1   Archaic Introgression Shaped Human Circadian Traits

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14   **ABSTRACT**

15   *Introduction:* When the ancestors of modern Eurasians migrated out of Africa and interbred with
16   Eurasian archaic hominins, namely Neanderthals and Denisovans, DNA of archaic ancestry
17   integrated into the genomes of anatomically modern humans. This process potentially
18   accelerated adaptation to Eurasian environmental factors, including reduced ultra-violet radiation
19   and increased variation in seasonal dynamics. However, whether these groups differed
20   substantially in circadian biology, and whether archaic introgression adaptively contributed to
21   human chronotypes remains unknown.
22   *Results:* Here we traced the evolution of chronotype based on genomes from archaic hominins
23   and present-day humans. First, we inferred differences in circadian gene sequences, splicing, and
24   regulation between archaic hominins and modern humans. We identified 28 circadian genes
25   containing variants with potential to alter splicing in archaics (e.g., *CLOCK*, *PER2*, *RORB*,
26   *RORC*), and 16 circadian genes likely divergently regulated between present-day humans and
27   archaic hominins, including *RORA*. These differences suggest the potential for introgression to
28   modify circadian gene expression. Testing this hypothesis, we found that introgressed variants
29   are enriched among eQTLs for circadian genes. Supporting the functional relevance of these
30   regulatory effects, we found that many introgressed alleles have associations with chronotype.
31   Strikingly, the strongest introgressed effects on chronotype increase morningness, consistent
32   with adaptations to high latitude in other species. Finally, we identified several circadian loci
33   with evidence of adaptive introgression or latitudinal clines in allele frequency.
34   *Conclusions*: These findings identify differences in circadian gene regulation between modern
35   humans and archaic hominins and support the contribution of introgression via coordinated
36   effects on variation in human chronotype.

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39   **Keywords:** circadian biology; chronotype; Neanderthals; adaptive introgression; gene
40   expression; adaptive evolution

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SIGNIFICANCE STATEMENT

Interbreeding between humans and Neanderthals created the potential for adaptive introgression as humans moved into environments that had been populated by Neanderthals for hundreds of thousands of years. Here we discover lineage-specific genetic differences in circadian genes and their regulatory elements between humans and Neanderthals. We show that introgressed alleles are enriched for effects on circadian gene regulation, consistently increase propensity for morningness in Europeans, and show evidence of adaptive introgression or associations between latitude and frequency. These results expand our understanding of how the genomes of humans and our closest relatives responded to environments with different light/dark cycles, and demonstrate a coordinated contribution of admixture to human chronotype in a direction that is consistent with adaptation to higher latitudes.

INTRODUCTION

All anatomically modern humans (AMH) trace their origin to the African continent around 300 thousand years ago (ka) (Stringer, 2016; Hublin *et al.*, 2017), where environmental factors shaped many of their biological features. Approximately seventy-thousand years ago (Bae, Douka, and Petraglia 2017), the ancestors of modern Eurasian AMH began to migrate out of Africa, where they were exposed to diverse new environments. In Eurasia, the novel environmental factors included greater seasonal variation in temperature and photoperiod.

Changes in the pattern and level of light exposure have biological and behavioral consequences in organisms. For example, *D. melanogaster* that are native to Europe harbor a polymorphism in *timeless*, a key gene in the light response of the circadian system, that follows a latitudinal cline in allele frequency (Sandrelli et al. 2007; Tauber et al. 2007). The ancestral haplotype produces a short TIM (S-TIM) protein that is sensitive to degradation by light because of its strong affinity to cryptochromes (CRY), photoreceptor proteins involved in the entrainment of the circadian clock. An insertion of a G nucleotide in the 5' coding region of the gene originated approximately 10 kya in Europe and created a start codon that produces a new long TIM isoform (L-TIM). The L-TIM variant has a lower affinity to CRY, creating a change in photosensitivity and altering the length of the period. L-TIM flies are at a higher frequency in southern Europe, while S-TIM flies are more prevalent in northern Europe. Another example is found in pacific salmon. Chinook salmon (*Oncorhynchus tshawytscha*) populations show a latitudinal cline in the frequency and length of repeat motifs in the gene *OtsClock1b*, strongly suggesting that this locus is under selection associated with latitude and photoperiod (O'Malley, Ford, and Hard 2010; O'Malley and Banks 2008). The evolution of circadian adaptation to diverse environments has also been widely studied in insects, plants (Michael *et al.*, 2003; Zhang *et al.*, 2008), and fishes, but it is understudied in humans. Adaptive processes could have helped to align human biology and chronotype to new natural conditions.

Previous studies in humans found a correlation between latitude and chronotype (morningness vs. eveningness) variation (Leocadio-Miguel et al. 2017; Lowden et al. 2018; Randler and Rahafar 2017) and a latitudinal cline in some circadian allele frequencies (Dorokhov *et al.*, 2018; Putilov, Dorokhov and Poluektov, 2018; Putilov *et al.*, 2019), highlighting the contribution of the environment to behavior and circadian biology. Many human health effects are linked to the misalignment of chronotype (Knutson and von Schantz 2018), including cancer, obesity (Gyarmati *et al.*, 2016; Papantoniou *et al.*, 2016, 2017; Gan *et al.*, 2018; Shi *et al.*, 2020; Yousef *et al.*, 2020), and diabetes (Gan *et al.*, 2015; Larcher *et al.*, 2015, 2016). There is also evidence of a correlation between evening chronotype and mood disorders, most notably seasonal affective disorder (SAD), depression, and worsening of bipolar disorder episodes (Srinivasan *et al.*, 2006; Kivelä, Papadopoulos and Antypa, 2018; Taylor and Hasler, 2018). Thus, we hypothesize that the differences in geography and environment encountered by early AMH populations moving into higher latitudes created potential for circadian misalignment and health risk.

Although AMHs arrived in Eurasia ~70 ka, other hominins (e.g., Neanderthals and Denisovans) lived there for more than 400 ka (Arnold *et al.*, 2014; Meyer *et al.*, 2014, 2016). These archaic hominins diverged from AMHs around 700 ka (Meyer *et al.*, 2012; Prüfer *et al.*, 2014, 2017; Nielsen *et al.*, 2017; Gómez-Robles, 2019; Mafessoni *et al.*, 2020), and as a result, the ancestors of AMHs and archaic hominins evolved under different environmental conditions. While there was substantial variation in the latitudinal ranges of each group, the Eurasian

101 hominins largely lived at consistently higher latitudes and, thus, were exposed to higher
102 amplitude seasonal variation in photoperiods. Given the influence of environmental cues on
103 circadian biology, we hypothesized that these separate evolutionary histories produced
104 differences in circadian traits adapted to the distinct environments.
105   When AMH migrated into Eurasia, they interbred with the archaic hominins that were
106 native to the continent, initially with Neanderthals (Green et al. 2010; Villanea and Schraiber
107 2019) around 60 ka (Sankararaman et al. 2012; Skoglund and Mathieson 2018) and later with
108 Denisovans (Jacobs et al. 2019). Due to this, a substantial fraction (>40%) of the archaic
109 variation remains in present-day Eurasians (Skov et al. 2020; Vernot and Akey 2014), although
110 each human individual carries only ~2% DNA of archaic ancestry (Vernot *et al.*, 2016; Prüfer *et*
111 *al.*, 2017). Most of the archaic ancestry in AMH was subject to strong negative selection, but
112 some of these introgressed alleles remaining in AMH populations show evidence of adaptation
113 (Racimo *et al.*, 2015; Gower *et al.*, 2021). For example, archaic alleles have been associated with
114 differences in hemoglobin levels at higher altitude in Tibetans, immune resistance to new
115 pathogens, levels of skin pigmentation, and fat composition (Huerta-Sánchez *et al.*, 2014;
116 Racimo *et al.*, 2015, 2017; Dannemann and Kelso, 2017; Racimo, Marnetto and Huerta-Sánchez,
117 2017; McArthur, Rinker and Capra, 2021). Previous work also suggests that introgressed alleles
118 could have adaptively influenced human chronotype. First, a phenome-wide association study
119 (PheWAS) in the UK Biobank found loci near *ASB1* and *EXOC6* with introgressed variants that
120 significantly associated with self-reported sleeping patterns (Dannemann and Kelso, 2017). One
121 of these alleles showed a significant association between frequency and latitude. Second,
122 summarizing effects genome-wide, introgressed alleles are also moderately enriched for
123 heritability of chronotype compared to non-introgressed alleles (McArthur, Rinker and Capra,
124 2021). These results suggest a potential role for introgressed alleles in adaptation to pressures
125 stemming from migration to higher latitudes.
126   Motivated by the potential for a role of archaic introgression in AMH circadian variation,
127 we explore two related questions: 1) Can comparative genomic analysis identify differences in
128 AMH and archaic hominin circadian biology?, and 2) Do introgressed archaic alleles influence
129 human circadian biology? Understanding the ancient history and evolution of chronotypes in
130 humans will shed light on human adaptation to high latitudes and provide context for the genetic
131 basis for the modern misalignment caused by the development of technology and night
132 shiftwork.
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136 **RESULTS**
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138 **Did archaic hominins and modern humans diverge in circadian biology?**
139 Following divergence ~700,000 years ago (ka) (Nielsen *et al.*, 2017; Gómez-Robles, 2019),
140 archaic hominins and AMH were geographically isolated, resulting in the accumulation of
141 lineage-specific genetic variation and phenotypes (Figure 1). In the next several sections, we
142 evaluate the genomic evidence for divergence in circadian biology between archaic hominin and
143 modern human genomes.
144
145 *Identifying archaic-hominin-specific circadian gene variation*

146   With the sequencing of several genomes of archaic hominins, we now have a growing, but
147   incomplete, catalog of genetic differences specific to modern and archaic lineages. Following
148   recent work (Kuhlwilm and Boeckx, 2019), we defined archaic-specific variants as genomic
149   positions where archaic hominins (Altai Neanderthal, Vindija Neanderthal, and Denisovan) all
150   have the derived allele while in humans the derived allele is absent or present at such an
151   extremely low frequency in the 1000 Genome Project (<0.00001) that it is likely an independent
152   occurrence. We defined human-specific variants as positions where all individuals in the 1000
153   Genomes Project carry the derived allele and all the archaics carry the ancestral allele.
154   We evaluated archaic-specific variants for their ability to influence proteins, splicing, and
155   regulation of 246 circadian genes (Methods). The circadian genes were identified by a
156   combination of literature search, expert knowledge, and existing annotations (Table S1; Figure
157   S1; Methods). The core circadian clock machinery is composed of a dimer between the CLOCK
158   and ARNTL (BMAL1) transcription factors, which binds to E-box enhancer elements and
159   activates the expression of the Period (*PER1/2/3*) and Cryptochrome (*CRY1/2*) genes (Figure
160   1C). PERs and CRYs form heterodimers that inhibit the positive drive of the CLOCK-BMAL1
161   dimer on E-boxes, inhibiting their own transcription in a negative feedback loop. CLOCK-
162   BMAL1 also drives the expression of many other clock-controlled genes (CCG), including
163   *NR1D1/2* (Nuclear Receptor Subfamily 1 Group D Member 1 and 2), *RORA/B* (RAR Related
164   Orphan Receptor A and B), and *DBP* (D-Box Binding PAR BZIP Transcription Factor). ROR
165   and REV-ERB are transcriptional regulators of BMAL1. CK1 binds to the PER/CRY
166   heterodimer, phosphorylating PER and regulating its degradation. Similarly, FBXL3 marks CRY
167   for degradation. Beyond the core clock genes, we included other upstream and downstream
168   genes that are involved in maintenance and response of the clock (Table S1; Figure S1).
169   We identified 1,136 archaic-specific variants in circadian genes, promoters, and
170   candidate distal cis-regulatory elements (cCREs). The circadian genes with the most archaic-
171   specific variants are *CLDN4*, *NAMPT*, *LRPPRC*, *ATF4*, and *AHCY* (125, 112, 110, 104, 102
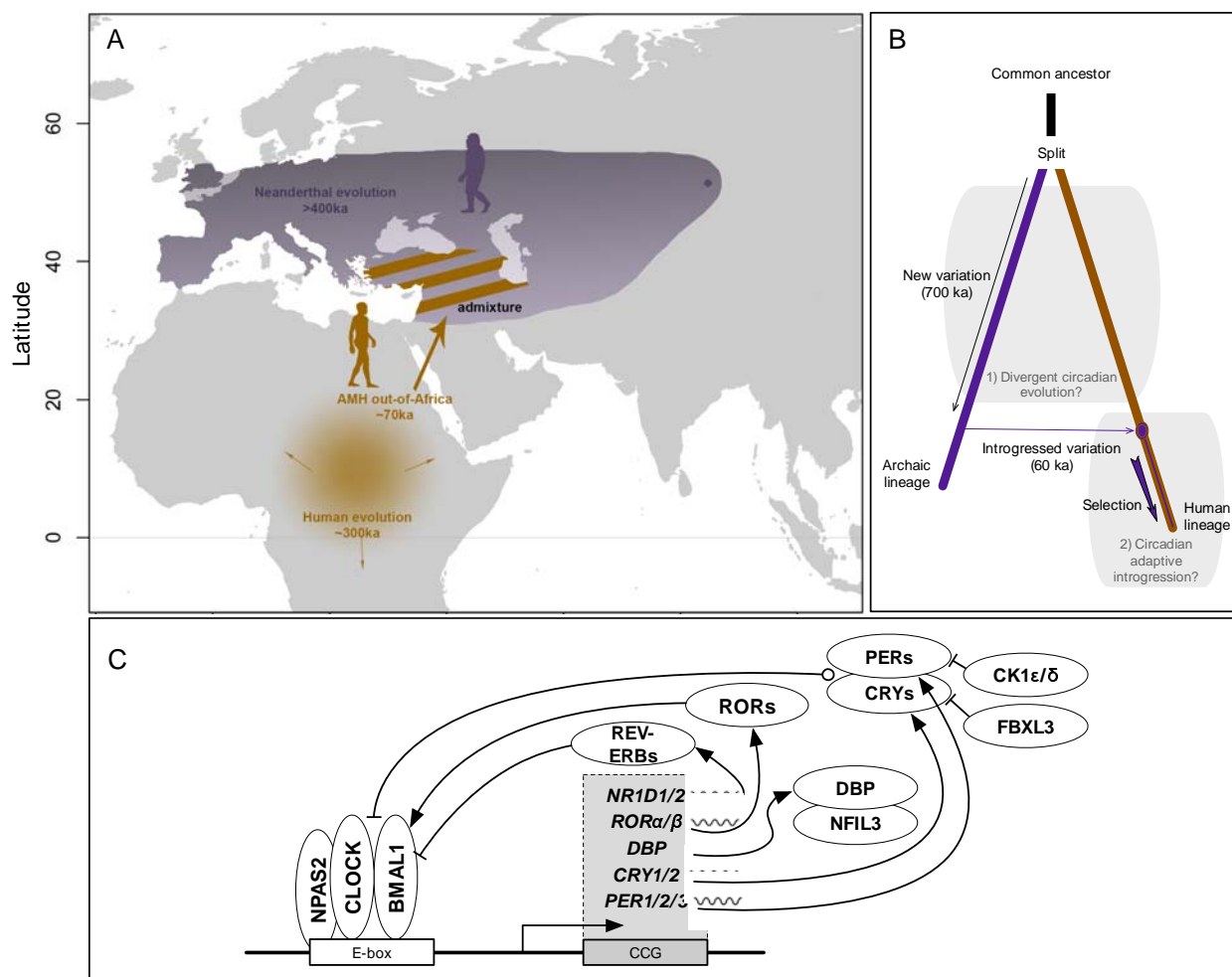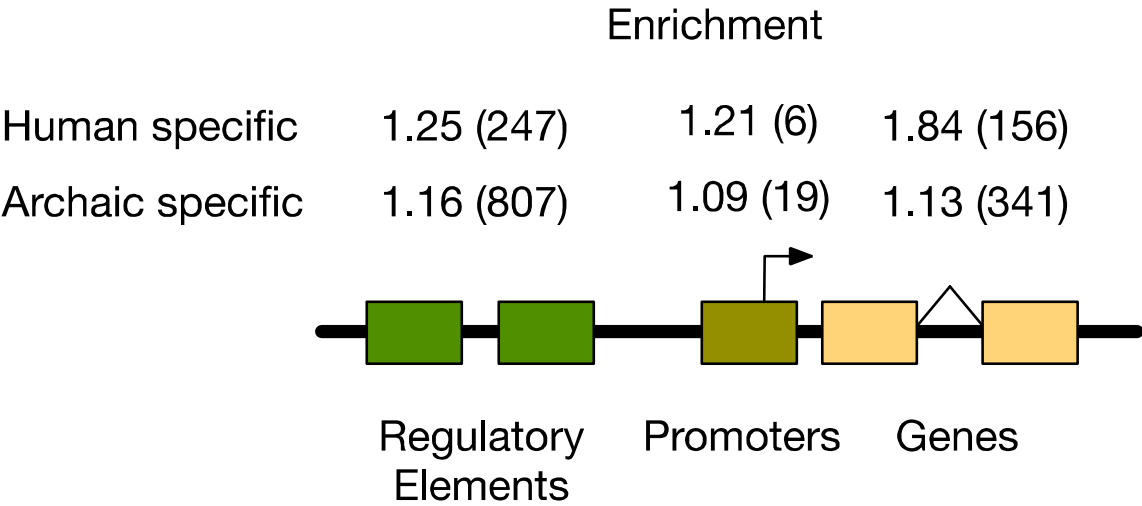172   respectively) (Table S2).
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Figure 1. Did the sharing of functionally diverged alleles from archaic hominins influence human circadian biology? A) Anatomically modern humans and archaic hominins evolved separately at different latitudes for hundreds of thousands of years. The ancestors of modern Eurasian humans left Africa approximately 70 thousand years ago (ka) and admixed with archaics, likely in southwestern Asia. The shaded purple range represents the approximate Neanderthal range. The purple dot represents the location of the sequenced Denisovan individual in the Altai Mountains; the full range of Denisovans is currently unknown. Silhouettes from phylopic.org. **B)** After the split between the human and archaic lineages, each group accumulated variation and evolved in their respective environments for approximately 700 ka. We first test for evidence for divergent circadian evolution during this time. Humans acquired introgressed alleles from Neanderthals and from Denisovans around 60 and 45 ka, respectively. These alleles experienced strong selective pressures; however, ~40% of the genome retains archaic ancestry in some modern populations. The second question we explore is whether introgression made contributions to human circadian biology. **C)** The core circadian clock machinery is composed of several transcription factors (ovals) that dimerize and interact with E-box enhancer elements and each other to create a negative feedback loop. We defined a set of 246 circadian genes through a combination of literature search, expert knowledge, and existing annotations (Table S1; Figure S1; Methods). Lines with arrows represent activation, and lines with bars represent suppression.

196 *Fixed human- and archaic-specific variants are enriched in circadian genes and associated*
197 *regulatory elements*
198 After the archaic and AMH lineages diverged, each group accumulated genetic variation specific
199 to each group. Variants fixed in each lineage are likely to be enriched in genomic regions that
200 influence traits that experienced positive selection. We tested whether human- and archaic-
201 specific fixed variants are enriched compared to other variants in circadian genes, their
202 promoters, and in annotated candidate cis-regulatory elements within 1 megabase (Mb) (Figure
203 2). We found that human- and archaic-specific fixed variants are enriched in circadian genes
204 (Fisher's exact test; human: OR=1.84, P=7.06e-12; archaic: OR=1.13, P=0.023) and distal
205 regulatory elements (Fisher's exact test; human: OR=1.25, P=8.39e-4; archaic: OR=1.16,
206 P=6.15e-5) compared to variants derived on each lineage, but not fixed. Promoter regions have a
207 similar enrichment pattern as that in gene and regulatory regions, but the p-values are high
208 (Fisher's exact test; human: OR=1.21, P=0.65; archaic: OR=1.09, P=0.63). This is likely due to
209 the small number of such variants in promoters. These results suggest that both groups had a
210 greater divergence in genomic regions related to circadian biology than expected.
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212
213 **Figure 2**. **Human- and archaic-specific fixed variants are enriched in circadian regulatory,**
214 **promoter, and gene regions.** Human-specific fixed variants are significantly enriched compared
215 to variants that are not fixed in circadian regulatory elements (Fisher's exact: OR=1.25, P=8.39e-
216 4) and gene regions (Fisher's exact: OR=1.84, P=7.06e-12). Promoters show a similar
217 enrichment, but the higher p-value is the result of the small number of variants (Fisher's exact
218 test: OR=1.21, P=0.65). Likewise, archaic-specific variants are enriched in circadian regulatory
219 regions (Fisher's exact: OR=1.16, P=6.15e-5) and gene regions (Fisher's exact: OR=1.13,
220 P=0.023), with the promoters showing a similar trend (Fisher's exact test: OR=1.09, P=0.63).
221 The numbers in parentheses give the counts of fixed variants observed in each type of element.
222 Regulatory elements were defined based on the ENCODE candidate cis-regulatory elements.
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225 *Several core circadian genes have evidence of alternative splicing between humans and*
226 *archaic hominins*

227　We find only two archaic-specific coding variants in circadian genes: one missense and one
228　synonymous. The missense variant (hg19: chr17_46923411_A_G) is in the gene *CALCOCO2*,
229　calcium-binding and coiled-coil domain-containing protein 2. SIFT, PolyPhen, and CADD all
230　predict that the variant does not have damaging effects. The second variant (hg19:
231　chr7_119914770_G_T) is in the gene *KCND2*, which encodes a component of a voltage-gated
232　potassium channel that contributes to the regulation of the circadian rhythm of action potential
233　firing, but it is synonymous and the variant effect predictors suggest it is tolerated.
234　　　To explore potential splicing differences in circadian genes between humans and
235　archaics, we applied SpliceAI to predict whether any sequence differences between modern
236　humans and archaics are likely to modify splicing patterns. Four archaic individuals were
237　included in this analysis (the Altai, the Vindija, the Chagyrskaya Neanderthals, and the Altai
238　Denisovan) (Meyer *et al.*, 2012; Prüfer *et al.*, 2014, 2017; Mafessoni *et al.*, 2020). We found that
239　28 genes contained at least one archaic-specific variant predicted to result in alternative splicing
240　in archaics. These included several of the core clock genes *CLOCK*, *PER2*, *RORB*, *RORC*, and
241　*FBXL13* (Figure 3A,C; Table S3). For example, the variant chr2:239187088-239187089 in the
242　1st intron of *PER2* is predicted to result in a longer 5' UTR. The splice-altering variants were
243　largely specific to the two different archaic linages (Figure 3A), with 13 specific to the
244　Denisovan, 8 shared among the three Neanderthals, and only one shared among all four archaic
245　individuals.
246
247　***Circadian gene regulatory divergence between humans and archaic hominins***
248　Given the enrichment of variants in regulatory regions of circadian genes, we sought to explore
249　the potential for differences in circadian gene regulation between humans and archaics with
250　causes beyond single lineage-specific variants. We leveraged an approach we recently developed
251　for predicting gene regulatory differences between modern and archaic individuals from
252　combinations of genetic variants (Colbran *et al.*, 2019). The approach uses PrediXcan, an elastic
253　net regression method, to impute gene transcript levels in specific tissues from genetic variation.
254　Previous work demonstrated that this approach has a modest decrease in performance when
255　applied to Neanderthals, but that it can accurately applied between humans and Neanderthals for
256　thousands of genes. Here, we quantify differences in predicted regulation of the 246 circadian
257　genes between 2,504 humans in the 1000 Genomes Project (1000 Genomes Project Consortium,
258　2010) and the archaic hominins. The predicted regulation values are normalized to the
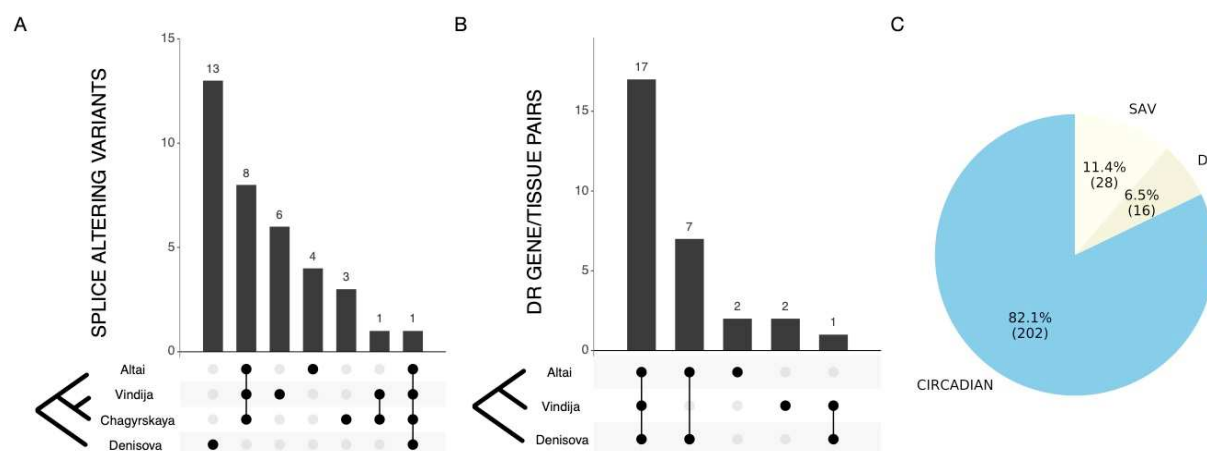259　distribution in the training set from the Genotype Tissue Expression Atlas (GTEx).
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Figure 3. Many circadian genes have evidence of alternative splicing and divergent regulation between modern and archaic hominins. A) The distribution of the 28 predicted archaic-specific splice-altering variants (SAV) in circadian genes across archaic individuals. Most are specific to either the Denisovan or Neanderthal lineage (Table S3). **B)** The sharing of predicted divergently regulated (DR) gene/tissue pairs across three archaic individuals. (Predictions were not available for the Chagyrskaya Neanderthal.) Seventeen divergently regulated gene/tissue pairs were present in all three archaics (representing 16 unique genes). Additionally, 7 gene/tissue DR pairs are shared between the Altai Neanderthal and the Denisovan individual. One pair is shared between the Vindija Neanderthal and the Denisovan (Table S4). **C)** The proportion of circadian genes containing archaic splice-altering variants predicted by SpliceAI (SAV; 11.4%) or divergently regulated circadian genes predicted by PrediXcan (DR; 6.5%). Thus, 17.9% of the circadian genes are predicted to contain differences to AMH via these mechanisms.

We first analyzed gene regulation predictions in the core circadian clock genes. Archaic gene regulation was at the extremes of the human distribution for many core clock genes including *PER2*, *CRY1*, *NPAS2*, *RORA, NR1D1* (Figure 4; Figure S2). For example, the regulation of *PER2* in the Altai and Vindija Neanderthals is lower than 2,491 of the 2,504 (99.48%) modern humans considered. The Denisovan has a predicted *PER2* regulation that is lower than 2,410 (96.25%). Expanding to all circadian genes and requiring archaic regulation to be more extreme than all humans (Methods), we identified 24 circadian genes across 23 tissues with strong divergent regulation between humans and at least one archaic hominin (Figure 3B; Table S4). For example, all archaic regulation values for *RORA*, a core clock gene, are lower than for any of the 2,504 modern humans. We found that 16 of these genes (Figure S3; Table S4), including *RORA*, *MYBBP1A*, and *TIMELESS*, were divergently regulated in all archaic individuals. This represents 6.5% of all the circadian genes (Figure 3C). Surprisingly, the two Neanderthals only shared one DR gene not found in the Denisovan, while the Altai Neanderthal and Denisovan shared seven not found in Vindija (Figure 3B). The Altai and Vindija Neanderthals represent deeply diverging lineages, and this result suggests that they may have experienced different patterns of divergence in the regulation of their circadian genes.

Given these differences in circadian gene regulation between humans and archaics, we tested whether circadian genes are more likely to be divergently regulated than other gene sets. Each archaic individual shows nominal enrichment for divergent regulation of circadian genes,

297   and the enrichment was stronger (~1.2x) in the Altai Neanderthal and Denisovan individual.
298   However, given the small sample size, the P-values are moderate (Permutation test; Altai:
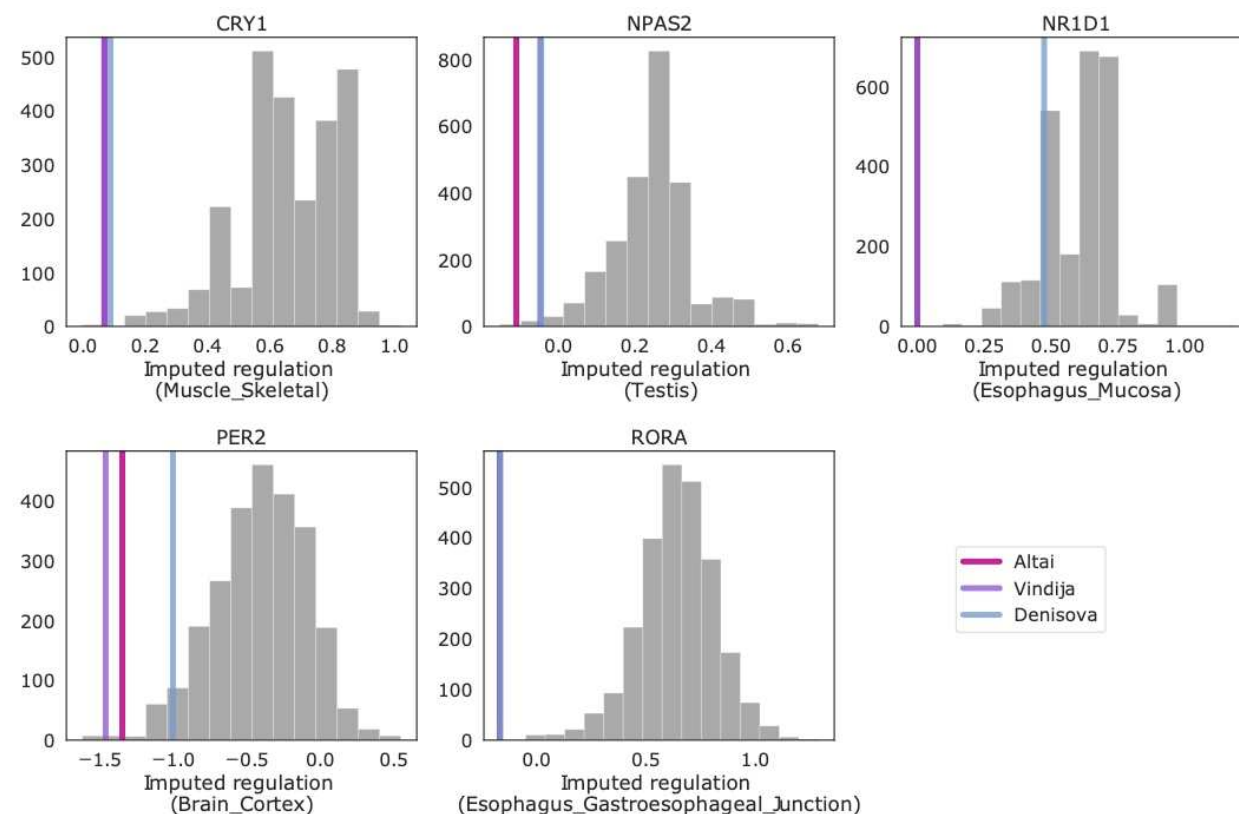299   OR=1.21, P=0.19, Vindija: OR=1.05, P=0.43, Denisovan: OR=1.20, P=0.24).
300
301

302
303   **Figure 4. Many circadian genes are divergently regulated between modern humans and**
304   **archaic hominins.** Comparison of the imputed regulation of core circadian genes between 2504
305   humans in 1000 Genomes Phase 3 (gray bars) and three archaic individuals (vertical lines). For
306   each core circadian gene, the tissue with the lowest average P-value for archaic difference from
307   humans is plotted. Archaic gene regulation is at the extremes of the human distribution for
308   several core genes: *CRY1*, *PER2*, *NPAS2, NR1D1 RORA*. See Figure S2 for all core clock genes
309   and Figure S3 for all divergently regulated circadian genes.
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312   **Did introgressed archaic variants influence modern human circadian biology?**
313   The previous sections demonstrate lineage-specific genetic variation in many genes and
314   regulatory elements essential to the function of the core circadian clock and related pathways.
315   Given this evidence of functional differences between archaic hominins and AMH in these
316   systems, we next evaluated the influence of archaic introgression on AMH circadian biology.
317

318   *Introgressed variants are enriched in circadian gene eQTL*
319   Given the differences between archaic and modern sequences of circadian genes and their
320   regulatory elements, we investigated whether Neanderthal introgression contributed functional
321   circadian variants to modern Eurasian populations. We considered a set of 863,539 variants with

322   evidence of being introgressed from archaic hominins to AMH (Browning *et al.*, 2018). These
323   variants were identified using the Sprime algorithm, which searches for regions containing a high
324   density of alleles in common with Neanderthals and not present or at very low frequency in
325   Africans. Since many approaches have been developed to identify introgressed variants, we also
326   considered two stricter sets: 47,055 variants that were supported by all of six different
327   introgression maps (Sankararaman *et al.*, 2014; Vernot *et al.*, 2016; Browning *et al.*, 2018;
328   Steinrücken *et al.*, 2018; Skov *et al.*, 2020; Schaefer, Shapiro and Green, 2021) and 755,653
329   variants that were supported by Sprime and at least one other introgression map. As described
330   below, our main results replicated on both of these stricter sets.
331   We first tested whether the presence of introgressed variants across modern individuals
332   associated with the expression levels of any circadian genes, i.e., whether the introgressed
333   variants are expression quantitative trait loci (eQTL). We identified 3,857 introgressed variants
334   associated with the regulation of circadian genes in modern non-Africans (Table S5). The genes
335   *PTPRJ*, *HTR1B*, *NR1D2*, *CLOCK*, and *ATOH7* had the most eQTL (304, 273, 262, 256, and 252
336   respectively). We found introgressed circadian eQTL for genes expressed in all tissues in GTEx,
337   except kidney cortex. Notably, several of these circadian genes (e.g., *NR1D2* and *CLOCK*) with
338   introgressed eQTL were also found to be divergently regulated in our comparison of modern and
339   archaic gene regulation. This indicates that some of the archaic-derived variants that contributed
340   to divergent regulation were retained after introgression and continue to influence circadian
341   regulation in modern humans.
342   Introgressed variants are significantly more likely to be eQTL for circadian genes than
343   expected by chance from comparison to all eQTL (Figure 5A; Fisher's exact test: OR=1.45,
344   P=9.71e-101). The stricter set of introgressed variants identified by Browning et al. plus at least
345   one other introgression map had similar levels of eQTL enrichment for circadian genes
346   (OR=1.47; P=2.4e-103). The highest confidence set of introgressed variants that were identified
347   by all six maps considered had even stronger enrichment (OR=1.68; P=6.5e-23).
348   Most core circadian genes are expressed broadly across tissues; the fraction expressed in
349   each GTEx tissue ranges from 57% (whole blood) to 83% in testis, and an average of 72%
350   (Table S6). As a result, we anticipated that the enrichment of introgressed variants among eQTL
351   for circadian genes would hold across tissues. Examining the associations in each tissue, we
352   found that introgressed eQTL showed significant enrichment for circadian genes in most tissues
353   (34 of 49; Figure 5B; Table S7) and trended this way in all but five. Given that tissues in GTEx
354   have substantial differences in sample size and cellular heterogeneity, statistical power to detect
355   enrichment differs. We anticipate that this is the main driver of differences in enrichment across
356   tissues.
357   These results suggest that circadian pressures were widespread across tissues. Given the
358   previously observed depletion for introgressed variants in regulatory elements and eQTL (Petr *et*
359   *al.*, 2019; Rinker *et al.*, 2020; Telis, Aguilar and Harris, 2020), this enrichment for circadian
360   genes among introgressed eQTL is surprising and suggests that the archaic circadian alleles
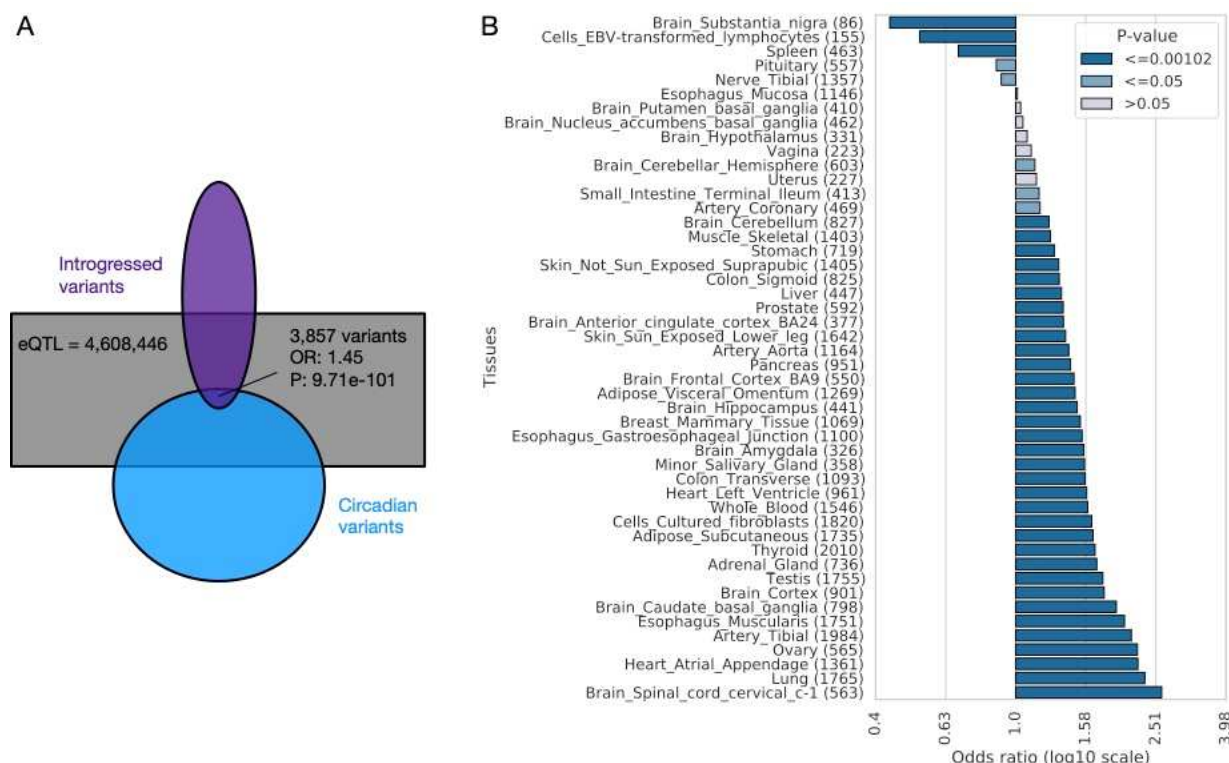361   could have been beneficial after introgression.
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Figure 5. Circadian genes are enriched for introgressed eQTL. A) Archaic introgressed variants are more likely to be eQTL for circadian genes in GTEx than for non-circadian genes (Fisher's exact test: OR=1.45, P=9.71e-101). Purple represents the set of introgressed variants, and blue represents the set of circadian variants. 3,857 are introgressed eQTL in circadian genes. Gray represents the universe of all GTEx eQTLs lifted over to hg19. The overlaps are not to scale. **B)** The enrichment for circadian genes among the targets of introgressed eQTLs in each GTEx tissue. Introgressed eQTL in most tissues show significant enrichment for circadian genes (Fisher's exact test; Table S7). Kidney cortex did not have any circadian introgressed eQTLs and thus is not shown. Numbers inside the parenthesis indicate the count of variants in each tissue. Gray bars indicate lack of statistical significance; light blue bars indicate nominal significance (p <= 0.05); and dark blue bars indicate significance at the 0.05 level after Bonferroni multiple testing correction (p <= 0.00102).

Introgressed variants predominantly increase propensity for morningness

After observing that circadian gene expression is influenced by archaic variants, we evaluated whether these effects are likely to result in a change in organism-level phenotype. To do this, we evaluated evidence that introgressed variants influence chronotype. The heritability of chronotype has been estimated in a range from 12 to 38% (Jones *et al.*, 2016, 2019; Lane *et al.*, 2016), and previous studies have identified two introgressed loci associated with sleep patterns (Dannemann and Kelso, 2017; Putilov *et al.*, 2019). We recently found modest enrichment for heritability of chronotype (morning/evening person phenotype in a GWAS of the UK Biobank) among introgressed variants genome-wide using stratified LD score regression (heritability enrichment: 1.58, P=0.25) (McArthur, Rinker and Capra, 2021). This analysis also suggested that introgressed variants were more likely to increase morningness.

390   To test for this proposed directional effect, we calculated the cumulative fraction of
391 introgressed loci associated with chronotype in the UK Biobank that increase morningness (after
392 collapsing based on LD at $R^2>0.5$ in EUR). The introgressed loci most strongly associated with
393 chronotype increase propensity for morningness (Figure 6; Table S8; Table S9). As the strength
394 of the association with morningness decreases, the bias begins to decrease, but the effect is
395 maintained well past the genome-wide significance threshold (P<5e-8). When focusing the
396 analysis on introgressed variants in proximity (<1 Mb) to circadian genes, the pattern becomes
397 even stronger. The bias toward morningness remains above 80% at the genome-wide
398 significance threshold. This result also held when limiting to introgressed variants found in
399 Browning plus one or all other introgression maps considered (Figure S4). This suggests that
400 introgressed variants act in a consistent direction on chronotype, especially when they influence
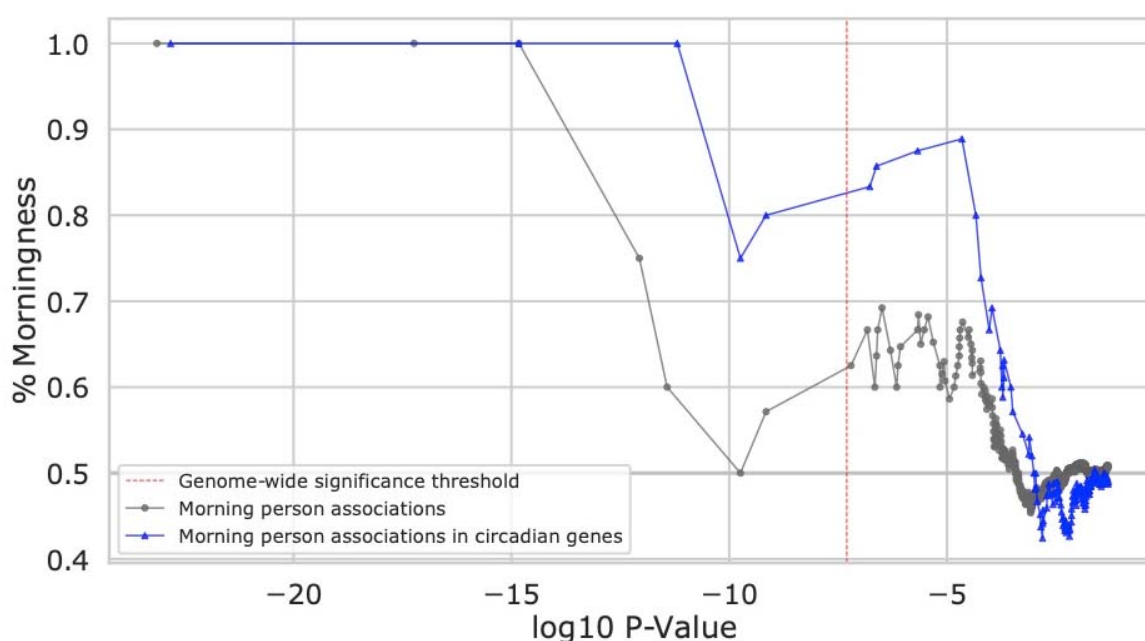401 circadian genes.
402
403

404
405 **Figure 6. Introgressed variants associate with increased morningness.** The cumulative
406 fraction of introgressed loci significantly associated with the morning vs. evening person trait in
407 the UK Biobank that increase morningness (y-axis) at a given p-value threshold (x-axis).
408 Introgressed loci associated with chronotype are biased towards increasing morningness, and this
409 effect is greatest at the most strongly associated loci. Introgressed variants nearby (<1 Mb)
410 circadian genes (blue) are even more strongly biased towards increasing morningness than
411 introgressed variants overall (gray). Each dot (triangle) represents an associated locus; variants
412 were clumped by LD for each set ($R^2>0.5$ in EUR).
413
414
415 Circadian rhythms are involved in a wide variety of biological systems. To explore other
416 phenotypes potentially influenced by the introgressed circadian variants, we evaluated evidence
417 for pleiotropic associations. First, we retrieved all the genome-wide associations reported for
418 introgressed variants in the Open Targets Genetics (https://genetics.opentargets.org) database,
419 which combines GWAS data from the GWAS Catalog, UK Biobank, and several other sources.

420 Introgressed circadian variants are associated with traits from a diverse range of categories
421 (Table S10). Associations with blood related traits are by far the most common; however, this is
422 likely because they have more power in the UK Biobank. Overall, circadian introgressed variants
423 are significantly more likely to have at least one trait association than introgressed variants not in
424 the circadian set (Fisher's exact test: OR=1.25, P=7.03e-25) (Figure S5A). The circadian variants
425 also associate with significantly more traits per variant than the non-circadian set (Mann-
426 Whitney U: P=9.93e-14) (Figure S5B; Table S11). These results suggest effects for introgressed
427 circadian variants beyond chronotype.
428
429 *Evidence for adaptive introgression at circadian loci*
430 The gene flow from Eurasian archaic hominins into AMH contributed to adaptations to some of
431 the new environmental conditions encountered outside of Africa (Racimo *et al.*, 2015). The
432 above analyses demonstrate the effects of introgressed variants on circadian gene regulation and
433 chronotype. To explore whether these circadian regions show evidence of adaptive introgression,
434 we considered three sets of introgressed regions predicted to have contributed to AMH
435 adaptation: one from an outlier approach based on allele frequency statistics (Racimo, Marnetto
436 and Huerta-Sánchez, 2017) and two from recent machine learning algorithms: *genomatnn*
437 (Gower *et al.*, 2021) and *MaLAdapt* (Zhang *et al.*, 2023). We intersected the circadian
438 introgressed variants with the adaptive introgression regions from each method.
439 We identified 47 circadian genes with evidence of adaptive introgression at a nearby
440 variant from at least one of the methods (Table S12). No region was supported by all three
441 methods; however, six were shared between Racimo and *MaLAdapt* and three were shared by
442 Racimo and *genomatnn*. The relatively small overlap between these sets underscores the
443 challenges of identifying adaptive introgression. Nonetheless, these represent promising
444 candidate regions for further exploration of the effects of introgressed variants on specific
445 aspects of circadian biology. For example, an introgressed haplotype on chr10 tagged by
446 rs76647913 was identified by both *MaLAdapt* and Racimo. This introgressed haploype is an
447 eQTL for the nearby *ATOH7* gene in many GTEx tissues. *ATOH7* is a circadian gene that is
448 involved in retinal ganglion cell development, and mice with this gene knocked out are unable to
449 entrain their circadian clock based on light stimuli (Brzezinski *et al.*, 2005).
450
451 *Latitudinal clines for introgressed circadian loci*
452 Motivated by the previous discovery of an introgressed haplotype on chr2 that is associated with
453 chronotype and increases in frequency with latitude (Dannemann and Kelso, 2017; Putilov *et al.*,
454 2019), we also tested each introgressed circadian variant for a correlation between allele
455 frequency and latitude in modern non-African populations from the 1000 Genomes Project.
456 The strongest association between latitude and frequency was a large chromosome 2
457 haplotype that contains the previously discovered introgressed SNP (rs75804782, R=0.85)
458 associated with chronotype. This haplotype is present in all non-African populations, and
459 rs61332075 showed the strongest latitudinal cline (R=0.87). The second strongest consisted of a
460 smaller haplotype of introgressed variants a few kb upstream of the previous haplotype (tagged
461 by rs35333999 and rs960783) that overlaps the core circadian gene *PER2*. These variants have a
462 correlation between latitude and frequency of ~0.68 They are also in moderate LD (R^2 of ~0.35
463 in EUR) with an additional introgressed variant (rs62194932) that has a similar latitudinal cline
464 of 0.70 (Figure S6; Table S13). These variants are each in very low LD with the previously
465 discovered haplotype (R^2 of ~0.01) and are each supported by multiple introgression maps.

466 Moreover, these introgressed variants are absent in all EAS populations, absent or at very low
467 frequency in SAS (<3%), and at higher frequency in EUR populations (~13%).
468   The EUR-specific introgressed variant rs35333999 causes a missense change in the PER2
469 protein (V903I) that overlaps a predicted interaction interface with PPARG. PER2 controls lipid
470 metabolism by directly repressing PPARG's proadipogenic activity (Grimaldi *et al.*, 2010). The
471 rs62194932 variant is an eQTL of *HES6* in the blood in the eQTLGen cohort (Võsa *et al.*, 2021).
472 *HES6* encodes a protein that contributes to circadian regulation of LDLR and cholesterol
473 homeostasis (Lee *et al.*, 2012).
474   Thus, this genomic region, that includes circadian genes and introgressed variants
475 associated with chronotype, has population-specific structure and at least two distinct sets of
476 introgressed variants with latitudinal clines and functional links to lipid metabolism. *PER2* is
477 also predicted to have lower gene regulation in archaic hominins than most humans (Figure 4).
478 and the Vindija Neanderthal carries a lineage-specific variant in this gene that has splice-altering
479 effects. These results together suggests that *PER2* may have experienced multiple functional
480 changes in different modern and archaic lineages, with potential adaptive effects mediated by
481 introgression.
482   We did not discover any other significant associations between latitude and frequency for
483 other introgressed circadian loci. The rapid migration and geographic turnover of populations in
484 recent human history is likely to obscure many latitude-dependent evolutionary signatures, so we
485 did not anticipate many circadian loci would have a strong signal.
486
487

488 **DISCUSSION**
489
490 The Eurasian environments where Neanderthals and Denisovans lived for several hundred
491 thousand years are located at higher latitudes with more variable photoperiods than the landscape
492 where AMH evolved before leaving Africa. Evaluating genetic variation that arose separately in
493 each of the archaic and AMH lineages after their split ~700 MYA, we identified lineage-specific
494 genetic variation in circadian genes, their promoters, and flanking distal regulatory elements. We
495 found that both archaic- and human-specific variants are observed more often than expected in
496 each class of functional region. This result suggests that, while each group evolved separately
497 during hundreds of thousands of years in divergent environments, both experienced pressure on
498 circadian related variation. Leveraging sequence-based machine learning methods, we identified
499 many archaic-specific variants likely to influence circadian gene splicing and regulation. For
500 example, core clock genes (*CLOCK*, *PER2*, *RORB*, *RORC*, and *FBXL13*) have archaic variants
501 predicted to cause alternative splicing compared to AMH. Several core genes were also predicted
502 in archaics to be at the extremes of human gene regulation, including *PER2*, *CRY1*, *NPAS2*,
503 *RORA, NR1D1*. Surprisingly, the Altai Neanderthal shared more divergent regulation in the
504 circadian genes with the Denisovan individual than the Vindija Neanderthals. The two
505 Neanderthals represent populations that were quite distantly diverged with substantially different
506 histories and geographical ranges. The Denisovan and Altai Neanderthal also come from the
507 same region in Siberia, while the Vindija Neanderthal came from a region in Croatia with
508 slightly lower latitude.
509   Introgression introduced variation that first appeared in the archaic hominin lineage into
510 Eurasian AMH. While most of this genetic variation experienced strong negative selection in
511 AMH, a smaller portion is thought to have provided adaptive benefits in the new environments

512 (Racimo *et al.*, 2015). Given the divergence in many circadian genes' regulation, we explored
513 the landscape of introgression on circadian genes. We first looked at introgressed circadian
514 variants that are likely to influence gene regulation in AMH. Variants in this set are observed
515 more often than expected, suggesting the importance of maintaining circadian variation in the
516 population. We also verified that these results held over variants identified by different methods
517 for calling archaic introgression.
518 We then evaluated the association of these introgressed variants with variation in
519 circadian phenotypes of Eurasians. We previously reported a modest enrichment among
520 introgressed variants for heritability of the morning/evening person phenotype (McArthur,
521 Rinker and Capra, 2021). Here, we further discovered a consistent directional effect of the
522 introgressed circadian variants on chronotype. The strongest associated variants increase the
523 probability of being a morning person in Eurasians.
524 While it is not immediately clear why increased morningness would be beneficial at
525 higher latitudes, considering this directional effect in the context of clock gene regulation and the
526 challenge of adaptation to higher latitudes suggests an answer. In present day humans, behavioral
527 morningness is correlated with shortened period of the circadian molecular clockworks in
528 individuals. This earlier alignment of sleep/wake with external timing cues is a consequence of a
529 quickened pace of the circadian gene network (Brown *et al.*, 2008). Therefore, the morningness
530 directionality of introgressed circadian variants may indicate selection toward shortened
531 circadian period in the archaic populations living at high latitudes. Supporting this interpretation,
532 shortened circadian periods are required for synchronization to the extended summer
533 photoperiods of high latitudes in *Drosophila*, and selection for shorter periods has resulted in
534 latitudinal clines of decreasing period with increasing latitude, as well as earlier alignment of
535 behavioral rhythms (Hut *et al.*, 2013). In addition, *Drosophila* populations exhibit decreased
536 amplitude of behavioral rhythms at higher latitudes which is also thought to aid in
537 synchronization to long photoperiods (Hut *et al.*, 2013).
538 Our finding that introgressed circadian variants generally decrease gene regulation of
539 circadian genes suggests that they could lead to lower amplitude clock gene oscillations.
540 However, when assayed in present day humans there is not a strong correlation between the
541 overall expression level of *NR1D1* and the transcriptional amplitudes of other clock genes within
542 individuals (Brown *et al.*, 2008), and quantitative modeling of the mammalian circadian
543 clockworks suggests that stable clock gene rhythms can result across a wide range of absolute
544 levels of gene expression as long as the stoichiometric ratios of key positive and negative clock
545 genes are reasonably conserved (Kim and Forger, 2012). Interestingly, lower transcriptional
546 amplitude of *NR1D1* does confer greater sensitivity of the present-day human clockworks to
547 resetting stimuli, a potentially adaptive characteristic for high latitudes (Brown *et al.*, 2008).
548 Thus, given the studies of latitudinal clines and adaptation from *Drosophila* and the
549 nascent understanding of clock gene contributions to behavioral phenotypes in present day
550 humans, the directional effects of introgressed circadian gene variants toward early chronotype
551 and decreased gene regulation we observed can be viewed as potentially adaptive. More
552 complex chronotype phenotyping and mechanistic studies of the variants of interest are needed
553 to fully understand these observations.
554 Finally, to explore evidence for positive selection on introgressed variants in AMH, we
555 analyzed results from three recent methods for detecting adaptive introgression. All methods
556 identified circadian loci as candidates for adaptive introgression. However, we note that the
557 predictions of these methods have only modest overlap with one another, underscoring the

558   difficulty of identifying adaptive introgression. Nonetheless, many of these loci, especially those
559   supported by both Racimo and *MaLAdapt*, are good candidates for adaptive introgression given
560   their functional associations with circadian genes
561   Several limitations must be considered when interpreting our results. First, it is
562   challenging to quantify the complexity of traits with a large behavioral component (like
563   chronotype) and infer their variation from genomic information alone. Nevertheless, we believe
564   our approach of focusing on molecular aspects (splicing, gene regulation) of genomic loci with
565   relevance to circadian biology, in parallel to GWAS-based associations, lends additional support
566   to the divergence in chronotype between archaic hominins and modern humans. Second, we also
567   note that circadian rhythms contribute to many biological systems, so the variants in these genes
568   tend to be associated with a variety of phenotypes. Thus, there is also the potential that selection
569   acted on other phenotypes influenced by circadian variation than those related directly to
570   chronotype. Third, given the complexity of circadian biology, there is no gold standard set of
571   circadian genes. We focus on the core clock genes and a broader set of expert-curated genes
572   relevant to circadian systems, but it is certainly possible that other genes with circadian effects
573   are not considered. Fourth, recent adaptive evolution is challenging to identify, and this is
574   especially challenging for introgressed loci. Nonetheless, we find several circadian loci with
575   evidence of adaptive introgression from more than one method. Finally, given the many
576   environmental factors that differed between African and non-African environments, it is difficult
577   to definitively determine whether selection on a particular locus was the result of variation in
578   light levels vs. other related factors, such as temperature. Nonetheless, given the observed
579   modern associations with chronotype for many of these variants, we believe it is a plausible
580   target.
581   In conclusion, studying how humans evolved in the face of changing environmental
582   pressures is necessary to understanding variation in present-day phenotypes and the potential
583   tradeoffs that influence propensity to different diseases in modern environments (Benton *et al.*,
584   2021). Here, we show that genomic regions involved in circadian biology exhibited substantial
585   functional divergence between separate hominin populations. Furthermore, we show that
586   introgressed variants contribute to variation in AMH circadian phenotypes today in ways that are
587   consistent with an adaptive benefit.
588
589
590
591   **METHODS**
592
593   **Circadian gene selection**
594   Circadian biology is a complex system due to its high importance in the functioning of biological
595   timing in diverse biological systems. For that reason, determining which genes are crucial for
596   selection to environment response related to light exposure is not a straight forward process. To
597   address this issue, we look at different sources of genome annotation databases and searched for
598   genes and variants associated with circadian related phenotypes. We considered all human
599   protein-coding genes in the Gene Ontology database annotated with the GO:0007623 ("circadian
600   rhythm") term or terms annotated with relationship "is_a", "part_of", "occurs_in", or "regulates"
601   circadian rhythm. We also considered genes containing experimental or orthologous evidence of
602   circadian function in the Circadian Gene Database (CGDB), the GWAS Catalog genes
603   containing "chronotype" or "circadian rhythm" associated variants, and a curated set of genes

604 available in WikiPathways [https://www.wikipathways.org/index.php/Pathway:WP3594,
605 https://doi.org/10.1093/nar/gkaa1024]. The final set of circadian genes was curated by Dr.
606 Douglas McMahon.
607   To select the candidate circadian genes with the highest confidence, we defined a
608 hierarchy system where genes annotated by McMahon or annotated in 3 out of 4 other sources
609 receive a "High" level of confidence. Genes with evidence from 2 out of 4 of the sources are
610 assigned a "Medium" level of confidence. Genes annotated as circadian only in 1 out of 4
611 sources are assigned to Low confidence and not considered in our circadian gene set. We then
612 defined our set of circadian variants from the 1000 Genomes Project using the official list of
613 circadian genes. The variants are included in analysis of coding, non-coding, regulatory, eQTL,
614 human-specific, archaic-specific, and introgressed variants.
615
616 **Definition of lineage-specific variants**
617 To identify candidate variants that are specific to the human and the archaic lineages, we used a
618 set of variants published by Kuhlwilm and Boeckx (Kuhlwilm and Boeckx, 2019)
619 (https://doi.org/10.1038/s41598-019-44877-x). The variants were extracted from the high-
620 coverage genomes of three archaics: a 122,000-year-old Neanderthal from the Altai Mountains
621 (52x coverage), a 52,000-year-old Neanderthal from Vindija in Croatia (30x coverage), and a
622 72,000-year-old Denisovan from the Altai Mountains (30x coverage). The variants were called in
623 the context of the human genome hg19/GRCh37 reference. The total number of variant sites
624 after applying filters for high coverage sites and genotype quality is 4,437,803. A human-specific
625 variant is defined as a position where all the humans in the 1000 Genomes Project carry the
626 derived allele and all the archaics carry the ancestral allele. An archaic-specific is defined as a
627 position where all the archaics carry the derived allele and the derived allele is absent or
628 extremely rare ($<= 0.00001$) across all human populations. Note that introgressed archaic alleles
629 are not included in the "archaic-specific" set. These criteria resulted in 9,424 human specific and
630 33,184 archaic-specific variants.
631
632 **Enrichment of lineage-specific variants among functional regions of the genome**
633 We intersected the sets of lineage-specific variants with several sets of annotated functional
634 genomic regions. Inside circadian gene regions (Gencode v29), we found 156 human-specific
635 variants and 341 archaic-specific variants. In circadian promoter regions, we found 6 human-
636 specific variants and 19 archaic-specific variants. Promoters were defined as regions 5 kb up- to
637 1 kb downstream from a transcription start site. In distal regulatory elements, we found 247
638 human-specific variants and 807 archaic-specific variants. For this last set, we considered
639 candidate cis-regulatory elements (cCREs) published by ENCODE (Moore *et al.*, 2020) within 1
640 Mb of the circadian genes.
641   To compute whether lineage-specific variants are more abundant than expected in
642 circadian genes, we applied a Fisher's exact test to the sets of human- and archaic-specific
643 variants in regulatory, promoter, and gene regions. Human and archaic-specific variants are
644 significantly enriched in both regulatory (Human: OR=1.25, P=8.39e-4; Archaic: OR=1.16,
645 P=6.15e-5) and gene (Human: OR=1.84, P=7.06e-12; Archaic: OR=1.13, P=0.023) regions. The
646 enrichment observed in the promoters of both lineages is not supported by a significant p-value
647 (Human: OR=1.21, P=0.65; Archaic: OR=1.09, P=0.63).
648
649 **Genes containing archaic variants with evidence of alternative splicing**

650 We used a set of archaic variants annotated with the splice altering probabilities to identify
651 circadian genes that may be differentially spliced between archaic hominins and AMH (Brand,
652 Colbran and Capra, 2023). We considered variants from four archaic individuals: the Altai,
653 Chagyrskaya, and Vindija Neanderthals and the Altai Denisovan. These archaic variants were
654 annotated using SpliceAI (Jaganathan *et al.*, 2019) and we considered any variant with a
655 maximum delta, or splice altering probability, > 0.2. We identified 36 archaic-specific splice
656 altering variants, defined as those variants absent from 1000 Genomes Project, among 28
657 circadian genes. Next, we tested for enrichment among this gene set using an empirical null
658 approach (McArthur *et al.*, 2022; Brand, Colbran and Capra, 2023). We shuffled the maximum
659 deltas among 1,607,350 variants 10,000 times and counted the number of circadian genes with a
660 splice altering variant each iteration. Enrichment was calculated as the number of observed genes
661 (N = 28) divided by the mean gene count among 10,000 shuffles. In addition to all genes with
662 archaic-specific variants, we considered six other subsets among these variants: 1) genes with
663 variants private to the Altai Neanderthal, 2) genes with variants private to the Chagyrskaya
664 Neanderthal, 3) genes with variants private to the Altai Denisovan, 4) genes with variants private
665 to all Neanderthals, 5) genes with variants shared among all archaic individuals, and 6) genes
666 with variants private to the Vindija Neanderthal. Finally, we considered a subset of splice
667 altering variants that were identified as tag SNPs by Vernot et al. (Vernot *et al.*, 2016).
668
669 **PrediXcan**
670 To understand the difference in circadian biology between present-day humans and archaic
671 hominins, we analyzed predictions on gene regulation. We considered the results from
672 PrediXcan gene regulation predictions across 44 tissues from the PredictDB Data Repository
673 (http://predictdb.org/). The models were trained on GTEx V6 using variants identified in 2,504
674 present-day humans in the 1000 Genomes Project phase 3 within 1 Mb of each circadian gene.
675 The original analysis includes predictions for 17,748 genes for which the models explained a
676 significant amount of variance in gene expression in each tissue (FDR < 0.05). The prediction
677 models were also applied to the Altai and Vindija Neanderthals and the Denisovan. The resulting
678 predictions are normalized values of the distribution observed in GTEx individuals used to train
679 the original prediction models. Each prediction contains an empirical P-value which was
680 calculated for each gene and tissue pair to define genes that are divergently regulated between
681 archaic hominins and humans. The P-value is obtained by calculating the proportion of humans
682 from the 1000 Genomes Project that have predictions more extreme compared to the human
683 median than the archaic individual. Significantly DR genes are defined as those where the
684 archaic prediction falls outside the distribution of humans in the 1000 Genomes Project
685 predictions.
686   We tested whether the circadian genes in our set are more likely to be DR compared to an
687 empirical null distribution from random gene sets of the same size. We account for the fact that
688 some genes are modeled in more tissues than others by matching the distribution of tissues in
689 which each gene could be modeled in the random sets to our set. Among 1,467 DR genes in the
690 Altai Neanderthal we find 23 DR circadian genes out of the total 236 genes in the circadian set.
691 We iterate through the permutation analysis 1,000,000 times and find an enrichment of 1.21
692 (P=0.19). A similar analysis is done in the Vindija Neanderthal (1,536 total DR, 21 circadian
693 DR, enrichment of 1.05, P=0.43) and the Denisovan individual (1,214 total DR, 19 circadian DR,
694 enrichment of 1.20, P=0.24). In this study, we define a set of DR genes as the intersection
695 between DR genes in all three archaics, resulting in a set of 16 genes.

696

Enrichment of introgressed variants in eQTL

We performed an enrichment analysis using Pearson's chi-squared test to evaluate if there is overrepresentation of introgressed alleles in our set of circadian variants using the GTEx dataset. We did a liftOver of the GTEx v8 dataset from hg38 to hg19. The original hg38 set contains 4,631,659 eQTLs across 49 tissues. After the LiftOver, 4,608,446 eQTLs remained, with the rest not mapping. We used the archaic introgressed variants dataset from Browning 2018. The set contains 863,539 variants that are introgressed in humans originating in archaic hominins. We performed an intersection between the set of genes containing evidence for eQTLs and our set of 246 circadian genes to retrieve a subset of variant sites with evidence of being eQTL in circadian genes. The resulting subset contained 97,441 circadian eQTLs in 49 tissues and 239 genes. We further intersected the introgressed variants and the set of eQTL, resulting in 128,138 introgressed eQTLs. The final set of eQTLs that are circadian and also introgressed is 3,857.

Direction of effect of chronotype associations

To explore the effect of archaic introgression in circadian dreams on human chronotype, we quantified the direction of effect of variants associated to a Morning/Evening person trait in a GWAS analysis of the UK Biobank (http://www.nealelab.is/uk-biobank/). The variants were LD clumped using PLINK v1.9 ($R^2>0.5$). We generated cumulative proportion values on the beta values assigned to each associated variant on an ascending order of P-values.

Detection of latitudinal clines in chronotype associations

To evaluate latitudinal clines in chronotype-associated variants, we assigned a latitude to each of the Eurasian 1000 Genomes Project populations. The latitude of diaspora populations was set to their ancestral country (GIH Gandhinagar in Gujarat: 23.223, STU Sri Jayawardenepura Kotte: 6.916667, ITU Amaravati in Andhra Pradesh: 16.5131, CEU: 52.372778). CEU was assigned a latitude in Amsterdam, following an analysis that shows that this group is more closely related to Dutch individuals (Lao *et al.*, 2008). We then used the LDlink API to retrieve allele frequencies for each introgressed morningness variant in Eurasian individuals (Machiela and Chanock, 2015). Variants that follow a latitudinal cline were identified using linear regression statistics requiring correlation coefficient ($R >= 0.65$) and P-value ($P <= 0.5$).

Detection of pleiotropy in the set of introgressed circadian variants

To understand the extent of different phenotypes associated with the introgressed circadian variants, we first extracted genome-wide associations from Open Targets Genetics (https://genetics.opentargets.org/) for each of the variants with evidence of introgression (Browning *et al.*, 2018). Only the variants with significant p-values were analyzed. The p-value threshold was set at the genome-wide significance level (P=5e-8). We split the variants in two sets: introgressed circadian and introgressed non-circadian. Many of these variants are not associated with any phenotype. We performed a Fisher's exact test to analyze which of the two sets contains a higher ratio of SNPs with at least one association versus SNPs with no association. The result showed that the circadian set had a significantly higher ratio (OR=1.36, P=5e-29). Then we calculated the total of unique traits associated with each of the variants, given that the SNP has at least one association. We used a Mann-Whitney U test to understand which set is represented by a higher level of traits per SNP. The circadian set was slightly more pleiotropic, and the result is supported by a significant p-value (P=5.4e-3).

742
743 **Identifying introgressed circadian variants with evidence of adaptive introgression**
744 We sought to identify circadian variants that contain evidence of adaptive introgression (AI). To
745 achieve this, we collected AI predictions from a method that applied various summary statistics
746 on 1000 Genomes Project data (Racimo, Marnetto and Huerta-Sánchez, 2017) and two sets of
747 genomic regions that were measured for their likelihood to be under AI by two machine learning
748 methods: *genomatnn* and *MaLAdapt*. *genomatnn* is a convolutional neural network trained to
749 identify adaptive introgression based on simulations (Gower *et al.*, 2021). *MaLAdapt* is a
750 machine learning algorithm trained to find adaptive introgression based on simulations using an
751 extra-trees classifier (ETC) (Zhang *et al.*, 2023). Following the thresholds used in each paper, a
752 region is considered to be under AI if the prediction value assigned to it meets a threshold of 0.5
753 or 0.9, respectively. To find the variants of interest that fall into AI regions, we intersected the
754 set of introgressed circadian SNPs with the Racimo et al. 2015, *genomatnn* and the *MaLAdapt*
755 regions individually. The set of introgressed circadian variants contains variants inside circadian
756 genes, in circadian promoter regions (5 kb up- and 1 kb downstream of the TSS), and variants
757 with regulatory function (cCREs) flanking circadian genes by 1 Mb.
758
759
760 DATA AVAILABILITY
761 The data underlying this article are available in the article and in its online supplementary
762 material.
763
764
765 DECLARATION OF INTERESTS
766 The authors declare that they have no competing interests.
767
768
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776
777
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782
783
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