BIOLOGY LETTERS

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Research

Cite this article: Ollivier M, Tresset A *et al.* 2018 Dogs accompanied humans during the Neolithic expansion into Europe. *Biol. Lett.* **14**: 20180286.
http://dx.doi.org/10.1098/rsbl.2018.0286

Received: 23 April 2018
Accepted: 24 September 2018

Subject Areas:
evolution

Keywords:
dog, ancient DNA, Neolithic, domestication

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Electronic supplementary material is available online at http://dx.doi.org/10.5061/dryad.h55p1q5.

THE ROYAL SOCIETY PUBLISHING

Evolutionary biology

Dogs accompanied humans during the Neolithic expansion into Europe

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

In Western Eurasia, settled agriculture and stock keeping first arose in the Fertile Crescent [1,2]. This Neolithic way of life then emerged in Europe between 9000 and 6000 BP, triggered by the arrival of immigrant farmers approximately

9000 BP who originated in the Near East and substantially replaced the local hunter–gatherer population except on the Western and northern margin of the continent, where Mesolithic societies persisted longer [3–5]. These farmers were accompanied by several domesticates including sheep and goats [6], pigs [7], cows [8–9] and cultigens including wheat, barley, peas, broad beans and lentils [10].

Ascertaining the geographical origins of the animals associated with this migration is not always straightforward. While the wild progenitors of neither sheep nor goats were ever present in Europe [6], the progenitors of both pigs and cattle were extant at the time of the arrival of the Neolithic [11,12] and some studies have claimed that these taxa were locally domesticated (e.g. [13]). Assessing whether the archaeological remains of these latter animals found in Neolithic contexts were derived from Near Eastern or European populations is complicated by the fact that imported domesticates often interbred with indigenous European wild populations [14–16].

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

To test this hypothesis, we analysed 99 published mitochondrial DNA sequences of ancient dogs (http://dx.doi.org/10.5061/dryad.h55p1q5 [21]) from 37 archaeological sites across Eurasia, from the Upper Palaeolithic to the Bronze Age (electronic supplementary material, table S1, figure S1 and §1–§6). We first assessed whether a specific mitochondrial dog haplogroup was associated with Neolithic farmers. We then ascertained whether that lineage was introduced to Europe by tracking its spatio-temporal frequency (electronic supplementary material, §6).

Each of the 99 sequences was assigned to previously established dog haplogroups (Hg) (electronic supplementary material, §6, table S2 and figure S2). Individuals were then grouped into seven temporally and geographically defined categories, and we tested the existence of a genetic structure congruent with the history of the Neolithization of Europe (electronic supplementary material, §2–§6 and table S3).

Prior to the Neolithic, all European dogs possessed mitochondrial Hg C (figure 1; electronic supplementary material, figures S1–S3). The subsequent Neolithic and post-Neolithic European dogs possessed Hg A (six samples), Hg D (21 samples) and Hg C (38 samples), thus suggesting the introduction of non-indigenous domestic dogs. An AMOVA analysis (electronic supplementary material, table S3) showed that inter-regional differences account for 44.3% of the total genetic variation (electronic supplementary material, tables S4 and S5).

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

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

Considering Southeastern Europe on its own, we can reject this null hypothesis ($p < 0.01$). Using a binomial confidence interval, the lowest possible post-Neolithic frequency of Hg D in Southeastern Europe is 69% (electronic supplementary material, table S6; 95% CI 69–94%), and it would have taken more than 700 dog generations (approx. 2800 years) for drift alone to explain this increase in Hg D after the Neolithic (with $p > 0.05$) (electronic supplementary material, figure S4B,C and §6). This is much longer than the duration of Neolithization in this region [22]. Moreover, our results show that a starting frequency of more than 41% of Hg D during the pre-Neolithic period in Southeastern Europe is required for drift alone to explain this transition, over a time period of 0–700 dog generations with probability greater than 5% (electronic supplementary material, figure S4B,C and §6). Considering that our binomial confidence interval for Hg D frequency in Southeastern Europe prior to the Neolithic is between 0 and 39% (electronic supplementary material, table S6), it is highly unlikely that the observed frequency of Hg D in this region (electronic supplementary material, §6) could result from drift.

Our results indicate that the appearance of dogs possessing Hg D resulted from a human-mediated introduction of dogs to Southeastern Europe. The haplogroup D largely replaced the haplogroup C in this region, though its frequency was far lower across the rest of Europe (20.8% in Central Western Europe and 3.8% in Northwestern Europe) (figure 1; electronic supplementary material, S1 and S3).

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

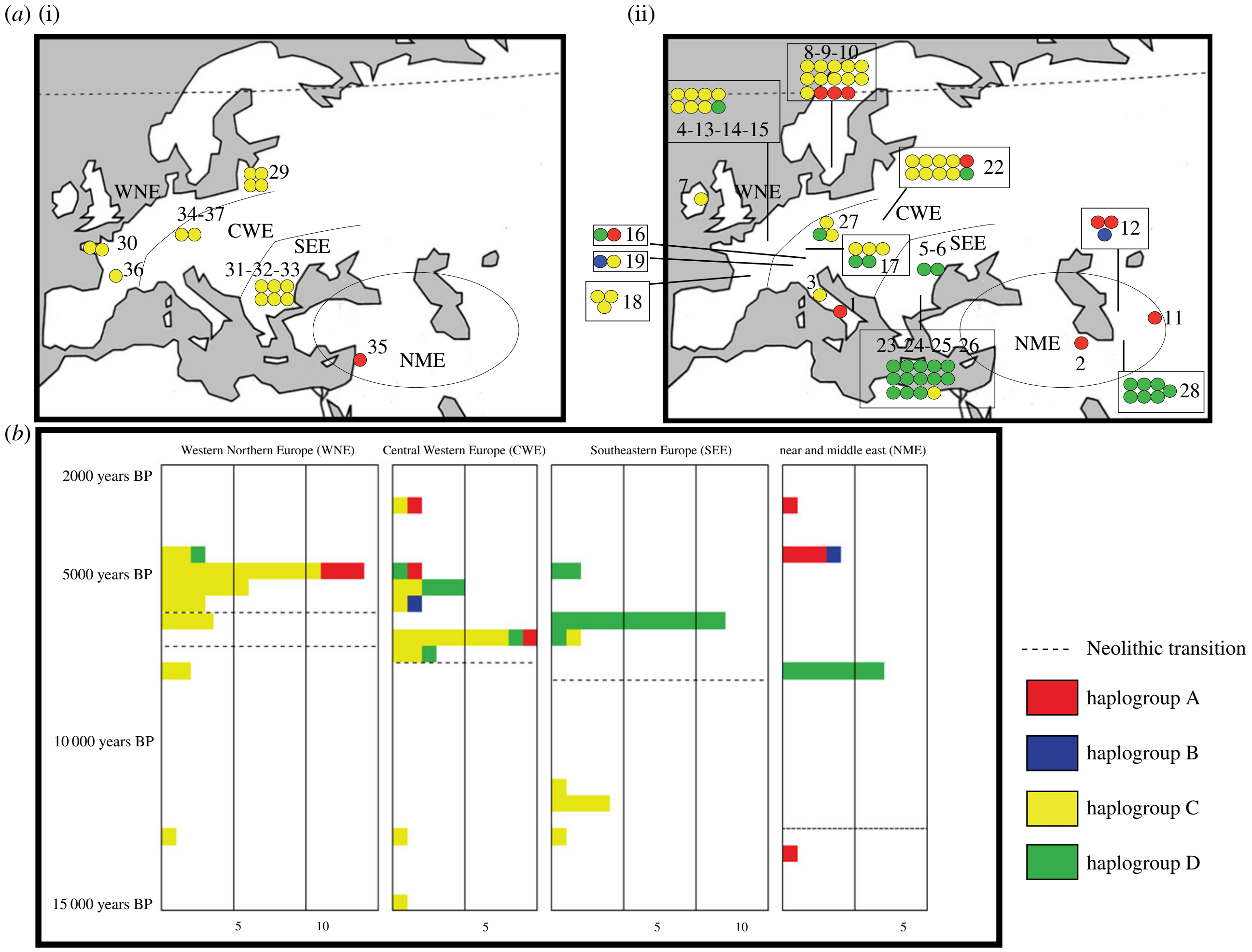

Figure 1. Genetic, geographical and chronological pattern of ancient dogs in the Middle East and Europe. *a*(i) Pre-Neolithic dogs' distribution. *a*(ii) Distribution during and after the Neolithic transition. Archaeological sites are numbered according to electronic supplementary material, table S1. (*b*) Chronological distribution of dog haplogroup frequencies among four geographical regions (according to electronic supplementary material, table S2). Red, haplogroup A; blue, haplogroup B; yellow, haplogroup C; green, haplogroup D; dashed line, Neolithic transition.

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

Data accessibility. DNA sequences are available from Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h55p1q5 [21].

Authors' contributions. M.O., A.T., L.A.F.F. and S.B. analysed the data, participated in the design of the study, coordinated the study and drafted the manuscript; G.L., C.H., C.Hi. and J.-D.V. designed the study and helped to draft the manuscript; A.Ba., M.M., A.B., M.P.-C., O.L., R.-M.A., L.B., K.D., R.R. and M.V.S. collected contextual data and edited the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We have no competing interests.

Funding. Nestlé Purina, Egide Econet Project no. 12676VE, CNRS, ENS de Lyon, Société Centrale Canine and the Romanian National Authority for Scientific Research, CNCS – UEFISCDI (no. PN-II-RU-TE-2014-4-0519) funded the project. M.P.-C. was supported by a CNRS-BDI grant. M.V.S.'s participation involved ZIN RAS funding (no. AAAA-A17-117022810195-3). L.A.F.F., O.L. and G.L. were supported by a European Research Council grant (ERC-2013-StG-337574-UNDEAD) and Natural Environmental Research Council grants (NE/K005243/1 and NE/K003259/1). L.A.F.F. was supported by a Junior Research Fellowship (Wolfson College, University of Oxford).

Acknowledgements. We thank V. Dumitraşcu (Romanian Academy of Sciences), D. Popovici (MNIR, Romania), C. Micu (ICEM Tulcea, Romania), H.O. Mollasalahi (Institute of Archaeology of University of Tehran), S. Pandrea ('Carol I' Brăila Museum, Romania), F. Haack and A. Zeeb (Germany's Directorate General for Cultural Heritage), M.S. Salehi (Institute of Archaeology of University of Tehran), Archaeological Museum of Lons-le-Saunier (France), J. Schibler (University of Basel), Cornelia Becker (Berlin University), A. Beeching (Lyon 2 University), S. Madeleine (MNP), C. & D. Mordant (Bourgogne University), A. Varlet, S. Grouard, P. Pétrequin, F. Valla, F. David, P. Chambon, O. Lecomte, M. Patou-Mathis, L. Salanova (CNRS), F. Poplin (MNHN) and Akira Tsuneki (University of Tsukuba) for their help and access to the material.

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