

Redefining the Evolutionary History of the Rock Dove, *Columba livia*, Using Whole Genome Sequences

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Associate editor: Joanna Kelley

Abstract

The domestic pigeon's exceptional phenotypic diversity was key in developing Darwin's Theory of Evolution and establishing the concept of artificial selection. However, unlike its domestic counterpart, its wild progenitor, the rock dove *Columba livia* has received considerably less attention. Therefore, questions regarding its domestication, evolution, taxonomy, and conservation status remain unresolved. We generated whole-genome sequencing data from 65 historical rock doves that represent all currently recognized subspecies and span the species' original geographic distribution. Our dataset includes 3 specimens from Darwin's collection, and the type specimens of 5 different taxa. We characterized their population structure, genomic diversity, and gene-flow patterns. Our results show the West African subspecies *C. l. gymnocyclus* is basal to rock doves and domestic pigeons, and suggests gene-flow between the rock dove's sister species *C. rupestris*, and the ancestor of rock doves after its split from West African populations. These genomes allowed us to propose a model for the evolution of the rock dove in light of the refugia theory. We propose that rock dove genetic diversity and introgression patterns derive from a history of allopatric cycles and dispersion waves during the Quaternary glacial and interglacial periods. To explore the rock dove domestication history, we combined our new dataset with available genomes from domestic pigeons. Our results point to at least 1 domestication event in the Levant that gave rise to all domestic breeds analysed in this study. Finally, we propose a species-level taxonomic arrangement to reflect the evolutionary history of the West African rock dove populations.

Key words: *Columba livia*, domestication, admixture, refugia theory, population genomics.

Introduction

The pigeon (*Columba livia*) is one of the most well-known birds worldwide due to the near ubiquitous distribution of its feral populations, and the extended breeding practices of its domestic form. The domestic pigeon, which on occasion has been bred to extreme exuberance, has been an important model organism for the study of evolution, behavior, and genotype expression, among other research areas (Helms and Bruggmann 2007). In contrast to the domestic pigeon, its parental species, the rock dove, has received relatively little attention and consequently, several unresolved questions remain. For instance, the intraspecific taxonomic classification of the rock dove is characterized by significant ambiguity, with several inconclusive or invalidated subspecies. The genetic diversity of

the rock dove remains largely unexplored, including its evolutionary history, ongoing evolutionary dynamics, and the extent of its admixture with feral pigeons (free-living birds mainly descended from domestics). Consequently, it is difficult to determine their conservation status, as feral pigeons may have replaced wild rock dove populations in several locations. Furthermore, the number of rock dove domestication events and their geographic locations remain uncertain.

Archaeological evidence suggests that the rock dove was first domesticated between 3,000 to 10,000 yr ago in either the Mediterranean Basin (Johnston 1992; Johnston and Janiga 1995; Stringham et al. 2012) or in the Fertile Crescent alongside the Neolithic Revolution (Driscoll et al. 2009). It has been argued that rock doves were probably domesticated several times and in different places

Received: May 03, 2023. Revised: October 10, 2023. Accepted: November 03, 2023

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spanning their natural distribution range, possibly over a period of thousands of years (Johnston 1992; Johnston and Janiga 1995; Shapiro and Domyan 2013). After an early domestication phase, humans spread domestic pigeons throughout Eurasia and North Africa where they diversified (Pacheco et al. 2020). Today, there are over 350 recognized domestic breeds, the majority of which are believed to have originated in the Middle East and South Asia. However, extensive admixture among domestic breeds and between domestic and wild populations make it difficult to study the rock dove domestication history (Shapiro et al. 2013).

The historical native range of rock doves covered extensive areas of Europe, North Africa, the Middle East, and South Asia, as well as the seacoasts from the Faroe Islands and Britain, to Madeira, the Canaries, and the Cape Verde islands, associated to nesting sites on rock faces and cliffs (Cramp 1985; Johnston 1992; Johnston and Janiga 1995; Gibbs et al. 2001; Shapiro and Domyan 2013; Urban et al. 2014). However, this long domestication history and the challenges distinguishing wild from feral populations make it difficult to accurately estimate their present and past distributions. For example, in some regions, such as most of Continental Europe, the extirpation of natural populations, or their complete absorption by the expanding feral birds, hinders the identification of the wild rock dove geographic range (Cramp 1985; Johnston and Janiga 1995; Gibbs et al. 2001). Furthermore, it has even been suggested that true wild rock dove populations only remain in locations that are outside of the range of feral populations (Johnston et al. 1988; Johnston and Janiga 1995). This is in line with a recent genomic study showing that rock doves from 1 of the regions believed to harbor wild populations in the British Isles had genetic admixture from feral and/or domestic pigeons (Smith et al. 2022).

Phenotypic variability such as the size and coloration of the rock dove is geographically structured (Cramp 1985), and has been used to categorize their populations into subspecies (Johnston 1992). At present, most authors recognize 9 subspecies: *C. l. livia*, *C. l. gaddi*, *C. l. palaestinae*, *C. l. schimperi*, *C. l. targia*, *C. l. dakhale*, *C. l. gymnocyclus*, *C. l. neglecta*, and *C. l. intermedia* (Goodwin 1977). Additionally, at least 3 more inconclusive subspecies have been proposed: *C. l. canariensis* from Canaries Islands, *C. l. atlantis* from Azores Islands, Madeira and Cape Verde, and *C. l. nigricans* from Mongolia and Northwest China (Cramp 1985; Gibbs et al. 2001; Urban et al. 2014). However, it has been debated whether these inconclusive subspecies have feral origins (Dickinson and Remsen 2013), or if they are simply morphological variants of the *C. l. livia* subspecies, as it is the case of the Subtropical Atlantic Islands rock doves (Murton and Clarke 1968; Cramp 1985; Gibbs et al. 2001).

Nevertheless, given that the morphological characteristics used to describe rock dove subspecies are clinally distributed, it is challenging to define the range limits of the subspecies, sometimes morphologically indistinguishable

between adjacent populations (Goodwin 1977). Thus, there is little consensus about the subspecies distribution ranges (Johnston 1992). Taking this into consideration, as well as the contiguously continental distribution of rock doves, the currently recognized subspecies could be questioned in terms of their evolutionary relevance (Mayr 1982).

Regarding their current conservation status, the IUCN Red List of Threatened Species lists the rock dove in the “Least Concern” status with decreasing populations (2022) due to its extremely large range and population size. However, it has been argued that it is difficult to accurately evaluate the rock dove’s conservation status, due to the high degree of admixture with feral populations (Johnston and Janiga 1995; Baptista et al. 1997). Furthermore, it has been suggested that the main threat to rock doves is the dissolution of their genetic pool, derived from genetic introgression from feral pigeons, which could lead to their imminent extinction within this century (Johnston et al. 1988; Johnston 1992; Johnston and Janiga 1995).

To explore the diversity and evolutionary history of the rock dove, we generated whole-genome sequencing data from 65 historical rock doves collected between 1865 and 1986, at localities spanning the species’ original distribution range. These samples represent all currently recognized subspecies. Our dataset includes 3 specimens from Darwin’s collection (collected in Madeira, Sierra Leone, and the Shetland Islands), and the type specimens of 5 different taxa: *C. l. atlantis* Bannerman, 1931, *C. l. canariensis* Bannerman, 1914, *C. l. butleri* Meinertzhagen, 1921 (currently merged with *C. l. shimperi*), *C. l. dakhlae* Meinertzhagen, 1928, and *C. l. lividor* Bates, 1932 (currently merged with *C. l. gymnocyclus*). We used these genomes to study the diversity of rock dove populations, taking advantage of the extensive available collections, and under the expectation of finding lower levels of admixture with feral pigeons in historical (as opposed to modern) specimens, particularly in populations that distributed far from significant human influence at the time of collection. Simultaneously, in combination with previously published genomes from domestic pigeons, we used our new dataset to re-investigate the origin of the domestic pigeon lineages. Moreover, we used our results to propose a model for the evolution of rock doves and their domestication. Finally, our results allowed us to confirm the feral origin of the subspecies *C. l. atlantis* and *C. l. canariensis*, identify the possible feral origin of *C. l. dakhlae* subspecies, and propose that *C. l. gymnocyclus* should be considered a full species, *Columba gymnocyclus* Gray, 1856.

Results

Rock Dove Genetic Population Structure Follows Geography

We produced whole genome sequencing data from 65 historical samples spanning their historical geographical range (Fig. 1) to a depth of coverage of 0.8 to 7.89×

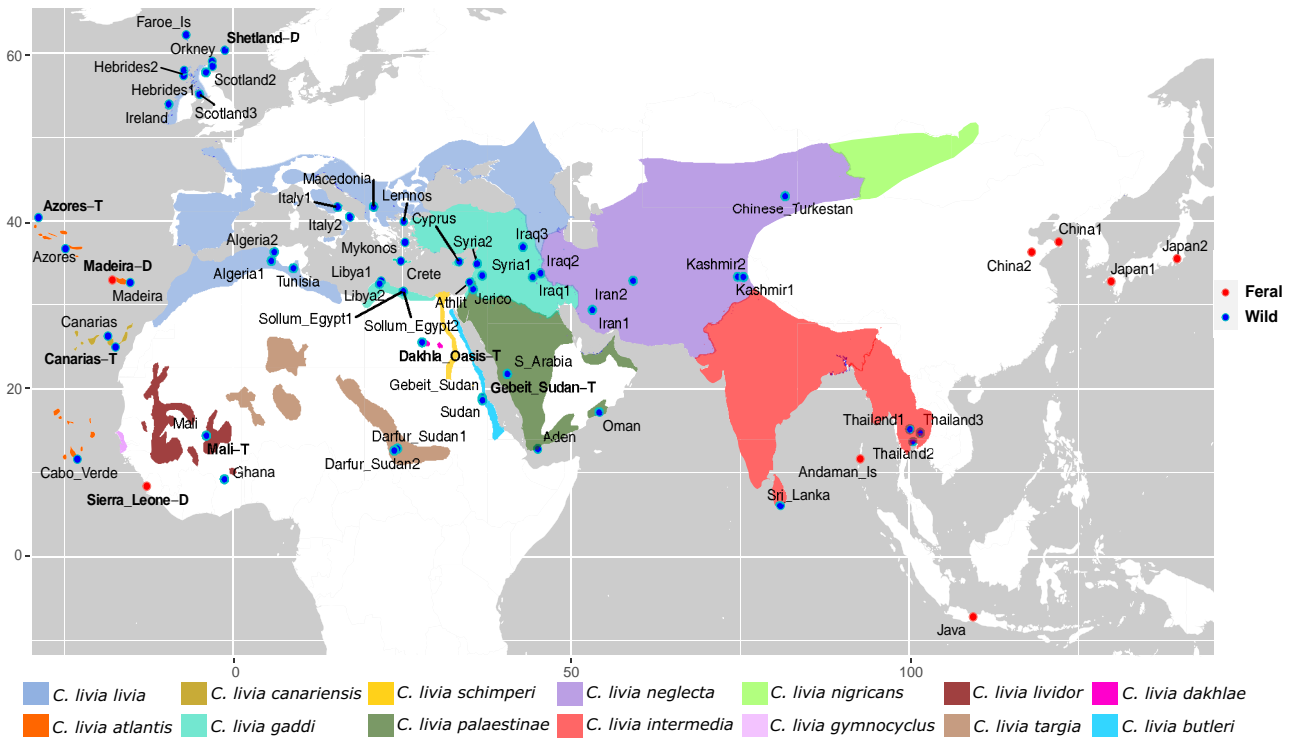


Fig. 1. Geographic distribution of rock dove subspecies, and location of the specimens sequenced in this study. The geographic distribution of currently recognized and not recognized subspecies of the rock dove (*C. l. atlantis*, *C. l. canariensis*, *C. l. lividor*, *C. l. nigricans*, and *C. l. butleri*) is shown in the map. The differently colored areas represent the estimated ranges of each subspecies, as described by Peters in 1931. The map displays the approximate locations of the specimens labeled as wild rock doves and feral pigeons according to the information from museum descriptions. Darwin's collection specimens (D) and the type specimens (T) are shown in bold font. To enhance clarity, the size of the Tropical Atlantic Islands has been increased.

(supplementary table S1, Supplementary Material online). To account for the low depth of coverage of some samples, a pseudo-haploid SNP (single nucleotide polymorphisms) panel was created using the historical samples together with genomic data from 39 publicly available domestic pigeons, 2 modern feral pigeons, 5 related Columbidae species (*Patagionas fasciata*, *C. palumbus*, *C. larvata*, *C. guinea*, *C. rupestris*) and 1 common pheasant (*Phasianus colchicus*) (supplementary table S2, Supplementary Material online). To avoid the introduction of potential biases associated with post-mortem DNA damage in the historical samples, transitions were removed, resulting in a final dataset of 1,642,881 transversion sites.

As an initial step to characterize the genetic diversity of rock doves, we performed a multidimensional scaling analysis (MDS). Broadly, the majority of the samples are distributed across dimension 1, with the domestic pigeons at 1 side and the historical rock doves at the opposite side of the distribution. Several of our historical samples that were originally labeled in the collection as “feral” are placed in the middle of the 2 clusters, as could be expected from admixed individuals. A divergent third cluster differentiates along dimension 2 and contains the historical rock doves from the western part of Africa (Mali, Ghana), including individuals of the *C. l. gymnocyclus* subspecies and the type

specimen for *C. l. lividor* (Mali-T) (Fig. 2A). Simultaneously, we conducted a principal components analysis (PCA) based on genotype likelihoods (supplementary fig. S2, Supplementary Material online) to evaluate the accuracy of the pseudo-haploid dataset. The obtained structure among the samples displayed remarkable similarity with the MDS analysis.

To explore the population structure of the rock dove and pigeon genomes in more detail, we estimated a second MDS plot excluding the West African rock doves (Fig. 2B). Similar to the previous MDS plot, domestic pigeons and historical rock doves are separated in dimension 1, with the historical feral pigeons placed between these 2 clusters. We observed that some of the historical rock doves, originally described as wild based on their geographic location and morphological data, fall within the distribution of feral pigeons, suggesting they are in fact feral or admixed (e.g. the *C. l. dakhlae* type specimen Dakhla_Oasis-T). Historical rock doves are separated by dimension 2, with the North Atlantic Islands rock doves (including the Shetland specimen from Darwin's collection) and the rock doves from the Red Sea and Arabian Peninsula (including the type specimen of *C. l. butleri*, Gebeit_Sudan-T) placed at opposite ends of the distribution. The remaining samples, placed at the center of the historical rock dove cluster are from the Mediterranean Basin, Sahara, Middle East, Central

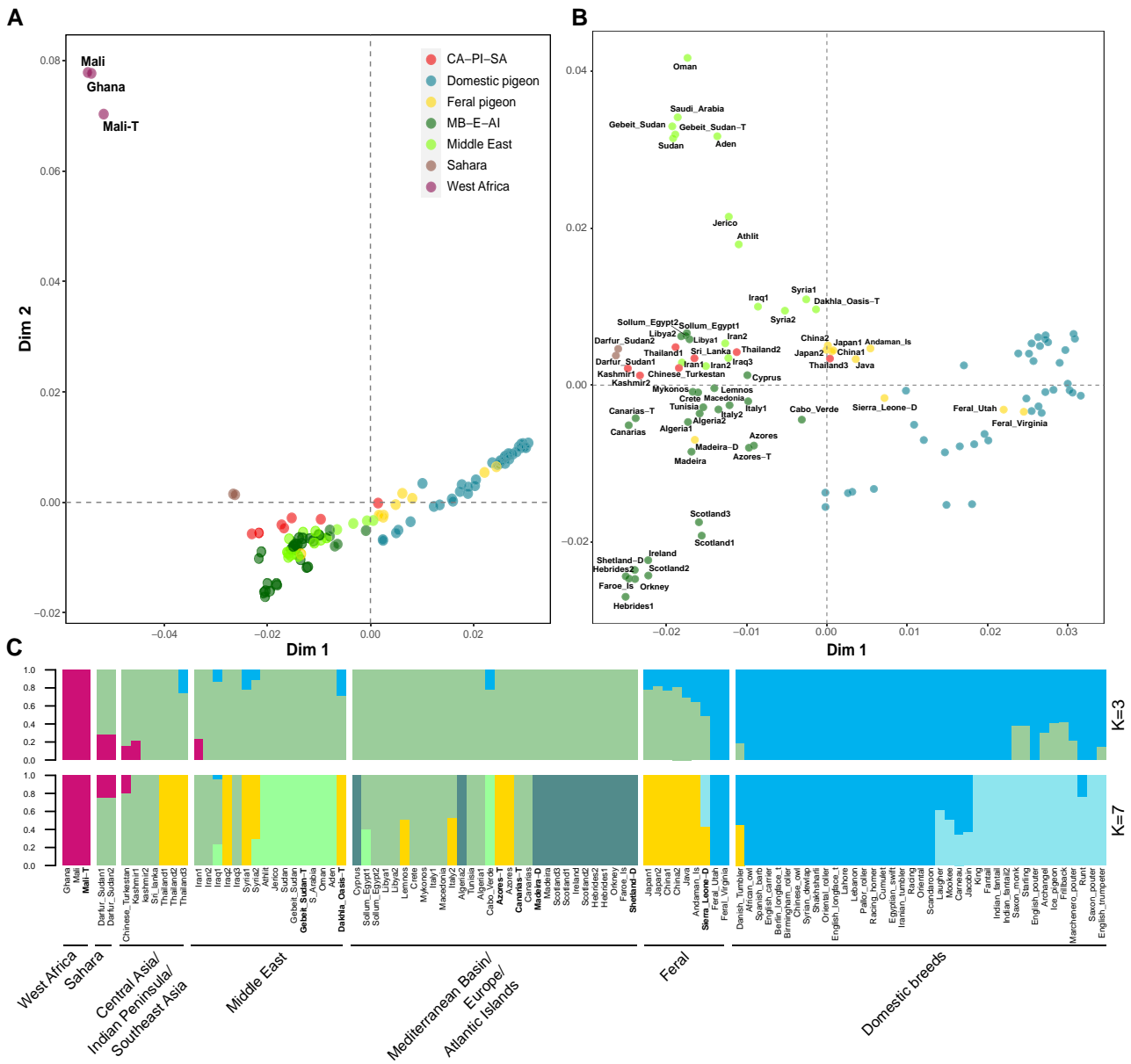


Fig. 2. Rock dove population structure reveals a divergent population in West Africa. A-B) MDS plots generated from pseudo-haploid data (1,642,881 transversion sites). a) MDS plot with all samples in our dataset, excluding the outgroups. Domestic breeds and historical rock doves are separated by dimension 1. West African rock doves appear divergent and separate on dimension 2. Colors represent the feral and domestic pigeons, as well as the geographic origin of the rock doves. CA-PI-SA stands for Central Asia, Peninsular India, and Southeast Asia; MB-E-AI stands for Mediterranean Basin-Europe-Atlantic Islands. Estimated *Stress* -1 value: 0.195. b) MDS plot excluding the divergent West African rock doves, showing the population structure among the rock doves. Estimated *Stress* -1 value: 0.221. c) Model-based clustering using ADMIXTURE assuming 3 and 7 ancestry components (K). Horizontal bars show different samples. Different colors show the estimated ancestry components and corresponding proportions. At K = 7. Darwin's collection specimens (D) and the type specimens (T) are shown in bold.

Asia, Sri Lanka, and Thailand. Rock doves from subtropical Atlantic Islands are placed at the bottom part of this central cluster, close to the north-western African rock doves, and separated by dimension 1. Notably, one of Darwin's collection specimens was described as feral (Madeira-D) and the subspecies *C. l. canariensis* (Canarias-T) and *C. l. atlantis* (Azores-T) type specimens, currently thought to be feral, are also placed close to the Subtropical Atlantic Islands rock dove group (Fig. 2B).

To further explore the population structure in the dataset, we performed an admixture analysis to estimate individual

ancestries assuming from 2 to 10 ancestry components (Fig. 2C and supplementary fig. S4, Supplementary Material online). When 2 ancestry components were estimated, we obtained 1 component that is mostly represented among domestic pigeons, and a second that is predominantly present among historical rock doves. Historical feral pigeons carry ancestry components from both clusters. In line with the MDS analysis, some of the historical rock doves (those placed close to the historical feral pigeons in the MDS plot) carry a fraction of ancestry from the same component identified in the domestic pigeons, including the type specimen

Dakhla_Oasis-T (supplementary fig. S4, Supplementary Material online). When we extended the analysis to 3 ancestry components, all the West African rock doves (whether labeled as *C. l. gymnocyclus* or *C. l. lividor*) formed a new independent cluster, consistent with the MDS analysis. Even though most samples retained similar ancestry proportions when assuming 2 or 3 components, some historical rock doves present a fraction of ancestry from the West African cluster, with the genomes of the rock doves from Darfur, in western Sudan exhibiting the highest proportions (Fig. 2C). One potential explanation for the absence of the West African rock dove ancestry component when estimating 2 ancestry components, as expected based on the MDS results, is the limited number of West African rock dove specimens. Finally, when estimating 7 ancestry components, the specimens are split into clusters that resemble the geographical patterns found in the MDS plot (Fig. 2B). The West African rock doves carry an ancestry component that is only shared with the samples from Darfur, Sudan (supplementary fig. S4, Supplementary Material online). The feral birds get their own ancestry component, potentially representing the mixture of wild and domestic ancestry, with the samples from Thailand, Cape Verde, Azores, the Dakhla Oasis (Egypt), and 1 Iraqi sample (Iraq2) also carrying a proportion of this ancestry. Some other samples share this feral ancestry component, which could reflect different levels of domestic pigeon admixture in wild populations. The other ancestry components recovered among the historical rock doves broadly correspond to rock doves from (i) the Near East and the Arabian Peninsula/Red Sea; (ii) the North Atlantic Islands and Madeira (including the samples Cyprus and Algeria2), and (iii) the Mediterranean Basin, Asia, and Canary Islands, as well as Darfur, Sudan (Fig. 2C).

The Evolutionary Relationships of Rock Doves Reveal Basal Placement of the West African Populations

To understand the evolutionary relationships among the historical rock doves, feral pigeons, and domestic breeds, we built a Neighbor-Joining (NJ) tree based on identity-by-state pairwise-distances (Fig. 3A and supplementary S5, Supplementary Material online). The internal branches of the rock dove and domestic breed clades are short, indicating low levels of differentiation among the populations (supplementary fig. S5, Supplementary Material online). Rock doves are grouped into clades that clearly reflect their geographic origin (Fig. 3A). The West African rock doves are placed as basal to all other rock doves, followed by the rock doves from the Sahara (Darfur, Sudan), Central Asia and Kashmir, and Sri Lanka, the Middle East and Red Sea region, and a clade including the Mediterranean Basin and Atlantic islands. In the tree, all domestic breeds appear as monophyletic and closely related to the Middle East and Red Sea rock dove's clade.

The historical feral pigeons form a clade together with the domestic pigeons, except for the sample Madeira_D, which appears among other Atlantic Islands rock doves. The specimens from Thailand are also placed within the feral pigeon's

clade, indicating their admixed status. The modern feral pigeons from the United States are closely related to the domestic pigeons as it was reported in a previous study by Shapiro et al. (2013). One notable observation is that the *C. l. dakhlae* type specimen is basal to all domestic and feral pigeons. The Near East, Red Sea, and Arabian Peninsula rock doves form a cluster that is placed as a sister clade of the domestic and feral pigeons. Also interesting is the placement of the rock doves from the Atlantic Islands, which are located within the Mediterranean Basin's clade, suggesting a close relationship between them. Rock doves from the Atlantic Islands are further divided into 2 clades: North Atlantic, and Subtropical Atlantic islands rock doves, with the *C. l. atlantis* and *C. l. canariensis* types specimens placed within the latter. Finally, the clade with the Atlantic Islands rock dove is placed as a sister clade of the rock doves from north-western Africa, Algeria, and Tunisia (Fig. 3A).

We additionally estimated a maximum likelihood (ML) phylogeny summarizing 1,000 independent trees. Overall, the estimated tree topology is similar to the NJ tree and shows short internal branch lengths (supplementary fig. S6, Supplementary Material online). The specimens from West Africa are placed at the base of the rock doves, and this partition is supported by a high bootstrap value. Another relevant similarity is that all domestic pigeons appear monophyletic, and are closely related to the feral birds, with the specimen Syria1 placed at the base of this clade. Some of the differences we observed between the NJ and ML approaches are the placement of the Saharan rock doves, which does not appear as basal, and previously observed geographic clades appear fragmented and mixed with rock doves from diverse geographical origins. These differences could be due to the low divergence in the genomic regions used for building the phylogeny and possible admixture with domestic or feral lineages (supplementary fig. S6, Supplementary Material online).

Extensive Introgression Between *C. rupestris* and the Rock Dove

Our phylogenetic results show western African rock doves are basal to all other rock doves in the dataset (Fig. 3A), which is unexpected given the proposed origin of the species in Asia (Johnston and Janiga 1995). We used the *D*-statistic (Patterson et al. 2012) to test the possibility of hybridization between the West African rock doves and other species from the *Columba* genus, as a possible explanation for their basal position in the tree. In particular, we investigated the possibility of hybridization with the speckled pigeon *C. guinea*, which overlaps in distribution with the West African rock doves and shares the highly developed red orbital ring phenotype (Baptista et al. 2009). We computed a *D*-statistic of the form $D(C. palumbus, C. guinea; \text{West African rock doves, X})$, where *C. palumbus* is used as an outgroup, the West African rock dove specimens are grouped into a single population, and X represents all rock dove, feral pigeons and domestic pigeons in our dataset. If there has been gene flow between the

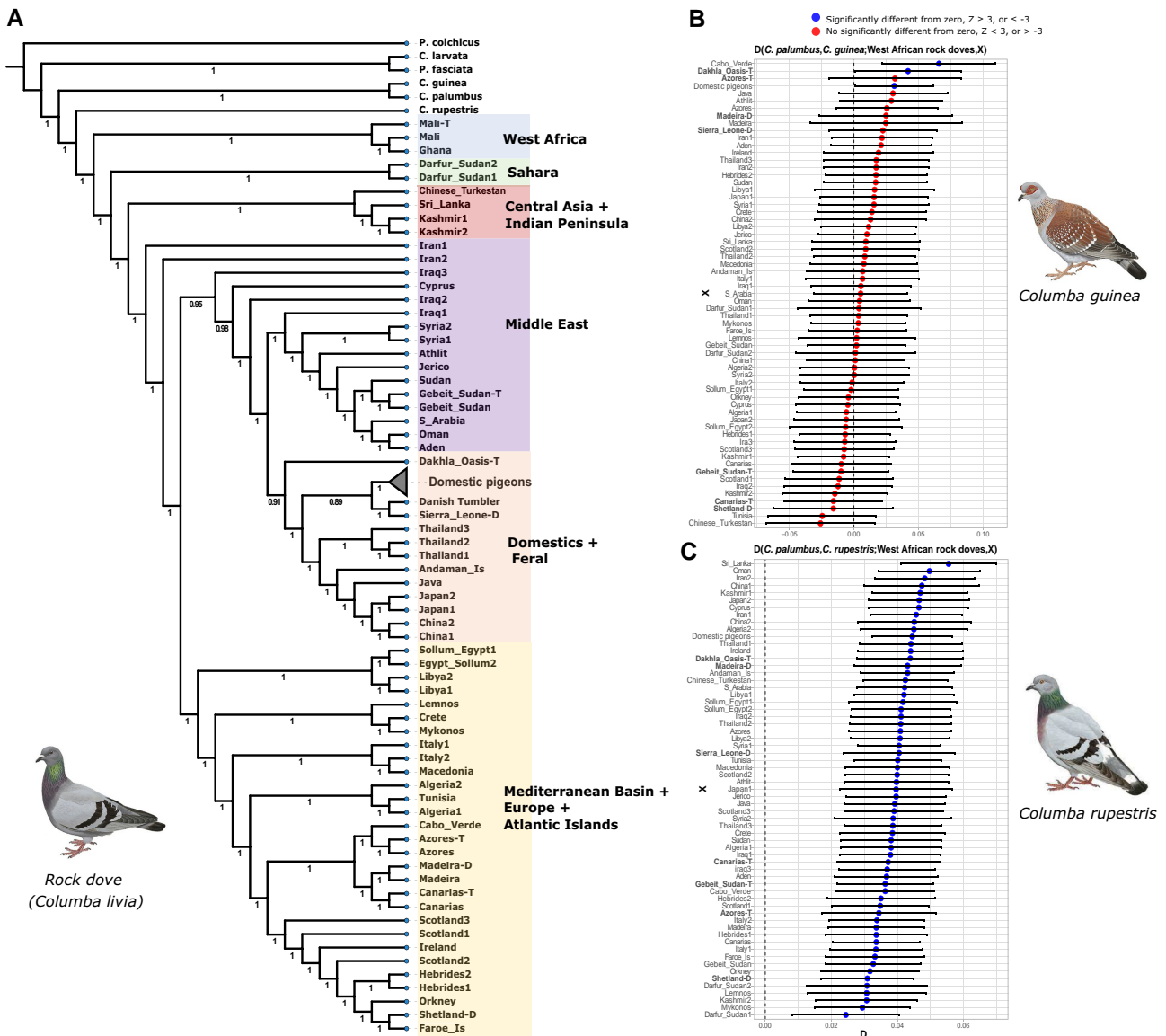


Fig. 3. Phylogenetic relationships among wild and domesticated pigeons. a) NJ tree based on genomic pairwise-distances. Branch length was disregarded in the tree representation (see also [supplementary fig. S5, Supplementary Material](#) online). Bootstrap values are shown next to each internal node. Different colors indicate the main geographical regions. The domestic pigeon breeds are shown as a collapsed clade. b and c) *D*-statistic analyses testing the possibility of hybridization in the West African rock doves that could explain its basal placement in the tree. b) *D*-statistic test in the form $D(C. palumbus, C. guinea; \text{West African rock doves, } X)$, where *C. palumbus* has been used as an outgroup, all West African rock doves are grouped in a single population, and *X* represents all other rock doves and domestic pigeons in the dataset. c) *D*-statistic of the form $D(C. palumbus, C. rupestris; \text{West African rock doves, } X)$ testing for hybridization between rock doves and its sister species *C. rupestris*. Horizontal bars show 3 standard errors estimated through a block jackknife approach. Tests with a resulting $|Z\text{-score}| > 3$ were considered statistically significant.

West African rock doves and *C. guinea*, we expect significant negative *D* values in all tests. Overall, our results provide no evidence of gene flow between the West African rock doves and *C. guinea* (Fig 3B). The only statistically significant tests suggest gene flow between *C. guinea* and the domestic pigeons, as well as between the specimens from Cape Verde and the Dakhla Oasis (Fig 3B), both closely related to domestic breeds according to our admixture results (Fig 2C). Patterns of admixture between *C. guinea* and domestic breeds were reported in Vickrey et al. (2018). To further explore possible hybridization in the West African rock doves that could explain its basal

position in the tree, we tested for gene flow with the hill pigeon *C. rupestris*. To do so, we computed a *D*-statistic of the form $D(C. palumbus, C. rupestris; \text{West African rock doves, } X)$. Our results indicate a clear signal of gene flow between *C. rupestris* and all samples in *X*, except for the West African rock doves (Fig 3C).

Sahara Rock Doves Show Admixture Signals With the West African Rock Doves

Saharan rock dove (*C. l. targia*) populations are isolated in the mountainous regions of the Sahara Desert. Our dataset includes 2 specimens from Darfur, Sudan, in the western

Sahara (Darfus_Suda1 and Darfur_Sudan2), which in previous results show affinity with the West African rock doves (Fig. 2A and 2C). In order to assess the possibility of gene flow between the Sahara and the West African rock doves, we performed a *D*-statistic test of form *D* (*C. palumbus*, West African; Sahara, X) (supplementary fig. S8, Supplementary Material online). If the Sahara rock doves are not admixed with the West African rock doves, we expect to obtain nonstatistically significant *D* values. Conversely, if the Sahara rock doves are admixed with the West African rock doves, the expected *D* values would be negative and statistically significant. Even though the majority of the test results are not statistically significant, we observe a trend where all tests resulted in negative *D*-statistic. These results suggest that the Sahara rock doves from Darfur, Sudan share more alleles than expected with the West African rock doves when compared to some rock doves from different geographic locations.

Domestic Pigeons are Closer to Wild Populations From the Middle East

The exact geographic location of the rock dove's domestication remains debated. Our phylogenetic results show domestic pigeons are closely related to the Middle East clade (that includes samples from the Red Sea region), followed by the Mediterranean Basin-Europe-Atlantic Islands (MB-E-AI) clade (Fig. 3A). We used the *D*-statistic to formally test if the domestic pigeons formed a clade with the Middle East (as suggested in the NJ tree) or with the MB-E-AI rock doves. If the topology obtained in the NJ tree is correct, we expect all tests of the form *D*(*C. palumbus*, MB-E-AI rock doves; Middle East rock doves, Domestic pigeons) to yield nonsignificant results ($D \sim 0$). Additionally, we expect significant results ($D > 0$) if we switch the positions of MB-E-AI and Middle East rock doves in the tests. We found that when Middle East rock doves are placed as a clade with the domestic pigeons, most tests yield nonsignificant results, particularly when comparing specimens from Syria, Palestine (Althlit and Jerico) and the specimen Iraq1 (Fig. 4). In contrast, when placing the MB-E-AI rock doves as a clade with the domestic pigeons, most tests yield statistically significant results. This pattern is especially clear when using the rock doves Syria1, Syria2, Althlit, Jerico, and Iraq1, suggesting these are the most closely related populations to the domestic breeds.

The Type Specimen *C. l. dakhlae* has an Admixed Origin

The *C. l. dakhlae* subspecies is restricted to the Dakhla and Kharga Oases in Central Egypt and is characterized by its very light homogeneous coloration (Cramp 1985; Dickinson and Remsen 2013; Urban et al. 2014). We leverage the fact that our dataset contained the *C. l. dakhlae* type specimen (Dakhla_Oasis-T) to investigate the ancestry of this subspecies. Our results show a close relationship of the *C. l. dakhlae* type specimen to the domestic breeds (Fig. 2B and 3A). We implemented a *D*-statistic analysis

to decipher if this similarity is due to (i) hybridization with domestic breeds, (ii) being ancestral to domestic breeds, or (iii) being an ancient domestic lineage. In this case, the *D*-statistic tests were performed of the form *D* (*C. palumbus*, X; Dakhla_Oasis-T, Domestic pigeons) and *D*(*C. palumbus*, Domestic pigeons; Dakhla_Oasis-T, X), where X represents all historical rock doves in the dataset. For the first set of tests, if Dakhla_Oasis-T belongs to the ancestral lineage of all domestic breeds or to an ancient domesticated lineage, we would expect to obtain nonstatistically significant *D* values. Alternatively, if the Dakhla_Oasis-T is a hybrid between domestic breeds and wild rock doves, we expect statistically significant results showing varying levels of affinity between the historical rock doves and the domestic pigeons. The results suggest that the Dakhla_Oasis-T is admixed, and shares more genetic drift with rock doves from similar geographic regions, mainly with specimens from the Red Sea region (S_Arabia, Aden, Sudan, Gebeit_Sudan, Gebeit_Sudan-T, and Oman) when compared with the domestic pigeons. However, in the remaining cases, the *D* values were nonstatistically significant, suggesting a higher affinity between the Dakhla_Oasis-T and domestic pigeons than with the other rock doves in the dataset (supplementary fig. S9, Supplementary Material online). For the second set of tests, if Dakhla_Oasis-T's main ancestry derives from wild rock doves, we expect to obtain statistically nonsignificant results. Alternatively, if Dakhla_Oasis-T main ancestry derives from domestic breeds, we expect to obtain negative statistically significant results when compared to the rock doves. The results show that Dakhla_Oasis-T shares more genetic drift with domestic breeds than with any other rock dove in the dataset. This confirms that the main ancestry of sample Dakhla_Oasis-T comes from domestic breeds.

Rock Doves' Genetic Diversity and Population Differentiation

To characterize the genetic differentiation of West African rock doves from other rock dove populations, we calculated pairwise weighted *F_{st}* among 12 populations from distinct geographic regions (North Atlantic Islands, Tropical Atlantic Islands, Northwest Africa, Mediterranean, Northeast Africa, Levant, Middle East, Arabian Peninsula, East Africa, Central Asia, and Thailand) (supplementary table S3, Supplementary Material online). The results reveal strong genetic differentiation ($F_{st} > 0.3$) between the West African and all other rock dove populations, confirming previous results (Fig. 2A). In contrast, when comparing the remaining rock dove populations, we observed low levels of genetic differentiation ($F_{st} < 0.1$). An exception was observed when comparing the Arabian Peninsula or East African rock dove populations to the Thailand or North Atlantic Island populations, which showed slightly higher *F_{st}* values ranging from 0.110 to 0.129, possibly due to geographic structuring.

Additionally, we calculated global per-sample heterozygosity based on genotype likelihoods for all the historical

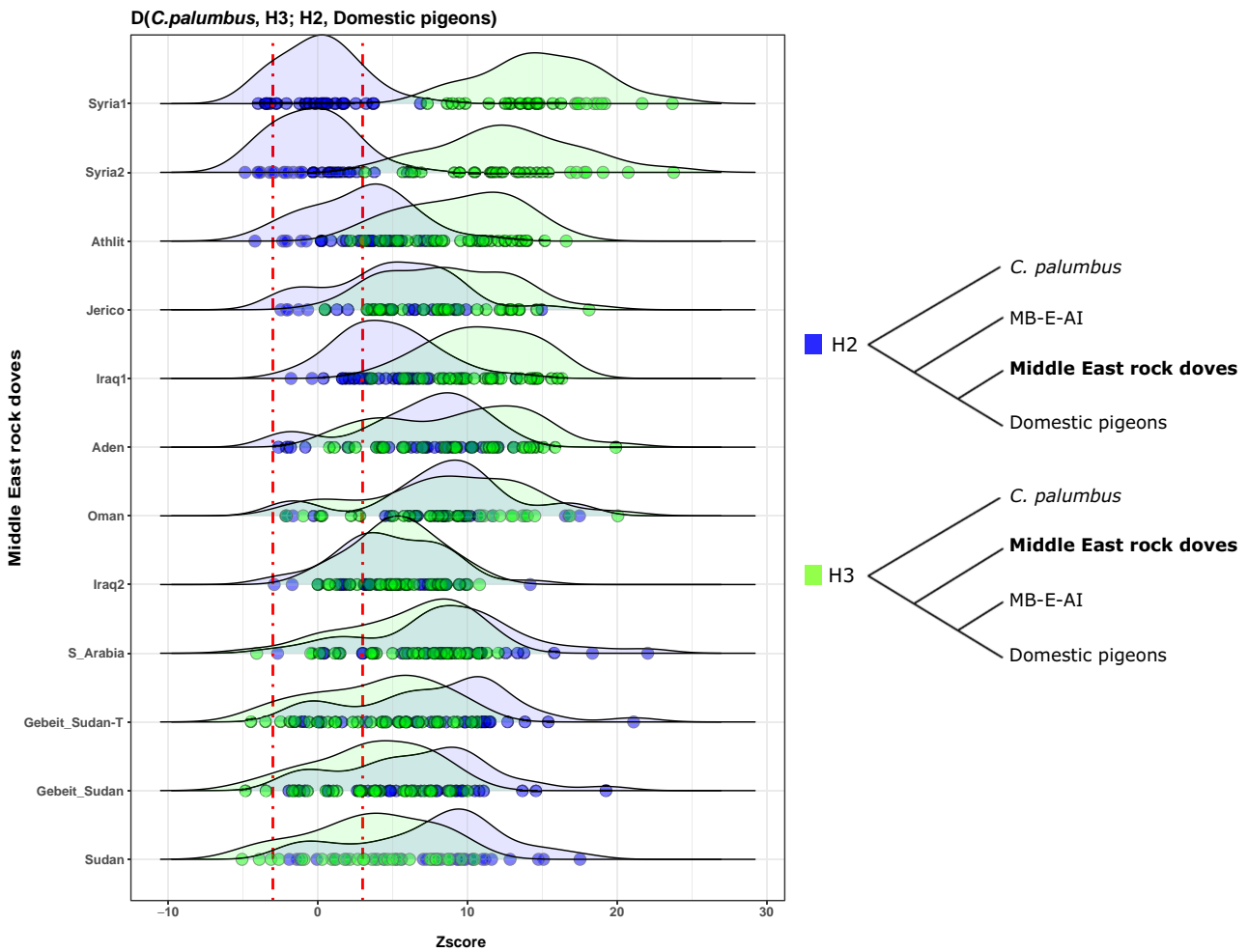


Fig. 4. Evaluating the position of the domestic pigeon breeds in the tree using D -statistic. Density plots showing the Z-scores distributions obtained from the D -statistic analyses testing the tree topology between the: domestic pigeons, Middle East rock doves, and rock doves from the MB-E-AI. The tests were performed for each sample individually, except for the domestic pigeons that were grouped in a single population. The 2 tree topologies tested are shown as diagrams in the right side of the figure. When Middle East rock doves were tested as an outgroup to the domestic pigeons and MB-E-AI rock doves, Z-scores were overall more positive and statistically significant, mainly for populations from Syria, Palestine, and Iraq. In contrast, when Middle East rock doves were placed as forming a clade with the domestic pigeons, Z-scores values were lower, and a higher proportion was nonstatistically significant. Dots represent the Z-score obtained for each MB-E-AI specimen tested. Dotted lines in the x axis show the range for nonstatistically significant values ($|Z| < 3$).

rock doves and feral pigeons in our dataset ([supplementary fig. S10A, Supplementary Material](#) online). Overall, the result showed consistently low global heterozygosity values across all samples (0.050 to 0.088), regardless of the sample age or the wild/feral status. Interestingly, the lower obtained global heterozygosity values correspond to the West African specimens (Ghana: 0.050; Mali: 0.056; and Mali-T: 0.059).

Individual inbreeding coefficients (F) based on genotype likelihoods were estimated for both historical rock doves and feral pigeons to evaluate their genetic diversity ([supplementary fig. S10B, Supplementary Material](#) online). While typical inbreeding coefficients fall within the range of 0 to 1, we also considered negative estimates due to their biological significance (Wang 2014). The results revealed a diversity of estimated F values, with some samples showing high inbreeding while others displayed outbreeding tendencies ($F = -0.045$ to 0.516). The West African rock doves exhibited the highest F values (>0.4), followed by

the Sahara rock doves and the sample Azores-T (>0.3). Certain geographical regions showed similar F values within their samples, such as Thailand ($F = 0.095$ to 0.157), Near East and Iran ($F = -0.043$ to 0.0004) (except for sample Syria2), Red Sea and Arabian Peninsula ($F = 0.078$ to 0.145), Mediterranean ($F = -0.045$ to 0.049), and Atlantic Islands ($F = -0.001$ to 0.078) (except for the samples Cabo_Verde and Azores, which obtained higher values). Notably, feral pigeons obtained similar F values above 0.1.

Discussion

Reconstructing the Evolutionary History of Rock Doves Through a Hypothetical Scenario based on the Refugia Theory

It has been proposed that the rock dove's geographic origin is in Asia based on the geographic distribution of its closest species, *C. rupestris* (Johnston and Janiga 1995). Therefore,

our finding that rock doves from West Africa are basal to all other rock doves is unexpected. Although this placement could be explained by hybridization of West African rock doves with *C. guinea*, our *D*-statistic tests suggest this is not the case. To explain this observation, we contextualized our results in light of the Pleistocene climate changes in the Sahara and the Sahel regions.

Global climate fluctuations during the Pleistocene glacial periods produced drastic changes in the distribution and abundance of flora and fauna, leading to fragmented species distributions, population reductions, high extinction rates, and speciation events (Mayr and O'Hara 1986; Hewitt 1996; Le Houérou 1997; Hewitt 2000; Weir and Schluter 2004; Lovette 2005; Nadachowska-Brzyska et al. 2015). Since the Pleistocene, the Sahara and Sahel underwent several dry and wet cycles associated with the glacial and interglacial periods (Le Houérou 1997; Larrasoña et al. 2013; Manning and Timpson 2014), with an estimate of 8 to 10 arid-wet periods during the last 125,000 yr alone (Le Houérou 1997). During the last glacial maximum (LGM), ca.18,000 yr ago, the Sahara region had a hyperarid period, where the desert area extended to the south (Larrasoña et al. 2013), and today's West African forest areas were covered by open woodlands and grasslands with a semi-arid climate (Nichol 1999). This hyperarid period was preceded and followed by what is known as Green Sahara Periods (GSPs) (Larrasoña et al. 2013).

We hypothesize that these cycles impacted the distribution of *C. livia* during the LGM, by expanding and contracting their geographic distributions. To explain our phylogenetic and admixture results we propose a 4-step scenario. First, we hypothesize that before the LGM, *C. livia* had a continental distribution similar to that of the modern wild members of species, including the Sahara region (Fig. 5A). While their geographic range previous to the LGM is unknown, in light of their high dispersal capacity and adaptability, it would not be surprising if the species were as widespread as it is today. Certainly, the basal position of West African rock doves in our results suggests that its distribution reached the Western region of Africa.

Second, during the LGM, the southward advance of the ice sheet in northern latitudes and the hyperarid conditions in the Sahara and Sahel, would have drastically diminished rock dove populations worldwide, leading to the formation of at least 2 main allopatric groups: 1 in West Africa and 1 in Central Asia (Fig. 5B). This is consistent with previous evidence showing a decrease in the effective population size of rock doves during the LGM (cf. Nadachowska-Brzyska et al. 2015). Several populations could have survived in different Pleistocene refugia; however, our data do not show evidence of populations that diverged in allopatry other than the West African populations. During this isolation period, we hypothesize that the Central Asian rock doves would have hybridized with *C. rupestris* (Fig. 5B), leading to the patterns observed in our *D*-statistic tests (Fig. 3C). These tests show that all rock doves (including domestic breeds) share significantly more alleles of *C. rupestris* when compared with the West

African populations. The fact that samples from distant locations show the same admixture pattern suggests that the *C. rupestris* admixture was acquired by common ancestry. The rock dove and *C. rupestris* have overlapping distribution in Central Asia, making hybridization possible (and indeed actual events of hybridization between *C. rupestris* and *C. livia* have been reported by Johnston and Janiga 1995).

Third, favorable environmental conditions after the LGM would have allowed the Central Asian rock doves to spread and recolonize previously occupied areas. Central Asian rock dove population spread after the LGM and gave rise to the modern rock dove populations in Asia, Europe, North Africa, the Sahara, East Africa, and the Atlantic Islands. The latter is supported by the low genetic diversity and differentiation observed in all except the West African populations. Furthermore, Central Asian rock doves may have reached the Sahara region, potentially during the Green Sahara Period (ca. 6,000 to 10,000 yr ago), producing a secondary contact and a hybrid zone with the West African rock dove population (Fig. 5C and supplementary fig. S8, Supplementary Material online). It has been proposed that during interglacial periods, rapid expansions in northern populations would produce increased homozygosity or low genomic diversity due to founder effects and continuous bottlenecks. In contrast, southern populations in more stable habitats tend to diverge by repeated allopatry during several glacial cycles and by being protected by hybrid zones (Hewitt 1996, 2000), as could be the case of West African rock doves. Refugia theory predicts the establishment of a secondary contact zone after the expansion allopatric populations and the development of hybrid zones (Mayr and O'Hara 1986). Our *D*-statistic results for the rock dove specimens from Darfur, Sudan, suggest the presence of such secondary contact and subsequent hybridization.

Fourth, the current arid Saharan period led to the isolation of the West African populations and the current *C. livia* distribution (Fig. 5D). The hybrid zone disappeared once the Sahara became arid, and the populations became allopatric. Genomes from rock dove populations from the Sahara will be necessary to confirm the previous existence of a hybrid zone and the complete or partial allopatry condition of the West African rock dove populations today. Our hypothetical scenario aims to explain in a broad sense the rock dove's evolutionary history. Future research studying the genomes of rock doves on a regional scale will be necessary to refine this model.

Insights into the Domestication of the Rock Dove

Most authors support the hypothesis that rock doves were independently domesticated at multiple locations in the Mediterranean Basin and the Near East (Johnston 1992; Johnston and Janiga 1995; Shapiro and Domyan 2013). Although today domestic breeds have low levels of genetic differentiation, this is likely due to recent intense crossing among breeds. Consequently, resolving the timing and number of domestication events is challenging (Shapiro

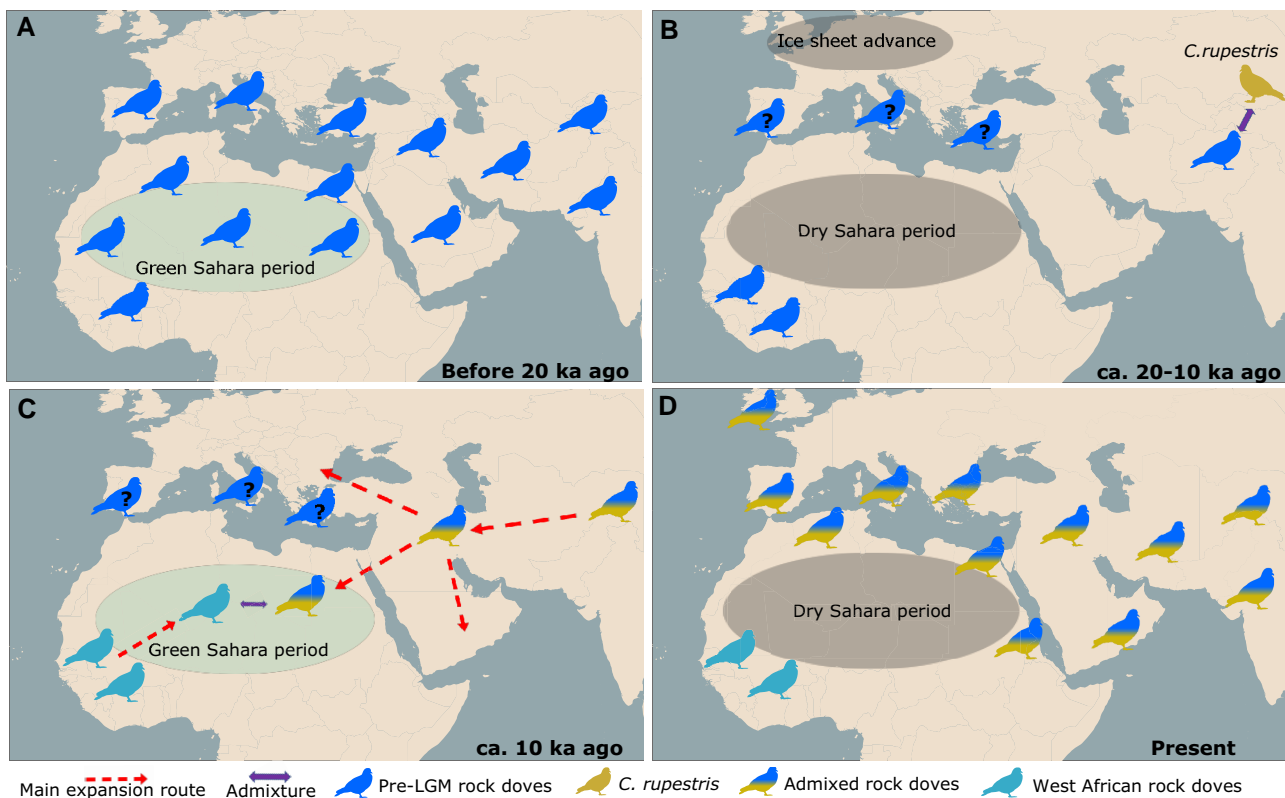


Fig. 5. Schematic representation of the proposed model of the rock dove evolutionary history under the refugia theory. a) Representation of the approximate geographic distribution of the rock dove previous to the LGM. b) An allopatric distribution of rock doves during the LGM. Two main populations survived in West Africa and in Central Asia, giving rise to the 2 differentiated clusters we observe in our data. Other populations may have also survived in other Pleistocene refugia, as exemplified by the question marks. During this time, we hypothesize that admixture occurred between *C. rupestris* and the rock dove population from Central Asia over the range where the 2 species cohabit, as indicated by the *D*-statistic results. c) Re-expansion of rock doves after the LGM. Due to isolation, West African rock doves diverged genetically, and possibly expanded to Central Sahara. Admixed rock doves from Central Asia expanded quickly throughout Eurasia and North Africa producing a secondary contact with the West African rock dove population, as also suggested by the *D*-statistic results (supplementary fig. S8, Supplementary Material online). Other rock dove populations may have become extinct or were replaced by the expansion of the admixed rock doves. d) Approximate modern rock dove geographic distribution (see Fig. 1 for the geographic distribution based on Peters 1931). West African rock dove populations are isolated from the rest of rock dove populations due to the current dry Sahara period.

and Domyan 2013). Under a scenario of multiple domestication events, we could expect that different breeds display different affinities with rock doves from distinct geographic locations, despite the high levels of crossing among breeds. Our results from the NJ and ML trees indicate that domestic pigeons form a closely related, monophyletic clade, as supported with high and moderate branch supports (bootstrap of 100% and 74%, respectively). Even if high levels of crossing among breeds have happened, it is somewhat unexpected to see such a level of genomic homogenization in all the breeds, effectively blurring other signals of ancestry across the different genomic regions. Our results also indicate that domestic pigeons are the sister clade of the Middle East rock dove populations. Similarly, the *D*-statistic analyses indicate that domestic pigeons are close to populations from the Levant (Syria, Iraq, and Palestine). These locations overlap with the Fertile Crescent, where several other species have been domesticated, and have also been proposed to be the geographic origin of domestic pigeons (Driscoll et al. 2009). Therefore, our results are consistent with at least 1 domestication

event in the Levant that gave rise to all domestic breeds analysed in this study. Although we cannot rule out the possibility of several domestication events, our data provides no evidence that domestication involved multiple genetically very distinct populations. We believe that rock dove synanthropy was common across a big part of its natural distribution, but complete domestications might be less common than previously assumed.

Furthermore, the anticipated continuous gene flow from wild rock dove populations to domestic pigeons aligns with the revised model of animal domestication proposed by Frantz and collaborators (2020). This model involves recurrent admixture between wild and domestic populations, contrasting with the traditional narrative that anticipates the maintenance of reproductive isolation of domestic populations. Future studies integrating wild rock dove and domestic pigeon genetic and phenotypic diversity, have the potential to explore the mechanisms that allowed the domesticated phenotypes maintenance in the presence of ongoing admixture with wild populations.

Taxonomic Implications

The intraspecific taxonomic classification of rock doves has been based on clinally distributed phenotypes that intergrade among populations (e.g. coloration, plumage patterns, and body size) (Murton and Clarke 1968). This classification leads to an oversimplification of their phenotypic diversity and complicates the defining of each subspecies' distribution range (Johnston 1992). Since the 19th and early 20th centuries, when the rock dove subspecies were defined, few major taxonomic changes have been implemented, most of which involved invalidating some subspecies due to their similitude with other adjacent subspecies (Murton and Clarke 1968; Cramp 1985; Gibbs et al. 2001). The rock doves from West Africa are distributed in Mauritania, Mali, Ghana, Senegambia, and Guinea (Urban et al. 2014), and are currently recognized as *C. l. gymnocyclus* Gray, 1856. This subspecies is characterized by its smaller size and very dark color, in comparison to the nominate *C. l. livia* Gmelin, 1789, and the extended bright scarlet orbital skin (Gibbs et al. 2001). Although Mali populations were initially separated based on their smaller size and paler plumage into the subspecies *C. l. lividor* Bates, 1932, they have subsequently been considered a synonym of the *gymnocyclus* subspecies (Gibbs et al. 2001). Our results show a close relationship between the specimens from Mali, including the type specimen for *C. l. lividor* (Mali-T), and specimens from Ghana described as *gymnocyclus*, further supporting the inclusion of *C. l. lividor* within *C. l. gymnocyclus*.

Subtropical Atlantic Islands rock doves have been historically classified into 2 subspecies, the populations from the Canary Islands as *C. l. canariensis* Bannerman, 1914, and the populations from Azores, Cape Verde, and Madeira as *C. l. atlantis* Bannerman, 1931. The *C. l. canariensis* subspecies has been described as smaller and darker than the nominate, but because the differences are considered very small, those populations are usually included in *C. l. livia* (Cramp 1985). On the other hand, *C. l. atlantis* is thought to have derived from feral pigeons due to its high variability in plumage patterns and coloration (Murton and Clarke 1968; Cramp 1985; Gibbs et al. 2001), or from melanistic mutants from the Canary Islands (Cramp 1985). Our results show the rock doves from the Subtropical Atlantic Islands are closely related to populations from northwest Africa and the North Atlantic Islands, indicating a natural origin for these Subtropical Atlantic Islands rock doves. However, we find signals of domestic pigeon admixture in the specimens from Cape Verde and Azores (Fig. 2C), which could explain the morphological variation that characterize these populations. The higher affinities of the Subtropical Atlantic Islands rock doves with other *C. l. livia* populations, suggests that the Subtropical Atlantic Islands derive from populations of the *C. l. livia* subspecies, despite their signals of admixture from feral birds. The description of *C. l. atlantis*, and *C. l. canariensis* subspecies was probably based on specimens belonging to populations with high levels of admixture with feral pigeons.

The Red Sea rock doves are described as *C. l. butleri* Meinertzhagen, 1921, are commonly included in *C. l. targia* Geyr von Schweppenburg, 1916 (Gibbs et al. 2001), in *C. l. gaddi* Zarudny and Loudon, 1906 (White 1965), or in *C. l. schimperi* Bonaparte, 1854 (Cramp 1985). According to our results, the rock doves from the Sudan Red Sea Province, which include the type specimen of *C. l. butleri*, are closely related to populations from the Arabian Peninsula (*C. l. palaestinae*) and the Levant (*C. l. palaestinae* and *C. l. gaddi*). Therefore, *C. l. butleri* cannot be included into *C. l. targia* subspecies. It will be necessary to include data from Egyptian *C. l. schimperi* specimens to confirm the inclusion of *C. l. butleri* into *C. l. schimperi* as suggested by Vauri (1965), or into *C. l. palaestinae* as suggested by our results.

An interesting case is the rock dove subspecies *C. l. dakhlae* Meinertzhagen, 1928 that is restricted to the Dakhla and Kharga Oases in Central Egypt and is characterized by a very light pale plumage. Throughout our analyses, the Dakhla_Oasis-T specimen showed a close affinity to the domestic breeds. The *D*-statistic results indicated that this specimen has mostly genetic components from domestic breeds, but also from rock doves from nearby regions. Therefore, it is likely that *C. l. dakhlae* derived from the hybridization between feral pigeons and wild populations. Consequently, we argue that the *C. l. dakhlae* subspecies status is invalid due to its apparent feral origin. Genomic data from additional specimens from this subspecies will be necessary to confirm this possibility. Nevertheless, we believe that rock doves from Dakhla and Kharga Oases offer an important opportunity to study the long-term effects of domestic introgression in wild populations.

Our genomic-based analyses showed that rock doves of the *C. l. gymnocyclus* subspecies form the basal sister clade to all other rock doves, potentially due to a long history of allopatry which derived in the divergence of its populations. For that reason, we suggest a taxonomic arrangement to reflect their evolutionary history. We propose that *C. l. gymnocyclus* Gray, 1856 should be considered a full species, *C. gymnocyclus* Gray, 1856, with *C. l. lividor* Bates, 1932 is being a junior synonym.

Finally, the geographically distributed diversity in the historic rock doves (excluding West African populations) in our study and the low differentiation among populations, suggest that rock doves form a continuum throughout their distribution range. This is in agreement with the clinally distributed phenotypic characters that have been used to previously criticize the rock dove subspecies classification. An extensive study based on the measurement of morphological characters of 222 male rock dove specimens that span the historical distribution of the species (Johnston 1992) concluded that some of the main subspecies (*C. l. gaddi*, *C. l. palaestinae*, *C. l. livia*, *C. schimperi*, and *C. l. neglecta*) were arbitrarily distinguished in the borders of their distributions, and suggested that such subspecies "have little potential evolutionary novelty in the absence of geographic isolation". Our genomic data indicates that

the rock dove subspecies are difficult to support and identify, especially considering adjacent populations. Although we are missing specimens from intermediate geographical locations among some populations, their inclusion most likely would strengthen the patterns observed. According to [Mayr \(1982\)](#), in populations with a contiguous distribution that displays clinal morphological variation, the subspecies concept loses evolutionary significance. Therefore, if *C. l. gymnocyclus* is raised to species status, *C. livia* can be considered as monotypic. Alternatively, 2 subspecies can be recognized considering the rump coloration, previously used to differentiate rock dove populations: the nominate *C. l. livia* Gmelin, 1789 (white rump coloration), and the remaining populations (which present mostly gray and occasionally white rumps) ([Johnston and Janiga 1995](#)) together into *C. l. intermedia*, Strickland, 1844, respecting the oldest subspecies name.

Conservation Implications

According to the IUCN Red List of [Threatened Species \(2022\)](#), the conservation status of rock doves is categorized as “Least Concern”, despite experts’ concerns regarding potential genetic swamping caused by the continuous admixture with feral pigeons ([Johnston et al. 1988, 1992](#); [Johnston and Janiga 1995](#); [Baptista et al. 1997](#)). Our dataset, which includes historical specimens, reveals evidence of admixture between feral and wild populations. Given that ongoing expansion of urbanization and environmental deterioration are conditions that favor the expansion of feral bird populations and ranges, we anticipate a further increase in admixture in contemporary times.

Genetic diversity measurements such as global per-sample heterozygosity and individual inbreeding coefficient are commonly used to assess the health of populations. Our results indicate that historical rock doves exhibit low heterozygosity, which aligns with our proposed evolutionary model that involves strong bottlenecks and fast expansions. However, when compared to other bird species listed as “Least Concern” by the IUCN Red List of Threatened Species, rock doves present considerably higher heterozygosity values, by at least 1 order of magnitude (e.g. the budgerigar = 0.004, or the little egret = 0.002) ([Li et al. 2014](#)). These differences could be associated with rock dove’s large population sizes, high mobility among populations (as suggested by estimated F_{st} values), and rising heterozygosity since the last relevant bottleneck during the Pleistocene. The West African rock doves show the lowest heterozygosity values, likely due to their long history of isolation from other rock dove populations, smaller population sizes, and subsequent genetic drift.

Regarding the estimated individual inbreeding coefficient, our findings show remarkably high coefficients in the West African rock doves, similar to those observed in endemic island populations ([Frankham 1998](#)). Although populations with such high coefficients might be considered endangered ([Reed et al. 2002](#)), it is possible that West African rock doves experience slow inbreeding, which is less detrimental than fast inbreeding ([Frankham 1998](#)). The Sahara rock doves

also present high coefficients as expected for their fragmented populations, and specimens from Azores and Cape Verde Islands show similar patterns, possibly for their isolation and admixture with domestic/feral birds. The remaining rock doves present low inbreeding or outbreed, likely due to large populations and high mobility.

Overall, our findings suggest that historical rock doves did not exhibit concerning values of heterozygosity or inbreeding, except for the West African and Sahara rock doves. This confirms that the main threat to worldwide rock dove populations is their replacement by the increasing feral pigeons. Considering the West African rock doves’ potential as a distinct species, their endemic condition to the West of Africa, and their high levels of inbreeding, a reevaluation of their conservation status may be necessary. Additionally, in the context of climate change, environment deterioration, and expansion of urbanization, it will be necessary to explore the genetics of contemporary rock doves, particularly of the West African populations. Finally, our results further highlight that the long history of admixture among rock dove, feral, and domestic pigeon populations provides an ideal model for investigating the process of extinction or extirpation by genetic swamping in terrestrial vertebrates and for exploring the consequences of gene flow from wild to domestic species in the establishment and adaptation of feral populations (exoferal), a key aspect in the study of invasive species ([Gering et al. 2019](#)).

Materials and Methods

Dataset

We obtained a total of 64 dried toe pads and 1 alcohol-preserved toepad from historical *C. livia* specimens dated from 1865 to 1986 that are held in the collections of the Natural History Museum at Tring, UK ([supplementary table S1, Supplementary Material](#) online). The specimens represent all currently recognized subspecies. The collection locations of these specimens cover most of the reported historical distribution of the species, including the Atlantic Islands, the Mediterranean Basin, the Saharan and West sub-Saharan Africa, Middle East, Central Asia, Sri Lanka, and Thailand. Eight of them are described as feral pigeons from China, Japan, Sierra Leone, Madeira Island, Java, and Andaman Islands. Among these specimens, 3 are from Darwin’s collection, and 5 are type specimens of different taxa: *C. l. atlantis* Bannerman, 1931, *C. l. canariensis* Bannerman, 1914, *C. l. butleri* Meinertzhagen, 1921, *C. l. dakhlae* Meinertzhagen, 1928, and *C. l. lividor* Bates, 1932. The rest of the samples, according to their taxonomic description, belong to the currently accepted subspecies ([supplementary table S1, Supplementary Material](#) online).

To complement our historical dataset, we included 41 reference pigeon genomes ([Shapiro et al. 2013](#)), 39 of them from different domestic pigeon breeds, and 2 feral pigeons from the USA. Additionally, 6 outgroups were added to the final dataset: 1 common pheasant (*Phasianus colchicus*) ([Liu et al. 2019](#)), 1 band-tailed pigeon (*Patagioneas fasciata*) ([Murray et al. 2017](#)), 1 cinnamon

dove (*C. larvata*), 1 common wood pigeon (*C. palumbus*), 1 speckled pigeon (*C. guinea*) (Vickrey et al. 2018), and 1 hill pigeon (*C. rupestris*) (Shapiro et al. 2013) (supplementary table S2, Supplementary Material online).

Laboratory Procedures

The historical samples were processed under strict clean (laboratory conditions at the Globe Institute, University of Copenhagen. DNA extractions were performed using a digestion buffer for keratin following Campos and Gilbert (2012). The samples mRD_1—mRD_10 were digested in 1 ml buffer and purified using a binding reservoir as in Dabney et al. (2013), combined with Monarch columns and a binding buffer as in Allentoft et al. (2015).

Samples mRD_11—mRD_65, followed the same overall approach. Tissue for each sample was digested overnight in 0.3 ml of the aforementioned digestion buffer, which was purified directly over Monarch DNA Clean-up Columns (5 µg) (New England Biolabs) using a 10:1 ratio of binding buffer (a modified version of Qiagen's phosphate buffer) to digestive mixture, repeated 3 times. PE buffer (Qiagen) was then used to wash the sample, followed by a dry spin to remove any remaining residue, and finalized with 2 consecutive elutions to increase DNA yield, each of 15 µL of EB (Qiagen) buffer incubated at 37 C for 10 min.

Double-stranded DNA sequencing libraries were constructed following BEST 2.0 (Carøe et al. 2018) although with modifications to allow sequencing using BGI (Beijing Genomics Institute) sequencing technology (Mak et al. 2017). Libraries were prepared using 32 µL of DNA in a final reaction volume of 80 µL. A maximum of 10 µL of each double stranded DNA library template was used for 1 round of PCR (polymerase chain reaction) amplification of 25 thermal cycles of: 30 s at 95°C (denaturation), 30 s at 60°C (annealing), and 110 s at 72°C (extension). Each 50 µL reaction contained 2.5 U PFU (*Pyrococcus furiosus*) Turbo CX Polymerase, 1x PFU Turbo buffer, 0.4 mg ml⁻¹ bovine serum albumin (BSA), 0.25 µM mixed dNTPs (deoxynucleoside triphosphates), 0.1 µM BGI forward index-primer, 0.1 µM BGI reverse index-primer. Amplified PCR products were purified using a 1.4X ratio of HiPrep PCR clean-up beads (Magbio Genomics), and were eluted in 28 µL of Elution Buffer with TWEEN (0.05%). Purified, amplified libraries were then quantified using the Qubit 2.0 Fluorometer (ThermoFisher Scientific, Inc) and Fragment Analyzer (Agilent Technologies) and were sequenced across 8 100 bp PE (paired-end) DNBseq-G400 lanes (BGI Europe).

Data Processing

PALEOMIX v.1.2.13.4 BAM pipeline (Schubert et al. 2014) was used to process the generated sequence reads and map them against the *C. livia* reference genome (Cliv_2.1) (Holt et al. 2018). In brief, this pipeline includes the following steps: (i) trimming of sequencing adapters, using AdapterRemoval v.2.2.0 (Schubert et al. 2016) discarding reads shorter than 25 bp after trimming

(—minlength default value (25 bp)); (ii) sequence alignment to the reference genome using BWA v.0.7.17 backtrack algorithm (Li and Durbin 2009), disabling the use of the seed parameter unmapped reads were discarded; (iii) identification and removal of PCR duplicates using Picard's MarkDuplicates (<https://github.com/broadinstitute/picard>); (iv) local realignment around indels using GATK v.3.8.3 IndelRealigner module (McKenna et al. 2010). The 3 most distant outgroups in our dataset (*P. colchicus*, *C. palumbus*, *C. guinea*) were mapped using BWA mem algorithm instead of backtrack given the reference data consisted of longer reads and we expect a higher proportion of mismatches to the reference genome due to their longer evolutionary distance.

Characterization of the DNA Damage Patterns in the Historical Genomes

We used mapDamage v.2.0.9 (Jónsson et al. 2013) with default parameters to estimate the patterns of nucleotide misincorporations and DNA fragmentation in the historical specimens and characterize their level of DNA damage (supplementary table S1, Supplementary Material online). In most cases, we do not observe a substantially high proportion of cytosine deamination at the end of the reads. This is consistent with previous observations showing historical specimens do not always show high C to T damage patterns (Sánchez-Barreiro et al. 2021). However, in order to avoid any potential bias caused by the small proportion of damage in the sequencing data, we restricted the analyses to transversion sites.

Sex Determination

To infer the biological sex of the rock dove and feral pigeon specimens lacking sex information in the museum description (Kashmir2, Libya1, Azores, Cabo_Verde, Orkney, Shetland-D, Sierra_Leone-D, Madeira-D, Java, Japan2, Oman), we mapped the data to the chromosome level *C. livia* reference genome (colliv2) (Damas et al. 2017) following the pipeline described above. Male specimens were identified if their Z sex chromosome (CM007524.1) depth of coverage was either similar to or exceeded the mean coverage of all the autosomal chromosomes. On the other hand, female specimens were identified if the Z chromosome depth of coverage was approximately half the mean coverage of all the autosomal chromosomes (supplementary table S1, Supplementary Material online).

SNP Calling

Due to the heterogeneity in the average depth of coverage obtained from our historical samples, we used a pseudo-haploid calling approach instead of performing genotype calling. We used ANGSD v.0.931 -dohaplocall 1, which randomly samples a single base per site for every sample (Korneliussen et al. 2014). We selected sites with the following quality filters and parameters: -doCounts 1, -minMinor 2, -maxMis 10, -C 50, -baq 1, -minMapQ 30, -minQ 20, -uniqueOnly 1, -remove_bads 1, -only_proper_pairs 1,

-skipTriallelic 1, -doMajorMinor 1, and -GL 2. Transitions were removed to avoid possible DNA damage in the historical rock doves and pigeon sequences. The SNPs calling was restricted to the first 128 scaffolds that are 1 Mb or longer. We then used Plink v1.90 (Chang et al. 2015) to discard sites with a minor allele frequency below 0.01 in order to exclude singletons that could derive from sequencing errors or aDNA-related errors. The final SNP panel contained 1,642,881 transversion sites and 112 samples.

Multidimensional Scaling Analysis

To explore the population structure in our dataset, an MDS analysis was implemented, allowing us to visualize the genetic structure in our samples. Pairwise-distances were estimated for the dataset described in the previous section using Plink v1.90. We estimated 2 MDS analysis, 1 including all samples (with exception of the outgroups) (Fig. 2A), and a second excluding the divergent rock doves from West Africa in order to observe in more detail the population structure among the rest of the genomes (Fig. 2B and supplementary S1, Supplementary Material online). The *Stress* -1 value for each analysis was estimated using the R library SMACOF (De Leeuw and Mair 2009). The *Stress* -1 value obtained for the first two-dimensional MDS was 0.195, while for the second two-dimensional MDS the obtained *Stress* -1 value was 0.221, close to the stress rules of thumb (lower than 0.20), but lower than the Sturrock and Rocha (2000) estimated two-dimensional stress for 100 objects matrix (0.396), indicating our result is not random or without structure. All the results were visualized using the ggplot2 library in R (Wickham 2016).

Principal Components Analysis

A PCA was estimated to explore the population structure in our dataset using genotype likelihoods. The read alignments were downsampled to 10 million mapped reads using Picard's tool DownsampleSam. Then, ANGSD v.0.931 was used to estimate genotype likelihoods implementing the parameters *-doGlf* 2 *-doMajorMinor* 1 *-doMaf* 2 *-doCounts* 1 *-SNP_pval* 1e-6 *-rmTrans* 1 *-minQ* 20 *-minmapq* 30 *-setMinDepth* 3. Finally, PCAnsd v.1.10 (Meisner and Albrechtsen 2018) was used to calculate the covariance matrix. R was used to compute eigenvalues and visualize the results.

Admixture Analysis

ADMIXTURE v.1.3.0 (Alexander et al. 2009) was used to estimate the ancestry components in our dataset, excluding the outgroup species. The pseudo-haploid dataset described in the previous section was used as input for this analysis. ADMIXTURE was run assuming from 2 to 10 ($K=\{2...10\}$) ancestry components and 20 independent replicas for each K value were performed. The replicas with the best likelihood value were selected. We performed cross-validation for each value of K (supplementary fig. S3, Supplementary Material online). R library Pophelper

v.2.3.1 (Francis 2017) was used to visualize the admixture results.

Neighbor-Joining Tree and Maximum Likelihood Phylogeny

We estimated an NJ tree based on 100 pairwise-distance matrices estimated from our pseudo-haploid dataset using Plink v1.90. Each pairwise-distance matrix was calculated based on 1 million randomly sampled SNPs from the initial pseudo-haploid dataset. The NJ trees were then constructed using the R library ape (Paradis and Schliep 2019). To summarize all the trees, Astral-III (Zhang et al. 2018) was employed. Finally, the Interactive Tree Of Life (iTOL) v4 online tool (Letunic and Bork 2019) was used to visualize it.

To infer a ML phylogeny, we generated genomic consensus sequences for each genome using ANGSD v.0.931 (*-dofasta* 2 option and applying quality filters *-minQ* 20 and *-minmapq* 20) and, using the *C. livia* reference genome (Cliv_2.1). Then, 1000 independent phylogenetic trees were estimated in RAXML-ng v.0.9.0 (Kozlov et al. 2019) under the GTR (generalised time reversible) + G evolutionary model, using 1000 random regions of 5000 bp taken from the previously created genomic consensus sequences. In the next step, all the gene trees were summarized to generate a species tree using ASTRAL-III (Zhang et al. 2018). The final tree was visualized using the Interactive Tree Of Life (iTOL) v4 online tool (Letunic and Bork 2019).

Weighted Pairwise Fixation Index (Fst)

To assess the genetic differentiation between the West African rock doves and the rest of the rock dove populations, we estimated weighted pairwise *Fst* based on our pseudo-haploid dataset using the Plink function *-fst*. The 3 West African rock doves' genomes in our dataset were grouped into a single population (Mali-T, Mali, Ghana). In order to correctly estimate the *Fst* values, 3 representative rock dove genomes were selected for each geographic region to form the distinct populations: North Atlantic Islands (Scotland1, Hebrides1, Ireland), Tropical Atlantic Islands (Azores, Madeira, Canarias), Northwest Africa (Algeria1, Tunisia, Algeria2), Mediterranean (Macedonia, Italy1, Lemnos), Northeast Africa (Egypt_Sollum1, Egypt_Sollum2, Libya2), Levant (Athlit, Jerico, Syria2), Middle East (Iraq2, Iran1, Iran2), Arabian Peninsula (S_Arabia, Oman, Aden), East Africa (Gebeit_Suda-T, Sudan, Gebeit_Sudan), Central Asia (Kashmir1, Kashmir2, Chinese_Turkestan), and Thailand (Thailand1, Thailand2, Thailand3).

qpWave

In consideration of the likely admixture between domestic pigeons and rock doves in our dataset, we implemented pairwise qpWave tests in ADMIXTOOLS v.5.1 (Patterson et al. 2012) to try to detect the minimum number of ancestral sources needed to explain the variation found in the domestic pigeon cluster. The test was done for all pairs of domestic pigeons (left populations) using the option

“allsnps = YES’. The Right populations used in the test included *C. palumbus* as the outgroup and samples representing the main clusters found in the NJ-tree: Mali, Darfur_Sudan2, Kashmir1, Thailand1, Iran1, and Iraq1. The analysis showed that most of the domestic pigeons can be grouped together, indicating their close relationships as was observed in the other obtained results (supplementary fig. S7, Supplementary Material online).

D-statistic

To evaluate the possible high levels of admixture between domestic and wild birds, we created a subset of our dataset including all historic samples and some domestic pigeons that represent the main genetic clusters identified in the qpWave analysis (Fantail, Indian fantail, Starling, Scandaroon, Lahore, English trumpeter, Racing, Oriental, Archangel, Cumulet) to perform SNP calling and try to avoid bias toward the domestic pigeons. For this purpose, genotype likelihoods were estimated (-GL 2 -doMaf 2) using ANGSD v.0.931 restricting to SNPs with a *P*-value threshold of 1e-6. The selected sites were extracted from the first pseudo-haploid dataset described above, and a minor allele frequency filter of 0.01 was applied using plink.

This new pseudo-haploid dataset was used to explore admixture patterns and test the NJ-tree and phylogenetic tree topologies using *D*-statistic as implemented in ADMIXTOOLS v.5.1. When a test in form D (Outgroup, A; B, C) deviates from 0, it suggests possible gene flow between A and B or A and C; if $D < 0$, A and B are sharing a higher level of genetic drift than expected, indicating possible gene flow; if $D > 0$, it indicates possible gene flow between A and C. Deviation from 0 was considered statistically significant when *Z*-score was under -3 or above 3. In all *D*-statistic tests, domestic pigeons were treated as a single population based on the previous results, and the qpWave analysis confirmed the majority of pigeon breeds derive from a common ancestral source when compared to a set of outgroups (supplementary fig. S6, Supplementary Material online). The significance of the test was estimated using a weighted block jackknife procedure over 1 Mb blocks. We performed the following tests:

- 1) To verify the basal position of the West African rock doves from Mali and Ghana, we tested the possibility of hybridization with closely related species *C. guinea* and *C. rupestris* in the form D(*C. palumbus*, *C. guinea*/*C. rupestris*; West Africa, X) where X represents all the other genomes in our dataset, and the West African rock doves were also grouped in a single populations.
- 2) In order to further investigate the previously observed affinities between the West African and the Saharan rock doves, we conducted a *D*-statistic test for each Saharan rock dove specimen (Darfur_Sudan1 and Darfur_Sudan2). The test was performed in form D(*C. palumbus*, West

Africa; Sahara, X), where West African rock doves were grouped as a single population, and X represents all the other rock dove genomes in our dataset.

- 3) We implemented 2 complementary *D*-statistic tests to explore these observed relationships between domestic breeds and historical rock doves from the Middle East group. First, we performed a test of form D (*C. palumbus*, MB-E-AI; Middle East rock doves, Domestic pigeons), where domestic pigeons were grouped as 1 population, each sample from the Middle East region was tested and MB-E-AI represented each specimen from the MB-E-AI. Then we estimated a second test of form D (*C. palumbus*, Middle East rock doves; MB-E-AI, Domestic pigeons), switching the wild populations with the goal to confirm the relationships and obtain some clues for the gene flow direction.
- 4) A *D*-statistic test was performed to assess the close relationship of the *C. l. dakhlae* type specimen with the domestic breeds found in the different results. The form of the tests was D(*C. palumbus*, X; Dakhale-Oasis-T, Domestic pigeons) and D (*C. palumbus*, Domestic pigeons; Dakhale-Oasis-T, X), where X represents all the historical rock doves in our dataset.

Heterozygosity and Inbreeding Coefficient

Heterozygosity per sample was estimated to explore the genetic diversity of the historical rock doves and feral pigeons. First, the read alignments were downsampled to 10 million mapped reads using Picard's tool DownsampleSam in order to avoid biases due to differences in depth of coverage. ANGSD v.0.931 was used to estimate genotype likelihoods for each sample at sites segregating in all the outgroups in our dataset (*P. colchinus*, *P. fasciata*, *C. palumbus*, *C. larvata*, *C. guinea*, and *C. rupestris*) using the parameters -minQ 20 -minmapq 30 -setMinDepth 3. The site frequency spectrum for each sample was estimated from the genotype likelihoods using ANGSD realSFS (Korneliussen et al. 2014) and used as a proxy for the individual heterozygosity.

Individual inbreeding coefficients (*F*) were estimated based on genotype likelihoods using the function—*inbreedSamples* as implemented in PCAnsd v.1.10 for each historical rock dove and feral pigeon. Genotype likelihoods were estimated using ANGSD v.0.931 with the parameters: -doGlf 2 -doMajorMinor 1 -doMaf 2 -doCounts 1 -SNP_pval 1e-6 -rmTrans 1 -minQ 20 -minmapq 30 -setMinDepth 3.

Supplementary Material

Supplementary material is available at *Molecular Biology and Evolution* online.

Acknowledgments

Funding for this research was provided by ERC award Extinction Genomics and D NRF143 Center for Evolutionary Hologenomics. G.H.-A. is supported by the Consejo Nacional de Ciencia y Tecnología from Mexico (CONACyT 576743).

Author Contributions

G.P. and M.T.P.G. conceived the study. H.v.G. provided the samples. Funding was obtained by M.T.P.G. M.M.C. led the processing of the samples in the laboratory with support from E.C., G.P., and M.-H.S.S. G.H.-A. perform the data analysis with support from J.R.-M. G.H.-A. wrote the manuscript with support from J.R.-M., and S.G., H.v.G., and M.T.P.G. All authors revised the final manuscript.

Conflict of interest statement. None declared.

Data Availability

Generated raw sequence reads, as well as the read alignment files mapped to the *C. livia* reference genome (Cliv_2.1) have been deposited at the European Nucleotide Archive (Project ID: PRJEB61000).

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