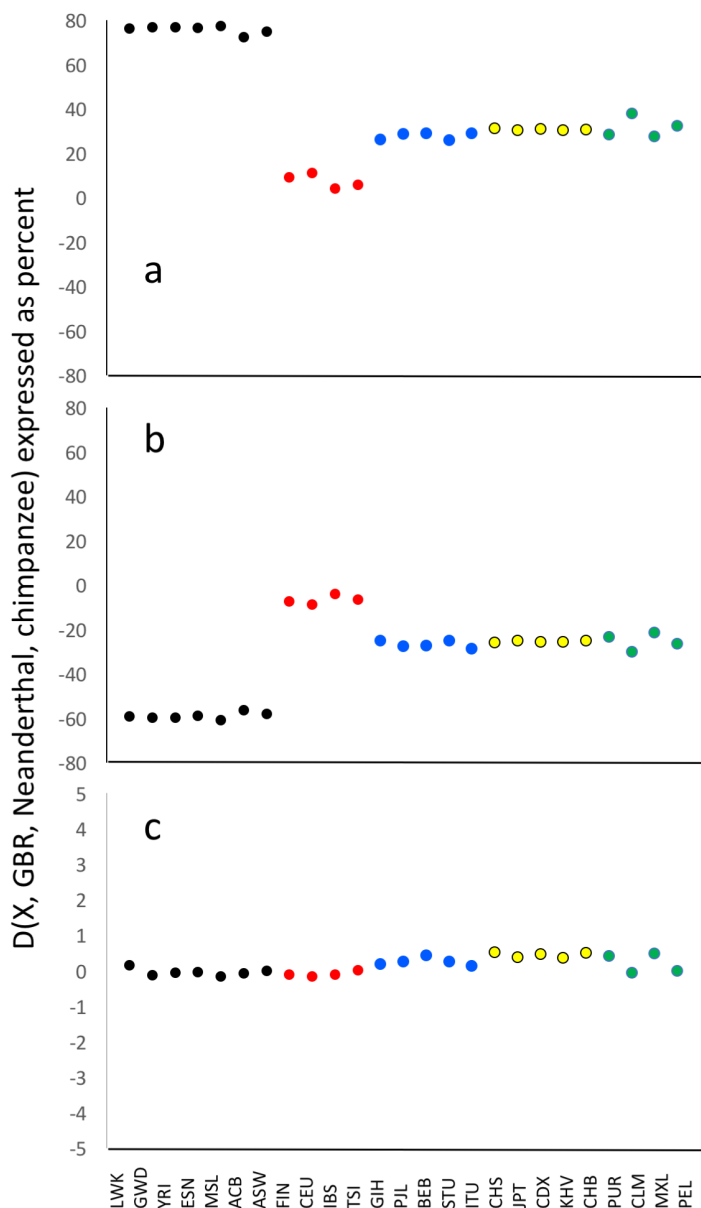
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222 **Figure 3. Dependence of D on the Neanderthal genome.** Each dot represents an autosomal  
223 ABBA-BABA test, expressed as  $D(X, GBR, Y, chimpanzee)$ , where X is one of 25 other 1000g  
224 populations, Y is either Neanderthal (panel 3a) or the inferred ancestral human allele (Panel  
225 3b). Populations are colour-coded by geographic region as in Figure 2, with populations  
226 appearing in the same order as listed in methods. Standard errors of the mean are of the order  
227 0.001 and are too small to show. All informative sites contributing to D in 3a were excluded  
228 from the analysis in 3b.  
229

### 230 Which allele is rare?

231 Inter-breeding and mutation slowdown also give opposing predictions for the frequencies of  
232 the 'A' and 'B' alleles. Under inter-breeding, positive D is driven by Neanderthal 'B' alleles  
233 entering human populations outside Africa. Under mutation slowdown, positive D is driven  
234 by an excess of 'A' alleles generated by back-mutation in Africa. In both cases, the key alleles  
235 will tend to be rare, having entered their respective populations after humans left Africa.  
236 However, the patterns are opposing. Inter-breeding and mutation slowdown predict that the  
237 'B' allele in Europe, will be rare and common respectively. To test this prediction I  
238 partitioned autosomal  $D(X, GBR, Neanderthal, chimpanzee)$  by the frequency of the 'B' allele in  
239 GBR. When X is African, D averages 75.7%, -59.1% and -0.05% depending on whether the 'B'  
240 allele is at high (>90%), low (<10%) or intermediate frequency respectively (Figure 4). Since  
241 positive D occurs when 'B' is common in GBR, these results support mutation slowdown over  
242 inter-breeding. The highly polarised patterns seen when one allele is rare / common and  
243 virtual lack of any signal at intermediate frequencies (mean D = 0.05%, Figure 4c) argues

244 against mechanisms where appreciable numbers of alleles are at intermediate frequency such  
 245 as very ancient inter-breeding.  
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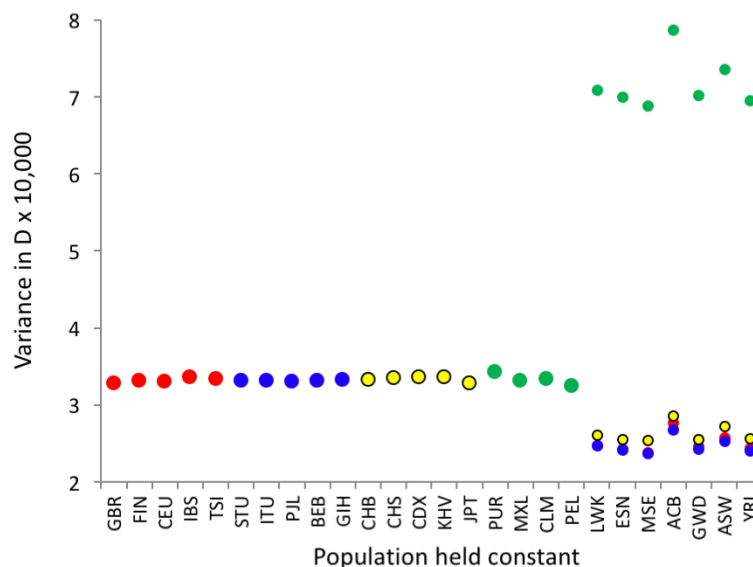
247  
 248 **Figure 4. Dependence of  $D$  on the frequency of the 'B' allele.** Data are subsets of those in  
 249 Figure 3, see that legend for details. The data are partitioned according to whether the  
 250 frequency of the 'B' allele in GBR is common (>90%), rare (<10%) or intermediate frequency,  
 251 shown in Figures 4a, 4b and 4c respectively. Note how the classic observation that  $D(\text{AFR}, \text{EUR},$   
 252  $\text{NEA}, \text{CMP}) \sim 4\%$  reflects an average of the extreme patterns seen in 4a and 4b. NB the vastly  
 253 different scales on the Y axis.  
 254

255 **Which populations drive most variation in  $D$ ?**

256 A further feature that could help distinguish the two hypotheses relates to variation in  $D$   
 257 among different population combinations. Both hypotheses predict one region drives  $D$  while  
 258 the other acts as a passive control. Consequently, across all African – non-African population  
 259 combinations, rotating populations from one region should cause more variation in  $D$  than



260 rotating populations from the other. Specifically, D should vary more when non-African and  
261 African populations are rotated under the inter-breeding mutation slowdown hypotheses  
262 respectively. Ignoring the highly admixed 'American' populations, variance in D is  
263 appreciably greater when African populations are rotated against non-African populations  
264 (mean variance =  $3.33 \times 10^{-4}$ ,  $\pm 1 \times 10^{-6}$  s.e.m.) than *vice versa* (mean variance =  $2.54 \times 10^{-4}$ ,  
265  $\pm 2.9 \times 10^{-6}$  s.e.m.), see **Figure 5**. If anything, this lends further support to the mutation  
266 slowdown hypothesis.  
267



268  
269 **Figure 5. Dependence of the variance in D on which populations are rotated for African -**  
270 **non-African population pairs.** *D*(H1, H2, Neanderthal, chimpanzee) values were generated for  
271 all African - non-African population pairs, H1, H2. For each population (X-axis) variance in *D*  
272 (Y-axis) is calculated for all comparisons in which it is included. Non-African variances are  
273 based on all comparisons to the African populations ( $N=7$ ) while the African comparisons are  
274 partitioned by each of the other four population groups (Europe,  $N=5$ ; Central Southern Asia,  
275  $N=5$ ; East Asia,  $N=5$ ; America,  $N=4$ ). Colour coding of non-African populations follows Figure 2.  
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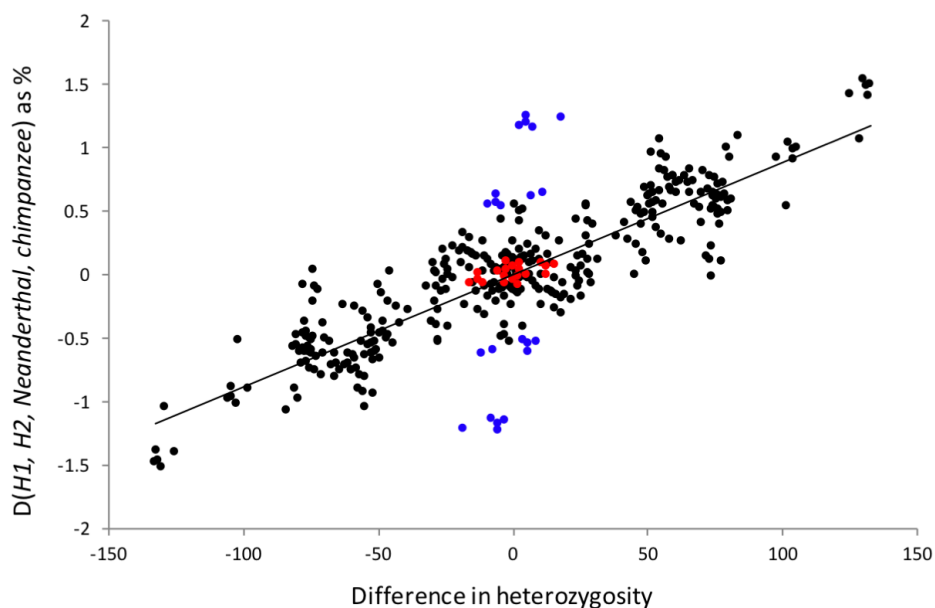
277 By far the highest variance is seen in African populations when American populations are  
278 rotated. Since the variances are so different from any of the other regions it seems likely that  
279 these extreme values relate to the high but varying levels of admixture. Arguments could be  
280 made in support of both hypotheses. Thus, the high variation in *D* could be driven by  
281 increased variation in the frequency of introgressed fragments. Equally, if mutation  
282 slowdown is driven by the loss of heterozygosity that occurred 'out of Africa', highly admixed  
283 populations would likely exhibit a counter-trend. Distinguishing these two possibilities is not  
284 trivial and has not been attempted here.

### 285 286 **Resolving the relationship between *D* and heterozygosity**

287  
288 Several studies have sought to understand how introgressed Neanderthal genes have been  
289 impacted by natural selection (2, 48). The action of selection can be captured by B-statistics, a  
290 derived measure of the extent to which patterns of heterozygosity have been distorted by  
291 selection (49). B and *D* are positively correlated across the genome, interpreted as selection

292 modulating the frequency of introgressed fragments (50). However, a correlation between D  
293 and heterozygosity is also expected under mutation slowdown. Here, the amount of diversity  
294 lost in the out of Africa bottleneck was modulated by natural selection, acting to accelerate  
295 loss of diversity in some genes and to reduce loss in others (51). As a result, strongly selected  
296 genes such as those in the immune system may have experienced more or less mutational  
297 slowdown, depending on whether they came under directional or balancing selection  
298 respectively. In turn, this could drive unusually high or low D values around selected genes.  
299

300 Although both hypotheses predict a relationship between D and heterozygosity, the exact  
301 nature of the relationship differs. Inter-breeding predicts a more or less simple relationship  
302 between heterozygosity and D in non-Africans only. In contrast, for mutation slowdown the  
303 key quantity is *difference* in heterozygosity and, while the main focus is on African - non-  
304 African comparisons, HI should drive non-zero D wherever heterozygosity differences have  
305 persisted for appreciable amounts of time. A second important difference is that, while  
306 selection acting on Neanderthal fragments may drive a correlation across regions within a  
307 genome, its impact on genome-wide values for individuals or populations should be minimal.  
308 Moreover, if Neanderthal fragments are common enough to increase heterozygosity, their  
309 presence would drive a negative relationship between heterozygosity difference and D. In  
310 contrast, HI should drive a positive correlation both within *and* between genomes.  
311  
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315 **Figure 6. Heterozygosity difference predicts D in pairwise population comparisons.**  
316 *Autosomal D(H1, H2, Neanderthal, chimpanzee) was calculated for all pairwise combinations of*  
317 *non-African populations (N=19 populations, black dots), non-admixed African population pairs*  
318 *(N=5 populations, red dots) and within Africa comparisons involving either admixed population,*  
319 *ACB or ASW. The X-axis is difference in mean number of heterozygous sites per megabase*  
320 *between populations, expressed as H1 - H2. The Y-axis is D which, being generally small, has*  
321 *been expressed in percent. The positive slope indicates that when H1 has higher heterozygosity,*  
322 *population H2 carries more bases in common with the Neanderthal. Note, each population pair*  
323 *is represented twice, with H1 and H2 reversed, making the pattern symmetrical about zero.*

324 To explore the impact of genome-wide heterozygosity difference on  $D(H1, H2, Neanderthal,$   
325 *chimpanzee*), I considered all pairwise population comparisons in the 1000g data. African –  
326 non-African combinations show high  $D$  and large differences in heterozygosity, so tell us little  
327 about the likely mechanism. I therefore focused on comparisons either inside or outside  
328 Africa, where differences in Neanderthal content are expected to be minimal or negligible  
329 (Africa). As expected,  $D$  values are small, rarely exceeding 1%. Nonetheless, outside Africa  $D$   
330 is strongly predicted by difference in heterozygosity (black symbols, **Figure 6**), with an  $r^2$  of  
331 81.2%. Since there is no *a priori* reason why a given population should appear as  $H1$  rather  
332 than  $H2$ , each comparison is included twice. This removes possible bias due to which  
333 population is made  $H1$  but means that the  $r^2$  value cannot be interpreted directly. Within  
334 Africa, non-admixed comparisons (red) conform quite closely to the outside Africa trend. In  
335 contrast, comparisons involving the two admixed populations (ACB, ASW, in blue) show a  
336 similar slope but much larger  $D$ , reinforcing the idea that admixture impacts  $D$  more than  
337 expected from passive mixing.

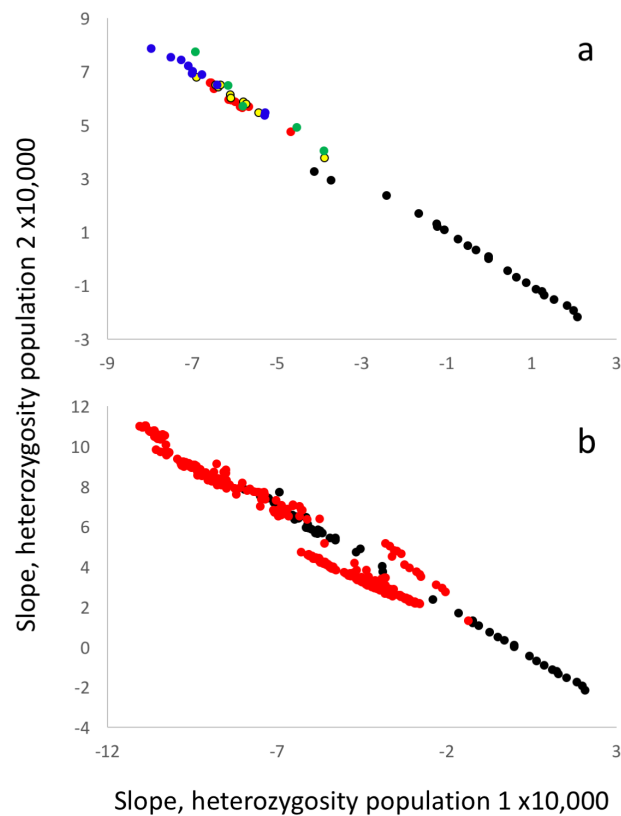
338  
339 A next examined the impact of heterozygosity difference on  $D$  across regions *within* the  
340 genome, I divided the autosomal genome into 1Mb windows. Within each window and for  
341 each of all possible pairwise population combinations I calculated heterozygosity in each  
342 population ( $Het_{H1}$  and  $Het_{H2}$ ) and counted ABBA and BABA for the tetrad  $H1, H2,$   
343 *Neanderthal, chimpanzee*. I then fitted multiple regressions of the form

$$\text{Proportion of ABBA} \sim Het_{H1} + Het_{H2}$$

344  
345  
346  
347 where the proportion of ABBA counts is fitted as a binomial response. 78 windows excluded  
348 where the total number of ABBA + BABA was less than one (fewer than 3% of windows, in  
349 effectively all cases windows with no chimpanzee alignment or including big overlaps with  
350 centromeres / telomeres). Apart from 20 non-significant, within Africa comparisons, the  
351 slopes of  $H1$  and  $H2$  are invariably significant ( $P < 0.01$ , though for a large majority  $P < < 10^{-10}$ )  
352 and show opposing slopes, being positive and negative respectively (**Figure 7**).

353  
354 Since previous studies only consider a single heterozygosity, expressed as a B statistic (49,  
355 50), I was interested to see if the two heterozygosity model (2HM) had similar or better  
356 explanatory power. For each population combination I compared the better fitting (lower  
357 AIC) single heterozygosity model (SHM) with the 2HM. No difference was found within Africa  
358 ( $N=21$  comparisons, mean AIC difference 0.1). In all other instances, the 2HM AIC was  
359 substantially lower (Table 2a). Apart from within Africa, the 2HM explained 2-9.5% of  
360 variation in  $D$ , 3-112 times more than the best SHM (Table 2b). Combined, the massive  
361 superiority of the 2HM and the highly significant, always opposing slopes are difficult to  
362 reconcile with a model based on selection, yet support a model where the key quantity is  
363 *difference* in heterozygosity.

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**Figure 7. Relationship between heterozygosity and D across the genome.** The autosomal genome was analysed in non-overlapping 1Mb windows in all pairwise combination of the 26 1000g populations. For each window in each pair I counted the numbers of ABBA and BABA and the expected number of heterozygous sites in each population (Het1, Het2). I then fitted a binomial general linear models of the form  $ABBA/BABA \sim Het1 + Het2$ . Slope estimates for all within-region population pairs are shown in Figure 7a, colour coded as in Figure 2. Figure 7b depicts the full set of comparisons, coded within region = black, between regions = red. All slopes are significant except for 20 within-Africa comparisons (righthandmost points in Figure 7a).

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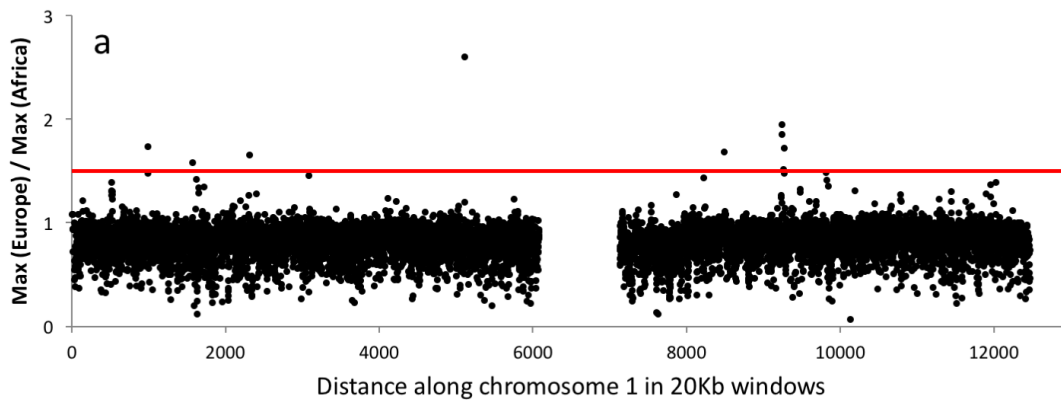
### **Do non-Africans carry unusual fragments?**

D is a relative measure and does not distinguish between non-Africans being more similar to Neanderthals and Africans being less similar. For a more objective estimate of the frequency of putative introgressed fragments I calculated all-against-all intra-population pairwise divergences for non-overlapping 20Kb windows across the genome, recording the maximum value in each population (MaxPD). With generally higher diversity, MaxPD(Africa) should be higher than MaxPD(outside Africa). However, even a single Neanderthal (or other archaic) fragment in a non-African population will tend to reverse this expectation. In other words, the ratio  $MaxPD(outside\ Africa)/MaxPD(Africa)$  should be  $<1$  when introgressed fragments are absent and  $>1$  when present. Since previous studies suggest that very few genomic regions carry zero Neanderthal ancestry (11, 50), the inter-breeding hypothesis predicts a ratio  $>1$  in most windows.

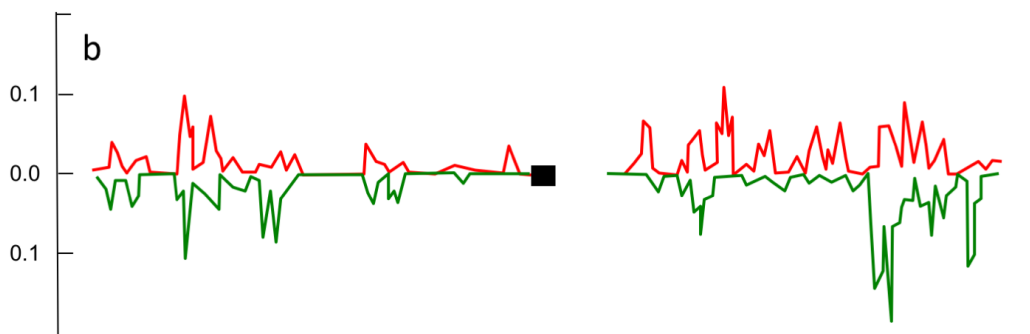
390  
391

Only ~5% of autosomal windows show a ratio  $>1$  (exemplified by chromosome 1, **Figure 8a**). Moreover, since a typical Neanderthal sequence is more divergent from modern humans than

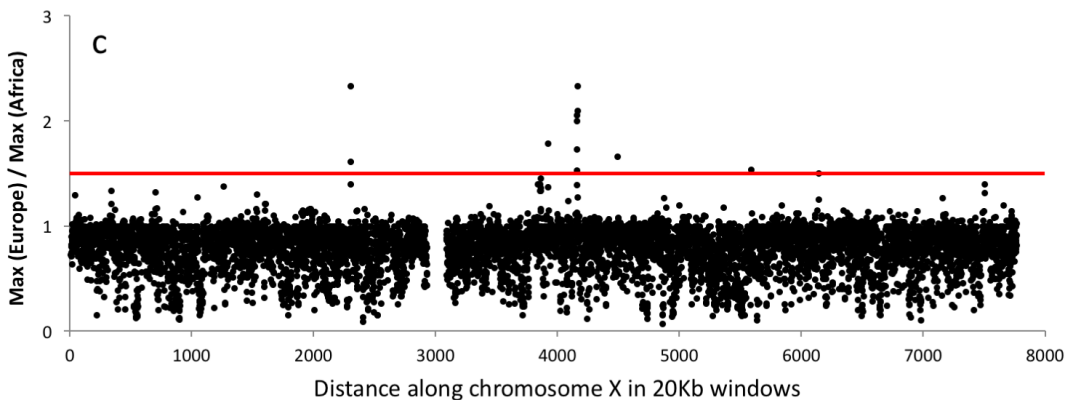
392 any two modern humans are from each other, genuine Neanderthal fragments should often  
393 yield even higher ratios. Setting the threshold ratio to 1.25 and 1.5 sees the proportion of all  
394



395



396



397

398

399 **Figure 8. A general test for presence of introgressed archaic fragments outside Africa.**

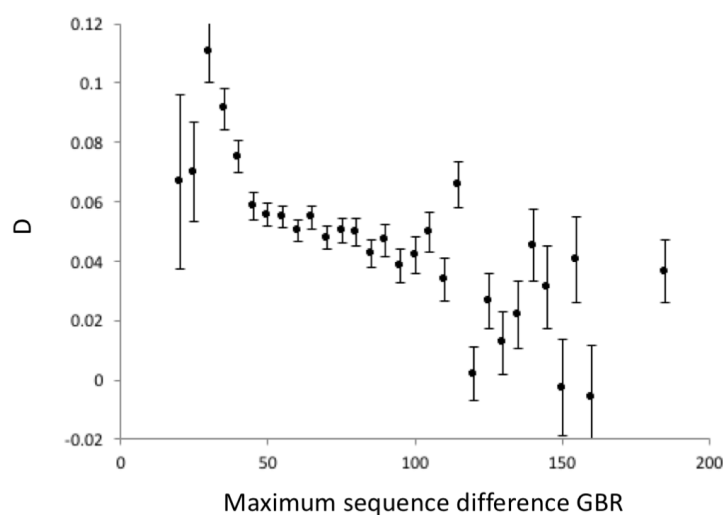
400 Figure 8a shows the ratio of maximum sequence divergence in Europe (GBR, FIN, CEU, TSI, IBS)  
401 to maximum sequence divergence in African (LWK, ESN, MDL, GWD, YRI) for 20Kb windows on  
402 chromosome 1. All other autosomes look very similar. Almost all windows give ratios less than  
403 the 1.5 expected if a Neanderthal fragment was present in one of the European samples (red  
404 line), and the average ratio is ~0.8, reflecting the greater diversity in Africa. For comparison,  
405 Figure 8b gives the landscape of inferred Neanderthal contribution to Europeans (red) and East  
406 Asians (green), redrawn from Sankararaman et al. (50). Published studies suggest little  
407 Neanderthal contribution to the X chromosome. If the few high ratios reflect introgressed  
408 fragments they should be rarer on the X but appear to be just as common (Figure 8c).

409

410 windows that qualify as putatively archaic fall to 0.3% and 0.08% respectively. Importantly,  
411 the locations of the few large ratios that are present do not correlate with peaks in the  
412 published Neanderthal landscape (**Figure 8b**) (50). Also, introgression has previously been  
413 inferred to be near-zero on the X chromosome (50). If high ratios do indicate introgressed  
414 fragments they should be rarer on the X. In fact, if anything, the X-chromosome carries more  
415 windows with ratios >1 than then autosomes (**Figure 8c**).

416  
417 Even though many fewer windows yield large MaxPD(outside Africa) compared with the  
418 published Neanderthal landscape (50), where regions lacking introgressed fragments appear  
419 rare, the high ratios could still reflect genuine archaic sequences. If so, MaxPD(outside Africa)  
420 and D should be positively correlated: high ratios should occur in windows that also  
421 contribute large D. Plotting D against MaxPD(Europe) for all autosomal 100kb windows  
422 reveals a highly significant *negative* correlation, with highest D being associated with the  
423 smallest MaxPD values and the highest MaxPD giving D's close to zero (**Figure 9**). Such a  
424 pattern is difficult to reconcile with a model where high D values are driven by divergent  
425 archaic fragments but is consistent with a model where the highest D values occur in regions  
426 where most diversity has been lost 'out of Africa'.

427  
428



429  
430

431 **Figure 9. Objectively unusual fragments in non-African populations are associated with**  
432 **low D.** For all autosomal data I calculated D(YRI, GBR, Neanderthal, chimpanzee) for each  
433 100Kb window and plotted the resulting values, binned by maximum divergence among  
434 fragments within GBR. The largest D values used to infer introgression are strongly associated  
435 with low Max(GBR), the opposite of what would be expected if large ratios genuinely reflect the  
436 presence of Neanderthal fragments. Windows with the very lowest Max(GBR) do not give the  
437 highest D values, plausibly because these include appreciable numbers of sequences that have  
438 very low diversity in all populations: these will have low D-values that depress the average.

439  
440

441 Finally, I attempted to estimate the proportion of unusual, presumed archaic origin fragments  
442 in individual non-African genomes. Within each window I define 'unusual fragments' as non-  
443 African sequences that exceed MaxPD(Africa) when compared with at least 10 intra-

444 population comparisons. This requirement reflects the rarity of most archaic fragments and  
445 also reduces the impact of comparisons between the most divergent non-African human  
446 fragments. Since Neanderthal fragments may occur in Africa, I take MaxPD(Africa)  
447 conservatively to be the smallest of the five individual African population maxima (ASW and  
448 ACB are not included because they have varying levels of European admixture). As an  
449 internal calibration, each non-African population was 'spiked' with a randomly selected  
450 African individual from GWD. In 33% of 20Kb windows this African sample qualified as  
451 'unusual'. Since Neanderthal fragments will be appreciably more divergent it is reasonable to  
452 assume that upwards of 50% of archaic fragments would be detected. Using the 50% value,  
453 the average archaic proportion in non-Africans is 0.14%, about 10 fold lower than currently  
454 estimated. Moreover, since divergent sequences but can arise through chance, selection and  
455 population mixing, even this figure should be treated as an upper limit.

456

## 457 **Discussion**

458

459 Neanderthals share more nucleotide bases with modern non-African humans than with  
460 Africans. This basic pattern can be explained either by historical inter-breeding or by the  
461 mutation rate having slowed in humans who left Africa. Here I test a series of opposing  
462 predictions aimed at distinguishing inter-breeding from mutation slowdown. By and large,  
463 the patterns I uncover favour the mutation slowdown model.

464

465 Mutation slowdown requires that mutations rates differ appreciably among human  
466 populations and that, since humans migrated out of Africa, the rate has been higher in Africa.  
467 Current evidence is inconclusive. Differences between Africans and non-Africans have been  
468 reported in mutation rate (8, 33), mutation type (31) and recombination rate (52), though the  
469 largest study finds a higher mutation rate outside Africa (33). On the other hand, the latter  
470 study does not distinguish between mutations that predate and postdate 'out of Africa'.  
471 When I analyse Complete Genomics data and 1000 genomes data I find significant but  
472 opposing trends, perhaps suggesting high sensitivity of the result to data filtering and or  
473 levels of imputation.

474

475 The question of whether Africans and non-Africans differ in their genome-wide mutation  
476 rates *since* humans left Africa thus remains unresolved. However, when mutation rate  
477 difference is estimated using  $D(\text{African}, \text{non-African}, \text{chimpanzee})$  (33) across the genome,  
478 there is a striking correlation with the amount of diversity lost: the more diversity was lost,  
479 the greater the excess mutation rate in Africa. Since the dominant mechanism driving  
480 heterozygosity difference is drift during the out of Africa bottleneck (37, 41), any causal  
481 relationship must be in the direction of changes in heterozygosity influencing mutation rate  
482 rather than *vice versa*. Having said this, the data do not formally rule out other, as yet  
483 unknown mechanisms. Such mechanisms are difficult to conceive because the most obvious  
484 way to change mutation rate would be through a variant polymerase, but this would only  
485 impact the genome-wide average, it would not drive a correlation between diversity lost and  
486 mutation rate.

487

488 A second important issue is the extent to which the mutation slowdown model can explain a  
489 raft of observations that support the inter-breeding hypothesis. That some inter-breeding  
490 occurred seems likely, based on discoveries of a genuinely hybrid skeleton and introgressed  
491 haplotypes (27, 53). However, the case for frequent mating leading to a larger legacy  
492 depends on genome-wide patterns which, though reported in many different forms (2, 12, 18,  
493 54), generally fail to distinguish between non-Africans being more similar to Neanderthals  
494 and Africans being less similar. Thus, while current evidence for inter-breeding appears  
495 compelling, until the various analyses are repeated in a way that distinguishes inter-breeding  
496 from mutation slowdown, the conclusion that inter-breeding offers the only possible  
497 explanation seems to me to be premature.

498  
499 It is of course desirable to assess the extent to which the panoply of recent observations  
500 currently interpreted as evidence of inter-breeding could be explained by a model based on  
501 mutation slowdown. If HI operates, it would drive variation in D (and related measures)  
502 wherever heterozygosity varies, either between populations, due to changes in population  
503 size (37), or across the genome, due to the action of selection (55). Moreover, recombination  
504 rate and mutation rate are correlated (56) and mutations occur non-independently to form  
505 clusters (20-22, 57). By interacting with HI, these phenomena appear capable of creating  
506 most of the patterns currently taken to indicate inter-breeding. Unfortunately, it is not yet  
507 possible to make a proper assessment of the extent to which this potential is fulfilled, if at all,  
508 because most of the component processes, although likely present, are too poorly understood  
509 to model effectively. It thus seems premature to claim either that HI can or cannot explain a  
510 given pattern.

511  
512 A key exception to the rule that most analyses fail formally to exclude HI appears in the  
513 original Green et al. study (1) and exploits the mosaic of sequences with European and  
514 African ancestry found in the human reference sequence. A small number of European but  
515 not African sequences show high similarity to Neanderthals yet low similarity to the Venter  
516 sequence (their Figures 5 and S39). This contradicts my observation that objectively unusual  
517 fragments are very rare. Where the truth lies remains to be determined. However, Green et  
518 al.'s analyses include multiple stages any one of which could be subject to a filtering bias of  
519 the sort that has changed interpretation elsewhere (doi:10.1038/nature.2016.19258). There  
520 is also an issue with the raw data. The length of the Neanderthal branch should be less than  
521 10% of the total hominin-chimpanzee divergence but Green et al.'s data suggest an  
522 implausibly high figure of ~75% (see their Table S51: counts of 5,827,247 and 8,156,936 for  
523 states AABA and BBBA respectively). In contrast, my analyses are based entirely on high  
524 quality (if low coverage) modern sequences, and include an internal calibration in the form of  
525 an African individual added to all non-African samples. That the African sample is identified  
526 as unusual in a third of all windows suggests that my approach should be capable of detecting  
527 ancient fragments, if present, in upwards of 50% of windows.

528  
529 Despite the above uncertainties, my analyses do uncover a number of additional patterns that  
530 appear difficult to reconcile with a model based entirely on inter-breeding. First, D actually  
531 strengthens when the Neanderthal genome is replaced by the inferred human ancestral  
532 bases. Interestingly, this observation agrees with data presented by Green et al., who report



533 756,324:689,594 BAAA:ABAA, a ratio of 1.097, similar to but larger than the 1.087 ratio seen  
534 in the same Table S51 for BABA:ABBA, and involving seven times as many bases. Green et al.  
535 ascribe their 66,730 excess BAAAs to sequencing errors, though this number seems  
536 implausibly large for high coverage sequences and it is unclear why sequencing errors would  
537 be so asymmetrically distributed. A more parsimonious explanation is that both the  
538 similarity of the BABA:ABBA and BAAA:ABAA ratios and the similarity between  $D(H1, H2,$   
539 *Neanderthal, chimpanzee*) and  $D(H1, H2, \textit{ancestral human, chimpanzee})$  reflect a common  
540 phenomenon, one that is inherent to all comparisons among modern humans and has little or  
541 no dependence on which outgroup is used as long as it lies close to the base of modern  
542 humans.

543  
544 A second problem for the inter-breeding story relates to which alleles are common and which  
545 are rare. Most early studies focused on individual high coverage genomes so were unable to  
546 consider allele frequencies. By using the 1000 genomes data I have been able to show that D  
547 is acutely sensitive to whether the 'B' allele is common or rare, only becoming positive when  
548 the 'B' allele frequency exceeds 90% in Europe. This is the exact opposite of what is expected  
549 under the inter-breeding hypothesis. Most Neanderthal fragments are likely neutral or near-  
550 neutral (55) and would have entered modern humans around 2,000 generations ago, too  
551 recently for any but a handful to drift to high frequency. If D is driven mainly by inter-  
552 breeding, positive D value should be associated with sites where the 'B' allele is rare in  
553 Europe, not common as I report. This analysis emphasises the extra information gained by  
554 considering allele frequencies.

555  
556 The third problem involves the relationship between heterozygosity and D, as discussed  
557 above. Variation in genome-wide heterozygosity is dominated by the out of Africa bottleneck  
558 (37, 41) and should be little impacted by a 1-2% Neanderthal legacy (if present). However,  
559 genome-wide D is almost perfectly predicted by heterozygosity difference across all  
560 population comparisons, the population with relatively lower heterozygosity invariably  
561 appearing closer to Neanderthals. If Neanderthal fragments did have an appreciable effect on  
562 heterozygosity this would, if anything, drive the opposite trend, because higher D would be  
563 linked to higher heterozygosity driven by introgression. The ubiquitous converse trend both  
564 within and outside Africa is therefore at odds with a model based entirely on inter-breeding.

565  
566 The relationship between heterozygosity and D *within* a genome also argues against inter-  
567 breeding. Previous work reveals a correlation between B statistics and D, interpreted as  
568 indicating selection acting on introgressed fragments. However, these studies only consider a  
569 single heterozygosity. When I fit models that include heterozygosity in each population  
570 separately (two heterozygosity models, 2HMs) a dramatically different picture emerges. All  
571 pairwise population comparisons, apart from those within Africa, yield a vastly superior fit  
572 for the 2HM, and the two slopes invariably go in opposite directions. Such opposing slopes fit  
573 well with a model where the key quantity is *difference* in heterozygosity, but seem at odds  
574 with a model based on selection, where all significant regressions should go in the same  
575 direction.

576

577 An interesting and perhaps telling feature of the 2HMs is the relationship between strength of  
578 correlation and genetic distance. In African – non-African comparisons, the 2HMs only  
579 explain 3-5 times as much variation in D compared with the best single heterozygosity model.  
580 In contrast, among non-African comparisons the single heterozygosity models are often non-  
581 significant while the 2HMs are at their strongest, particularly in comparisons between the  
582 three Eurasian regions EUR, CSA and EAS. This is difficult to understand using a model based  
583 on selection, where the strongest correlations should always be the African – non-African  
584 comparisons. A plausible explanation based on HI can be made but needs more work to  
585 determine whether it works in practice. Thus, populations within a region show little  
586 variation in D across the genome because they are too similar in heterozygosity and have not  
587 been separated long enough for many mutations to accumulate. Similarly, African – non-  
588 African comparisons show strong D but the correlation with heterozygosity is somewhat  
589 degraded due to complicated patterns of mixing and demographic change since humans left  
590 Africa. This leaves the strongest signal to form over an intermediate timescale where the best  
591 balance lies between developing a strong signal and this signal being degraded by complex  
592 and often contrasting demographic histories.

593  
594 Finally, a number of other observations sit somewhat uncomfortably with the idea of  
595 sufficient inter-breeding to leave a 2% legacy. Estimates of introgression are near-zero and  
596 zero for the X chromosome and mitochondrial DNA respectively. This pattern could be  
597 explained by some combination of unidirectional gene flow, mediated by a preponderance of  
598 male Neanderthals mating with female humans, and selection acting against the Neanderthal  
599 sex chromosomes. However, both mechanisms are speculative and lack empirical support.  
600 Were human men unable or unwilling to defend their wives against (presumed) rape and, if  
601 defence was not possible, why did the populations not move apart to reduce contact? It is  
602 also unclear that mixed offspring would thrive and be accepted readily by society. Here, the  
603 context of the hybrid skeleton (9) may be relevant. People generally use caves either for  
604 burials, when many remains would be found, or for living, when no skeletons would usually  
605 be found. The finding of just two skeletons is not consistent with either but might reasonably  
606 be interpreted as the ostracising or separation of individuals deemed uncomfortably  
607 different, implying lower fitness.

608  
609 In conclusion, I explore an alternative explanation for relatively greater base-sharing  
610 between Africans and non-Africans, based on mutation slowdown out of Africa. Although the  
611 mutation slowdown model is speculative and its ability to account for patterns used to infer  
612 inbreeding are largely untested, this new model does make a number of predictions that  
613 appear to be fulfilled. In comparison, the inter-breeding struggles to account for the patterns  
614 I find. Clarifying which model fits best across all observations will require a lot more work. I  
615 hope that future studies will seek both to determine how well mutation slowdown fits for the  
616 many published studies, and to find reasonable explanations for why the interbreeding model  
617 fits so poorly in the analyses I have conducted. Future inferences about possible inter-  
618 breeding should consider mutation slowdown as a viable alternative explanation that needs  
619 to be eliminated before introgression can confidently be inferred.

620  
621 **Methods**

622

623 **Data:** Modern human sequences were obtained from the 1000 genomes project, Phase 3, and  
624 downloaded as composite vcf files. These comprise low coverage genome sequences for 2504  
625 individuals drawn from 26 modern human populations spread across five main geographic  
626 regions: Africa (**LWK**, Luhya in Webuye, Kenya; **GWD**, Gambian in Western Division, The  
627 Gambia; **YRI**, Yoruba in Ibadan, Nigeria; **ESN**, Esan in Nigeria; **MSL**, Mende in Sierra Leone;  
628 ACB, African Caribbean in Barbados; ASW, African Ancestry in Southwest US), Europe (**GBR**,  
629 British from England and Scotland; **FIN**, Finnish in Finland; CEU, Utah Residents (CEPH) with  
630 Northern and Western Ancestry; **TSI**, Toscani in Italy; **IBS**, Iberian populations in Spain),  
631 Central Southern Asia (GIH, Gujarati Indian in Texas; **PJL**, Punjabi in Lahore, Pakistan; **BEB**,  
632 Bengali in Bangladesh; STU, Sri Lankan Tamil in the UK; ITU, Indian Telugu in the UK), East  
633 Asia (**CHS**, Han Chinese South; **JPT**, Japanese in Tokyo, Japan; **CDX**, Chinese Dai in  
634 Xishuangbanna, China; **KHV**, Kinh in Ho Chi Minh City, Vietnam; **CHB**, Han Chinese in Beijing,  
635 China) and the Americas (PUR, Puerto Rican from Puerto Rico; CLM, Colombian in Medellin,  
636 Colombia; MXL, Mexican ancestry in Los Angeles, California; PEL, Peruvian in Lima, Peru).  
637 Codes in bold are populations sampled from their geographic origin, assumed to be less  
638 admixed than those sampled from elsewhere, unbolded. Although in the 1000 genomes  
639 dataset singleton variants are probably correctly called, to guard against possible sequencing  
640 errors and to be maximally conservative singletons were excluded from all analyses. A list of  
641 informative bases for the Altai – chimpanzee (PanTro4) – Hg19 alignment was kindly  
642 provided by Andrea Manica and Marcos Llorente. Where required, human – chimpanzee  
643 alignments were extracted from the Ensembl-Compara eight primate alignments  
644 (<http://www.ensembl.org/info/data/ftp/>). To avoid alignment ambiguities, bases were only  
645 accepted if they lay within blocks of at least 300 aligned bases and were 10 or more bases  
646 from the nearest gap, even if this was a single base indel.

647

648 **Analyses:** All analyses were conducted using custom scripts written in C++, available on  
649 request. All statistical analyses were conducted in R 3.3.0 (<https://cran.r-project.org/>). To  
650 be conservative, singleton variants were excluded from all analyses. **ABBA-BABA:** Since the  
651 1000 genomes data are low coverage and include much imputations, population allele  
652 frequencies are determined with far greater reliability than individual genotypes.  
653 Consequently, ABBA-BABA counts were determined probabilistically based on population  
654 allele frequencies assuming Hardy-Weinberg. Thus, if allele 'A' had frequencies of 0.2 and 0.4  
655 in populations one and two, this site would contribute  $0.2 * (1 - 0.4) = 0.12$  ABBAs and  $(1 -$   
656  $0.2) * 0.4 = 0.32$  BABAs, the numbers expected if alleles were drawn at random from each  
657 population. **Heterozygosity:** heterozygosities were calculated similarly: if 'A' and 'B' were at  
658 frequencies 0.3 and 0.7,  $0.3 * 0.7 * 2 = 0.42$  of a heterozygous site was counted, the probability  
659 of drawing a heterozygous genotype from that locus if the population were in Hardy-  
660 Weinberg equilibrium. **Genetic distance:** since the 1000g data are unphased, genetic  
661 distances between haplotypes cannot be calculated. Instead I used an approximation. All  
662 variable sites in each individual were recoded as 0, 1 and 2 for 'AA', 'AB' and 'BB' respectively.  
663 In pairwise comparisons between individuals, zero and one difference were recorded at sites  
664 with the same and different codes, equivalent to assuming that bases that could be identical  
665 are identical and that, wherever a difference must exist this always occurs in the most

666 dissimilar haplotype pair. Genetic distance was then taken as the sum of differences within a  
667 given window.

668  
669 **Detecting unusual fragments:** For the general analysis, for a given genomic window of  
670 20Kb, all pairwise intra-population distances (PIPD) were calculated (for distance measure,  
671 see above). Across the genome, mean PIPD was 45.7 +/- 15 s.e.m. To reduce noise,  
672 uninformative windows with mean PIPD < 15 were excluded. Each population then yields a  
673 maximum divergence and I compared the maximum maximum among the seven African  
674 populations with the maximum maximum found across Europe, East Asia and Central  
675 Southern Asia. American populations were excluded due the high levels of admixture, though  
676 inclusion would have had negligible impact.

677  
678 To estimate the frequency of objectively unusual fragments in individuals I used a more  
679 conservative approach. For the African maximum I used the 'minimum maximum', i.e. the  
680 smallest of the four maxima across LWK, ESN, MSL & YRI. This guards against the possibility  
681 of an occasional Neanderthal fragment in Africa. ASW and ACB were excluded because these  
682 populations include appreciable non-African ancestry, while GWD was chosen at random to  
683 be reserved as an independent source of control individuals. Each non-African population  
684 had one random GWD individual added as an internal control so that allowance could be  
685 made for windows with too little differentiation for a Neanderthal sequence to stand out: if  
686 the African sequence qualifies as unusual, so too should a more divergent Neanderthal  
687 sequence. I further assumed that introgressed fragments were not at high frequency such  
688 that any introgressed fragment would yield many (at least 10) comparisons with individuals  
689 carrying modern human DNA.

690  
691 **Acknowledgements**

692  
693 I have had a number of useful and informative discussions Anders Eriksson, Andrea Manica,  
694 Mathias Meyer, Rob Foley, Marta Lahr, Eske Willerslev, Martin Sikora, Simon Martin and  
695 Mathias Currat. This work was not funded.

696  
697 **Competing interests:** I have no competing interests

698  
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833 **Tables**

pop	difference	s.e.	t	diff_adj
GBR	0.0131	0.0008	17.0	0.023
FIN	-0.0014	0.0009	-1.6	0.019
CEU	0.0181	0.0008	23.5	0.022
IBS	0.0388	0.0008	46.6	0.020
TSI	0.0324	0.0006	53.3	0.018
CHB	0.0201	0.0006	31.3	0.019
CHS	0.0133	0.0005	25.4	0.021
CDX	0.0187	0.0005	34.7	0.023
KHV	0.0357	0.0005	69.8	0.022
JPT	0.0153	0.0006	26.5	0.019
STU	0.0109	0.0005	21.8	0.020
ITU	0.0205	0.0004	51.3	0.020
PJL	0.0177	0.0005	38.9	0.019
BEB	0.0296	0.0005	56.0	0.021
GIH	0.0271	0.0007	40.0	0.025
LWK	0.0308	0.0004	77.5	0.022
ESN	0.0149	0.0006	24.2	0.024
MSL	0.0538	0.0005	104.3	0.026
ACB	0.0126	0.0007	18.5	0.021
GWD	0.0107	0.0005	23.1	0.019
ASW	0.0183	0.0011	16.4	0.038
YRI	0.0322	0.0006	51.5	0.024
PUR	0.0039	0.0011	3.6	0.032
MXL	0.0967	0.0007	137.1	0.020
CLM	0.1716	0.0009	187.7	0.019
PEL	-0.1757	0.0016	-111.0	0.026

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**Table 1. The impact of heterozygosity on mutation probability.** Recent mutations, defined as variants present in only two copies, both in a single population ('pop', for full names see methods), were identified and used to define a 2kb window, 1kb either side of the variant. 'Difference' is the average of the difference in heterozygosity between the population where the doubleton occurs minus heterozygosity in the same window averaged over all other populations from the same major geographic region. In all but two cases the average difference is massively significantly positive, the two exceptions being FIN (non-significantly negative) and PEL (massively significantly negative). 'Dif\_adj' is 'difference' adjusted for the difference genome-wide heterozygosity across all windows, revealing a very similar positive values of just over 2% in all populations.



847 **Table 2a**

$\Delta$ AIC	AFR	EUR	CSA	EAS	AMR
AFR	0.1	239	123	555	235
EUR		38.5	490	1818	339
CSA			40.1	860	288
EAS				43.6	872
AMR					144

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849 **Table 2b**

R <sup>2</sup>	AFR	EUR	CSA	EAS	AMR
AFR	0.003	0.029	0.017	0.044	0.026
EUR		0.04	0.088	0.12	0.061
CSA			0.048	0.095	0.053
EAS				0.038	0.087
AMR					0.046

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851 **Table 2c**

R <sup>2</sup> ratio	AFR	EUR	CSA	EAS	AMR
AFR	26.6	3.2	3.4	3.9	4.5
EUR		57.5	18.3	19.4	15.4
CSA			78.8	15.9	42.2
EAS				112	17.8
AMR					39.1

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853 **Table 2. Relationship between heterozygosity and D.** For each possible pairwise population  
854 comparison ABBA, BABA and heterozygosity counts were made for all 1Mb autosomal windows.  
855 General linear models were fitted with binomial response ABBA and BABA counts and predictors  
856 either both population heterozygosities (2HM) or just one (SHM). Each 2HM was compared  
857 with the better fitting SHM and summary statistics averaged by regional comparison: for Africa  
858 (AFR), Europe (EUR), Central Southern Asia (CSA), East Asia (EAS) and America (AMR): Table  
859 2a is difference in AIC; Table 2b is proportion of variance in D explained by the 2HM; Table 2c  
860 gives the ratio of variance explained by the 2HM divided by variance explained by the best SHM.