THE UNIVERSITY OF
WARWICK

University of Warwick institutional repository: http://go.warwick.ac.uk/wrap

This paper is made available online in accordance with publisher policies. Please scroll down to view the document itself. Please refer to the repository record for this item and our policy information available from the repository home page for further information.

To see the final version of this paper please visit the publisher's website. Access to the published version may require a subscription.

Author(s): Sarah A. Palmer, Jonathan D. Moore, Alan J. Clapham, Pamela Rose and Robin G. Allaby
Article Title: **Archaeogenetic Evidence of Ancient Nubian Barley Evolution from Six to Two-Row Indicates Local Adaptation.**
Year of publication: 2009
Link to published version:
http://dx.doi.org/10.1371/journal.pone.0006301/
Publisher statement:

Archaeogenetic Evidence of Ancient Nubian Barley Evolution from Six to Two-Row Indicates Local Adaptation

Sarah A. Palmer[1], Jonathan D. Moore[1], Alan J. Clapham[2], Pamela Rose[3] and Robin G. Allaby[1]

[1] Warwick HRI, The University of Warwick, Wellesbourne, Warwickshire, United Kingdom, CV359EF

[2] Worcestershire Historic Environment & Archaeology Service, Woodbury, University of Worcester, Henwick Grove, Worcester, United Kingdom, WR2 6AJ

[3] The McDonald Institute for Archaeological Research, University of Cambridge, Downing Street, Cambridge, United Kingdom, CB2 3ER

Corresponding author: Robin Allaby

Email: r.g.allaby@warwick.ac.uk

Abstract

Background. Archaeobotanical samples of barley (*Hordeum vulgare* L.) found at Qasr Ibrim display a two-row phenotype that is unique to the region of archaeological sites upriver of the first cataract of the Nile, characterised by the development of distinctive lateral bracts. The phenotype occurs throughout all strata at Qasr Ibrim, which range in age from 3000 to a few hundred years.

Methodology and Findings. We extracted ancient DNA from barley samples from the entire range of occupancy of the site, and studied the *Vrs1* gene responsible for row number in extant barley. Surprisingly, we found a discord between the genotype and phenotype in all samples; all the barley had a genotype consistent with the six-row condition. These results indicate a six-row ancestry for the Qasr Ibrim barley, followed by a reassertion of the two-row condition. Modelling demonstrates that this sequence of evolutionary events requires a strong selection pressure.

Conclusions. The two-row phenotype at Qasr Ibrim is caused by a different mechanism to that in extant barley. The strength of selection required for this mechanism to prevail indicates that the barley became locally adapted in the region in response to a local selection pressure. The consistency of the genotype/phenotype discord over time supports a scenario of adoption of this barley type by successive cultures, rather than the importation of new barley varieties associated with individual cultures.

33 **Introduction**

34 Barley (*Hordeum vulgare*) was among the earliest cereal crops to be exploited during the
35 Mesolithic Neolithic transition (1) and the first to become domesticated, about 9500 years
36 before present (BP) (2). The primary role of barley in the origins of agriculture can be
37 attributed to its resilient qualities such as rapid development to maturation (3), xerophytic
38 adaptations such as long awns (4), as well as tolerance of a wide range of edaphic factors
39 such as pH and salinity (5). These features made it well suited to the early Holocene
40 climate in which the spring growing season was shorter than it is now, and the summer
41 longer, hotter and drier (3). Subsequently, barley became the most widespread of the
42 cereal crops being grown in northern extremities of the British Isles and Scandinavia (6),
43 as well as the hot, dry climate of Egypt by the seventh millennium BP (7). The robust
44 nature of this crop ensures its importance in the future, as crop varieties need to be
45 developed to increase the potential areas amenable to arable agriculture to sustain the
46 expanding human population.

47 An important architectural feature of barley concerns the spikelet morphology which
48 gives rise to 'two-row' and 'six-row' forms of the crop. The spikelet is composed of a
49 central floret and two lateral florets. In the two-row forms only the central florets develop
50 grains whereas in six-row both central and lateral florets develop grains. Within these two
51 broad classes a number of morphologies are possible depending on the extent of lateral
52 floret development (8-10). Wild barley (*H. vulgare* ssp. *spontaneum*) occurs in the two-
53 row form, whereas cultivated barley (*H. vulgare* ssp. *vulgare*) has varieties of both two-
54 row and six-row forms. Six-row barley has higher protein content than two-row and is
55 often favoured as a food source as a result (11). In comparison, the biomass productivity

56 differs little between the two forms due to the ability for compensatory growth, typical of
57 cereals (12); two-row barleys tend to have fewer, larger grains relative to six-row barleys.
58 However, the two forms have notably different fecundities as a result of the differing
59 number of grains produced; six-row plants typically produce 1.5-2.0 times as many grains
60 as two-row plants (13).

61 On the African continent six-row barley is mostly grown where it is typically used as a
62 food source, often for animal feed. The archaeological record shows that six-row varieties
63 were available to farmers by 8800 years BP (*2*) and are present at the earliest African
64 archaeological sites dating to the seventh millennium BP (*7, 16*) (7,14).

65 Archaeobotanical remains of barley have been recovered from the archaeological site
66 at Qasr Ibrim (Fig 1), which was occupied from around 3000 years ago to several
67 hundred years ago (15). This site is interesting because it was a boundary settlement on
68 the edge of the Nubian and Roman Empires located between the first and second
69 cataracts of the Nile, and was occupied by five successive cultures: Napatan, Roman,
70 Meroitic, Christian and Islamic. Throughout all of the cultural stages the people of Qasr
71 Ibrim grew barley without engineered irrigation but using the natural cycle of hydrology
72 provided by the Nile, so called basin irrigation. The phenotype of archaeobotanical
73 remains of barley from all of the cultural stages at Qasr Ibrim and from Nauri, another
74 archaeological site further upriver of Qasr Ibrim (Fig 1), is surprising because of its two-
75 row appearance (SI 1, 16). Contemporaneous archaeobotanical remains found
76 downstream of the first cataract, from sites such as Tell el-Amarna (Fig 1), are of the six-
77 row type indicating that six-row varieties were available to the people of Qasr Ibrim and
78 indeed were typical of Egypt then as now (14). Barley was used as animal feed at these

79 sites (15), it is therefore a mystery why the typically preferable 6-row types that were
80 available were not grown, and it is unknown why or how the 2-row phenotype occurred.
81 Did the people of Qasr Ibrim import two-row barley from outside the region? It is a
82 further mystery that this state of affairs was propagated through five successive cultures.

83 Recently, the genetic mechanism that causes the switch from two to six-row in barley
84 was described (9). A homeodomain-leucine zipper I-class homeobox gene *Vrs1* produces
85 a transcription factor that inhibits lateral bud growth leading to the two-row condition.
86 The six-row condition is a derived state in which a loss of function mutation occurs in the
87 *Vrs1* gene. Three different loss of function mutations have been identified in the *Vrs1*
88 gene, all of which result in the loss of lateral bud suppression resulting in the six row
89 varieties seen in cultivated barley (9). Two of these mutations are geographically
90 restricted to the Western Mediterranean and East Asia respectively. The third, defined by
91 the *vrs1.a1* clade in (9), occurs worldwide, is responsible for most of the six-row barley
92 varieties, and is considered the most ancient of the three mutations. The presence of any
93 one of these recessive alleles was found to be sufficient to cause the six-row phenotype
94 by itself (9).

95 The remarkable biomolecular preservation of archaeobotanical material at Qasr Ibrim
96 makes it highly suited to ancient DNA analysis (17). In order to investigate the cause of
97 the curious 2-row phenotype present at Qasr Ibrim we amplified the *Vrs1* gene from
98 ancient DNA retrieved from archaeobotanical remains of barley spanning the entire range
99 of occupancy of the site.

100

101 **Results and Discussion**

102 Consistent with previous findings from Qasr Ibrim, relatively large amounts of DNA
103 were retrieved from the barley grains (SI 2). The quantities of DNA retrieved appear to
104 decrease in a time dependent manner (Fig 2). This suggests that the bulk of DNA
105 extracted from these samples is endogenous because one would not expect secondary
106 DNA sources (such as bacteria or human) to correspondingly reduce in amount. The
107 amount of DNA present per seed closely follows an exponential curve with a half-life of
108 approximately 350 years. Under these assumptions we would predict from our empirical
109 data that there would be little chance of retrieving DNA from archaeobotanical remains
110 much older than 3000 years under the preservation conditions at Qasr Ibrim (SI 3). The
111 principal process of DNA degradation is through hydrolytic depurination. A number of
112 factors influence DNA diagenesis including humidity, pH, temperature and the
113 availability of oxygen (18, 19). While conditions at Qasr Ibrim are hot, which would
114 serve to speed up oxidative and hydrolytic processes and shorten the preservation time of
115 DNA, they are also very dry, which would greatly reduce the rate of hydrolysis. The
116 preservation of DNA in ancient Egyptian contexts has been the matter of recent debate
117 (20-22). It has been asserted that when Egyptian archaeological sites are subject to
118 occasional flooding or increased humidity caused by flooding, as is the case with some
119 tomb sites, in combination with such high temperatures it is unlikely that DNA would
120 persist for even a few hundred years. Our results are therefore only consistent with a
121 completely dry history at Qasr Ibrim given our knowledge of DNA diagenesis. Qasr
122 Ibrim is a raised settlement, 60 metres above the Nile valley floor and therefore not
123 subject to flooding or increased levels of annual humidity, so it would appear the context

124 is consistent with the preservation. As such, our data may prove a useful empirical
125 baseline in measuring DNA decay in the virtual absence of water at high temperatures.

126 We amplified the *Vrs1* gene in a series of amplicons that ranged in size from 64-235
127 base pairs (bp). A block of two amplicon targets which spanned an AT rich region
128 between bases 205-461 did not amplify from any of our samples. All the remaining
129 amplicon targets were amplified from the Islamic and Late Christian samples (200 and
130 1000 years BP respectively). Amplicons were produced containing thirteen single
131 nucleotide polymorphisms (SNPs) previously observed in (9) from the Meroitic (1450-
132 1800 years BP) sample strata (Fig 3A). Eleven of these thirteen SNPs were recovered
133 from the Classic Christian (1000-1450 years BP) and Napatan (2400-2900 years BP)
134 sample strata and 5 SNPs (including the 2 *vrs1.a1* clade specific SNPs) were recovered
135 from the Pre-Meroitic (1900-2000 years BP) stratum sample (SI 4).

136 To our surprise a single base pair deletion in exon 3 causing a frame shift and the loss
137 of function associated with the *vrs1.a1* worldwide clade for the six-row condition (9) was
138 present in all the samples we tested. Furthermore, the remaining phylogenetically
139 informative sites placed the Qasr Ibrim *vrs1* sequences in clade *vrs1.a1*, (Fig 3). Eight
140 additional sample specific mutations were found in introns (SI 2, SI 5). These were all G-
141 >A and C->T mutations, consistent with post-mortem damage common of ancient DNA
142 (23-26) and are therefore unlikely to represent allelic variants. It is notable in this respect
143 that the oldest sample (Napatan) had the most such mutations and samples from the most
144 recent strata (Islamic) had none, again consistent with base modification over time. The
145 sample from the Classic Christian stratum does not follow the same time-dependent
146 relationship seen in the other samples in terms of the amount of DNA recovered and the

147 number of post-mortem changes. This result could be due to a different diagenetic history
148 in this sample (perhaps the sample was exposed to excessive heat from cooking, for
149 instance), or these barley grains may in fact have originated in an earlier stratum.

150

151 Qasr Ibrim barley was derived from a six-row ancestor

152 The barley at Qasr Ibrim carries the non-functional *vrs1* allele and therefore is derived
153 from an ancestor that was six-row. This unravels at least part of the mystery; the people
154 of Qasr Ibrim did not necessarily import a two-row type from outside of the region, but
155 that this barley was more likely derived from local six-row barleys. Supporting this
156 scenario is the fact that the phenotype found at Qasr Ibrim and its neighbouring sites
157 differs to that found anywhere else outside the region, because of the characeristic
158 development of lateral bracts. As far as we are aware, this is the first report of a two-row
159 form developing from a six-row ancestor, rather than a two-row condition being
160 reasserted from a six-row one through backcrossing with two-row. However, this
161 sequence of evolutionary events adds to the mystery considerably. Six-row barley
162 produces 1.5-2.0 times as many grains as two-row (13), and has the potential to produce
163 threefold more grains. Consequently, six-row barley has a natural advantage in the
164 population relative to two-row; one would not expect a two-row variety to prevail under
165 normal neutral circumstances. The graph in Fig 4 demonstrates the output of a model in
166 which a six-row individual is introduced into a population of 10^6 two-row plants with the
167 properties of barley (2% out-crossing and a six- to two-row fecundity ratio of 2.0). Under
168 these conditions (2% out-crossing and a six- to two-row fecundity ratio of 2.0) the *vrs1*

169 allele conferring the six-row condition would be expected to sweep through the
170 population very rapidly, within the lifetime of a single farmer. The rise of six-row types
171 in early agriculture may be simply explained by fecundity with no selective pressure,
172 conscious or unconscious, derived from farming practice. Under these conditions it is not
173 possible for two-row barley to become established in a six-row population unless the
174 mortality ratio of two to six-row grains is greater than the fecundity ratio of six to two-
175 row; each grain of six-row must be at least twice as likely to die as each two-row grain. A
176 switch from six to two-row would have required extreme selective pressure in favour of
177 the 2-row condition, with a selection coefficient (s) equal to a value of 2.

178 The consistency of the two-row phenotype throughout all the strata spanning three
179 millennia indicates that the mechanism of lateral floret inhibition is more likely to be
180 genetic, not environmental. Consequently, the two-row condition has probably resulted
181 from a gain of function mutation at another locus that reasserted the two-row condition
182 from a six-row ancestor. It is known that the *int-c* gene, which has not yet been isolated
183 and sequenced, can interact with *Vrs1* to modify phenotype (*27-30*), but this is an
184 unlikely candidate to have obtained such a gain of function and be causative of the Qasr
185 Ibrim phenotype since it is thought to interact with the functional *Vrs1* product (29).
186 Alternatively, a novel mutation at another unknown gene may have been responsible for
187 reasserting lateral floret inhibition.

188 The type of selection pressure that caused the shift from six-row to two-row is
189 currently a matter for speculation. It is unlikely that it came from conscious action by
190 farmers since such an explanation requires a premeditated awareness of an outcome that,
191 without experience, the farmers could not have had. Alternatively, there may have been a

192 natural selection pressure that strongly favoured the two-row condition. One such
193 possible cause we are currently investigating is water stress. Qasr Ibrim is located in the
194 upper Nile which is very arid relative to the lower Nile where six-row remains are found,
195 and studies have shown that two-row can survive water stress better than six-row (*31,*
196 *32*).

197

198 **Local adaptation at Qasr Ibrim**

199 Perhaps the most striking feature about the Qasr Ibrim barley is that successive cultures
200 have the same distinctive two-row phenotype. The discordant combination of phenotype
201 and anomalous *vrs1* genotype is consistent throughout all strata suggesting that the same
202 barley was grown *in situ* despite the cultural transitions, some of which were peaceful,
203 and others more forcible. It may have been the case that six-row varieties of barley were
204 imported with new cultures, but performed poorly against the indigenous two-row
205 phenotype, which itself was ultimately derived from a six-row ancestor. Whatever the
206 reason, successive cultures appear to have adopted the preceding culture's barley
207 agriculture. If this was the case, the Qasr Ibrim barley may represent a 'lost variety' that
208 was cultivated continually for thousands of years. Such a long period of cultivation in the
209 same location would give opportunity for the crop to become locally adapted. Primitive
210 landraces of wheat and barley in Europe and maize in South America have shown
211 phylogeographic evidence associated with the Neolithic expansion, and later population
212 movements that are dated to past millennia (33-36). In order for such ancient human
213 movements to be evident, these crops must have existed *in situ* for thousands of years.

214 Under such circumstances adaptation to local environmental conditions would be
215 expected, as appears to be borne out at Qasr Ibrim. A tendency for local adaptation in
216 primitive varieties has important implications in two ways. Firstly, such strong selection
217 pressure is likely to have affected many genes in terms of adaptation, which if identified
218 through archaeogenetics could confirm the nature of the selection pressure and be
219 valuable in the development of new varieties. Secondly, such temporal stability offers an
220 insight into how past cultures are likely to have interacted in terms of adopting
221 indigenous agriculture or importing agriculture with new cultures.

222

223 Materials and Methods

224 **Archaeobotanical material.** Barley samples were collected during excavations at
225 Qasr Ibrim between 1984 and 1986.

226 **Ancient DNA extraction.** DNA extraction was carried out in a dedicated, chambered,
227 ancient DNA extraction laboratory using suitable precautions to avoid contamination by
228 foreign contaminants. This laboratory had not been previously used to extract DNA from
229 modern barley samples and was physically removed from areas where PCR is carried out.
230 Extraction blanks were carried out in parallel with each sample extraction to ensure
231 authenticity. DNA was extracted from 3-6 seeds, ground in a mortar and pestle, and
232 incubated in 1 ml of CTAB buffer (2 % cetyltrimethylammoniumbromide [CTAB], 0.1
233 M trishydroxymethylaminomethane [Tris-HCl] pH 8.0, 20 mM
234 ethylenediaminetetraacetic acid [EDTA], 1.4 M sodium chloride) (37) mixed by agitation
235 for 24 hours at 37 ºC. The DNA was purified by chloroform:isoamyl alcohol (24:1)

236 extraction, concentrated using Amicon® concentrators (Centricon® plus-70 with a 30 kDa
237 Ultracel-PL membrane; Millipore) and further purified using a DNeasy® silica column
238 (Qiagen). Double stranded DNA was quantified using a Qubit® Fluorometer (Invitrogen).

239 **Sequencing of *vrs1* locus.** Primer pairs (SI 6) were designed to amplify overlapping
240 segments of *Vrs1*, up to 235bp in length, capturing known SNPs (SI 5). DNA
241 amplifications were carried out in 20 µL reactions containing 15 mM Tris-HCl pH 8.3, 50
242 mM KCl (pH 8.0), 2 mM $MgCl_2$, 100 µM of each dNTP, 0.5 U Platinum® Taq DNA
243 polymerase (low DNA; Applied Biosystems), 0.65 µM of each primer and 50 – 100 pg
244 of the extracted DNA product. PCR and extraction blank reactions were carried out in
245 parallel to the sample PCRs. Thermocycling conditions were as follows: 94 ºC for 3 min,
246 then 70 cycles of 94 ºC for 30 s, 60 ºC for 1 min and 72 ºC for 1 min, followed by 7 min
247 at 72 ºC. Blanks and amplification products were confirmed by gel electrophoresis in 2%
248 agarose stained with gel red. QIAquick® (Qiagen) columns were used to purify the PCR
249 products. Sequencing was performed using Big Dye terminator v3.1 Cycle Sequencing
250 kit (Applied Biosystems) and the products separated on a 3130xl Genetic Analyzer at the
251 Genomics Laboratory, HRI, University of Warwick.

252 ***Vrs1* population model.** Expectations of fixation of dominant two- and recessive six-
253 rowed alleles in crop populations were calculated by modelling the average number of
254 homozygous and heterozygous individuals at each generation in a population of diploid
255 plants with an initial single heterozygous individual. Harvest seed proportions were
256 predicted with conservative (20%) and realistic (2%) panmictic out-crossing rates (38)
257 and fecundity ratios between six-and two-rowed plants of 1.5 and 2.0 (13). Seed for each

258 generation were drawn at random from the previous harvest and cumulative probability
259 of extinction through stochastic harvest sampling was calculated at each generation.

260

261 **Acknowledgements**

262 We would like to thank the Egypt Exploration Society for access to the material from
263 Qasr Ibrim, Takao Komatsuda for sequencing data of *Vrs1* clade specific SNPs, and
264 Dorian Fuller for useful discussions.

265

266

267 **References**

268 1. Weiss E, Wetterstrom W, Nadel D, Bar-Yosef O (2004) The broad spectrum
269 revisited: Evidence from plant remains. *Proc Natl Acad Sci USA* 101:9551-9555.
270 2. Zohary D & Hopf M (2000) *Domestication of Plants in the Old World*, 3rd
271 Edition, (Oxford University Press, Oxford), pp 67-69.
272 3. Blumler MA (1996) in *The Origins and Spread of Agriculture and Pastoralism in*
273 *Eurasia*, ed Harris DR (UCL Press, London), pp 25-50.
274 4. Grundbacher FJ (1963) The physiological function of the cereal awn. *Bot Rev*
275 29:366-381.
276 5. Willcox G (2005) The distribution, natural habitats and availability of wild cereals
277 in relation to their domestication in the Near East: multiple events, multiple
278 centres. *Veg Hist Archaeobot* 14:534-541.

279 6. Jarman RJ (1996) Bere barley – a living link with 8th Century? *Plant Var Seeds*
280 9:191-196.
281 7. Wetterstrom W (1993) in *The archaeology of Africa: food, metals and towns,* eds
282 Shaw T, Sinclair P, Andah D, Okpoko A (Routledge, London), pp 165-226.
283 8. Necas J (1963) Inheritance of Development of Lateral Florets in Spikelets of
284 Barley Spike. *Biologia Plantarum (Praha)* 5:89-99.
285 9. Komatsuda T, Pourkheirandish M, He C, Azhaguvel P, Kanamori H, Perovic D *et*
286 *al*. (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine
287 zipper I-class homeobox gene. *Proc Natl Acad Sci USA* 104:1424-1429.
288 10. Wiebe GA, Reid DA (1961) Classification of barley varieties grown in the United
289 States and Canada in 1958. *Technical bulletin 1224, US Dept of Agriculture*.
290 11. Knüpffer H, Terentyeva I, Hammer K, Kovaleva O, Sato K (2003) in *Diversity in*
291 *Barley* eds von Bothmer R, van Hintum T, Knüpffer H and Sato K (Elsevier,
292 Amsterdam), pp 53-76.
293 12. Evans LT, Wardlaw IF (1976) Aspects of the comparative physiology of grain
294 yield in cereals. *Adv Agron* 28:301-359 .
295 13. Kjaer B, Jensen J (1996) Quantitative trait loci for grain yield and yield
296 components in a cross between a six-rowed and a two-rowed barley. *Euphytica*
297 90:39-48.
298 14. Murray MA (2000) in *Ancient Egyptian Materials and Technology*, eds Nicholson
299 P, Shaw I (Cambridge University Press, Cambridge), pp 505-536.
300 15. Clapham AJ, Rowley-Conwy PA (2007) in *Fields of Change – Progress in*
301 *African Archaeobotany*, ed Cappers R (Groningen) *Groningen Archaeological*

302 *Studies* 5:157-164.

303 16. Fuller DQ, Edwards DN (2001) Medieval plant economy in Middle Nubia:
304 preliminary archaeobotanical evidence from Nauri. *Sudan and Nubia* 5:97-103.

305 17. O'Donoghue K, Clapham A, Evershed R, Brown TA (1996) Remarkable
306 preservation of biomolecules in ancient radish seeds. *Proc Roy Soc Ser B*
307 263:541-547.

308 18. Lindahl T, Nyberg B (1972) Rate of depurination of deoxyribonucleic acid.
309 *Biochemistry* 11:3610-3618.

310 19. Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature*
311 362:709-715.

312 20. Marota I, Basile C, Ubaldi M, Rollo F (2002) DNA decay rate in papyri and
313 human remains from Egyptian archaeological sites. *Am J Phys Anthropol*
314 117:310-318.

315 21. Zink A and Nerlich AG (2003) Molecular analyses of the "Pharaos": Feasibility
316 of molecular studies in ancient Egyptian material. *Am J Phys Anthropol* 121:109-
317 111.

318 22. Gilbert MTP, Barnes I, Collins M, Smith C, Eklund J *et al.* (2005) Long term
319 survival in ancient Egypt: response to Zink and Nerlich. *Am J Phys Anthropol*
320 128:110-114.

321 23. Binladen J, Wiuf C, Gilbert MTP, Bunce M, Barnett R *et al.* (2006) Assessing the
322 fidelity of ancient DNA sequences amplified from nuclear genes. *Genetics*
323 172:733-741.

324 24. Helgason A, Pálsson S, Lalueza-Fox C, Ghosh S, Sigurdardóttir S *et al.* (2007) A
325 statistical approach to identify ancient template DNA. *J Mol Evol* 65:92 - 102.
326 25. Stiller M, Green RE, Ronan M, Simons JF, Du L *et al.* (2006) Patterns of
327 nucleotide misincorporations during enzymatic amplification and direct large
328 scale sequencing of ancient DNA. *Proc Natl Acad Sci USA* 103:13578-13584.
329 26. Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E *et al.* (2007)
330 Recharacterization of ancient DNAmiscoding lesions: insights in the era of
331 sequencing-by-synthesis. *Nucleic Acids Re.* 35:1-10.
332 27. Lundkvist U, Franckowiak JD (1997) BGS 178; Intermedium spike-c. *Barley*
333 *Genet News* 26:200-201.
334 28. Leonard WH (1942) Inheritance of fertility in the lateral spikelets of barley.
335 *Genetics* 27:299-316.
336 29. Komatsuda T, Mano Y (2002) Molecular mapping of the intermedium spike-c
337 (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). *Theor*
338 *Appl Genet* 105: 85-90.
339 30. Gymer PT (1978) The genetics of the six row/tow row character. *Barley Genet*
340 *News* 8:44-46.
341 31. Forster BP, Ellis RP, Moir J, Talamè V, Sanguineti MC *et al*. (2004) Genotype
342 and phenotype associations with drought tolerance in barley tested in North
343 Africa. *Ann Appl Biol* 144:157-168.
344 32. Demissie A, Bjørnstad Å (1996) Phenotypic diversity of Ethiopian barleys in
345 relation to geographical regions, altitudinal range, and agro-ecological zones: as
346 an aid to germplasm collection and conservation strategy. *Hereditas* 124:17-29.

347 33. Brown TA, Linsday S, Allaby RG (2006) in *Darwin's Harvest*, eds Motley TJ,
348 Zeregra N, Cross H (Columbia University Press, New York), pp 197-212.
349 34. Jones H, Leigh FJ, Mackay I, Bower M, Smith L *et al.* (2008) Population based
350 re-sequencing reveals that the flowering time adaptation of cultivated barley
351 originated east of the fertile crescent. *Mol Biol Evol* 25:2211-2219.
352 35. Freitas FO, Bendel G, Allaby RG, Brown TA (2003) DNA from primitive maize
353 landraces and archaeological remains: Implications for the domestication of maize
354 and its expansion into South America. *J Archaeol Sci* 30:901-908.
355 36. Lia VV, Confalonieri VA, Ratto N, Cámara Hernández JA, Miante Alzogaray
356 AM *et al.* (2007) Microsatellite typing of ancient maize: insights into the history
357 of agriculture in southern South America. *Proc R Soc B* 274:545–554.
358 37. Rogers SO, Bendich AJ (1985) Extraction of milligram amounts of fresh,
359 herbarium and mummified plant-tissues. *Plant Mol Biol* 5:69-76.
360 38. Abdel-Ghani AH, Parzies HK, Omary A, Geiger HH (2004) Estimating the
361 outcrossing rate of barley landraces and wild barley populations collected from
362 ecologically different regions of Jordan. *Theor Appl Genet* 109:588-595.
363

363 **Figure 1.** Map of Nubia. Locations of archaeological excavations at Tell el-Amarna, Qasr
364 Ibrim, and Nauri and the cataracts along the River Nile are indicated.

365

366 **Figure 2.** Recoverable archaeological DNA quantity over time. DNA extracted per
367 Barley seed plotted on a logarithmic scale against the years BP of the assemblage from
368 which each sample was recovered. Error bars indicate the range of years BP of each strata
369 from which samples were taken.

370

371 **Figure 3.** Characterisation of *Vrs1* locus in Qasr Ibrim barley. *(A)* Analysis of Qasr Ibrim
372 barley for haplotype specific polymorphisms (9). SNPs coloured in red indicate *vrs1* loss
373 of function mutations. – indicates where sequencing data were not available. *(B)*
374 Phylogenetic tree of *Vrs1* alleles indicating the position of the Qasr Ibrim barley within
375 the vrs1.a1 clade.

376

377 **Figure 4.** Six-row introduction to two-row populations. Modelling of generations to
378 fixation of recessive six-rowed alleles in homozygous two-rowed barley crops by
379 automatic selection using realistic constraints (Population size: 10^6; Out-crossing rate:
380 2%; Six- to two-rowed fecundity ratio: 2.0) the a six-row individual is introduced into a
381 population of 10^6 two-row plants.

382

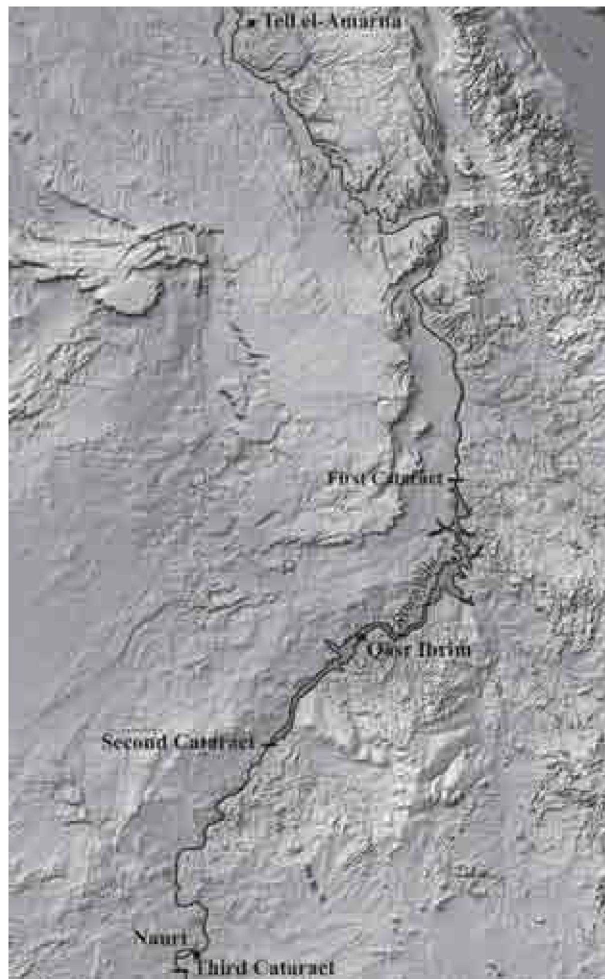


Fig. 1

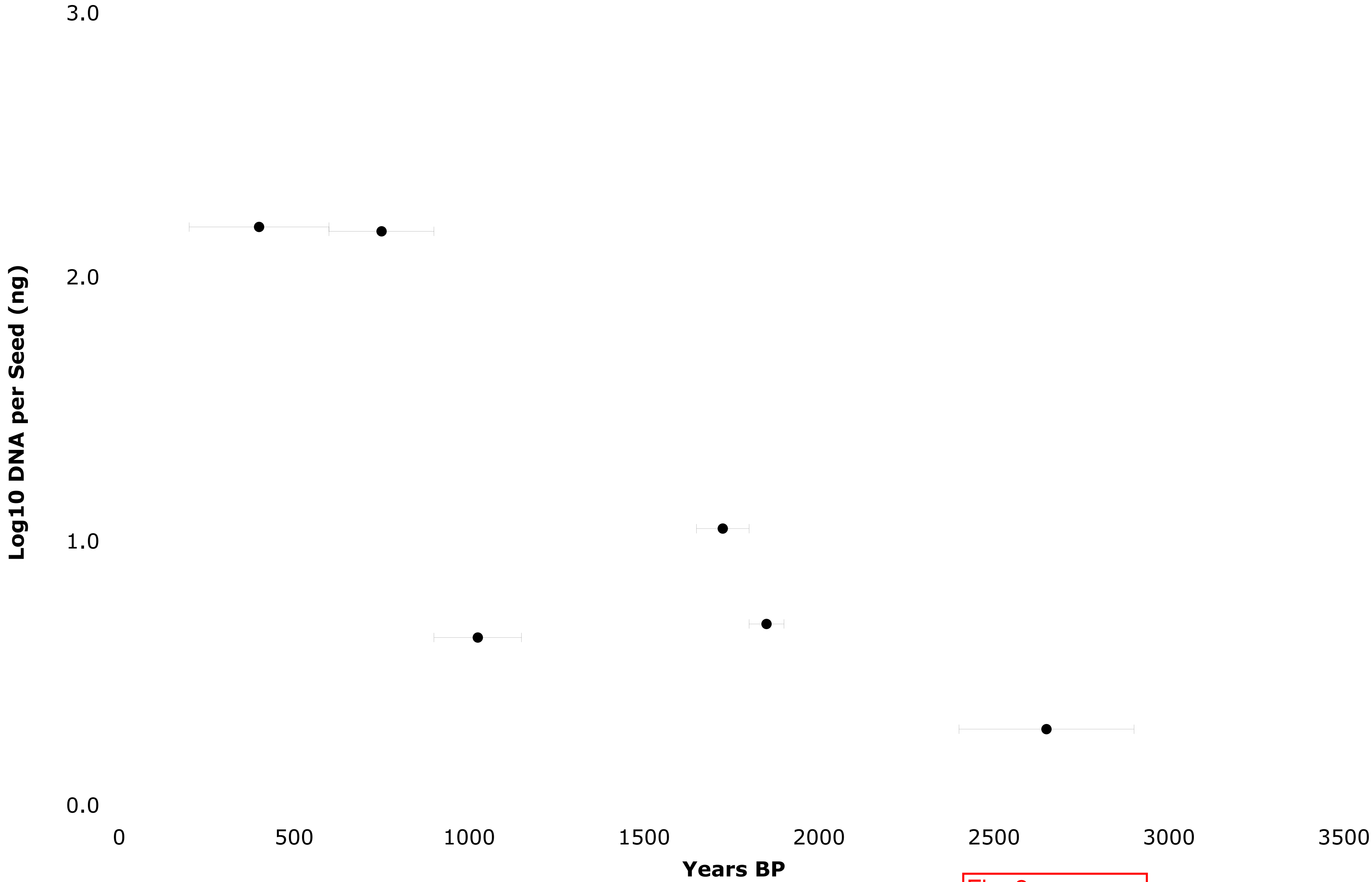


Fig. 2

A

	Base Position	73	139	157	483	604	694	873	914	1020	1235	1352	1589	1668
	SNP	G->C	G->T	C->T	A->G	A->G	G->A	G->T	CG->CTG	C->G	C->T	GAG->GA-	C->T	G->T
	Clade Specificity	2 specific	2 specific	2 specific	a1 haplotype specific	a1 haplotype specific	3 specific	3 specific	a2 specific	a3 specific	2 specific	a1 specific	1 specific	2 specific
Sample Assemblage	**Years BP**													
Islamic	197 - 600	G	G	C	A	A	G	G	CG	C	C	GA-	T	G
Late Christian	600 - 900	G	G	C	A	A	G	G	CG	C	C	GA-	T	G
Classic Christian	900 - 1150	G	-	-	A	A	G	G	CG	C	C	GA-	T	-
Meroitic	1650 - 1800	G	G	C	A	A	G	G	CG	C	C	GA-	T	G
Pre-Meroitic	1800 - 1900	-	-	-	-	-	-	G	CG	C	-	GA-	T	-
Napatan	2400 - 2900	G	-	-	A	A	G	G	CG	C	C	GA-	T	G

B

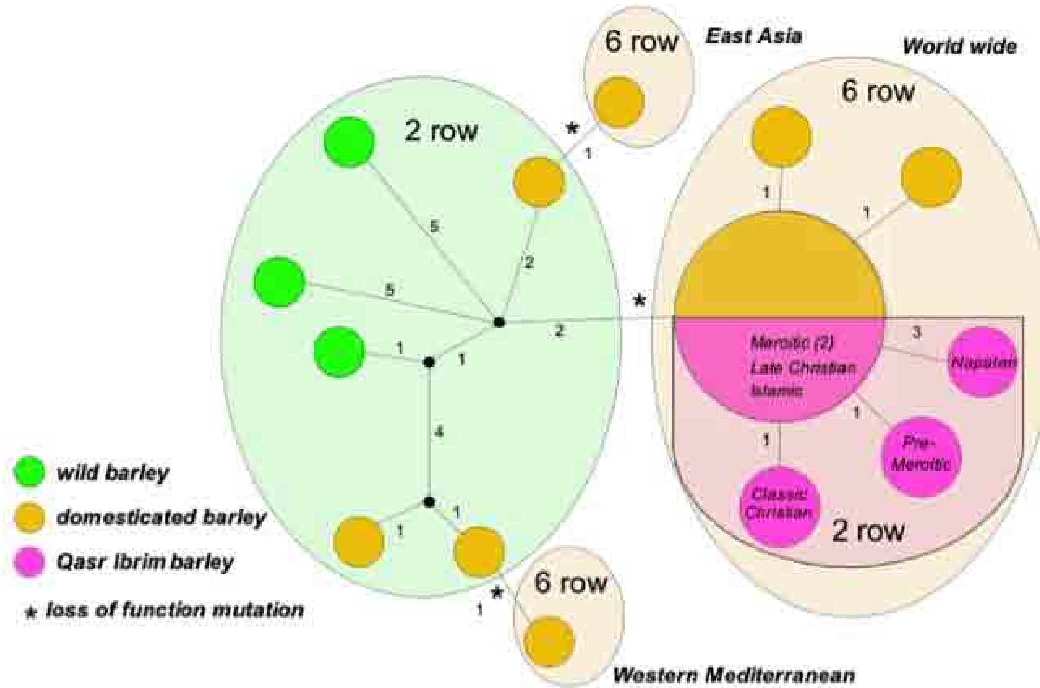


Fig. 3

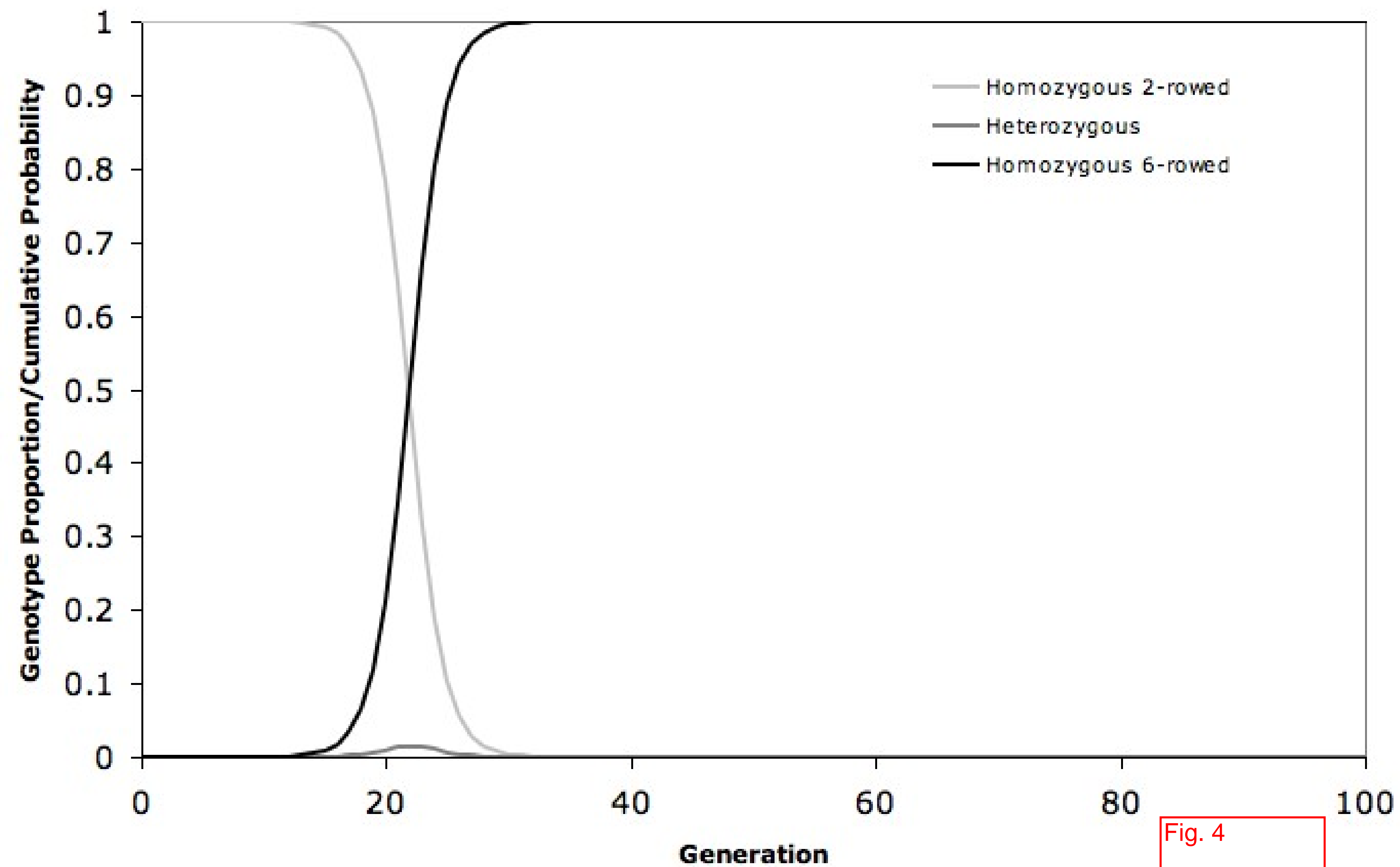


Fig. 4

Figure S1. Barley spikelet morphology from Qasr Ibrim. Abbreviations: fs (fertile spikelet), slb (sterile lateral bract), g (glume), r (rachis). Scale: divisions = 1mm. a. Unattached spikelet and bract, b. Spikelet and bract attached to rachis. The central fertile spikelet contains a barley grain, sterile lateral bracts do not. The ventral groove of the grain remains untwisted, typical of two-row barley rather than six-row. The resulting barley ear has only two rows of grains

Table S2. Qasr Ibrim Barley DNA studies summary table. DNA extracted per seed, length range of amplicons and C->T and G->A base pair modifications recovered from each of the Qasr Ibrim assemblages.

Figure S3 . Recoverable archaeological DNA quantity over time. DNA extracted per Barley seed plotted against the years BP of the assemblage from which each sample was recovered. Error bars indicate the range of years BP of the strata from which each sample was taken. The exponential fitted model predicts that DNA will cease to be recoverable from samples over the age of 4500 years.

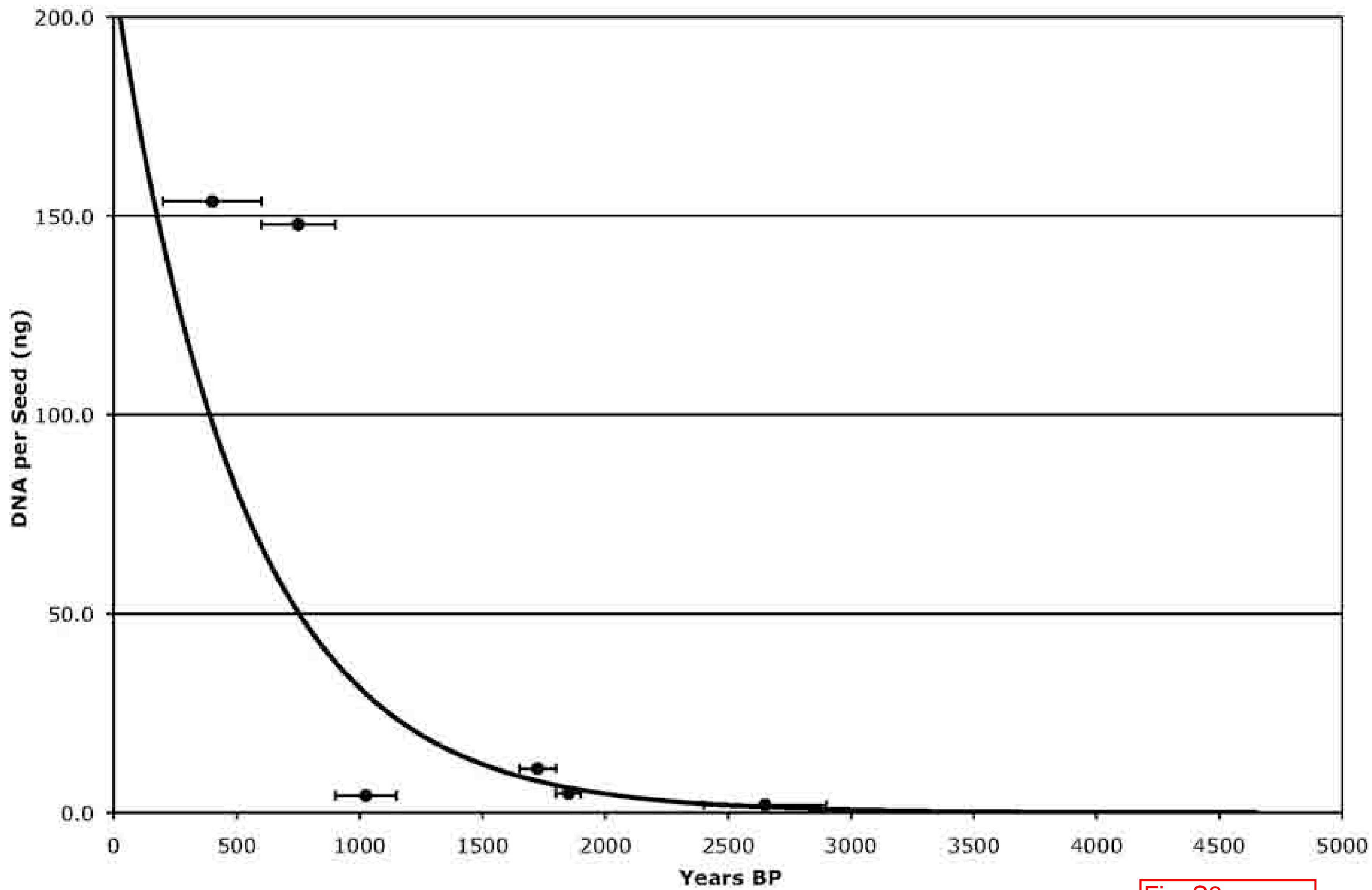
Figure S4. Qasr Ibrim barley amplifications of *Vrs1.* Regions of the *Vrs1* locus amplified (indicated in green) from archaeobotanical remains of barley from Qasr Ibrim and the location of priming sites and the phylogenetically informative SNPs described by (9). Position of exons in *Vrs1* shown.

Figure S5. Alignment of Qasr Ibrim barley sequences against published *Vrs1* alleles (9). The vrs1.a1 clade single base-pair deletion can be seen at position 1353.

Text S6. Priming sites used to amplify 1747bp of the *vrs1* locus from the archaeobotanical remains of barley from Qasr Ibrim. * Indicates previously published (9).

Fig. S1

Sample Assemblage	***Years BP***	***DNA per seed (ng)***	***Amplicon lengths (bp)***	***C->T***	***G->A***
Islamic	197 - 600	153.6	106 - 235		
Late Christian	600 - 900	147.8	106 - 235		
Classic Christian	900 - 1150	4.3	60 - 182	3	1
Meroitic	1650 - 1800	11.1	106 - 235		
Pre-Meroitic	1800 - 1900	4.8	100 - 215		1
Napatan	2400 - 2900	1.9	54 - 215		3


200.0
150.0
100.0
50.0
0.0
DNA per Seed (ng)
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Years BP
Fig. S3

Exon 1 Exon 2 Exon 3

Assemblage	Age (yBP)
Islamic	197 - 600
Late Christian	600 - 900
Classic Christian	900 - 1150
Meroitic	1650 - 1800
Pre-Meroitic	1800 - 1900
Napatan	2400 - 2900

Priming site
SNP
vrs1.a1 clade specific SNP

150 bp

Fig. S4

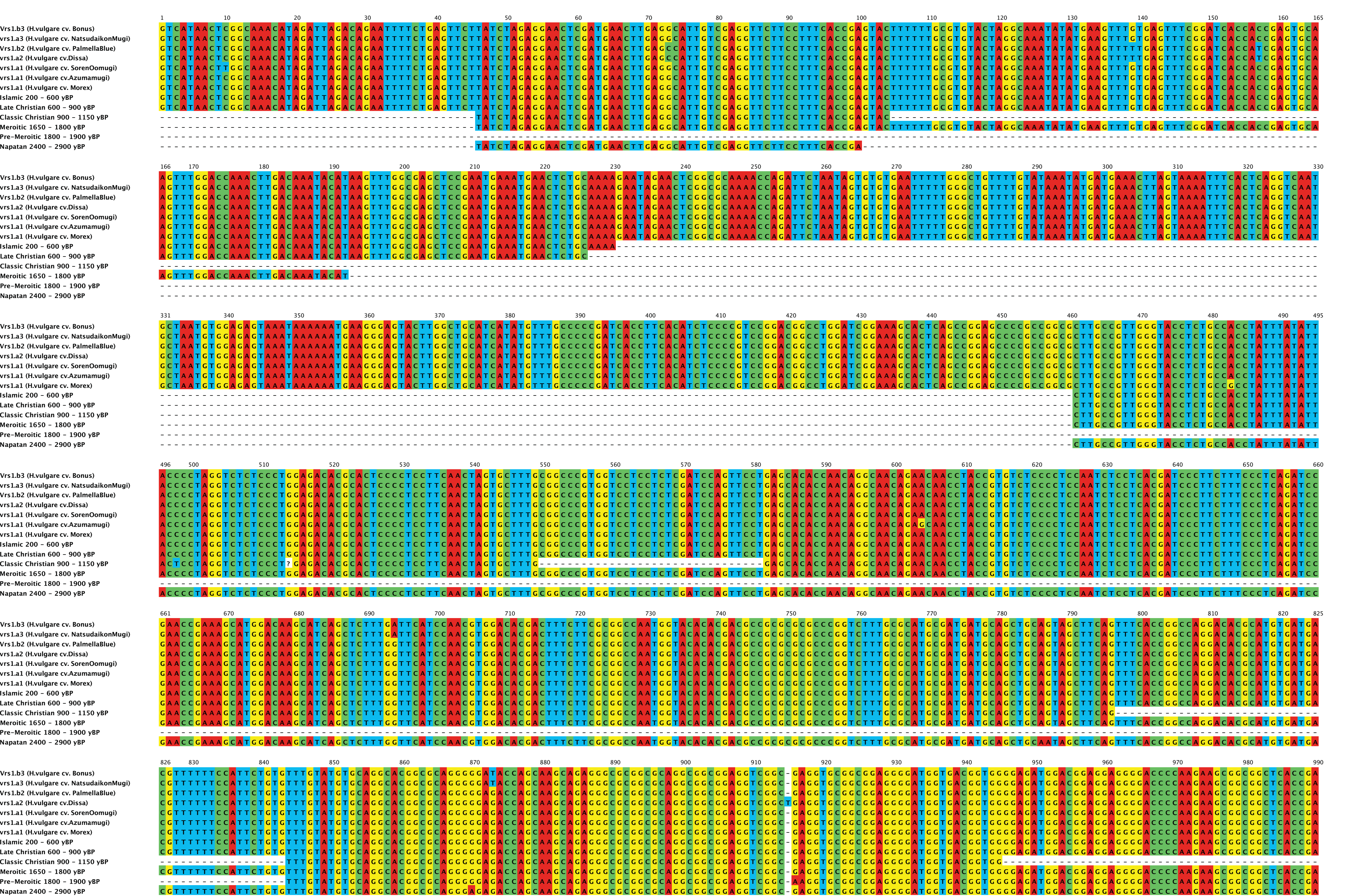


Fig. S5

5' GTCATAACTCGGCAAACATAG 3' *

5' TATCTAGAGGAACTCGATGAACTT 3'

5' GTTCTTCCTTTCACCGAGTAC 3'

3' GTACTCGGTGAAAGGAAGAAC 5'

5' GCGTGTACTAGGCAAATATATG 3'

3' ATGTATTTGTCAAGTTTGGTCCA 5'

5' CTCCGAATGAAATGAACTCTGC 3'

3' GCAGAGTTCATTTCATTCGGAG 5'

5' CTCAGGTCAATGCTAATGTGG 3'

3' CCACATTAGCATTGACCTGAG 5' *

5' TGGATCGGAAAGCACTCAGC 3'

3' GCTGAGTGCTTTCCGATCCA 5'

5' CTTGCCGTTGGGTACCTCT 3'

3' CCAGGGAGAGACCTAGGG 5'

5' CCTCCTTCAACTAGTGCTTTG 3'

3' CAAAGCACTAGTTGAAGGAGG 5'

5' GAGCACACCAACAGGCAACA 3'

3' GGAGGGGAGACACGGTAG 5'

5' TCAGATCCGAACCGAAAGCAT 3'

3' ATGCTTTCGGTTCGGATCTGA 5'

3' GCGAAGAAAGTCGTGTCCAC 5'

5' CAGCAAGCAGAGGGCGC 3'

3' GCGCCCTCTGCTTGCTG 5'

5' GAGGGGATGGTGACGGTG 3'

3' CACCGTCACCATCCCCTC 5'

5' GCCGAGATTCTGGAGCTGA 3'

5' GAGAACGAGGTATGCTTGCTC 3'

3' CAGCGCCATATGTAAGCCAG 5'

5' GAGAGACTGGGAGCGACTG 3'

3' CCCAGCTGCCGACCTGAG 5'

5' CATGAATTAGAGTTTATGCTGG 3'

3' AACACTCGACCACGCTGCTA 5'

5' ATAGCCGAGATAGCTGCTGC 3'

Text S6