

1 **Dogs accompanied humans during the Neolithic expansion into Europe**

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35 **Abstract**

36 Near Eastern Neolithic farmers introduced several species of domestic plants and
37 animals as they dispersed into Europe. Dogs were the only domestic species present in
38 both Europe and the Near East prior to the Neolithic. Here, we assessed whether early
39 Near Eastern dogs possessed a unique mitochondrial lineage that differentiated them
40 from Mesolithic European populations. We then analysed mitochondrial DNA sequences
41 from 99 ancient European and Near-Eastern dogs spanning the Upper Palaeolithic to the
42 Bronze Age to assess if incoming farmers brought Near Eastern dogs with them, or
43 instead primarily adopted indigenous European dogs after they arrived. Our results show
44 that European pre-Neolithic dogs all possessed the mitochondrial haplogroup C, and that
45 the Neolithic and Post-Neolithic dogs associated with farmers from Southeastern Europe
46 mainly possessed haplogroup D. Thus, the appearance of haplogroup D most likely
47 resulted from the dissemination of dogs from the Near East into Europe. In Western and
48 Northern Europe, the turnover is incomplete and C haplogroup persists well into the
49 Chalcolithic at least. These results suggest that dogs were an integral component of the
50 Neolithic farming package and a mitochondrial lineage associated with the Near East
51 was introduced into Europe alongside pigs, cows, sheep, and goats. It got diluted into the
52 native dog population when reaching the Western and Northern margins of Europe.

53

54 **Key words:** dog, ancient DNA, Neolithic, domestication

55

56 **Main text**

57 In Western Eurasia, settled agriculture and stock keeping first arose in the Fertile
58 Crescent [1, 2]. This Neolithic life way then emerged in Europe between 9,000 and 6,000
59 BP, triggered by the arrival of immigrant farmers ~9,000 BP who originated in the Near
60 East and substantially replaced the local hunter-gatherer population except on the
61 western and northern margin of the continent, where Mesolithic societies persisted
62 longer [3-5]. These farmers were accompanied by several domesticates including sheep
63 and goats [6], pigs [7], cows [8-9], and cultigens including wheat, barley, peas, broad
64 beans and lentils [10].

65

66 Ascertaining the geographic origins of the animals associated with this migration is not
67 always straightforward. While the wild progenitors of neither sheep nor goats were ever
68 present in Europe [6], the progenitors of both pigs and cattle were extant at the time of

69 the arrival of the Neolithic [11,12] and some studies have claimed that these taxa were
70 locally domesticated [e.g. 13]. Assessing whether the archaeological remains of these
71 latter animals found in Neolithic contexts were derived from Near Eastern or European
72 populations is complicated by the fact that imported domesticates often interbred with
73 indigenous European wild populations [14-16].

74

75 Dogs are even more problematic since both wolves and domestic dogs were present in
76 the Near East and Europe prior to, during, and after the arrival of Neolithic farmers into
77 Europe [11,17]. A recent analysis suggested that dogs may have been domesticated
78 independently from geographically and genetically differentiated wolf populations in
79 Western Eurasia and East Asia [18]. This study also demonstrated a turnover in the
80 proportion of mitochondrial haplotypes in Europe, though it lacked the power to
81 establish when the turnover took place. Given the close relationship between dogs and
82 people, as for example demonstrated by the increase in *AM2YB* gene copy number
83 related to an increase in the efficiency of starch digestion and coincidental with the
84 regional advent of agriculture [19, 20], it is possible that dogs associated with Near
85 Eastern farmers were brought into Europe alongside other domestic animals.

86

87 To test this hypothesis, we analysed 99 ancient dog published mitochondrial DNA
88 sequences [21] from 37 archaeological sites across Eurasia, from the Upper Palaeolithic
89 to the Bronze Age (Table S1, Figure S1, SI-§1-§5-§6). We first assessed whether a
90 specific mitochondrial dog haplogroup was associated with Neolithic farmers. We then
91 ascertained whether that lineage was introduced to Europe by tracking its spatiotemporal
92 frequency (SI-§6).

93

94 Each of the 99 sequences was assigned to previously established dog haplogroups (Hg)
95 (SI-§6, Table S2, Figure S2). Individuals were then grouped into seven temporally and
96 geographically defined categories and we tested the existence of a genetic structure
97 congruent with the history of the Neolithization of Europe (SI-§2-§6; Table S3).

98

99 Prior to the Neolithic, all European dogs possessed mitochondrial Hg C (Figures 1-S1-
100 S3). The subsequent Neolithic and post-Neolithic European dogs possessed Hg A (6
101 samples), Hg D (21 samples) and Hg C (38 samples), thus suggesting the introduction of

102 non-indigenous domestic dogs. An AMOVA analysis (Table S3) showed that inter-
103 regional differences account for 44.3% of the total genetic variation (Table S4, S5).

104

105 Following the dominance of Hg C, the appearance of Hg D during the Neolithic and
106 Post-Neolithic period could have resulted from either an influx of Hg D from separate
107 source population(s), or potentially by drift alone. To evaluate the likelihood of these
108 scenarios, we simulated genealogies under a previously described demographic model
109 for dogs [18] and computed the probability (SI-§6) that Hg D reached the frequencies
110 observed during the Neolithic and Post-Neolithic in both the entirety of Europe and just
111 in South-Eastern Europe through either drift alone, or as a result of an influx of dogs
112 from elsewhere.

113

114 When considering all of Europe at once (81 samples), the simulation showed that a
115 starting frequency for Hg D of 21% would have been sufficient to obtain the frequency
116 observed in the Neolithic-Post-Neolithic period (33%) by drift alone in a few hundred
117 dog generations (Figure S4A). All of our pre-Neolithic European samples possessed Hg
118 C, but because our dataset consisted of 15 samples, we cannot reject the null hypothesis
119 of drift alone (SI-§6, Table S6).

120

121 Considering Southeastern Europe on its own, we can reject this null hypothesis ($p < 0.01$).
122 Using a binomial confidence interval, the lowest possible post-Neolithic frequency of Hg
123 D in Southeastern Europe is 69% (Table S6, 95% CI, 69-94%) and it would have taken
124 >700 dog generations ($\sim 2,800$ years) for drift alone to explain this increase in Hg D after
125 the Neolithic (with $p > 0.05$) (Figure S4B-C, SI-§6). This is much longer than the duration
126 of Neolithization in this region [22]. Moreover, our results show that a starting frequency
127 of $>41\%$ of Hg D during the pre-Neolithic period in Southeastern Europe is required for
128 drift alone to explain this transition, over a time period of 0-700 dog generations with
129 probability greater than 5% (Figure S4B-C, SI-§6). Considering that our binomial
130 confidence interval for Hg D frequency in Southeastern Europe prior to the Neolithic is
131 between 0 and 39% (Table S6), it is highly unlikely that observed frequency of Hg D in
132 this region (SI-§6) could result from drift.

133

134 Our results indicate that the appearance of dogs possessing Hg D resulted from a human-
135 mediated introduction of dogs to Southeastern Europe. The D haplogroup largely

136 replaced the C haplogroup in this region, though its frequency was far less across the rest
137 of Europe (20.8% in Central-Western Europe and 3.8% in Northern-Western Europe)
138 (Figures 1, S1, S3).

139

140 Our study did not include wolves from either the Near-East or Europe, which prevented
141 us from assessing whether admixture with wolves played a role in the pattern described
142 above. The overall spatiotemporal pattern of haplotype distribution, however, is highly
143 congruent with early human population dynamics during the Neolithic expansion from
144 Near-East (SI-§3, [22]). It also reflects the versatile nature of the European Neolithic,
145 owing to exogenous inputs in the South-East and incorporating more and more
146 Mesolithic elements toward the North and the West (SI-§2, [5, 22]). In addition, like the
147 modern global dog population, Neolithic and post-Neolithic European dogs also
148 possessed Hg A, although in smaller proportions than Hg D. This haplogroup may have
149 been brought into Europe at a later period than the early Neolithic [18] potentially
150 during migrations from the Pontic steppe (SI-§4, [3, 23]).

151

152 Overall, the evidence presented here suggests that, like domestic ungulates, cereals and
153 pulses [24-25], mtDNA dog lineages indigenous to Near-East were brought to Europe
154 during the Neolithic from the beginning of the 9th millennium BP before later spreading
155 west and north. Ancient nuclear DNA studies will further reveal the spatiotemporal
156 spread of specific dog populations in Europe and across the globe.

157

158

159 **Ethical statement**

160 No ethical approval was required.

161

162 **Permission to carry out fieldwork**

163 This heading does not apply. All the data have been previously published.

164

165 **Data accessibility**

166 DNA sequences: doi:10.5061/dryad.h55p1q5

167

168 **Competing interests**

169 We have no competing interests.

170

171 **Author's contributions:**

172 M.O., A.T., L.F., S.B. analysed the data, participated in the design of the study,
173 coordinated the study and drafted the manuscript; G.L., C.H., C.Hi and J.D.V. designed
174 the study and helped to draft the manuscript; A.Ba., M.M., A.B., M.P.C., O.L., R.M.A.,
175 L.B., K.D., R.R., M.S. collected contextual data and edited the manuscript. All authors
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269 **Figure legends**

270

271 **Figure 1:** Genetic, Geographic and chronological pattern of ancient dogs in Middle East
272 and Europe

273 **A1-** Pre-Neolithic dogs distribution; **A2-** distribution during and after the Neolithic
274 transition

275 **B-** chronological distribution of dog haplogroup frequencies among 4 geographic regions
276 (according to Table S2)

277 Archaeological sites are numbered according to Table S1.

278 Red: Haplogroup A, Blue: Haplogroup B, Yellow: Haplogroup C, Green: Haplogroup D.

279 Dashed line: Neolithic transition

280

281