Extinction by hybridisation in the wild Rock Doves of the British Isles

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Declaration

I declare that this thesis was composed by myself and that the work contained herein is my own except where explicitly stated in the text. The work has not been submitted for any degree or professional qualification except as specified.

William J. Smith

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Publications and contributions

The following chapters consist of manuscripts which are either currently in peer-review, or have now been accepted for publication. My contribution to each is outlined.

- **Chapter 2.** Smith, W.J., Quilodrán, C.S., Jezierski, M.T., Sendell-Price, A.T., and Clegg, S.M. (2022) The wild ancestors of domestic animals as a neglected and threatened component of biodiversity. *Conservation Biology 36*, e13867**. I conceived of the manuscript, carried out the literature review, and wrote the first draft. All authors edited the manuscript.**

- **Chapter 3.** Smith, W.J. (2023) The undomesticated Rock Dove in Britain and the Isle of Man. *British Birds* 116, 70-76. **I conceived of the manuscript and carried out the literature review. I wrote the first draft, and edited the manuscript.**

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with advice from ATSP and SK. I wrote the first draft with feedback from SMC, ALF, and LAT. All authors edited the manuscript.

- **Chapter 5.** Smith, W.J. & Clegg, S.M. (2023) Gene flow and genomic diversity in British and Irish pigeons *Columba livia*. *Conservation Genetics* (in review). **I conceived of the manuscript and carried out the literature review. I carried out data analysis. I wrote the first draft, and both authors contributed to editing the manuscript.**

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- **Chapter 7.** Smith, W.J., Jezierski, M.T, Dunn, J.C., and Clegg, S.M. (2023) The interplay of parasite exchange and hybridisation at a wild-feral-domestic interface. *International Journal for Parasitology* (in review following revisions). **I conceived of the manuscript with input from SMC and JCD, and I carried out the literature review. I carried out data collection, DNA extraction, and PCR jointly with MTJ. I led bioinformatic analysis with advice from MTJ. I wrote the first draft of the paper, and all authors contributed to editing the manuscript.**

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Abstract

Hybridisation can lead to genomic admixture between populations that were previously isolated. Increased anthropogenic transportation of species, along with habitat and climate change mediating contact of populations, has caused multiple cases of 'extinction by hybridisation'. This is when introgression leads to the loss of a population or species as a form distinct from its hybridising relative. In this thesis, I use the undomesticated Rock Dove (*Columba livia*) and its widespread conspecific, the feral pigeon, to explore the process of genomic replacement that characterises 'extinction by hybridisation'. Specifically: (i) I review the scientific and conservation importance of the wild forms of domestic animals, and discuss the threats they face following gene flow from their feral conspecifics; (ii) I introduce the Rock Dove, outlining its status in the British Isles, and highlight its currently poor conservation prospects given ongoing interbreeding with the superabundant feral pigeon; (iii) I carry out the first population genomic study of the wild Rock Dove, identifying both hybrid populations as well as those exhibiting negligible signs of feral pigeon introgression; (iv) I show that interbreeding correlates with increased genomic diversity in hybridising populations, and discuss the potential benefits of gene flow between the lineages; (v) I show that plumage patterns can be used, at a population level, to make inferences about the extent of hybridisation in different Rock Dove colonies, and (vi) I examine variation in the prevalence and diversity of the disease-causing parasite *Trichomonas* among wild, feral and hybrid populations of *Columba livia*, and suggest that disease transmission may influence patterns of gene flow. Overall, this body of work extends our understanding of the process of extinction by hybridisation, specifically relating to scattered, relictual undomesticated populations which are in contact with conspecific feral domestic populations. It also provides a greater understanding of the wild Rock Dove's genomic status and basic biology. Characterisation of the wild Rock Dove will extend the utility of the domestic pigeon as a major model organism in urban evolution, developmental genetics, and behavioural science, by allowing for a greater understanding of trait evolution and natural behaviour in a species which has been predominantly studied in a laboratory setting.

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Introduction

Chapter 1: Introduction

Hybridisation was traditionally considered a problem for understanding species and reproductive boundaries, largely due to the widespread endorsement of the biological species concept and its requirement for species to be reproductively separate entities (Harrison & Larson, 2014). We now know that it is a common process, and studying introgression between different lineages is recognised as a useful tool to help understand evolution (Taylor & Larson, 2019). Hybridisation and subsequent gene flow can occur between species, subspecies, or genetically diverged populations, which can be distinguished on the basis of one or more heritable characters (Futuyma & Antonovics, 1992; Harrison & Larson, 2014). Hybrid zones, where interbreeding taxa meet, have been referred to as 'natural laboratories' that are valuable for exploring the processes of divergence and speciation (Hewitt, 1988). Hybridisation, and subsequent backcrossing leading to introgression of alleles from one form to another, is so interesting to biologists because it can be both a constructive and homogenising force, which can lead to various evolutionary outcomes (Arnold, 1992). On the one hand, it can provide genetic diversity for selection, or lead to the formation of hybrid lineages (Mallet et al., 2007; Abbott et al., 2013; Harrison & Larson, 2014; Payseur & Rieseberg, 2016; Zecherle et al., 2021). On the other hand, it can act as a homogenising force, stalling or even reversing the speciation process (Rhymer & Simberloff, 1996; Todesco et al., 2016; Kearns et al., 2018). Hybridisation has become more common with increasing human influence on the natural world (Ottenburghs, 2021). Direct introduction of non-native species, either accidentally or deliberately, can bring them into contact with native relatives with whom they can

interbreed (Rhymer & Simberloff, 1996). Similarly, habitat or climate change can lead to distributional shifts, bringing previously isolated taxa into contact (Chunco, 2014).

Within the field of conservation biology, the dichotomy between the potential outcomes of hybridisation is well-recognised. Genetic rescue, the introduction of individuals from a different but related population as a conservation measure, can be successful in restoring small and inbred populations by reducing inbreeding (Chan et al., 2019). For example, the introduction of genetically diverged Mountain Pygmy Possums (*Burramys parvus*) into a declining population led to hybrid fitness and rapid population recovery (Weeks et al., 2017). Contrastingly, hybridisation can lead to wasted reproductive potential. For example, Fin Whales (*Balaenoptera physalus*) hybridising with Blue Whales (*Balaenoptera musculus*) leads to a reproductive loss for the latter, threatening its recovery in the Atlantic Ocean (Pampoulie et al., 2021). Even in examples where animal hybrids are successfully born and are fertile, they may be maladapted. For example, the introduction of a Turkish subspecies of ibex to assist the demographic recovery of the subspecies found in Czechoslovakia led to hybrids calving too early and having reduced survival, contributing to the critical decline of the population (Waller, 2015). Another major impact, which requires successful introgression and genomic admixture, is the 'extinction by hybridisation' or 'genetic replacement' of one or both pure parental lineages (Rhymer & Simberloff, 1996). Such biotic homogenisation (Olden, 2006), representing a loss of overall biodiversity, has proven difficult to study due to there often being limited phenotypic differences between the interacting populations. It has therefore long been difficult to assess the contemporary status of many populations threatened by extinction by hybridisation. Genetic, and, increasingly, genomic approaches, combined with

phenotypic analyses now offer a window into these dynamics but require model systems amenable for study. In this thesis, I examine hybridisation among highly divergent forms (wild and feral domestic) of the pigeon *Columba livia* in the British Isles. This will inform conservation efforts to protect its undomesticated lineage, the Rock Dove, and provide a tractable case study with which to explore extinction by hybridisation from both an evolutionary and conservation perspective.

Introgression as a constructive process

The role of hybridisation and subsequent introgression as an important constructive force in evolution and conservation is increasingly appreciated (Grant & Grant, 1992; Michael, 2016). The introduction of genetic diversity can fuel selection and therefore facilitate adaptation, and this can in turn, enable speciation (Abbott et al., 2013). In the Coyote (*Canis latrans*), hybridisation with domestic dogs (*Canis familiaris*) or Grey Wolves (*Canis lupus*) is a source of novel adaptive genetic variation. This potentially allowed adaptation both to human-dominated environments, and to fill the niche of wolves following their decline (Monzón et al., 2014). In many other systems the adaptive potential of hybridisation has been noted (Godwin et al., 2020) and this includes *Homo sapiens*, which received potentially adaptive traits relating to brain size following introgression from archaic human groups they encountered as they spread from Africa (Evans et al., 2006). Similarly, North American Grey Wolves with black coat colour originating from gene flow from domestic dogs are more resistant to canine distemper than their grey (wild-type)

relatives (Cubaynes et al., 2022). In *Heliconius* butterflies, introgression among species is considered to be a major factor in their evolutionary radiation (Edelman et al., 2019). As well as supplementing genetic diversity and enabling the transfer of adaptively useful alleles between lineages, the process of hybridisation can also lead to speciation more directly. In Darwin's Galápagos finches (*Geospiza* spp.), a self-perpetuating population of hybrid origin has been identified (Lamichhaney et al., 2018). Such hybrid speciation is particularly common in plants, leading to novel lineages such as the Arran whitebeams (*Sorbus arranensis*, *S. pseudofennica* and *S. pseudomeinichii*) (Robertson et al., 2004; Mchaffie et al., 2011) and Oxford Ragwort (*Senecio squalidus*) (Abbott et al., 2002).

The constructive evolutionary role of hybridisation has been utilised by conservation biologists via the successful implementation of genetic rescue – the practise of supplementing genetically depauperate or inbred populations, subspecies, or species with genetic variation from closely related forms (Frankham, 2015). Successful examples include the Adder (*Vipera berus*), where the introduction of new individuals reversed a population decline (Madsen et al., 1999), and the 'Florida panther', a population of the North American Cougar (*Puma concolor*), where survival of offspring to adulthood was improved following the introduction of Texan conspecifics (Pimm et al., 2006). Another example is reciprocal translocation between two inbred populations of the South Island Robin (*Petroica australis*) leading to increased juvenile survival and recruitment, with hybrids having higher immunocompetence (Heber et al., 2012). On the other hand, some cases of genetic rescue have failed to lead to positive demographic results. This is illustrated by the natural arrival of a new Grey Wolf to Isle Royale, leading to a population decline (following a brief population increase), potentially due to the introduction of

deleterious alleles (Hedrick & Garcia-Dorado, 2016). The use of hybridisation as a conservation management tool has also been suggested to have applications at the level of ecosystem, for example in enhancing the resilience of coral reef communities to anthropogenic-induced environmental changes (Chan et al., 2019). Taking all these examples together it is clear that hybridisation is increasingly being seen as a tool for managing the adaptability of populations and ecosystems to climatic and habitat changes in the future (Chan et al., 2019).

Introgression as a homogenising process

Despite the evident potential of hybridisation to be a constructive force in evolution and conservation, it can also have the opposite effect. Hybridisation can stall the speciation process and even reverse it, as has been proposed in Common Ravens (*Corvus corax*). In this example, the ravens were found to have mosaic genomes resulting from the fusion of two lineages that diverged ~1.5 million years ago (Kearns et al., 2018). This process of admixture between lineages leading to the collapse of a species pair into a single species has also been noted in the Three-spined Stickleback (*Gasterosteus aculeatus*) (Taylor et al., 2006). Below species level, interbreeding between populations, such as those of the Marine Iguana (*Amblyrhynchus cristatus*), can prevent incipient speciation by maintaining gene flow (MacLeod et al., 2015). In the same way that the generative evolutionary role of hybridisation is of relevance to conservation, this homogenising effect is also important and can contribute to the extinction of species, infraspecific taxa and

populations (Rhymer & Simberloff, 1996). This is because hybridisation can facilitate the formation of hybrid swarms where all individuals have mixed ancestry, leading to the loss of one or both (as in 'natural' reverse speciation) distinct ancestral lineages (Senn et al., 2019). This process is called 'extinction by hybridisation' or 'genetic replacement' and leads to biotic homogenisation, contributing to the global decline of biodiversity (Olden, 2006). The risk of genetic replacement is greatest when the entities have diverged recently, and reproductive isolation is therefore far from complete (Rhymer & Simberloff, 1996). It is also likely when there is a demographic imbalance between the forms involved (Nussberger et al., 2018; Quilodrán et al., 2019). The reduction in the pool of potential mates for the less common population or species, and the subsequent increase in hetero-specific matings, has been referred to as the 'desperation hypothesis' (McCracken & Wilson, 2011).

One group that are at particular risk of genetic replacement are those which are restricted geographically, often to islands, but are weakly diverged from their relatives. Even if they do not have species status, the divergent populations and subspecies in this category contribute often disproportionately to biodiversity (Des Roches et al., 2018). They are also prone to extinction for a variety of reasons, with islands hosting 61% of known extinctions despite covering only 5.3% of global land area (Spatz et al., 2017). Examples of divergent island forms threatened by hybridisation span a range of taxonomic groups and represent both full species and divergent infraspecific populations. The Lesser Antillean Iguana (*Iguana delicatissima*) is Critically Endangered and threatened by hybridisation with its introduced sister species the Green Iguana (*Iguana iguana*) (Vuillaume et al., 2015). *Argyranthemum coronopifolium* is an endemic plant from

Tenerife that now exists as a hybrid swarm after road building facilitated contact with the widespread *A. frutescens* (Brochmann, 2008). The endemic Seychelles subspecies of the Malagasy Turtle Dove (*Nesoenas picturata rostrata*) went extinct in a genomically distinct form following contact with the widespread nominate subspecies *N. p. picturata* (Cade, 1983). These examples show that extinction by hybridisation impacts a geographically varied group of organisms. Within individual countries, for example the United Kingdom, there are often many examples of conservation issues relating to hybridisation, impacting culturally, ecologically, and economically important species (**Table 1**).

Table 1. Comprehensive list of animal and plant populations whose genetic integrity is known to be threatened by hybridisation in a UK context.

Native form	**Non-native form**	**Status in the U.K.**	**References**
European Wildcat *Felis silvestris*	Domestic cat *Felis catus*	Following extensive interbreeding with feral domestic cats, the European Wildcat in Scotland now exists only as part of a population of individuals with mixed feral-wild ancestry. Reintroduction of captive European wildcats, especially those from continental Europe (which have also experienced wild-feral admixture, albeit to a lesser extent than the Scottish population) is underway. Such projects aim to regenerate a genomically and ecologically distinct 'wildcat' conservation unit in the country.	(Daniels et al., 1998; Beaumont et al., 2001; Fredriksen, 2015; Meredith et al., 2018; Senn et al., 2019)
European Polecat *Mustela putorius*	Domestic ferret *Mustela furo*	Polecat populations have been increasing for several decades, following historic persecution which had driven them back to relict populations, mostly located in central Wales. As they have expanded outwards into England, there has been significant interbreeding with feral ferrets. It is now difficult to identify wild polecats due to extensive phenotypic and genetic overlap between the wild and feral populations.	(Davison et al., 1999; Costa, 2014; Croose et al., 2018)
Red Deer *Cervus elaphus*	Sika Deer *Cervus nippon*	There are Red Deer on many Scottish islands (e.g. the Outer Hebrides) which are isolated from non-native Sika Deer populations. On the other hand, 'mainland' Scottish Red Deer have experienced	(Smith et al., 2018)

		introgression from their relative, and in certain locations hybridisation is extensive. Certain Hebridean islands act as refugia, where Red Deer are protected from any contact with Sika Deer.	
Wild Boar *Sus scrofa*	Domestic pig *Sus domesticus*	Boar have been (mostly accidentally) reintroduced to the UK following escapes from captivity. Some of these populations have significant proportions of domestic ancestry. Questions have been raised regarding whether the British Boar populations can therefore be considered to represent a reintroduced native species.	(Frantz et al., 2012)
Rock Dove *Columba livia*	Feral pigeon *Columba livia*	The spread of the feral pigeon and subsequent gene flow has obscured the true distribution of its rarer relative, the Rock Dove. It has been claimed that all Rock Dove populations in the British Isles have now experienced extensive genomic mixing with feral pigeons, rendering the undomesticated form of the species extinct as a discrete evolutionary entity. Putative wild Rock Dove populations persist in northern Scotland, especially on islands. These populations are the focus of this thesis.	(Hewson, 1967; Johnston et al., 1988)
Greylag Goose *Anser anser*	Domestic goose *Anser anser*	The British Greylag Goose population consists of native residents (e.g. in the Northern Isles and Western Isles of Scotland), migratory flocks which visit in the winter, re-introduced birds which predominate in southern Britain, and escaped domestic birds. The latter two populations in particular often interbreed, resulting in mixed semi-feral populations which are common in towns and cities around the country. The complex population genetics of the UK's Greylag Geese has so far not been studied, but feral-wild goose hybridisation is common elsewhere.	(Mitchell et al., 2012; Heikkinen et al., 2020)
Crucian Carp *Carassius carassius*	Goldfish *Carassius auratus*	Crucian Carp are dubiously native to the UK, but are threatened in continental Europe. In the UK, hybridisation with 'feral' domestic Goldfish could threaten the genetic integrity of the Crucian Carp. There is however some evidence of hybrid sterility which would prevent this issue.	(Hänfling et al., 2005)

Atlantic Salmon *Salmo salar*	Domestic salmon *Salmo salar*	Large-scale farming of domestic salmon can result in escapes which can hybridise with native stocks. Globally, such introgression is a significant problem for wild salmon, leading to maladaptation. There has been less work focussed on British stocks, compared to those of Norway and North America.	(Wringe et al., 2018; Sylvester et al., 2019; Willoughby & Christie, 2019; Solberg et al., 2020)
Black honeybees *Apis mellifera*	Feral honeybees *Apis mellifera*	The wild 'black honeybee' populations of parts of the Inner Hebrides have been understudied, and their true origin is debated. There have also been similar 'wild' populations identified in various regions (e.g. the grounds of Blenheim Palace in Oxfordshire). Hybridisation with honeybees from hives, and their feral offshoots, potentially threatens the 'wild' bees with genomic replacement. There is little understanding of the population genomics of the various types of wild honeybee in the UK.	(Carreck, 2008; Muñoz et al., 2015)
Wild Apple *Malus sylvestris*	Domestic apple *Malus domestica*	Wild Apples have strongholds in northern England and in Scotland, but elsewhere have experienced extensive hybridisation with their domestic relative. Populations of 'feral' domestic apples, which have escaped from hedgerows or orchards, are now more common than their wild relatives.	(Ruhsam et al., 2019)
English Bluebell *Hyacinthoides non-scripta*	Spanish Bluebell *Hyacinthoides hispanica*	Hybridisation between the native English and introduced Spanish Bluebells has long been claimed to pose a threat to the genetic integrity of the former. Hybrid bluebells are common in areas where the two species exist in proximity. New research has questioned the risks of this interbreeding, with recent studies suggesting that hybrid bluebells have lower fitness than pure English stock (and therefore English Bluebell populations will maintain their genetic integrity following English-Spanish hybridisation).	(Kohn et al., 2019)

As well as island populations, the wild forms of domestic animals and plants are especially vulnerable to extinction by hybridisation. Domestic forms have exceptionally important roles in historical and contemporary society, and their wild relatives are often endangered (with weak or essentially non-existent wild-feral-domestic reproductive

boundaries) (Zeder, 1982). Perhaps surprisingly, there is often a lack of understanding of the contemporary status of wild ancestral-type forms, which are almost universally either extinct or endangered in a form lacking significant domestic introgression (Redford & Dudley, 2018; Smith, Quilodrán, et al., 2022). There is increasing recognition that this is a problem, as the genetic diversity of ancestral-type undomesticated lineages contributes to global biodiversity, and has potential in supplying the raw material for future domestication or genetic rescue of inbred domestic strains (Riordan & Nabhan, 2019). Particular attention has focused on plants (Perrino & Perrino, 2020), where identification of crop wild relatives may provide a source of important genetic variation to help combat the global food crisis, for example by facilitating the development of agriculture in arid or saline regions (Riordan & Nabhan, 2019). Despite such importance, our knowledge of the wild forms of domesticates is often insufficient and their taxonomic status debated. We know that the ancestral lineage of the domestic dog is extinct, with contemporary Grey Wolves representing a sister lineage (Freedman et al., 2014); the Aurochs (*Bos primigenius*), ancestor of domestic cattle (*Bos taurus*), has been extinct since 1627 (Mona et al., 2010; Park et al., 2015; Upadhyay et al., 2017) ; and the African Wild Ass (*Equus africanus*) is Critically Endangered (Tesfai et al., 2019). In other cases, debate over the wild, feral or hybrid status of contemporary populations continues (Smith, Quilodrán, et al., 2022). The existence of unadulterated populations of Red Junglefowl (*Gallus gallus*) is unclear, with extensive evidence of introgression from feral domestic chickens (Peterson & Brisbin, 1998; Thakur et al., 2018). A genomic study argued that the putatively wild Przewalski's Horses (*Equus ferus przewalskii*) were an ancient feral domestic population (*Equus caballus*), and that the wild horse is actually extinct (Gaunitz et al., 2018). This discovery was challenged by more recent osteological work which argued that

Przewalski's Horse has not been through domestication and therefore does represent a relictual population of the undomesticated wild horse (Taylor & Barrón-Ortiz, 2021). Further research will be needed to determine the provenance and evolutionary history of these populations.

The study of extinction by hybridisation

In recent decades, our ability to quantify the extent of hybridisation at the level of both population and individual has increased (Randi, 2008). Early work depended heavily on phenotypic assessments and assumptions regarding which traits reflect a genetic contribution from a particular parental lineage. Such works often acknowledged both the limitations of inferences based solely on physical characteristics, and the complexities of the biological systems involved. With the European wildcat (*Felis silvestris*) and the feral cat (*Felis catus*), one of the most famous examples of extinction by hybridisation, inferring wild or feral provenance based on pelage and morphology is fraught with issues relating to the lack of a known phenotypic baseline for an unadmixed wildcat and the inability to account for natural deviation from the 'wildtype' appearance (Randi, 2008; Mattucci et al., 2019; Senn et al., 2019). Whilst some interpretations could be made regarding phenotypic patterns of introgression, recent genomic approaches and statistical hybrid analyses have allowed more precise quantification of the level of mixed ancestry within widespread hybrid swarms (Randi, 2008). Such analyses follow the same basic process as phenotypic analyses, wherein differentiation between unadmixed

populations of each parental lineage is quantified and then the relative contributions of each to the ancestry of individuals is estimated (Payseur & Rieseberg, 2016). The use of Single Nucleotide Polymorphism (SNP) markers in the estimation of the level of admixture of individuals is now the dominant method with which to characterise situations where genetic replacement is unfolding. It has been applied with the wildcat, junglefowl, and horse wild-feral-domestic case studies and is considered significantly less subjective than phenotypic methods (Gaunitz et al., 2018; Senn et al., 2019; Wu et al., 2020). Although issues regarding the determination of non-introgressed genomic and phenotypic baselines persist, our ability to quantify hybrid ancestry has vastly improved over the past decade. Nevertheless, much of our knowledge of extinction by hybridisation comes from research focussed on a handful of species. In fact, most of our understanding of wild-feral hybridisation, an economically and ecologically important subset of extinction by hybridisation issues, comes from studies of wildcats (Daniels et al., 1998; Beaumont et al., 2001; Witzenberger & Hochkirch, 2014; Gil-Sánchez et al., 2015; Quilodrán et al., 2019; Senn et al., 2019; Beugin et al., 2020; Tiesmeyer et al., 2020; Quilodrán et al., 2020) and junglefowl (Peterson & Brisbin, 1998; Johnsson et al., 2016; Thakur et al., 2018; Wu et al., 2020). The study of extinction by hybridisation (and especially between wild and feral domestic entities) in other species would contribute to understanding global variation in the process. We would be better able to explore the factors determining the prevalence, extent, and outcomes of extinction by hybridisation. In a world increasingly influenced by anthropogenic activity, it is important to assess whether extinction by hybridisation is a common or rare outcome of interbreeding, and to determine whether hybridisation could in some cases provide beneficial conservation outcomes.

DPhil Research

This thesis explores the dynamics of hybridisation using a novel case study consisting of a feral domestic animal and its undomesticated form. The pigeon *Columba livia* is primarily represented worldwide by captive domestic breeds (e.g. racing pigeons or fancy breeds for shows) and their feral descendants (Johnston & Janiga, 1995). The species was domesticated as early as 10,000 years ago to the east of the Mediterranean (Kiple et al., 2000) and received a genetic contribution from the African Speckled Pigeon (*Columba guinea*) during the domestication process (Vickrey et al., 2018). Pigeons have had a long relationship with humanity and have deep practical, traditional, and spiritual significance in many cultures worldwide, within birds potentially only matched by the Red Junglefowl and its domestic chicken offshoot (Johnston & Janiga, 1995). Darwin himself used the many domestic breeds of pigeon as a source of inspiration with which to explore his ideas about selection (Pacheco et al., 2020). The undomesticated and wild form of the species, the Rock Dove, has a cryptic genetic status, and the extent to which truly wild populations still exist over their original range (covering parts of Europe, Asia, and north Africa) has long been unclear (Johnston et al., 1988; Stringham et al., 2012). This is due to widespread replacement with feral pigeons and hybrids following extensive interbreeding (Johnston & Janiga, 1995). It has been suggested that Rock Doves lacking any feral genetic contribution are now extinct (Stringham et al., 2012; Blasco et al., 2014). In the British Isles, putative Rock Dove populations are restricted to the coasts of western Ireland and north-west Scotland, and especially their offshore islands (Hewson, 1967). The level of feral pigeon presence within Scotland and Ireland varies, which likely impacts the

magnitude of wild-feral hybridisation experienced (Hewson, 1967). Within the British Isles, the Rock Dove-feral pigeon population mosaic has the potential to represent a tractable example of interbreeding and gene flow at the wild-domestic interface. The Rock Dove is an ancestral-type entity, with a contemporary distribution that is mostly insular (at least in Europe), where we can sample from putatively unadmixed, newly mixing and hybrid swarm populations. Throughout this thesis, I will be using Rock Doves to explore the dynamics of extinction by hybridisation, and to characterise this system to enable future research. Chapters two to seven are as follows:

2. A review which explores the scientific importance of the wild forms of our domestic animals (Smith, Quilodrán, et al., 2022). Historically neglected compared to the undomesticated forms of crop plants, these entities are often endangered and pose specific conservation challenges relating to their distinctiveness from domestic conspecifics. We outline these difficulties and argue that undomesticated populations of domesticated animals are a conservation priority. This chapter is now published as: **Smith, W.J. et al. (2022) The wild ancestors of domestic animals as a neglected and threatened component of biodiversity. *Conservation Biology 36*, e13867.**

3. A review which outlines the history of British populations of undomesticated Rock Doves (Smith, 2023b). I highlight their decline and contemporary conservation status as described in existing literature, before assessing their prospects. I explain what information is needed to conserve the remaining wild populations, and what research should therefore be prioritised. This chapter is now published as: **Smith,**

W.J. (2023) The undomesticated Rock Dove in Britain and the Isle of Man. *British Birds* 116, 70-76.

4. An empirical analysis of the population genomics of Rock Doves and feral pigeons in the British Isles (Smith, Sendell-Price, et al., 2022). This project is the first to highlight the extensive wild-feral gene flow that threatens relictual Rock Dove populations. It located what is currently the only Rock Dove population (in the Outer Hebrides) which has been genomically proven to have experienced negligible feral pigeon gene flow. This paper is now published as: **Smith, W.J. et al. (2022) Limited domestic introgression in a final refuge of the wild pigeon. *iScience* 25 (7), 104620.**

5. A study which explores the impact of wild-feral gene flow on genomic diversity. We show that contemporary hybridising populations, where Rock Doves are in contact with feral pigeons, have higher genomic diversity and reduced inbreeding, potentially granting conservation benefits. We use these results to discuss the potential positive role of hybridisation. **This chapter, entitled 'Gene flow and genomic diversity in British and Irish pigeons *Columba livia*', is currently in review at *Conservation Genetics*.**

6. After characterising the populations genomically, I examined plumage variation among different populations of Rock Doves and feral pigeons in the British Isles (Smith, 2023a). I show that is it possible to use variation in plumage polymorphism to distinguish between Rock Doves and feral pigeons, and to infer

variation in the progression of the extinction by hybridisation process among Rock Dove colonies. This method is amenable to a citizen science approach. This paper is now published as: **Smith, W.J. (2023) Rapid assessment of the extent of feral introgression in British and Irish Rock Dove (*Columba livia*) populations. *Ibis* in press.**

7. I characterised the prevalence and diversity of the parasite *Trichomonas* in three populations of *Columba livia* in the UK. These represent Rock Dove, feral pigeon and hybrid populations. We show that prevalence and diversity vary among the populations, and we discuss the possibility that disease transmission could increase the risk of extinction by hybridisation. **This chapter, entitled 'The interplay of parasite exchange and hybridisation at a wild-feral-domestic interface', is currently in review (following revisions) at the *International Journal for Parasitology*.**

Finally, in the General Discussion, I outline the ways in which my work fits into the broader range of studies focussed on genomic introgression, and the advancements my work makes for the field. I then outline future directions for scientific research on extinction by hybridisation. I finish by proposing that the Rock Dove-feral pigeon system presents an excellent future model system to understand the dynamics of hybridisation, and outline ways that increased understanding of Rock Dove biology has the potential to benefit research focussed on domestic pigeons in a laboratory setting.

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The wild ancestors of domestic animals as a neglected and threatened component of biodiversity

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ESSAY

The wild ancestors of domestic animals as a neglected and threatened component of biodiversity

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Article Impact Statement
The wild ancestors of domestic animals should be studied and conserved to afford scientific, practical, and ecological benefits.

Abstract
Domestic animals have immense economic, cultural, and practical value and have played pivotal roles in the development of human civilization. Many domesticates have, among their wild relatives, undomesticated forms representative of their ancestors. Resurgent interest in these ancestral forms has highlighted the unclear genetic status of many, and some are threatened with extinction by hybridization with domestic conspecifics. We considered the contemporary status of these ancestral forms relative to their scientific, practical, and ecological importance; the varied impacts of wild–domestic hybridization; and the challenges and potential resolutions involved in conservation efforts. Identifying and conserving ancestral forms, particularly with respect to disentangling patterns of gene flow from domesticates, is complex because of the lack of available genomic and phenotypic baselines. Comparative behavioral, ecological, and genetic studies of ancestral-type, feral, and domestic animals should be prioritized to establish the contemporary status of the former. Such baseline information will be fundamental in ensuring successful conservation efforts.

KEYWORDS
conservation genetics, domestic animals, extinction, feral populations, hybridization, wild–domestic interface

Los Ancestros de Animales Domésticos como un Componente Descuidado y Amenazado de la Biodiversidad

Resumen: Los animales domésticos tienen un inmenso valor económico, cultural y práctico y han jugado un papel muy importante en el desarrollo de la civilización humana. Muchos animales domesticados tienen, entre sus parientes silvestres, formas no domesticadas representativas de sus ancestros. Un interés renovado en estas formas ancestarles ha destacado el estatus genético poco claro de muchas, y algunas están amenazadas de extinción por hibridación con conespecíficos domesticados. Consideramos el estatus contemporáneo de estas formas ancestrales en relación con su importancia científica, práctica y ecológica; los impactos diversos de la hibridación silvestre-domesticado; y los retos y soluciones potenciales involucrados en los esfuerzos de conservación. La identificación y conservación de formas ancestrales, particularmente en relación con desenredar patrones de flujo génicos, es compleja debido a la carencia de líneas de base genómicas y fenotípicas. Se deben priorizar estudios conductuales, ecológicos y genéticos comparativos de los animales ancestrales, ferales y domésticos para establecer el estatus contemporáno de los primeros. Tal información de base será fundamental para asegurar esfuerzos de conservación exitosos.

PALABRAS CLAVE
Animales domésticos, extinción, genética de la conservación, hibridación, interfase silvestre-doméstico, poblaciónes ferales

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家畜的野生祖先物种是生物多样性中被忽视的受威胁组成部分

【摘要】家畜具有巨大的经济、文化和实用价值, 在人类文明的发展中发挥了关键作用。许多家畜的野生近缘物种中都有代表其祖先型的未驯化动物。重新关注这些祖先型的动物, 会发现其中许多的遗传状态不明确, 还有些因为与同种的家畜发生杂交而面临灭绝的威胁。我们分析了这些祖先型动物目前在科学、实用和生态方面的重要性、野生动物与家畜杂交的各种影响, 以及保护工作中的挑战和潜在的解决方案。由于缺乏可用的基因组和表型基线数据, 识别和保护祖先型动物仍是十分复杂的问题, 特别是解析其受到来自家畜的基因流模式。我们建议应优先考虑对祖先型动物、野生动物和家畜的行为学、生态学和遗传学进行比较研究, 以确定前者当前的状态。这些基线信息是确保保护工作成功的基础。

【翻译: 胡怡思; 审校: 聂永刚】

关键词: 保护遗传学, 灭绝, 家畜, 杂交, 野生种群, 野生动物-家畜的接触

INTRODUCTION

Among animals, domesticated species have developed an unparalleled relationship with humanity, playing fundamental economic and sociocultural roles (Zeder, 1982). They hold a unique position in our collective culture, and many have central roles in human mythologies and major world religions (Lodrick, 2005). As well as this cultural importance, there is growing recognition of their ecological role (Doherty et al., 2017). The current global biomass of mammalian domestic livestock and chickens surpasses that of all wild mammals and birds, respectively, resulting in significant climatic and ecological impacts (Bar-On et al., 2018). In addition, predation, competition, and disease transmission occur at the wild–domestic interface—the latter being of particular interest in the context of emerging zoonotic disease threats (White & Razgour, 2020). Despite the importance of domestic animals, many of their undomesticated forms are relatively poorly understood. This lack of knowledge is a major problem because many undomesticated forms are at risk of extinction, and the failure of these entities to persist represents a loss of scientific, socioeconomic, and ecological opportunity (Taberlet et al., 2008).

The origin of domestic animals is often obscure. Most are presumed to originate from a primary ancestor with genetic contributions from closely related species (Thakur et al., 2018). Domestication can be considered a process rather than an outcome, and porous reproductive boundaries frequently allow extensive gene flow with related wild populations (McHugo et al., 2019). Currently, the status of undomesticated forms of many domestic animals is uncertain due to a lack of clear phenotypic demarcations between them and their relatives of domestic origin, which is often exacerbated by hybridization between the forms (Redford & Dudley, 2018) (Figure 1, Table 1, & Appendix S1). We use the term *undomesticated forms* to refer to the variously labeled truly wild or ancestral populations thought to be the closest extant wild relatives of a domesticate, descending from the wild populations from which the original stock was domesticated. This definition is used to prevent confusion when discussing feral entities, for which both domestic and the original wild populations are 'ancestral'. Our use of *undomesticated forms* excludes other wild relatives. For example, we do not consider the speckled pigeon (*Columba guinea*) and grey junglefowl (*Gallus sonneratii*), which made small genetic contributions to domestic pigeons (*Columba livia)* (Vickrey et al., 2018) and chickens (*Gallus gallus)* (Lawal et al., 2020), respectively, undomesticated forms. We focused specifically on the extant representatives of the primary wild ancestors of domestic animals and the issues associated with their conservation. Although extant gray wolves (*Canis lupus*) and European wildcats (*Felis silvestris*) are not the ancestral lineages of domestic dogs (*Canis familiaris*) and cats (*Felis catus*), respectively, with these being an extinct wolf population and the African wildcat (*Felis lybica*), respectively, we considered them where appropriate given their ecological and cultural importance and the parallel conservation issues associated with them.

One of the main benefits of maintaining wild relatives is as a source of genetic variation for domestic forms. There has been a particular focus on wild crop relatives, given their potential contribution of beneficial genetic variation to modern crops (Zhang et al., 2016). The extinction of the undomesticated forms of domestic animals may similarly lead to the loss of genetic variation for potential introduction into domestic populations and, therefore, to a reduction in the ability to assure the resilience of domestic populations to disease and environmental threats (Redford & Dudley, 2018). Yet, they receive far less attention despite the importance of domestic animals as food sources, major ecological players, and model organisms. We sought to increase awareness of the importance of conserving the undomesticated forms of domestic animals. We expanded on the multiple practical and scientific benefits of their protection and considered the threat to undomesticated lineages, particularly with respect to hybridization with feral and domestic relatives. We also explored the challenges of their conservation, potential resolutions to these challenges, and priorities for research in these systems.

IMPORTANCE OF MAINTAINING WILD ANCESTORS

Scientific opportunity

The undomesticated forms of domestic animals provide multiple avenues of scientific opportunity. Teasing apart the complexity of gene flow at the wild–domestic interface will be

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TABLE 1 Examples of domestic animals and their International Union for the Conservation of Nature (IUCN) status in an undomesticated form

Domestic form and use	Undomesticated form	Contemporary feral status	Conservation status of undomesticated form	Reference
Companions				
Dog (*Canis familiaris*)	extinct population of wolves	globally distributed and abundant; two ancient feral populations of note (dingo and the New Guinea singing dog)	generally accepted as an extinct population of wolves; domestic hybridization locally threatens extant populations of gray wolf, which is widely distributed across Eurasia and North America and is recovering in its range in some regions (e.g., in Europe)	Bergström et al., 2020; Freedman et al., 2014; Perri et al., 2021
Cat (*Felis catus*)	wildcat *Felis lybica* (*Felis silvestris* sister species)	globally distributed and abundant	least concern and widely distributed across Afro-Eurasia; threatened with extinction by hybridization with feral and domestic cats; many wildcat populations, particularly in Europe, have experienced extensive introgression, and some (e.g., in Scotland) have become extinct as a separate phylogenetic entity and replaced by hybrids and feral cats	Beugin et al., 2020; Quilodrán et al., 2019; Senn et al., 2019
Food source[a]				
Cattle (*Bos taurus*)	aurochs (*Bos primigenius*)	globally distributed and abundant	extinct; originally present across Asia, Europe, and North Africa	Decker et al., 2014; Mona et al., 2010; Upadhyay et al., 2017
Sheep (*Ovis aries*)	mouflon (*Ovis gmelini*)	globally distributed and abundant; famous example is Soay sheep on St Kilda, Scotland, used by biologists to study evolution; European mouflon represent a feral population of early domestic sheep	near threatened and distributed in the Near East and Middle East; most significant conservation issue is poaching; no genomic studies have assessed global variation in extent of domestic introgression in the wild populations	Barbato et al., 2017; Bleyhl et al., 2019; Ciani et al., 2020; Eydivandi et al., 2020
Goat (*Capra aegagrus* or *Capra hircus*)	probably the bezoar ibex (*Capra aegagrus*)	globally distributed and abundant	near threatened and distributed from the Near East to south Asia; threatened by habitat change, hunting, and competition with livestock; no genomic studies have assessed global variation in extent of domestic introgression in wild populations	Alberto et al., 2018; Dong et al., 2015; Kuemmerle et al., 2020
Water buffalo (*Bubalus bubalis*)	wild water buffalo (*Bubalus arnee*)	regionally common in southeast Asia and Oceania, including Australia; present elsewhere, including South America	endangered; very restricted in range and almost completely found in India; threatened by habitat change, competition, and disease transmission from domestic livestock, hunting, and domestic introgression	Flamand et al., 2003; Kierstein et al., 2004; Zhang et al., 2020
Bali cattle (*Bos javanicus*)	banteng (*Bos javanicus*)	locally common where the domestic form is kept	endangered; native to southeast Asia; threatened by poaching, habitat change, and disease transmission from domestic livestock; subject to hybridization with domestic bovids; an introduced population in Australia is potentially of conservation significance because it is more isolated from these risks	Corey et al., 2006
Gayal (*Bos frontalis*)	gaur (*Bos gaurus*)	relatively rare and limited to regions in which gayal are commonly kept	vulnerable; native to Southeast Asia and south Asia; threatened by habitat loss and poaching; there are few data regarding hybridization between gaur and domestic bovids	Choudhury, 2002; Nguyen et al., 2007

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TABLE 1 (Continued)

Domestic form and use	Undomesticated form	Contemporary feral status	Conservation status of undomesticated form	Reference
Pig (*Sus scrofa*)	wild boar (*Sus scrofa*)	globally distributed and abundant	least concern; widely distributed throughout Afro-Eurasia; experiencing gene flow from feral domestic pigs	Giuffra et al., 2000; Iacolina et al., 2018
Chicken (*Gallus gallus*)	red junglefowl (*Gallus gallus*)	globally distributed and abundant; common in the United States and elsewhere, including throughout the range of the ancestral junglefowl	least concern; widely distributed throughout Southeast Asia and South Asia; threatened with extinction by hybridization with feral and domestic chickens; most populations have been subjected to extensive introgression	Lawal et al., 2020; Thakur et al., 2018; Wu et al., 2020
Domestic pigeon (*Columba livia*)	rock dove (*Columba livia*)	cosmopolitan distribution and superabundant	least concern; originally present across Afro-Eurasia; threatened with extinction by hybridization with feral and domestic pigeons; appears restricted to relict populations in more remote regions; most populations have been subjected to extensive introgression; unclear whether populations exist that have not been extensively influenced by gene flow from domestic pigeons	Johnston et al., 1988; Johnston & Janiga, 1995
Domestic goose (*Anser anser*)	greylag goose (*Anser anser*)	globally distributed and abundant, including in native range of Greylag goose	least concern; distributed across Eurasia; very widespread and abundant; evidence of bidirectional gene flow with the domestic goose	Heikkinen et al., 2020
Guinea pig (*Cavia porcellus*)	Montane guinea pig (*Cavia tschudii*)	little evidence of substantial self-sustaining feral populations	least concern; native to the Andes; no major threats to the species as per IUCN	Lord et al., 2020
Domestic Atlantic salmon	Atlantic salmon (*Salmo salar*)	substantial escaped populations from fish farms	least concern; native to the North Atlantic; widespread domestic introgression in existing wild populations leading to negative demographic impact populations lacking domestic influence rare	Glover et al., 2020; McGinnity et al., 2003; Solberg et al., 2020
Western honey bee (*Apis mellifera*)	wild honey bee (*Apis mellifera*)	feral colonies can occur and are the dominant form in the wild in many regions	data deficient; extent to which population trends are driven by feral or wild form unclear; some wild populations threatened by domestic introgression (although domestic status and origin of different lineages of honey bee is debated)	Carreck, 2008; Muñoz et al., 2015; Tihelka et al., 2020
Practical utility				
Horse (*Equus ferus*)	possibly the tarpan (*Equus ferus*)	globally distributed and abundant; famous examples are mustangs of the western United States and semiferal mountain and moorland breeds of pony in the British Isles; potential ancient feral population (Przewalski's horse, see Discussion)	extinct or represented by Przewalski's horse, which is restricted to the steppes of Central Asia (see Discussion)	Gaunitz et al., 2018; Kavar & Dovč, 2008; Librado et al., 2016
Donkey (*Equus africanus*)	African wild ass (*Equus africanus*)	globally distributed and abundant; famous examples are burros of North America and albinistic Asinara donkeys of Sardinia	critically endangered and currently found on the Horn of Africa; threatened by hunting, habitat change, and competition with domestic livestock; some putative subspecies, such as the Atlas wild ass, are extinct; extent of domestic introgression in surviving populations unclear	Tesfai et al., 2019; Utzeri et al., 2016

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TABLE 1 (Continued)

Domestic form and use	Undomesticated form	Contemporary feral status	Conservation status of undomesticated form	Reference
Domestic yak (*Bos grunniens*)	Wild yak (*Bos mutus*)	relatively rare and almost completely limited to regions where domestic yaks are commonly kept; domestic yaks are often kept in a manner allowing contact with wild yaks	vulnerable and found in the Himalayas; threatened by poaching; some evidence of interbreeding with domestic yaks suggests that introgression may be a problem	Chen et al., 2018; Wang et al., 2016
Domestic Bactrian camel (*Camelus bactrianus*)	direct ancestor extinct, but a closely related species (wild Bactrian camel [*Camelus ferus*]) exists	relatively rare; there were once populations of feral domestic Bactrian camels in deserts of the United States	critically endangered and restricted to the steppes of Central Asia; threatened by poaching and genetic introgression from domestic Bactrian camels	Fitak et al., 2020; Kaczensky et al., 2014
Dromedary (*Camelus dromedarius*)	ancestral dromedary	patchy global distribution; significant population in Australia	extinct	Almathen et al., 2016; Orlando, 2016
Llama (*Lama glama*)	guanaco (*Lama guanicoe*)	rare and localized	least concern and common in southern South America; abundant, with an introduced population in the Falkland Islands; unclear to what extent hybridization with llamas occurs	Bustamante et al., 2002; González et al., 2014
Alpaca (*Vicugna pacos*)	vicuna (*Vicugna vicugna*)	rare and localized	least concern and common in the central Andes; abundant, although some subpopulations are at risk from poaching and habitat loss; extent of hybridization with alpacas unclear	Marín et al., 2007; Wakild, 2020
Domestic ferret (*Mustela putorius*)	European polecat (*Mustela putorius*)	patchy global distribution; often occurs on islands, including New Zealand and Guernsey	least concern; widely distributed throughout western Eurasia; locally and regionally threatened by persecution and domestic introgression from feral ferrets	Croose et al., 2018
Domestic silk moth (*Bombyx mori*)	wild silk moth (*Bombyx mandarina*)	survival of domestic silk moths outside captivity apparently poor	not assessed by IUCN; occurs over a wide range in Asia; extent of domestic introgression to the wild moth unclear, although the two forms can hybridize	Sun et al., 2012; Xiang et al., 2018

[a]Includes those originally domesticated for food but now mostly kept for recreation.

valuable in elucidating the evolutionary role of hybridization. Due to the often-cosmopolitan presence of feral animals, there are usually multiple contact zones with undomesticated populations, providing the opportunity to study geographic variation in patterns of introgression across the genome. For example, different populations of the European wildcat (Senn et al., 2019), wild greylag goose (*Anser anser*) (Heikkinen et al., 2020), and wild mallard (*Anas platyrhynchos*) (Söderquist et al., 2017) are subject to varying extents of gene flow with their domestic counterparts. Studies of such scenarios contribute to the development of an understanding of the homogenizing effect of hybridization, which could in turn improve the understanding of diversification and evolution of novel lineages (Taylor & Larson, 2019). Genomic regions of very low introgression may represent "barrier loci," which are important in the development of reproductive isolation (Liu et al., 2020). Therefore, studying introgression in wild–domestic systems could also advance the understanding of speciation.

Characterization of undomesticated lineages informs understanding of the processes of domestication and feralization, both important processes in an increasingly anthropogenic world (Gering et al., 2019). Feralization, in particular, is understudied and cannot be seen as a reverse of domestication, given that it involves novel selective pressures and evolutionary trajectories. Studies of feral chickens in Kauai show that feralization and domestication target different genomic loci (Johnsson et al., 2016). Such an understanding enhances the ability to comprehend the biology of economically and practically important domestic animals, as well as economically and ecologically important feral pests (Henriksen et al., 2018). Furthermore, domestic animals are important model organisms in biomedical and zoological research (Bähr & Wolf, 2012). Characterization of the genetics, behavior, and physiology of undomesticated lineages would allow investigation of trait evolution and selection in a wild setting. This context will increase the relevance of conclusions drawn from experiments carried out using model organisms. For example, studies of domestic pigeons to understand collective behavior (Sasaki & Biro, 2017) could benefit from parallel information in wild rock doves, allowing inferences about the evolution of the observed traits and their

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adaptive roles in a natural setting. Although it is important to recognize that extant undomesticated forms are not perfectly representative of the original ancestral populations (Bosse, 2019), such comparative studies of undomesticated, domestic, and feral forms would allow identification of traits of scientific, practical, and economic interest in understudied wild populations.

Reservoir of genetic diversity

Many domestic animals, including our most economically important livestock species, such as cattle and sheep, are genetically impoverished following many generations under artificial selection. Consequently, many livestock species could be considered "genetically endangered" due to their low adaptive potential and concomitant vulnerability to developing climatic and disease threats (Taberlet et al., 2008). Undomesticated populations may act as reservoirs of genetic diversity and harbor useful traits that could be introduced into domestic stock. This has been well recognized with respect to plants (Zhang et al., 2016), For example, a lack of genetic diversity in the apple (*Malus pumila*) and barley (*Hordeum vulgare*) gene pools led to suggestions that wild relatives could alleviate this threat (Dempewolf et al., 2017). Animal ancestors have similar utility in terms of harboring useful genetic diversity. For example, introgression of genes from wild sheep into domestic breeds, such as Soay sheep (*Ovis aries*), has likely enhanced innate immunity (Barbato et al., 2017). Similarly, it is thought that introgression from (now-extinct) aurochs (*Bos primigenius*) conveyed parasite resistance to domestic cattle (Decker et al., 2014). The loss of distinct undomesticated lineages would mean a missed opportunity to introduce favorable traits into domestic stocks, though some ancestral genomic variation may be recoverable from older domestic lineages. For example, Highland cattle have a greater genetic contribution from ancestral aurochs than many extant breeds (Park et al., 2015). Nevertheless, the maintenance of genetically diverse populations of extant undomesticated lineages would be the optimal strategy to ensure that adaptive introgression remains an option for domestic animals.

Ecological function

The ecological repercussions of the loss of wild ancestors and the extent to which hybrid or domestic forms could replace them are important topics of discussion. For wildcats, it has been proposed that hybrid cats of mixed domestic–wild ancestry could approximate the ecological role of true wildcats in Scotland as predominantly solitary mesopredators of forested regions (Fredriksen, 2015). However, individuals of mixed ancestry may have an ecological role intermediate between their feral and wild forms. Feral domestic cats are ecologically damaging because they prey on birds and small mammals (Trouwborst et al., 2020), whereas wildcats have more specific habitat and dietary requirements (Széles et al., 2018). Hence, it would be an undesirable outcome if hybrid individuals behaved more like feral cats than wildcats. Where there is no other option, domestic animals have been used as ecological proxies for their extinct ancestors to apparent success in landscape-level conservation efforts. An example of this is the substitution of domestic cattle for extinct aurochs (Marris, 2011). It is likely that aurochs played a broadly similar ecosystem engineering role, grazing and fertilizing grassland, although extent of overlap with the ecological functionality of domestic cattle is unknown. The degree to which individuals of mixed ancestry, or domesticates, are adequate ecological replacements for undomesticated forms depends on the domestic animal involved and requires species-specific research. It would seem wise, without good evidence for the ecological utility of hybrid or domestic forms, to adhere to the precautionary principle with respect to ecological function.

OUTCOMES OF HYBRIDIZATION BETWEEN DOMESTIC ANIMALS AND THEIR UNDOMESTICATED FORMS

The outcomes of hybridization, whether homogenizing or diversifying, have attracted significant attention from conservationists. With respect to hybridization between domestic animals and their undomesticated forms, it is likely that spatially and temporally varying gene flow has occurred since domestication began (Lawal et al., 2020). To explore the varying impacts of wild–domestic hybridization, we considered two gene-flow scenarios that need not be mutually exclusive across the range of a species. In the first, the distinction between the lineages breaks down. In the second, the two lineages remain distinct despite transfer of genetic material. Both scenarios are of conservation relevance.

Extensive gene flow leading to the formation of a hybrid swarm

Extensive gene flow between undomesticated and domestic forms can lead to the formation of a hybrid swarm as seen in Scotland's European wildcats (Senn et al., 2019). Hybrid swarms are populations in which most or all individuals are of mixed ancestry (Beninde et al., 2018). Because the undomesticated population is often less populous than its domestic relative, the formation of hybrid swarms can lead to the "extinction by hybridization" of the former (Senn et al., 2019). This process can be defined as the genetic replacement of a distinct population or species with a relative following introgression. When it occurs naturally, without either human introductions of species or anthropogenic habitat alteration leading to range-shifts, this breakdown in the distinctiveness of different lineages has been referred to as "reverse speciation" (Kearns et al., 2018). It is possible that extinction by hybridization is of particular interest to conservationists following population reductions driven by various demographic threats. For example, there is evidence that domestic cattle and horses hybridized with their respective undomesticated forms (now extinct, primarily due to

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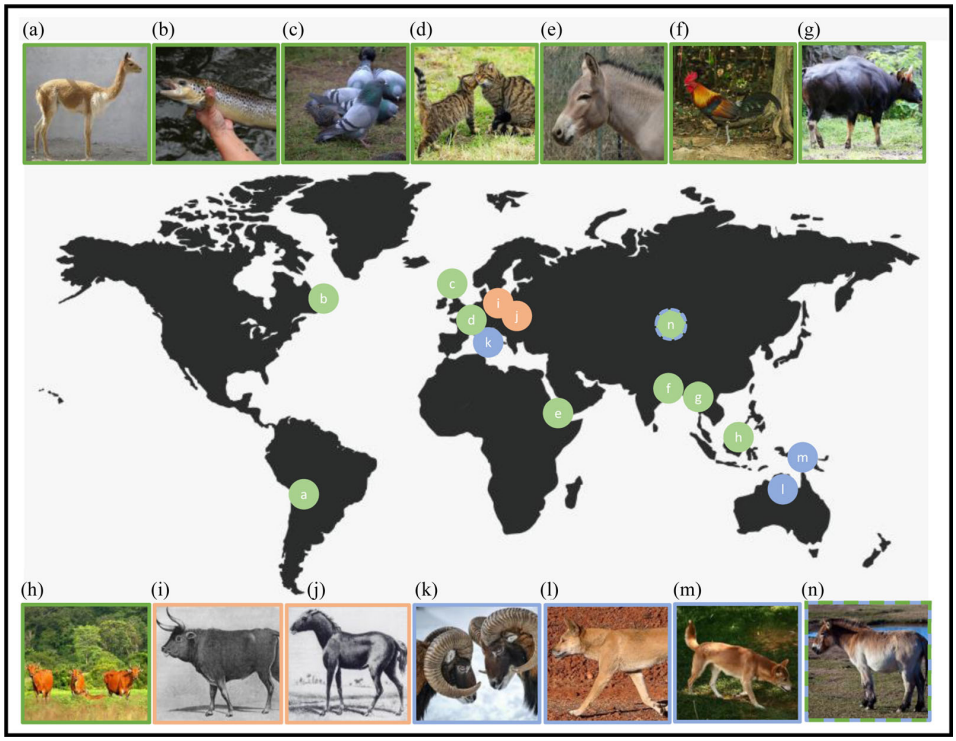


FIGURE 1 Distribution of some of the undomesticated and ancient feral forms of domestic animals: (a) vicuna (*Vicugna vicugna*), (b) Atlantic salmon (*Salmo salar*), (c) rock dove (*Columba livia*), (d) wildcat (*Felis silvestris*), (e) African wild ass (*Equus africanus*), (f) red junglefowl (*Gallus gallus*), (g) gaur (*Bos gaurus*), (h) banteng (*Bos javanicus*), (i) aurochs (*Bos primigenius*), (j) tarpan (*Equus ferus ferus*), (k) European mouflon (*Ovis aries*), (l) dingo (*Canis familiaris*), (m) New Guinea singing dog (*Canis familiaris*), and (n) Przewalski's horse (*Equus ferus przewalski*). Animals represented in photographs (a–h) are extant. Those in (i) and (j) are extinct and those in (k–m) are of ancient feral origin. The undomesticated or ancient feral status of Przewalski's horse (n) is disputed. Photographers, respectively, are Thomas Quine (CC BY 2.0), Greg Thompson/USFWS (public domain), Mike Pennington (CC BY-SA 2.0), Peter Trimming (CC BY 2.0), Greg Goebel (CC BY 2.0), Francesco Veronesi (CC BY-SA 2.0), Mahbob Yusof (CC BY 2.0), Rochmad Setyadi (CC BY-SA 2.0), Charlie Hamilton Smith (public domain), Public Domain Image, Andrea Schieber (CC BY-NC-ND 2.0), Jarrod Amoore (CC BY 2.0), Patti McNeal (CC BY 2.0), and Lawrence Schaefer (CC BY 2.0).

habitat loss and overhunting) when those undomesticated forms were geographically restricted and demographically imperiled (Decker et al., 2014).

The process of extinction by hybridization can proceed in a geographically restricted manner, as appears to be the case for the polecat (*Mustela putorius*) (Croose et al., 2018) and wild boar (*Sus scrofa*) (Iacolina et al., 2018), and can also be common across the entire range of the undomesticated form, as is the case for the Atlantic salmon (*Salmo salar*) (Solberg et al., 2020) and red junglefowl (Thakur et al., 2018). For species in the latter category, which also includes the rock dove (Baldaccini, 2020) and wild water buffalo (*Bubalus arnee*) (Flamand et al., 2003), there is often uncertainty regarding the continued persistence of 'pure' undomesticated populations. Even in the absence of data on genomic-level introgression, the geographic arrangements of populations strongly suggest that introgression is occurring. For example, African wild ass (*Equus africanus*) populations are very small, fragmented, and in sympatry with domestic populations (Tesfai et al., 2019). In such situations, individuals in undomesticated populations may have more difficulty finding mating partners and thus become less choosy, which increases their likelihood of mating with a domestic individual (the "desperation hypothesis" [Hubbs, 1955]). Such demographic imbalances and mate choice opportunities are also relevant for expanding undomesticated populations. In the Swiss Jura, European wildcats are undergoing a spatial demographic recovery following conservation efforts and are at much lower density than feral cats at the expanding front. Here, wildcats are more likely to mate with their domestic counterparts than with others of their kind, resulting in extensive introgression (Quilodrán et al., 2019). This highlights that any conservation efforts for undomesticated populations should give special attention to the potential for introgression from domestic relatives upon range expansion (Quilodrán et al., 2020) and should survey the extent to which introgression is geographically and temporally limited to the expansion front.

The evolutionary consequences of hybrid swarm formation can vary depending on the fitness of hybrid individuals in the local environment. In many situations, hybrids may be at a selective advantage. Such hybrid vigor has been noted in nondomestic situations. For example, hybrid individuals of the medium tree finch (*Camarhynchus pauper*) and small tree finch

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(*C. parvulus*) have fewer parasites in their nests compared with their parental species (Peters et al., 2019). This could accelerate hybrid swarm formation and the genetic replacement of the rarer parental lineage. Conversely, hybrid populations may also be maladapted compared with their parental lineages. Although this should limit introgression and, therefore, the formation of hybrid swarms, in many cases, the population imbalance between undomesticated and domestic forms is so great that extensive introgression still occurs. For example, in Atlantic salmon (*S. salar*), domestic escapees often far outnumber undomesticated populations (Sylvester et al., 2019). Selection for increased growth rate (and perhaps relaxed selection in a captive context) in domestic populations has been hypothesized to have reduced their environmental sensitivity, rendering wild–domestic hybrids more prone to risk-taking behavior. This can cause increased predation of wild–domestic hybrids in natural settings (Solberg et al., 2020). Such unwitting introduction of maladaptive traits may pose a risk, especially to small and disjunct wild populations.

Low levels of introgression with maintenance of distinct lineages

The formation of hybrid swarms is not inevitable, and in many cases, hybridization is limited spatially and temporally (Iacolina et al., 2018). In such situations, introgression can introduce traits of interest without complete admixture of the entire genome and a subsequent breakdown in the genomic distinctiveness of the two lineages involved. Multiple conditions could foster such a limitation. First, if both populations are large enough, then heterospecific mating will be rare, especially if assortative mating occurs (Hubbs, 1955). Assortative mating could be reinforced when wild populations and those of domestic origin have different social systems. Wolves exhibit a level of social complexity and cooperation that is not seen in wildcats (Cordoni & Palagi, 2019). It is possible that feral dogs are, therefore, less able to interbreed with wolves than feral cats are with wildcats, and this may help to explain why the latter has been more prone to hybrid swarm formation. The risk of hybrid swarm formation may also be reduced when hybrids are less fit than parental lineages, and undomesticated population sizes are substantial. However, the production of hybrids that are less fit than individuals from the parental lineages is not always sufficient to maintain lineage distinction, especially when there are large disparities in population sizes (as in salmon). Even when the overall level of hybridization is insufficient for the formation of hybrid swarms and the genetic replacement of the undomesticated lineage, traits that have been introgressed into undomesticated populations can still be of evolutionary and conservation significance. For example, it has been hypothesized that genes providing immunological benefits to North American wolves were introduced via introgression from domestic dogs (Coulson et al., 2011; Smith et al., 2020). This highlights the potential of hybridization with the domestic form to have some positive outcomes for undomesticated forms.

CHALLENGES OF STUDYING AND CONSERVING ANCESTORS OF DOMESTIC ANIMALS

Quantifying mixed ancestry

All domestic animals that have been examined have been in contact with their undomesticated forms to some extent since their initial divergence (Larson & Fuller, 2014). In this respect, they differ from examples of the process of extinction by hybridization not involving a domestic entity, such as the Lesser Antillian iguana (*Iguana delicatissima*), which is threatened by the green iguana (*Iguana iguana*) (Vuillaume et al., 2015). In such cases, prior to secondary contact, populations have often diverged in isolation (Schield et al., 2019). Reproductive barriers are, therefore, likely to be less permeable on average than at the wild–domestic interface. For the latter situation, this means there is sometimes no useful baseline for nonintrogressed ('pure') individuals, making it difficult to quantify the degree of mixed ancestry. This situation occurs for Scottish European wildcats, in which introgression with domestic cats has been so extensive that it caused a breakdown in the linkage disequilibrium between genes used in genetic hybrid indices developed by conservation scientists to estimate wildcat purity and those coding for traits used in phenotypic hybrid indices (that mostly relate to pelage patterning). Thus, there is no identifiable pure wildcat to use as a baseline for hybrid identification (Senn et al., 2019). In this case, further debate aiming to identify an acceptable cutoff level of introgression to be considered a wildcat should be coupled with research efforts to establish how to maintain demographically viable and genetically diverse wild populations and mediate wild–domestic hybridization where feasible. More generally, conservation aims must be coupled with strategies to manage the undomesticated and domestic populations and must acknowledge that some level of introgression is inevitable. This has relevance to legislative issues regarding whether individuals of hybrid ancestry receive protection. In many cases, they do not, and this risks the loss of important ancestral variation, especially in situations where hybrid swarms predominate (Trouwborst, 2014).

Unbalanced population sizes and genetic diversity

In contrast to feral and semicaptive domestic animals, which can sustain superabundant and globally distributed populations due to their commensal relationships with humans, undomesticated lineages are often subject to habitat change and other demographic pressures in their natural environments, resulting in numerically small and geographically restricted populations. For example, the wild banteng (*Bos javanicus*) in Vietnam is the endangered wild ancestor of domesticated Bali cattle. They exist in disjunct populations that have experienced significant declines in their geographic range following poaching, disease, and habitat loss (Pedrono et al., 2009). Small populations often harbor less genetic diversity than large ones, limiting their

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evolutionary potential (Hoffmann et al., 2017). Conservation of undomesticated lineages in the face of such an imbalance in population size and low genetic diversity is challenging. Where logistically possible, demographic and genetic reinforcement of undomesticated populations could alleviate the risk of population imbalance. For instance, the banteng was introduced to Australia in 1849, and this non-native population could potentially be used for reintroduction projects back in its native range (Bradshaw et al., 2006). Such approaches must be informed by an appreciation of the factors affecting the populations of the domestic and undomesticated lineages, including disease, persecution, and habitat change. For example, reintroductions to regions where feral populations have not been removed would lead to massive introgression (Nussberger et al., 2018). Overall, a greater understanding of demography and genetic diversity via comparative studies at different spatial scales should allow for a more holistic approach to conservation of undomesticated lineages.

Ancient feral populations

Feral populations of some previously domesticated animals, for example, the Australian dingo (*C. familiaris*) and New Guinea singing dog (*C. familiaris*), have existed for thousands of years (Zhang et al., 2020). These forms are often far less dependent on close association with humans than other feral animals, and we call them *ancient feral* populations. Their existence can make it difficult to identify true undomesticated populations because older feral populations are often phenotypically convergent with their ancestors (Johnston, 1992). Although it has long been known that the canid lineages mentioned above are of domestic origin, other cases have only recently been revealed or are contentious. For example, it is likely that European mouflon (*Ovis aries musimon*) descended from early domestic sheep (Barbato et al., 2017). Przewalski's horse (*Equus ferus przewalskii*), long thought to be the last undomesticated horse, was identified, based on genomic data, as an ancient feral population (Gaunitz et al., 2018), but a recent zooarchaeological study disputed this conclusion on the basis of osteological traits (Taylor & Barrón-Ortiz, 2021). Using ancient DNA could further elucidate the origins of such populations, and it is probable that no wild-living populations of any domesticated species are fully representative of the original undomesticated populations. In many examples, ancient ferals are among the earliest diverging extant groups in their species' phylogenies (Stephens et al., 2015). Hence, we argue that from a conservation perspective, the existence of ancient feral populations is best viewed as an opportunity for conservation scientists studying the ancestors of domestic animals, in that they may represent among the closest extant analogues to the ancestor in situations where undomesticated forms have become extinct. More genomic research is needed to quantify the extent to which they harbor ancestral genetic variation originating from undomesticated populations that has been lost in contemporary domestic populations. This could involve identifying "ghost" introgression, where admixture is detected from an extinct species or population (Ottenburghs, 2020).

CONCLUSION

Extinction by hybridization of the undomesticated forms of domestic animals poses unique conservation challenges. Recent divergence, population imbalances, and long-standing contact make undomesticated forms vulnerable to extinction by hybridization. The complex spatial and temporal variation in the level of introgression within and between wild–domestic systems can make it difficult to resolve their status. Despite these challenges, we argue that the importance, socioeconomically and via the contribution they can make to science, of undomesticated forms cannot be overstated. Preventing their extinction will provide the opportunity for a greater understanding of processes, such as extinction by hybridization, adaptation, domestication, and feralization. This research will also provide valuable context, allowing for a better understanding of some of the most important model organisms and enhancing the ability to protect valuable sources of genetic diversity that could be introduced to domestic populations. Although there are many outstanding questions to be addressed, a priority should be to compare the genetics and behavior of undomesticated, ancient feral, contemporary feral, domestic, and hybrid forms. This variation underpins the evolutionary, scientific, socioeconomic, and practical opportunities these animals provide, which allow both the identification of valuable traits and the design of efficient conservation strategies.

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SUPPORTING INFORMATION

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Supporting Information/Appendix: Literature Cited for Table 1

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3

The undomesticated Rock Dove in Britain and the Isle of Man

The undomesticated Rock Dove in Britain and the Isle of Man

William J. Smith

Abstract The domestic pigeon *Columba livia* has a close relationship with humans, both in captivity and in a feral state, the Feral Pigeon. The domestic pigeon's ancestor, the wild Rock Dove, is, however, something of an enigma: following extensive hybridisation with Feral Pigeons across its range (with many populations of Rock Doves becoming extinct by hybridisation with Feral Pigeons), little is known of its contemporary distribution, nor of the genetic purity of the remaining populations. Putative populations of wild Rock Doves have long been known to inhabit coastal regions of Britain and the Isle of Man but they have been subject to little research. This paper outlines the existing knowledge regarding the distribution and genetic status of Rock Doves in Britain and the Isle of Man, and assesses the likely impact of ongoing hybridisation on the continued persistence of the Rock Dove.

Introduction

The Rock Dove *Columba livia* is native to the Old World, including Europe, northern Africa, the Middle East, India and Central Asia (del Hoyo *et al.* 1997), and was amongst the earliest animals to be domesticated. The domesticated form was transported worldwide and, following releases and escape from captivity, the feral form, Feral Pigeon, became established across the globe. Feral Pigeons can be extremely numerous, especially in areas around human settlement, and have now replaced wild Rock Doves across most of their former native range (Johnston & Janiga 1995). Although Feral Pigeons occur in a multitude of different colours (and some individuals can exhibit plumage features common in captive domestic pigeons, such as fanned tails or feathered legs), some birds can closely resemble wild, ancestral-type Rock Doves (hereafter simply 'Rock Doves'). Rock Doves have slimmer bills, smaller ceres, less exposed skin on the face, and rounder heads than Feral Pigeons (Smith *et al.* 2022). Rock Doves are associated with coastal cliffs and rocky mountainous regions, but genetic contamination from Feral Pigeons – which can occur in similar habitats, especially in areas close to urban or agricultural areas – is threatening Rock Doves with extinction by hybridisation.

The pre-domestication range and population size of the Rock Dove is uncertain. The situation is complicated, since Rock Doves were likely to have been associated with agriculture – and thus areas of human habitation – even before their domestication. It has therefore been suggested that the species may have spread to much of what we consider to be its native range in the wake of the expansion of agricultural activity (Murton & Westwood 1966; Goodwin & Gillmor 1970), much like the House Sparrow *Passer domesticus* (Ravinet *et al.* 2018). Despite this, excavations in Gibraltar suggest coexistence with *Homo neanderthalensis*, which did not practise agriculture (Blasco *et al.* 2014). It is nonetheless likely that anthropogenic activities led to an increase in the range and population of the Rock Dove to at least some degree, especially in more northern regions; for example, the species is reported

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to have become extinct on St Kilda, Outer Hebrides, following the evacuation of the human population (Hewson 1967).

Rock Doves have been categorised into various subspecies, which primarily occur in a contiguous range from western Europe and northwest Africa to southern Asia (Vaurie 1961). Variation is mostly clinal and future genetic studies may lead to redesignation and the loss of a number of the more tentative subspecies. The nominate subspecies, *C. l. livia*, found wild in Europe, North Africa and the Near East, has a white rump, while the easternmost subspecies, the 'Indian Rock Dove' *C. l. intermedia*, has darker plumage and a grey rump (Goodwin & Gillmor 1970). Most other subspecies are intermediate between these two subspecies, although the desert forms (notably *C. l. dakhlae*) are distinctly paler, and *gymnocycla* of western Africa is dark grey with a broader red orbital ring than the other subspecies (Vaurie 1961). Subspecies described from Macaronesia (*atlantis* and *canariensis*) and Mongolia (*nigricans*), which are from areas disjunct from the species' main distribution, are now considered to be Feral Pigeons (Vaurie 1961; Goodwin & Gillmor 1970; del Hoyo *et al.* 1997), although it seems possible that Rock Doves were once native to parts of Macaronesia at least.



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69. Rock Dove *Columba livia*, South Ronaldsay, Orkney, July 2019. This bird was shown to have a degree of Feral Pigeon ancestry and is part of a feral-wild mixed population.

Estimation of the current range of 'genetically pure' Rock Doves – i.e. those populations that have not experienced significant genomic admixture with Feral Pigeons – is difficult, and even European populations of apparent Rock Doves have an unclear conservation status given the uncertainty of hybridisation with Feral Pigeons (Johnston *et al.* 1988; Baldaccini 2020). In Scotland, Hewson (1967) characterised populations as Rock Doves rather than Feral Pigeons if more than 75% of birds showed a wild-type plumage (blue-grey with two black wingbars and no black chequering on the wing). Similar inferences, most of which have not been followed up by genomic analyses, have led to the generally accepted understanding that, in Europe, populations of Rock Doves are restricted to relictual populations in north and west Scotland, western Ireland, the Faroe Islands and the Mediterranean region (particularly populations on coastal cliffs in Crete, Sardinia, Cyprus and Croatia) (Fr *et al.* 1949; Johnston *et al.* 1988). Apparent Rock Doves in Scotland make up a significant proportion of the European population and, as a consequence, are of conservation interest. Further study is required in the rest of Europe, but Rock Doves are reported to persist along the western coast of Ireland, and these populations are likely also of conservation importance, although the presence of Feral Pigeons within these areas was noted by Hutchinson (2010).

In this paper, I outline the evidence to support the identification of such populations as 'true' wild pigeons, and discuss what is known regarding their historical and

contemporary distribution. I highlight variation in the level of hybridisation occurring throughout the Rock Dove's range in Britain and the Isle of Man, including through work with genetics, and outline its relevance from a conservation perspective.

The changing status of the Rock Dove in Britain and the Isle of Man

Historical confusion with Stock Doves *Columba oenas*, as well as the ongoing issue of confusion with Feral Pigeons displaying a wild-type plumage and poor recording of this 'uninteresting' species, makes it difficult to accurately map the former distribution of Rock Doves (or Feral Pigeons, for that matter) in Britain and Ireland (Brown and Grice 2005; see also Balmer *et al.* 2013). Despite this, it is likely that, before the widespread presence of Feral Pigeons, Rock Doves were present along all suitable coastlines, including the cliffs of southern England and eastern Ireland. The species' range may have been almost contiguous around the British coast, with gaps only in regions such as East Anglia, where there is a lack of suitable nesting habitat. It is also possible that Rock Doves bred away from the coast in rocky habitat, such as the mountainous regions of inland Scotland and Wales, as they are known to do in other parts of their global range (Baldaccini 2020).

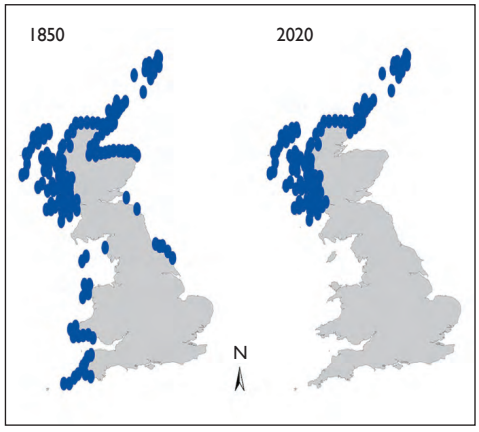


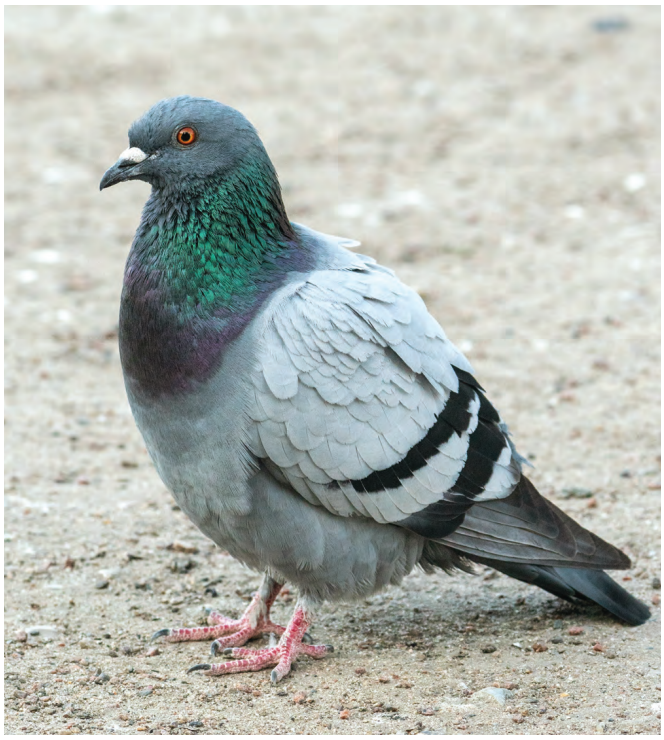
Fig. 1. The estimated range of the Rock Dove *Columba livia* in Britain and the Isle of Man in 1850 and in 2020. See text for discussion. Ireland's Rock Dove populations require further study in order to more accurately characterise the range of populations that now show signs of hybridisation with Feral Pigeons.

Despite some confusion over exactly which populations were wild and which were of domestic origin, Feral Pigeons were certainly well established in Europe by the early 1900s (Johnston & Janiga 1995) and Rock Doves had already been lost by hybridisation along vast tracts of European coastline, including in Britain and the Isle of Man (fig. 1). Saunders (1889) considered Rock Doves to be 'few and far between' in southern and eastern England and the Channel Islands, but present along the Welsh coastline, on the Isle of Man and at St Bees Head, Cumbria. The Rock Dove was also described as present at Flamborough Head/Bempton Cliffs, Yorkshire, which was historically likely to have been the largest colony in Britain, and in Northumberland, as well as at Bass Rock, Fife, and northwards, with the species reaching its highest densities in Scotland in Shetland, Orkney and the Outer Hebrides.

Populations in Devon and Cornwall persisted through the 1800s but were 'all but extinct' by the end of that century (Brown & Grice 2005). Despite extensive hybridisation with Feral Pigeons, the population at Flamborough/Bempton has retained many Rock Dove-like birds through the twentieth and into the twenty-first century. In Wales, the Rock Dove is described as having an unclear status during the late nineteenth century, with the last populations described as truly wild having been those on the cliffs of Pembrokeshire and Ramsey Island in the 1930s and 1940s (Lovegrove *et al.* 1994). Certainly, by the twenty-first century, the wild Rock Dove can be considered extinct in England and Wales following hybridisation with Feral Pigeons.

In Scotland, the range of the Rock Dove has undergone a significant reduction during the past 100 years, with extinction by hybridisation occurring along almost the entirety of Scotland's east coast, as far north as Caithness, where hybrid populations now predominate (Davey *et al.* 2015). However, Rock Doves are described as persisting on the islands of northern and western Scotland, as well as on the mainland from west of Caithness to Argyll (Thom 2010).

In the Isle of Man, Morris & Sharpe (2021) said that 'the "substitution" of this



Stephen Menzie

70. Feral Pigeon, Sweden, June 2021. A rather Rock Dove-like bird but which shows a swollen cere and feathered legs.

wild species [Rock Dove] by its domesticated form, Feral Pigeon, is one of the British Islands' overlooked ornithological tragedies', and Rock Dove appears to now be exinct from the island.

Ongoing hybridisation with Feral Pigeons

A genetic study by Stringham *et al.* (2012) showed that wild Rock Doves sit on their own branch of the species' phylogenetic tree, being genetically close to but distinct from domesticated forms and Feral Pigeons. The Rock Dove sampled in that study was from Skye, Highland; the study used just short fragments of genes (microsatellites) and did not look at possible hybridisation with Feral Pigeons.

In Smith *et al.* (2022), we examined genetic differences from across the full genome (rather than the short fragments of genes used in earlier work) of Rock Doves in Britain to determine which birds showed genetic influence from Feral Pigeons (i.e. clear signs of hybridisation) and which birds were pure Rock Doves (i.e. with little or no signs of hybridisation).

The results of this genetic study showed that a number of populations previously thought to consist of Rock Doves in fact consisted mostly of hybrids (individuals with mixed feral-wild ancestry, and not necessarily first-generation Rock Dove × Feral Pigeon hybrids): this was the case in Caithness and Orkney, where the populations appear to be 'hybrid swarms' – populations where hybridisation between the two parent forms is not necessarily ongoing but has resulted in a population consisting entirely of birds that have a mixture of Rock Dove and Feral Pigeon genes.

Despite this, some areas of Scotland still hold populations of Rock Doves that show little or no evidence of hybridisation with Feral Pigeons. The majority of birds in Shetland, Mull, Skye, Tiree and Islay showed only limited genetic evidence of hybridisation, despite a growing population of Feral Pigeons in, for example, Lerwick, Shetland. Birds sampled from the Outer Hebrides showed the least degree of hybridisation, with no individual sampled showing any significant evidence of past hybridisation with Feral Pigeons. This is reflected by visual observations: it is extremely rare to find any Rock Dove in the Outer Hebrides with plumage traits that deviate from the typical wild-type.

Given how little is known about the status and distribution of wild Rock Doves in Europe, and recent evidence of hybridisation in populations that were previously considered to be untainted by Feral Pigeons (e.g. Caithness and Orkney), the Outer Hebridean population is potentially of significant global importance for the conservation of the Rock Dove.

Factors affecting hybridisation

The complex pattern of geographic variation in the level of genetic admixture caused by hybridisation across Britain requires further study in order to understand its progression



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71. Rock Doves foraging on agricultural land, Berneray, Outer Hebrides, November 2021.

and predict its future dynamics. For example, gene flow can occur in two different contexts. In the first, lost racing or dovecote pigeons join wild Rock Dove flocks. This is observed across the entire range of the Rock Dove, including remote areas such as Shetland and occasionally the Outer Hebrides, although escaped birds typically do not persist in the population for long. In the second, range expansion by Feral Pigeons can bring their populations into contact with Rock Dove populations, as has happened in Caithness. It is unclear which mechanism is the most prevalent (although both will occur in tandem), and whether one is quicker at leading to feral-wild genetic admixing than the other. It is also unclear which factors might accelerate the rate of 'genetic replacement'.



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72. Rock Doves gathered on a ruined building, Vallay, Outer Hebrides, August 2020.

Disease transmission may also be a driving factor in the balance between Rock Doves and Feral Pigeons. *Trichomonas gallinae* is a disease that can be of great concern in endangered pigeons and doves globally (Bunbury *et al.* 2008; Marx *et al.* 2017). If infected Rock Doves experience a greater decline in their fitness than the potentially more genetically diverse, Feral Pigeon × Rock Dove hybrids, then this would give a selective advantage to the latter, which could facilitate the genetic replacement of Rock Doves with hybrids and Feral Pigeons.

Further study of the genomics and population dynamics of British and Irish Rock Doves will be necessary to resolve the complex progression of their gradual extinction by hybridisation. This should include research characterising the basic biology of the Rock Dove. For example, it is often written that Rock Doves are sedentary and rarely move. This is supported by BTO ringing recoveries, which have rarely been further than 10 km. However, extremely few Rock Doves are ringed every year (Robinson *et al.* 2022) and it's likely that many ringed birds are simply overlooked as likely being escapes from pigeon fanciers, which often also carry rings, although these are different in style from those used by the BTO ringing scheme. Consequently, we lack information on aspects such as juvenile dispersal and potential winter movements, though there is some anecdotal evidence for a degree of movement; for example, large flocks of Rock Doves have long been observed passing Fair Isle, perhaps moving between Shetland and either Orkney or Caithness (Lack 1986).



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73. Colour-ringed Rock Dove *Columba livia*, Vallay, Outer Hebrides, November 2021.

Future prospects

It seems inevitable that gene flow between Rock Doves and Feral Pigeons will continue. This, in turn, will cause further contraction of the range of 'true' Rock Dove populations. Already, Rock Doves in Caithness and much of Orkney can be considered to have gone extinct following hybridisation, as has previously happened in eastern Scotland, England, Wales, and many other areas of the Rock Dove's former native range. Attempting to reverse such trends would, given the significant populations of Feral Pigeons already in these areas, be exceptionally difficult. It is likely that efforts to reintroduce wild Rock Doves to locations where they have become extinct and could feasibly now sustain populations (e.g. the coasts of Pembrokeshire or Yorkshire) would be unsuccessful without continual removal of Feral Pigeons and continual monitoring to prevent new incursions. Any such populations would be completely conservation-dependent, which is not a desirable outcome. The only hope for expanding the British Rock Dove population would require a significant decline in the Feral Pigeon population.

Despite these challenges, it might be possible to conserve at least some of the existing Rock Dove populations. It is likely that in some regions, and particularly with respect to the large and relatively isolated population in the Outer Hebrides, monitoring for Feral Pigeon incursions and bans on keeping free-flying domestic pigeons would be sufficient to reduce gene flow and prevent extensive hybridisation. In regions where substantial Feral Pigeon populations are localised, such as Skye (in Portree), Mull (in Craignure and Tobermory) and Shetland (in Lerwick), complete removal of Feral Pigeons could be feasible. In Scotland, there is already a precedent for such conservation actions aimed at preventing



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74. Colour-ringed Rock Dove roosting in a ruined building, South Uist, Outer Hebrides, November 2021.

undesirable gene flow: Colonsay, Argyll, is designated as a protected area for a lineage of Honey Bee *Apis mellifera mellifera*, which is threatened by hybridisation with conspecifics in the rest of Britain (Muñoz *et al.* 2015). Likewise, various Hebridean islands act as designated 'refugia' for Red Deer *Cervus elaphus*, which are protected from gene flow with its introduced relative the Sika Deer *C. nippon* (Smith *et al.* 2018). Similar efforts could, relatively simply, maintain genetically diverse and self-sustaining populations of wild Rock Doves in Britain.

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4

Limited domestic introgression in a final refuge of the wild pigeon

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Limited domestic introgression in a final refuge of the wild pigeon



William J. Smith, Ashley T. Sendell-Price, Annette L. Fayet, ..., Steven Kelly, Lindsay A. Turnbull, Sonya M. Clegg

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Highlights

The Rock Dove is the wild ancestor of today's domestic and feral pigeons

The status of the Rock Dove was unclear owing to gene flow from its domestic relatives

We used genetic and morphological data to identify Rock Doves in the British Isles

Outer Hebridean populations experience the least gene flow with domestic pigeons

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Limited domestic introgression in a final refuge of the wild pigeon

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SUMMARY

Domesticated animals have been culturally and economically important throughout history. Many of their ancestral lineages are extinct or genetically endangered following hybridization with domesticated relatives. Consequently, they have been understudied compared to the ancestral lineages of domestic plants. The domestic pigeon *Columba livia*, which was pivotal in Darwin's studies, has maintained outsized cultural significance. Its role as a model organism spans the fields of behavior, genetics, and evolution. Domestic pigeons have hybridized with their progenitor, the Rock Dove, rendering the latter of dubious genetic status. Here, we use genomic and morphological data from the putative Rock Doves of the British Isles to identify relictual undomesticated populations. We reveal that Outer Hebridean Rock Doves have experienced minimal levels of introgression. Our results outline the contemporary status of these wild pigeons, highlighting the role of hybridization in the homogenization of genetic lineages.

INTRODUCTION

The genetic status of the undomesticated Rock Dove is ambiguous throughout its original Afro-Eurasian range (Baldaccini, 2020; Stringham et al., 2012). The "wildtype" plumage of the Rock Dove regularly manifests in feral domestic pigeons, hindering the discrimination of the two forms at an individual level (Goodwin and Gillmor, 1970; Johnston et al., 1988). Subtle morphological differences have been proposed but not rigorously assessed, particularly with respect to the effects of hybridization (Johnston et al., 1988; Johnston and Janiga, 1995). In many regions where Rock Doves were once present (e.g., England, Wales, Norway, and the Italian peninsula) they have long been fully replaced by feral pigeons (Baldaccini, 2020; Brown and Grice, 2005; Lovegrove et al., 1994; Michaelsen and Refvik, 2003). In previous European strongholds (e.g., Sardinia and the Faroe Islands) observations of plumage strongly suggest that wild-domestic hybridization has been occurring for decades (Baldaccini, 2020; Fr et al., 1949; Johnston, 1992). African and Asian populations are understudied, and the status of any of their Rock Doves is, therefore, unknown (Johnston and Janiga, 1995). Although the Macaronesian (*C. l. atlantis*) and Central Asian (*C. l. nigricans*) subspecies are now considered to be of feral provenance, for those thought not to have a history of domestication (e.g., the central Egyptian *C. l. dakhlae* and west African *C. l. gymnocyclus*), the extent to which they have hybridized with feral pigeons is unclear. Given the prolonged history of domestic populations, and the porous geographical and reproductive boundaries between the two forms, there are doubts as to whether any unadmixed Rock Dove populations remain. This is a concern of particular importance with regard to the ancestral type (as the primary progenitor of the domestic pigeon) *C. l. livia* (Baldaccini, 2020; Stringham et al., 2012). Nevertheless, there is likely to be variation in the level of feral hybridization among different Rock Dove populations, and those that have experienced minimal feral introgression are of scientific and conservation interest. The caves and cliffs of certain regions of the British Isles, including the Hebrides, Shetland, Orkney, parts of the Scottish Highlands, and parts of western Ireland (e.g. Cape Clear Island), have been proposed to harbor ancestral-type Rock Dove populations that are minimally impacted by hybridization, based on the predominance of "wildtype" plumage traits (Johnston, 1992). However, feral and free-flying domestic pigeon populations frequent these regions, and Rock Dove colonies in the Highlands and Northern Isles show "feral" plumage traits (Hewson, 1967). Using a combination of whole-genome sequencing data and morphometric assessments, we first explore the contemporary status of these putative Rock Doves; and then examine patterns of domestic introgression

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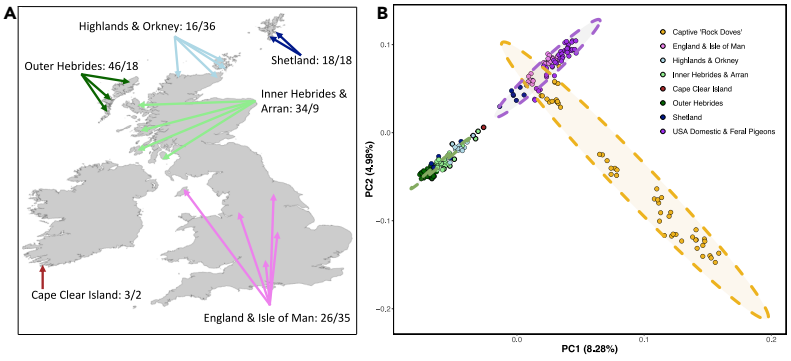


Figure 1. Rock Dove populations

(A) Rock Doves and feral pigeons were sampled from various locations around the British Isles. Rock Dove capture sites were categorized according to geography. Numbers indicate: birds sampled for DNA/adults measured for morphological data. Expanded location and sampling information is provided in Table S1.

(B) Principal component analysis shows genetic differentiation of wild Rock Doves from both domestic/feral birds and captive "Rock Doves." The analysis was carried out using PCAngsd, based on a genotype likelihood file filtered for linkage disequilibrium with 2,583,744 polymorphic nuclear sites, and 232 individual birds. The variance explained by PC1 and PC2 is shown in parentheses on the axes. 95% confidence interval ellipses show the wild Rock Dove (green), feral/domestic pigeon (purple), and captive "Rock Dove" (amber) groups. The size and shape of the Rock Dove ellipse reflect the close genetic relationships between different populations within the British Isles. Color of points represents the population grouping to which the individual bird belongs (see legend).

among populations, among individuals, and along the genome, to assess the value of Rock Dove populations in the British Isles as one of the last bastions of ancestral variation in this species.

RESULTS

We characterized genetic structure among pigeons from three different sources. First, we used wild-caught Scottish and Irish ancestral-type Rock Doves (meaning those which putatively represent the undomesticated form of the species). These birds were sampled in various locations and then categorized geographically as belonging to either the "Outer Hebrides" (N = 44), "Inner Hebrides & Arran" (N = 34), "Highlands & Orkney" (N = 16), "Shetland" (N = 9) or "Cape Clear Island" (N = 3) populations (Figure 1A and Table S1). Next, we sampled birds in captive collections that were claimed to be ancestral-type Rock Doves (N = 48). These came from eight private and public zoological collections in the Netherlands. The provenance of all of these "Rock Doves" was unclear, and many of the collections are known to have indulged in recreational inter-species hybridization in the past (including with the Stock Dove *Columba oenas*), as is common in aviculture (Ottenburghs et al., 2015). Despite this, the captive "Rock Doves" conformed to the phenotype of the nominate subspecies *C. l. livia* (Johnston and Janiga, 1995). Finally, we used samples from pigeons of domestic origin, including fancy breeds (N = 38) as well as feral pigeons from the USA (N = 2), England (N = 26), Isle of Man (N = 3), and Lerwick, Shetland (N = 9) (Table S1). The captive/fancy domestic pigeon breeds have varying provenance, including various regions of continental Europe, Asia, Africa, and the Middle East. Wild Rock Doves were genetically differentiated from both the fancy/feral pigeons and captive "Rock Doves." This was supported by groupings resolved by Principal Component Analysis (PCA) based on genotype likelihoods at 2,583,745 polymorphic nuclear sites filtered for linkage disequilibrium (see STAR Methods), where feral pigeons from England, the Isle of Man, and Scotland were genetically closer to American feral pigeons, and fancy pigeons originating from various global regions, than their Scottish and Irish Rock Dove neighbors (Figure 1B). Nine feral pigeons from Lerwick in Shetland clustered with the fancy/feral pigeons rather than the Rock Doves with which they share their island, which provides further support to the idea of a wild/feral population split rather than, for example, geographic structure owing to different colonization waves or differentiation between feral populations. Furthermore, maximum likelihood estimation of individual ancestries using NGSadmix (Skotte et al., 2013) indicated that $K = 3$ was the optimal model fit according to the Evanno method ($K = 1$ to $K = 7$ tested), where the first group corresponded to wild Rock Doves, the second to fancy/feral pigeons, and the third to the captive "Rock Doves" (Figures S1A and 3). $K = 2$ had the second highest support and split the Rock Doves from all other birds

(Figure S1B). When depicted as a maximum likelihood phylogenetic tree using IQTree from a dataset of 871,968 SNPs (rather than genotype likelihoods) and 121 birds, both the Rock Dove clade and the fancy/feral/captive clade showed 100% bootstrap support (Figure S2). The identification of wild Rock Doves as a distinct genomic group, which is an outgroup to the clade containing all sampled domestic pigeons (including very ancient breeds such as the ice pigeon (Figure S1B)), provides evidence that remnant populations of ancestral-type Rock Doves persist (although more global sampling and the use of ancient DNA would be required to provide a more accurate understanding of ancestry, given the possibility of today's Rock Doves representing an ancient feral pigeon population). Feral pigeons grouping with domestic pigeons in both PCA, NGSadmix, and the phylogeny reveals a close relationship with the homing/racing pigeons that make up a significant portion of their ancestry (Johnston and Janiga, 1995). The captive "Rock Doves" provenance remains unclear. About a quarter of these birds were likely of domestic origin, grouping with the fancy/feral population according to PCA and NGSadmix. The remaining birds had an unknown ancestry component, potentially the result of extensive hybridization with different subspecies of Rock Dove and other pigeon species. Further genetic structure was evident within the wild Rock Doves, with a strong distinction between the Outer Hebridean birds and those from elsewhere in Scotland (Figure S3).

Genomic distinctiveness of Rock Doves from their feral domestic conspecifics was mirrored by morphological differences, though intermediates were observed (Figures 2A and 2B). We quantified morphological variation using PCA of 149 birds caught around the British Isles, including English feral pigeons (n = 66) and Rock Doves from Scotland and Cape Clear Island (n = 83). Morphological differences primarily relate to head shape—for example, Rock Doves have smaller ceres, and the angle of the head rising from the base of the bill is smaller than in feral pigeons (Tables S2 and S3, Figure 2A). Contrary to prior opinion, we show that these differences are sufficient to allow ancestral-type Rock Doves (at least in the British Isles) to be distinguished from feral pigeons in the field, even among those with similar plumage patterns (Figure 2C) (Blasco et al., 2014). These differences also support early suggestions (based on skeletal comparisons using very small sample sizes) that the Rock Dove was morphologically distinct from feral pigeons at the level of avian subspecies (Johnston et al., 1988). Individuals with intermediate morphology were more common in regions with more opportunities for hybridization (e.g., doves from the "Highlands & Orkney" region) (Hewson, 1967) (Figure 2B). The attenuation of a distinctive Rock Dove morphology in these regions suggests that hybridization is leading to a breakdown in the genetic integrity of the Rock Dove lineage, and will also complicate phenotypic identification of non-hybrids in such regions.

Despite being both genetically and morphologically differentiated from feral pigeons, the Scottish and Irish Rock Dove populations did show evidence of introgressive hybridization with feral pigeons, but to different extents. We characterized the pattern of gene flow each Rock Dove population has experienced from feral domestic pigeons. First, we used NGSadmix at $K = 3$ (the best supported value of K) to examine the level of mixed ancestry in each individual (using the LD-filtered dataset of genotype likelihoods at 2,583,745 polymorphic nuclear sites). This analysis revealed negligible admixture in the Outer Hebrides, some in the "Inner Hebrides & Arran" group and Shetland, and higher levels in all individuals sampled from Cape Clear Island and the "Highlands & Orkney" group (Figure 3). The NGSadmix plot also shows that, when assessing both the feral pigeons of Lerwick in Shetland and the "England & Isle of Man" group, Rock Dove ancestry was highest in the Isle of Man, Yorkshire, and Lerwick. At these sites, Rock Doves were present in the last century (Brown and Grice, 2005; Fr et al., 1949). The identification of introgression between feral pigeons and Scottish/Irish Rock Doves was further supported by TreeMix analysis (Pickrell and Pritchard, 2012), which identified gene flow between the domestic and Rock Dove clades. Examining multiple runs for each number of putative migration events suggested that four migration edges (three between the fancy/feral group and the Rock Dove group, and one within the Rock Dove group) best explained the sample covariance (Figure S4A). There was a notable lack of migration events to or from the Outer Hebrides group (Figure S4B). TreeMix also supported the topology of the maximum likelihood phylogeny.

Population-level, genome-wide estimates of introgression from feral pigeons into the Scottish/Irish Rock Doves were estimated using ABBA-BABA SNP patterns (using the genomic dataset of 871,968 SNPs, the sister species the Hill Dove *Columba rupestris* as an outgroup, and calculating Patterson's D-statistic using *Dtrios* in the *Dsuite* software package (see STAR Methods)). Introgression was detected from the "England & Isle of Man" feral pigeons into the "Inner Hebrides & Arran" ($D = 0.005$, $Z = 6.21$, f4-ratio = 3.86%, p-value < 0.001), Cape Clear Island ($D = 0.012$, $Z = 3.86$, f4-ratio = 10.35%, p-value < 0.001), "Highlands

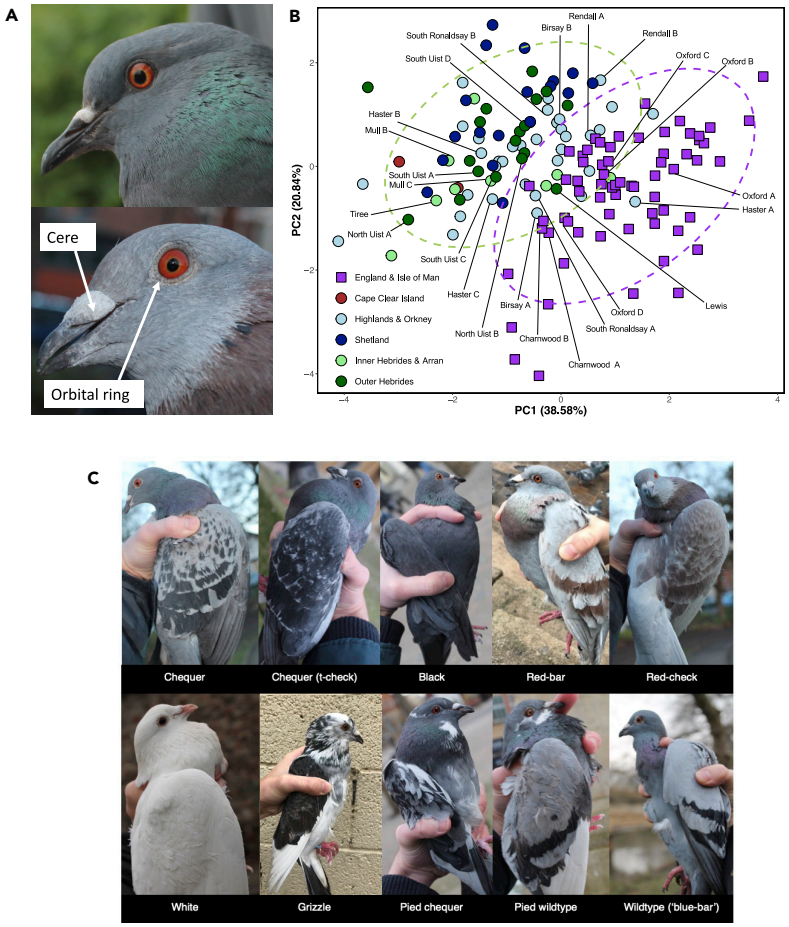


Figure 2. Rock Dove morphology

(A) The large cere, thick bill, flattened forehead and pale/thick orbital ring of the feral pigeon (bottom: from Leicester, England) distinguish it from the undomesticated Rock Dove (top: from the Inner Hebrides, Scotland).

(B) Rock Doves and feral pigeons in the British Isles are morphologically differentiated from each other, albeit with overlap, according to Principal Components Analysis of morphological variation based on six traits (wing length, cere width, tarsus length, bill length, head angle, and mass) in 149 birds. 95% confidence interval ellipses show feral pigeons (purple) and Rock Doves (green). Variance explained by PC1 and PC2 is shown in parentheses on the axes. Labeled individuals are birds that are also part of the genomic dataset.

(C) Although Rock Doves naturally exhibit only the "wildtype" ("blue-bar") pattern, feral domestic pigeons can exhibit identical plumage, as well as a variety of other patterns and colors. All birds in the photographs are feral pigeons from Oxford and Leicester, England. Note the "feral pigeon" head shape is evident in all the birds pictured here, even the one with wildtype plumage.

& Orkney" (D = 0.015, Z = 11.41, f4-ratio = 12.59%, p-value < 0.001), and, to a lesser extent, Shetland (D = 0.003, Z = 2.58, f4-ratio = 2.11%, p < 0.001) populations relative to the Outer Hebridean Rock Doves. Whilst introgression detected in Rock Dove populations depends on the level of local contact with feral pigeons, the broader population genetic structure reflected the degree of geographic isolation from the core of the feral pigeon range in Great Britain. Genome-wide weighted F_{ST} estimates between the feral pigeons of "England & Isle of Man" and the Rock Doves of the British Isles were lowest for the more proximate regions ("Inner Hebrides & Arran" (F_{ST} = 0.084), "Highlands & Orkney" (F_{ST} = 0.077)) and highest for the most peripheral regions (Cape Clear Island (F_{ST} = 0.148), Shetland (F_{ST} = 0.105) and Outer Hebrides (F_{ST} = 0.099)).

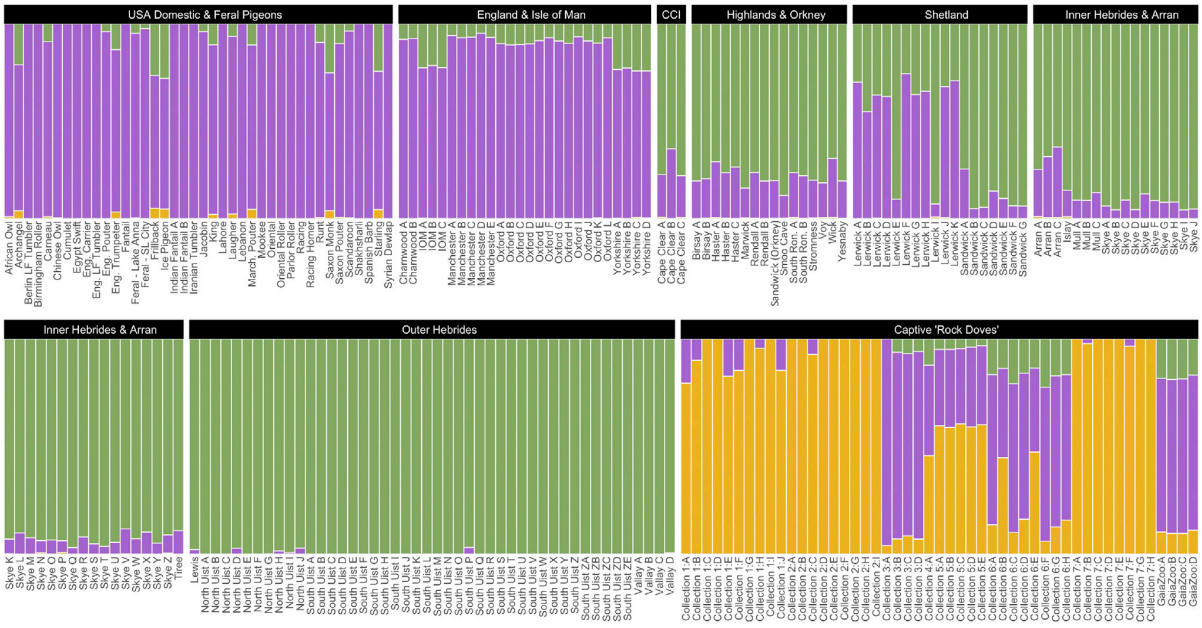


Figure 3. Maximum likelihood estimation of individual ancestries for feral/domestic pigeons, wild Rock Doves, and captive "Rock Doves"

Calculated using NGSadmix using a linkage disequilibrium-filtered dataset of genotype likelihoods containing 2,583,744 nuclear sites, and the most likely value of K = 3 (see Figure S1A). There are variable levels of admixture between the feral/domestic pigeon group (purple) and different wild Rock Dove populations (green), with the least admixed Rock Dove individuals occurring in the Outer Hebrides. The third cluster (amber) was primarily found in captive "Rock Doves", although some of these individuals also showed high levels of admixture from feral-domestic pigeons (purple).

The distribution of introgression across the genome was quantified using a *D*-statistic derivative, f_d, considered more robust to small sample sizes (here, windows of 40 informative SNPs) (see STAR Methods for more information regarding window-based analyses) (Martin et al., 2015). This revealed that, relative to the Outer Hebridean Rock Doves, feral pigeon introgression was distributed heterogeneously throughout the genome in the Rock Doves of the "Inner Hebrides & Arran," "Highlands & Orkney," Shetland, and Cape Clear Island (Figure 4A). The degree of genetic differentiation (F_{ST}) between each Rock Dove population and the feral pigeons was also distributed heterogeneously throughout the genome (Figure 4B). Windows of high introgression and differentiation were often tightly clustered; however, the position of most of these clusters varied between populations. The relative divergence of chromosome Z was higher than that observed in autosomes, a pattern seen in many avian divergence studies, and hypothesized to relate to the presence of a disproportionate number of genes involved in sexual selection and reproductive isolation, as well as the smaller effective population size of sex chromosomes and reduced recombination relative to the autosomes (Oyler-McCance et al., 2015; Sendell-Price et al., 2020). Most genes associated with peaks of introgression or differentiation differed between populations, reflecting the varied impacts of gene flow, drift, and selection (Table S4). Exceptions were the *CBFB* gene, associated with skeletal system development (Ng et al., 2015), which was associated with peaks of differentiation with the "Inner Hebrides & Arran," "Highlands & Orkney" and Shetland groups; and *PAX9* and *NKX2-1*, which play roles in morphogenesis (Li et al., 2017; Puelles et al., 2000), associated with introgressed regions in Cape Clear Island and differentiated regions in Shetland. Given their known roles in shaping phenotypes, these and other shared genes could contribute to the morphological differentiation described here between Rock Doves and feral pigeons. Together, the results for individual levels of admixture, alongside population-level introgression statistics, support the identification of the Outer Hebridean Rock Doves as being the least introgressed. As the extent of hybridization across the global Rock Dove population appears to be very high (Baldaccini, 2020), the results described here mark the Outer Hebrides as a population of conservation significance. Populations from other parts of Scotland, particularly in the Highlands and Orkney, are more introgressed and unlikely to experience a reprieve from ongoing hybridization (Murton and Westwood, 1966). Without intervention, it is likely that Scottish Rock Doves will follow the fate of their now-extinct English and Welsh Rock Dove neighbors (Brown and Grice, 2005; Lovegrove et al., 1994). Overall, the presence of birds with

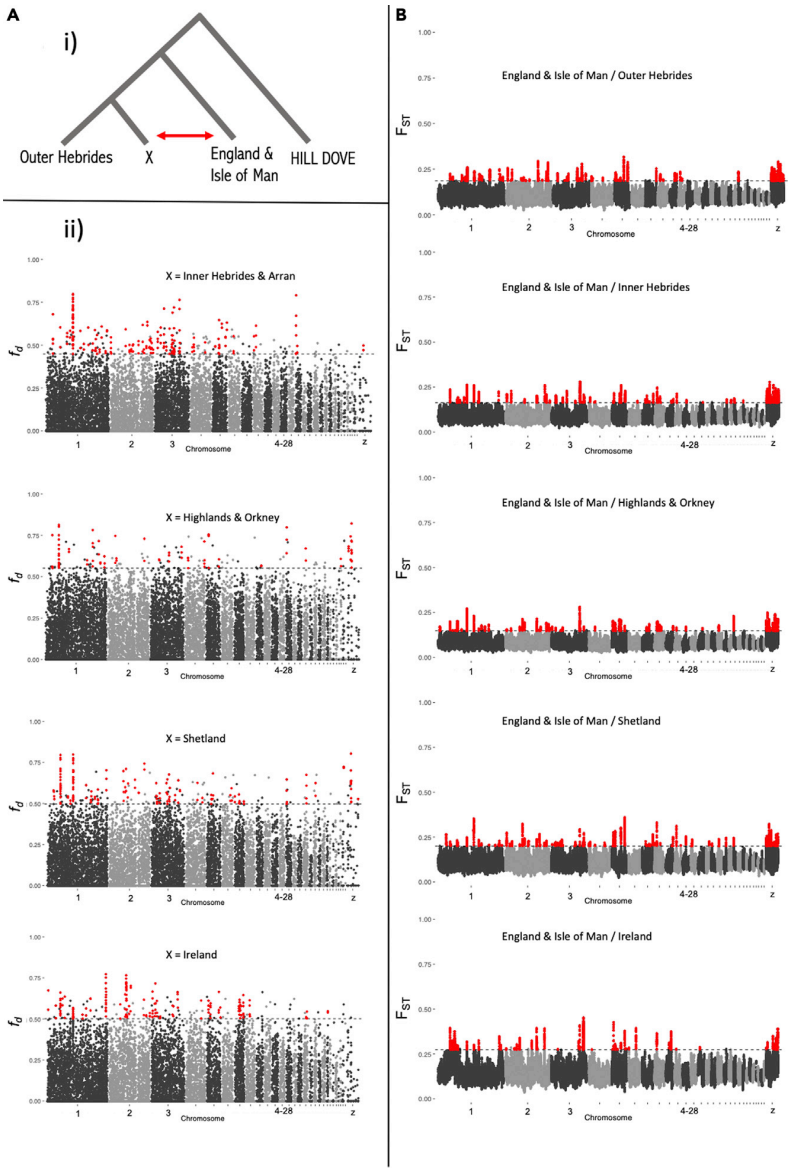


Figure 4. Genome-wide variation in introgression and population differentiation

(A) (i.) Tests for introgression (using f_d) included the Hill Dove *C. rupestris* as an outgroup, with fixed positions for the Outer Hebrides and "England & Isle of Man" populations. Estimates of introgression were made between the "England & Isle of Man" and each remaining Rock Dove population (X) (as indicated by the red arrow). f_d was calculated using a window size of 40 informative SNPs (i.e., SNPs that change the numerator of the statistic for a trio of populations) and step size of 20 SNPs, with the VCF of 871,968 SNPs. (ii.) The f_d statistic showed that introgression has occurred across the genome in all population combinations tested. Chromosomes are depicted in alternating colors. The dashed line indicates +3 standard deviations above the mean (points above this are classified as outlier windows) and red points indicate "outlier clusters" where outlier windows fall within 10 windows of one another.

(B) Pairwise F_{ST} comparisons of each Rock Dove population with the "England & Isle of Man" feral population showed that peaks of genetic differentiation occur throughout the genome. Chromosomes are depicted in alternating colors. The dashed line indicates +3 standard deviations above the mean (points above this are classified as outlier windows) and red points indicate "outlier clusters" (where outlier windows fall within 10 windows of one another). F_{ST} was calculated with a window size of 100,000 and a step size of 10,000 base pairs.

mixed ancestry, and the genome-wide nature of introgressed genetic material, highlights the ongoing genetic replacement of the ancestral-type lineage, and the real risk of their global extinction by hybridization.

DISCUSSION

The occurrence of extinction by hybridization is proliferating in an increasingly anthropogenic world, particularly involving feral domesticates and their wild relatives (Ottenburghs, 2021; Smith et al., 2022). Indeed, the wild-domestic interface is viewed as an increasingly important component of biodiversity (Clark et al., 2018). The Rock Dove populations we have identified have a primarily insular distribution, with populations on different islands have experienced varying levels of introgression ranging from the least admixed Outer Hebrides populations to sites where the ancestral-type Rock Dove may now be considered to be extinct as a distinct lineage (e.g., parts of Orkney). Most ancestors of domestic forms, such as junglefowl and wildcats, have primarily continental distributions and often occur as mixed wild-domestic populations (Senn et al., 2019; Wu et al., 2020). In contrast, Rock Doves present an unusual geographic arrangement of a wild-domestic interface with varying levels of isolation experienced, providing a tractable system to explore the spatial dynamics of incipient extinction by hybridization as a contributor to biotic homogenization and declining global biodiversity (Olden, 2006; Rhymer and Simberloff, 1996). The rarity of such populations is underscored by our discovery that putative captive populations of Rock Doves are genetically closer to domesticated populations.

The documentation of genetically and morphologically distinct Rock Dove populations provides an opportunity to understand the biology of an entity representing an undomesticated form, in its original setting (Smith et al., 2022). This will greatly enhance the utility of the domestic pigeon as a model organism. As well as its more traditional role in behavioral and neuroscience studies (Sasaki and Biro, 2017), the species is emerging as a valuable genetic model system (Shapiro et al., 2013) and, in its superabundant and cosmopolitan feral form, one used to understand microevolution in urban environments (Carlen and Munshi-South, 2021). In terms of the latter, understanding the wild context in which *Columba livia* evolved should facilitate the identification of traits that are derived adaptations for urban dwelling. More generally, a more nuanced understanding of how the species evolved, and the selection pressures it is exposed to in a contemporary environment, will enhance our ability to make inferences about the evolution of the behavioral, developmental, and genetic phenomena studied in domestic pigeons.

The Rock Dove offers an unprecedented opportunity to study an undomesticated form in a natural setting, providing the opportunity to enhance our understanding of the domestic pigeon as a model organism in the biological sciences. Concerningly, relict populations are at real risk of replacement with feral pigeons following extinction by hybridization. Future work should focus on comparing the ancestral-type, feral and captive domestic lineages of *C. livia* from around the world, to better explain phenomena studied in domestic pigeons, explore the extent to which Rock Doves represent the ancestral genetic variation, and unlock the potential of the species as a tractable model organism with which to explore domestication and hybridization.

Limitations of the study

To explore the ancestry of the sampled populations in more detail, and to place our study into a global context, more widespread geographic sampling would be required. The use of ancient DNA would also be useful. This could be used to establish the extent to which contemporary Rock Dove populations are representative of the original populations from which domestic pigeons originated, given the fact that domestication first happened thousands of years ago. Nevertheless, the Rock Doves identified here form an outgroup in phylogeny to domestic breeds originating from all over the world, including ancient breeds from the Middle East where the species was originally domesticated. They are, therefore, of significant conservation and scientific interest as wild representatives of the cosmopolitan domestic and feral pigeons. As well as this, it is probable that other populations of wild pigeons exist, particularly of subspecies other than the ancestral-type *livia*, which are relatively isolated from gene flow with contemporary feral and domestic pigeons. These could be in northern Africa for example (subspecies *gymnocyclus* or *dakhlae*). Again, more global sampling would be needed to establish this.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104620.

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AUTHOR CONTRIBUTIONS

Conceptualization: WJS, LAT, SK, and SMC, Data curation: WJS, ASP, and CVDK, Formal analysis: WJS, ASP, SK, and MTJ, Funding acquisition: WJS, LAT, SMC, ALF, and SK, Investigation: WJS, ASP, and MTJ, Methodology: TMS and KCR, Project administration: WJS and CVDK, Resources: SMC, KCR, and BCS, Supervision: SMC, BCS, ALF, LAT, and SK, Visualization: WJS, MTJ, SMC, and ASP, Writing – original draft: WJS, SMC, and ASP, Writing – review & editing: WJS, MTJ, SMC, ASP, BCS, ALF, LAT, SK, KCR, TMS, and CVDK.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Feathers from wild-living and captive *Columba livia*	See Table S1	N/A
Pectoral muscle tissue from freshly dead *Columba livia*	See Table S1	N/A
Chemicals, peptides, and recombinant proteins		
Glycogen	Life Technologies	Cat# R0561
Proteinase K	Stratech Scientific	Cat# K1037
Dithiothreitol	Merck	Cat# D9779-5G
Ethanol	Enzo Life Sciences	Cat# SV-39556-01
Sodium acetate	Merck	Cat# 32319-500G-R
Phenol/Chloroform/Isoamyl alcohol	Fisher Scientific	Cat# 327111000
Molecular Biology Grade Water	VWR International	Cat# 436912C
NaCl	Insight Biotechnology	Cat# SC-295832
Sodium dodecyl sulfate	Merck	Cat# L5750-500G
EDTA	Merck	Cat# 03620-50G
Tris-HCl	Promega UK	Cat# A2641
AMPure XP	Beckman Coulter	Cat# A63881
Critical commercial assays		
DNeasy Blood & Tissue Kit	QIAGEN	Cat# 69506
Tagment DNA TDE1 Enzyme and Buffer Kit	Illumina	Cat# 20034198
Qubit 2.0 Fluorometer	Thermofisher	Cat# Q32850
TapeStation	Agilent	Cat# G2991BA
Deposited data		
Raw sequencing reads	This study	ENA: PRJEB52260
Software and algorithms		
FastQC v0.11.9	(Andrews, 2015)	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Trimmomatic v0.39	(Bolger et al., 2014)	http://www.usadellab.org/cms/?page=trimmomatic
ANGSD v0.933	(Korneliussen et al., 2014)	http://www.popgen.dk/angsd/index.php/ANGSD
VCFtools v0.1.16	(Danecek et al., 2011)	https://vcftools.github.io/index.html
Samtools v1.12	(Li et al., 2009)	http://www.htslib.org/
Bowtie 2 v2.4.1	(Langmead and Salzberg, 2012)	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
GATK v4.1.8.1	(McKenna et al., 2010)	https://gatk.broadinstitute.org/hc/en-us
Picard v2.26.10	(Picard, http://broadinstitute.github.io/picard/)	https://broadinstitute.github.io/picard/Picard
PLINK v2.00	(Purcell et al., 2007)	https://zzz.bwh.harvard.edu/plink/
ngsLD v1.1.1	(Fox et al., 2019)	https://github.com/fgvieira/ngsLD

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
PCAngsd v0.98	(Meisner and Albrechtsen, 2018)	http://www.popgen.dk/software/index.php/PCAngsd
NGSadmix v32	(Skotte et al., 2013)	http://www.popgen.dk/software/index.php/NgsAdmix
OptM v0.1.6	(Fitak, 2021)	https://cran.r-project.org/web/packages/OptM/index.html
CLUMPAK	(Kopelman et al., 2015)	http://clumpak.tau.ac.il/
DSuite v0.4	(Malinsky et al., 2021)	https://github.com/millanek/Dsuite
IQTREE v2.1.3	(Minh et al., 2020)	http://www.iqtree.org/
Stacks v 2.41	(Catchen et al., 2013)	https://catchenlab.life.illinois.edu/stacks/
Treemix v1.13	(Pickrell and Pritchard, 2012)	https://bitbucket.org/nygcresearch/treemix/wiki/Home
Dendroscope v3	(Huson et al., 2007)	https://uni-tuebingen.de/en/fakultaeten/mathematisch-naturwissenschaftliche-fakultaet/fachbereiche/informatik/lehrstuehle/algorithms-in-bioinformatics/software/dendroscope/
R v4.0.2	(R Development Core Team, 2010)	https://www.r-project.org/
Pophelper v2.3.1	(Francis, 2017)	http://www.royfrancis.com/pophelper/articles/index.html
Other		
Columba livia reference genome colLiv2	(Damas et al., 2017)	GenBank: GCA_001887795.1
Columba livia reference genome Cliv_1.0	(Shapiro et al., 2013)	GenBank: GCA_000337935.1
Columba livia reads from fancy domestic pigeon breeds and USA feral pigeons	(Shapiro et al., 2013)	GenBank: PRJNA167554
NCBI Genome Remapping Service	(NCBI Genome Remapping Service, https://www.ncbi.nlm.nih.gov/genome/tools/remap)	https://www.ncbi.nlm.nih.gov/genome/tools/remap

RESOURCE AVAILABILITY

Lead contact

Requests for further information should be directed to and will be fulfilled by the Lead Contact, William Smith (william.smith@queens.ox.ac.uk).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The accession number for the sequencing data reported in this paper is publicly available (ENA: PRJEB52260). No original code was written for the analysis of this project. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Our dataset contains 232 *Columba livia* individuals. Of these, 40 come from publicly available sequencing reads (NCBI: SRA054391) from fancy and feral pigeons (we also used one individual of the Hill Dove *Columba rupestris* for use as an outgroup in phylogenetic and introgression analyses) sampled in the USA as part of a previous study (Shapiro et al., 2013). A further 49 samples were from the feathers of birds claimed to be ancestral-type Rock Doves in both public zoological and private avicultural collections. The remaining 143 birds were sampled from feral pigeon and Rock Dove populations across the United

Kingdom, Isle of Man and Ireland (117 within the traditionally accepted range of the undomesticated Rock Dove according to existing literature, and 26 outside of it (Brown and Grice, 2005; Hutchinson, 2010; Lovegrove et al., 1994; Thom, 2010)). The Scottish and Irish samples were categorized according to geographical region for analyses. These categories were: 'Shetland', 'Highlands & Orkney', 'Inner Hebrides & Arran', 'Outer Hebrides' and 'Cape Clear Island'. Nine of eighteen birds caught in Shetland were feral pigeons and were excluded from introgression analyses to prevent a biased overestimate of the extent of gene flow into Shetland Rock Doves. Table S1 provides further detail on sample collection. All samples were stored in a freezer at −20°C before DNA extraction. All collection of samples responsible for the newly generated sequencing data was carried out between 2017 and 2020. Sampling and bird handling was approved by both the British Trust for Ornithology's Special Methods Technical Panel (SMTP) and the University of Oxford's Department of Zoology Animal Welfare and Ethical Review Body (AWERB). Sex of birds was unknown (the species cannot be reliably sexed in the field) and none were squabs (i.e. all were post-fledging age and no chicks in the nest were sampled).

METHOD DETAILS

Data collection – Captive populations

CVDK liaised with aviculturists responsible for seven different private avicultural collections, and WJS liaised with staff at GaiaZOO in the Netherlands. Ten flank feathers were taken from each of the 49 individual birds claimed to be ancestral-type Rock Doves within these collections. Birds were confirmed to conform to the phenotype expected of an ancestral-type Rock Dove (*C. l. livia*) according to existing literature (Goodwin and Gillmor, 1970).

Data collection – Wild populations

Birds were caught across various sites in the British Isles, by both WJS and other qualified British Trust for Ornithology bird ringers. Three flank feathers were taken from each individual bird. WJS liaised with pest controllers in Yorkshire, Manchester and the Isle of Man, and acquired twelve fresh feral pigeon carcasses (no birds were killed for this study). A further eight freshly dead birds, found opportunistically (e.g. casualties of window collisions), were sent to WJS by volunteers local to Rock Dove colonies.

Birds caught in 2019 were measured for biometric analysis. Birds were first classified as juveniles or adults based on plumage (Baker, 2016), and only adults (n = 149) were included in the analyzed dataset to avoid including juveniles and chicks at variable growth stages. Six morphological traits were recorded by WJS: Maximum wing chord and tarsus length to the claw were measured with a butted metal ruler (±1mm); cere width and bill length (to feather edge) were measured with dial calipers (±0.1mm); mass was measured with a digital pesola scale (±0.1g) and head angle (from the top line of the bill to the frontal rise of the forehead) was measured with a computerized protractor (±1°) (Protractor v20.6.1) from a lateral-view photograph of each bird. Overlap between birds sampled for morphological and genetic data collection is highlighted in Figure 2.

DNA extraction

For the single carcass sample from Tiree, DNA was extracted from 25mg of pectoral muscle tissue using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands), following the manufacturer's instructions. For feather samples, and the pectoral muscle samples from additional carcasses, DNA was extracted using the following phenol-chloroform extraction protocol. Samples were assigned randomly to an extraction batch, rather than extracting batches according to fieldwork location or putative Rock Dove/feral pigeon status. For each bird sampled, three to five mm from the end of each feather calamus was cut and split lengthwise with a scalpel on a clean laboratory tile to expose the tissue inside, then added to a microcentrifuge tube containing 250 μL of DIGSOL extraction buffer (0.02 M EDTA, 0.05 M Tris-HCl (pH 8.0), 0.4 M NaCl, 0.5% sodium dodecyl sulphate (SDS)), 4 μL of Proteinase K and 30 μL of 1 M Dithiothreitol (DTT) solution. Samples were incubated at 55°C for at least 5 h to overnight. Following incubation, 400 μL of phenol:chloroform:isoamyl alcohol (25:24:1) was added and samples centrifuged at 15,000 rpm for 15 min. The aqueous layer was transferred to a new microcentrifuge tube containing 35 μL of 3 M sodium acetate, vortexed for 10 s and chilled for 10 min at −20°C. DNA was precipitated with addition of 700 μL of ice-cold 100% ethanol and 2 μL of glycogen, chilled overnight at −20°C, centrifuged for 30 min at 15,000 rpm and the supernatant was removed. The precipitate was rinsed with 900 μL of ice-cold 70% ethanol, placed in a refrigerated centrifuge at 4°C for 30 min at 15,000rpm, the supernatant removed and the precipitated

DNA left to dry at room temperature. Once dried, the DNA was resuspended in 40 μL of Low-EDTA TE (Tris-EDTA) buffer (0.01 M Tris-HCL (pH 8.0), 0.0001 M EDTA).

Library preparation and sequencing

Library preparation and sequencing of the Tiree sample was conducted at BGI Genomics (Hong Kong). Library preparation used the MGISP-100 automated system, and sequencing was conducted using the BGISEQ-500 platform (Huang et al., 2017), using paired end 150-bp sequence reads. Library preparation of all other samples was carried out by TMS using a protocol designed for increased efficiency when using low-input DNA (Schweizer et al., 2021). DNA samples were quantified using a Qubit 2.0 (Invitrogen, Waltham, Massachusetts, USA). Nine feather DNA samples with a concentration below 1 ng/μL were concentrated from 40 μL total volume to 5 μL total volume using a 1:1 bead ratio with Ampure XP beads (Beckman Coulter, Brea, CA, USA) and quantified again using the Qubit. DNA samples were normalized to 2.5 ng/μL, with an accepted range from 0.3 ng/μL to 4.0 ng/μl 1 μL DNA was then fragmented and adapters were annealed (called Tagmentation) using Illumina's Tagment DNA TDE1 Enzyme and Buffer Kit. DNA then went through a two-step PCR amplification totaling 12 cycles to add unique Nextera dual indices (Illumina, San Diego, CA, USA) and amplify tagmented DNA, called an 'Indexing PCR' and 'Booster PCR'. Individual DNA libraries were then size selected and cleaned using a 0.7:1 bead to DNA ratio of Ampure XP beads, and eluted into 30 μL of 10 mM Tris-HCl. Individual cleaned DNA libraries were quantified using Qubit and pooled in equal amounts based on the library with the lowest total DNA yield. Ampure XP beads were used to complete a double size selection on pooled library to remove fragments outside of desired range of 320-500 bp, and library was eluted into 50 μL of 10mM Tris-HCl. Final library quantification was 1.55 ng/μL (4.95 nM) and final TapeStation showed an average fragment size of 474 bp (insert size: 361bp). The library was sequenced on four Illumina Hiseq 4000 lanes (Illumina, San Diego, CA, USA) at Novogene USA, using paired end 150-bp sequence reads.

QUANTIFICATION AND STATISTICAL ANALYSIS

Mapping

The quality of all sequencing reads was assessed using FASTQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/). Based on quality reports we removed the first 10bp of each read using Trimmomatic v0.39 (HEADCROP:10) (Bolger et al., 2014). Trimmed reads were aligned to the *Columba livia* chromosomal genome assembly 'colliv2' (NCBI Assembly GCA_001887795.1) using Bowtie 2 v2.4.1 (Langmead and Salzberg, 2012) with end-to-end alignment and default settings (allowing for a maximum of two mismatches in the seed (-n 2)) and resulting BAM files sorted using SAMtools v1.12 (samtools sort) (Li et al., 2009).

Genotype likelihood estimation and SNP calling

We carried out SNP calling on all 233 birds (i.e. including all *Columba livia* individuals plus *Columba rupestris*, the Hill Dove) using HaplotypeCaller in GATK v4.1.8.1 with the *Columba livia* genome assembly v2 (NCBI Assembly GCA_001887795.1), the genotyping_mode 'DISCOVERY' and a confidence threshold of 20 (-stand-call-conf 20). This was to generate data for IQTREE, TreeMix and Dsuite, which take a VCF as an input. SNP-based analyses can be significantly affected by variable sequencing depth in different individuals. To achieve a sensible range of sequencing depths of 4-10x, we used DownSampleSam in Picard (java -jar picard.jar DownsampleSam) to reduce the sequencing depth of the Tiree sample (p = 0.2), and of all the USA Domestic & Feral Pigeon samples (p = 0.5). The outputted VCF file was filtered with VCFtools v0.1.16 (Danecek et al., 2011) to get only biallelic sites (–min-alleles 2, –max-alleles 2) remove indels (–remove-indels) and only include sites where the minor allele count was ≤5% (–maf 0.05); genotype quality was >20 (–minGQ 20), and where genotypes were called in at least 50% of individuals (–max-missing 0.5). Individual data missingness was then calculated using VCFtools v0.1.13 (Danecek et al., 2011) and individuals with >50% missing data removed (–remove) from the VCF file. The resulting VCF contained 121 individuals and 871,968 biallelic Single Nucleotide Polymorphisms (SNPs). For TreeMix analysis, we filtered this file using PLINK (Purcell et al., 2007) with the settings –indep-pairwise 1000kb 1 0.8, to reduce the impact of linkage disequilibrium. This led to the retention of 675,581 SNPs in the linkage-disequilibrium-filtered VCF.

For the population structure analyses, we used ANGSD v0.933 (Korneliussen et al., 2014) to estimate genotype likelihoods (-doGlf) using the chromosomal *Columba livia* genome assembly v2 (colLiv2) (NCBI Assembly GCA_001887795.1) and the GATK genotype likelihood model (-GL 2), inferring major and minor

from GL data (-doMajorMinor 1) and including only proper pairs (-only_proper_pairs 1), using qscore recalibation with the BAQ model (baq -1) (Li, 2011). We set thresholds of base quality of at least 30 (-minQ 30) and mapping quality of at least 25 (-minMapQ 25), and discarded 'bad' reads (-remove_bads 1) and those that did not map uniquely (-uniqueOnly 1). We repeated this genotype likelihood estimation twice; once for all 232 *Columba livia* individuals (using -minInd 116), and once for all 108 of the British and Irish undomesticated Rock Doves (117 birds from Rock Dove regions minus nine individuals from Lerwick in Shetland which were feral pigeons) using -minInd 54. Because the files were destined for NGSadmix and PCAngsd, which required low linkage between sites, we then ran ngsLD (Fox et al., 2019) on both output files to estimate pairwise linkage disequilibrium. We filtered the outputted file using the prune_graph.pl script provided for ngsLD, with the settings –max-kb-dist 5 and –min_weight 0.2. For the file of 232 birds, the outputted list contained 7,742,273 sites. For the file of 108 birds, it contained 7,353,590 sites. We then repeated the generation of genotype likelihoods as before, but restricted to the low-LD sites using the ANGSD -sites flag. Following this filtering for linkage disequilibrium, the final 232 bird genotype likelihood file contained 2,583,745 sites, and the 108 bird British/Irish genotype likelihood file contained 2,554,226 sites.

PCA analyses

We examined the patterns of population structure by Principal Component Analysis (PCA) of genotype likelihoods using PCAngsd's (Meisner and Albrechtsen, 2018) 'pcangsd.py' script for the full dataset of all 232 *Columba livia* individuals. We repeated this separately for the genotype likelihood file containing only the 108 birds from the British Isles (Figure S3A).

NGSadmix

We performed estimation of individual admixture proportions for all 232 birds using the genotype likelihood file and NGSadmix (Skotte et al., 2013). We tested *K* values of between 1 and 7 to determine which grouping had the most support, using sites where at least 116 individuals had NGS data (-minInd 116). For each value of *K* we conducted 10 runs, and summarized these runs using CLUMPAK (Kopelman et al., 2015) with the Evanno method (Evanno et al., 2005). $K = 3$ had the highest ΔK value (meaning $K = 3$ is the uppermost level of structure) between 1 and 7. We plotted the $K = 3$ and $K = 2$ (which had the second highest ΔK value) results using pophelper (Francis, 2017) in R (R Development Core Team, 2010). We repeated this for the file with 108 of the British Isles birds using -minInd 54 (Figure S3). Here, $K = 2$ had the highest Delta K value (Figure S3B).

TreeMix

We used TreeMix (Pickrell and Pritchard, 2012) as a further test of hybridization. TreeMix models population relationships as a tree in which positions can be connected by migration edges representing genetic admixture between lineages. We used the LD-filtered VCF of 675,581 SNPs and converted it to a 'treemix' file using the Stacks –treemix flag (Catchen et al., 2013). We then performed the analysis varying the number of migration edges (*M*) from one to twelve. To identify the number of migration events that optimized the fit of the data to the tree, we used OptM (Fitak, 2021). OptM allows one to judge the optimal number of migration edges by outputting the Δm statistic, which represents the second order rate of change across values of *M*, peaking at the correct number of simulated migration edges. We ran TreeMix 50 times ($i = 50$) per *M* value, using *Columba rupestris* as a root and a randomly selected window size (k) of between 100 and 1000 SNPs (varying in 50 SNP increments). Varying the window size is necessary to prevent the standard deviation across runs being zero, which would make Δm undefined. OptM identified four migration events as optimizing the fit of the data to the tree (Figure S3C).

D statistics

Using *Dsuite* (Malinsky et al., 2021) and the VCF of 871,968 SNPs generated by GATK, along with the *-g* flag instructing the program to use the genotype likelihoods in the VCF, we carried out *D* statistic introgression analyses for the geographical groupings of Scottish and Irish Rock Doves. We carried out both genome-wide and within-genome introgression estimates. We first assessed the genome-wide levels of feral introgression using the *D* statistic (Durand et al., 2011; Patterson et al., 2012; Soraggi et al., 2018). To do so, we used the *Dtrios* program of *Dsuite* with all British and Irish populations (excluding the Lerwick feral pigeons from the Shetland population to prevent a biased overestimate of introgression between feral pigeons and the Shetland Rock Doves). *Dtrios* outputs a *D* statistic, *Z*-score, p-value and an f4-ratio (of admixture) for

each trio of populations (setting populations as 'P1', 'P2' and 'P3'). If a *D* statistic is not zero and has a *Z score* greater than 3, then it is deemed significant (Zheng and Janke, 2018). A positive *D* statistic implies introgression between P2 and P3, and a negative *D* statistic implies introgression between P1 and P3.

Following this genome-wide 'global' introgression analysis, to determine how the pattern of introgression varied across the genome, we calculated windowed *D* statistics using *Dinvestigate* (also from *Dsuite*). Rather than the *D* statistic itself, we used f_d, which is also used to detect loci that have experienced introgression, but is more reliable when basing calculations on smaller genomic regions, as is the case with window-based analyses (Martin et al., 2015). Unlike with *Dtrios*, for *Dinvestigate*, in each calculation P1, P2, P3 (phylogeny positions) and the outgroup must be set manually (see Figure 4A). We kept P1 as the 'Outer Hebrides' group (shown to have experienced negligible introgression by NGSadmix and TreeMix), P3 as the "England & Isle of Man' feral pigeon group and the outgroup as *Columba rupestris*. We then varied P2 (shown as 'X' in Figure 4A) and carried out four calculations, enabling us to judge introgression between feral pigeons and all of the Scottish and Irish Rock Dove populations where significant gene flow with feral pigeons was identified by *Dtrios* (Highlands & Orkney, Shetland, Inner Hebrides & Arran and Cape Clear Island), relative to the Outer Hebrides. In each case, the analysis quantified the pattern of feral pigeon introgression with the population set as P2. We used a window size of 40 informative SNPs (i.e. SNPs that change the numerator of the statistic for a trio of populations) and a step size of 20 (-w 40,20), and plotted f_d along the genome. We plotted the midpoints of genomic windows in R, and classified 'outlier regions' as those where the f_d value was more than 3 standard deviations above the mean. We classified 'outlier clusters' as being present where outlier regions were within 10 windows of each other.

F_{ST} calculation

We estimated both genome-wide and window-based genetic differentiation between the 'England & Isle of Man' feral pigeons and each of the Scottish/Irish Rock Dove populations (excluding Lerwick's feral pigeons from the Shetland population to prevent a biased underestimate of differentiation between them and the feral pigeons). Firstly, we generated a list of variable sites. To do so, we ran ANGSD using a list of all the 'England & Isle of Man' birds plus all the birds in the comparison population (meaning that F_{ST} calculation was carried out five times to compare the feral pigeons to Rock Doves from the Outer Hebrides, Inner Hebrides & Arran, Highlands & Orkney, Shetland and Cape Clear Island) with the following settings: -remove_bads 1, -only_proper_pairs 0, -trim –0, -minMapQ 30, -minQ 20, -minInd 10, -baq 1, -doMaf 1, -doMajorMinor 1, -doPost 2, -GL 2 and -minMal 0.01. We then extracted the first two columns from the mafs.gz output file and indexed this file of variable sites using ANGSD with the 'sites index' flags. Using ANGSD, we next calculated allele frequency likelihood for both the 'England & Isle of Man' population and the comparison population using the reference assembly instead of an ancestral state reference, and the flags -remove_bads, -only_proper_pairs, -trim 0, -minMapQ 30, -minQ 20, -baq 1, -dosaf 1 and -gl 2. The outputted saf.idx files were then used to calculate the folded site frequency spectrum (using -fold 1) with realSFS, outputting folded.sfs files. Following this, we were able to use realSFS fst index with the saf.idx files for both populations and -sfs with the folded.sfs file to output F_{ST} on a per site basis with -fstout (giving a fst.idx file). Finally, we could get global F_{ST} estimates with realSFS fst stats, and use the fst.idx file to get a global.fst file. We also calculated Fst estimates in a sliding window using realSFS fst stats2 and the fst.idx file with a window size of 100,000 and a step size of 10,000 (which led to a broadly similar window size to *Dsuite*'s 'informative SNP's method). We plotted the midpoints of each window along the genome and classified 'outlier regions' as being 3 standard deviations above the mean. We classified 'outlier clusters' as being regions where the outliers fall within 10 windows of each other.

Identifying genes associated with regions of interest

We mapped the genome annotation of the scaffold-based Cliv_1.0 assembly (Shapiro et al., 2013) to the chromosomal (unannotated) assembly colliv2 using NCBI's Genome Remapping Service (https://www.ncbi.nlm.nih.gov/genome/tools/remap). The subsequent gff file contained the annotations associated with Cliv_1.0 mapped to the colliv2 genome. We first filtered this gff for the mRNA feature (which contains the names of genes and their location along the genome). We then added two columns to the list of 'outlier clusters' of f_d (i.e. the *Dinvestigate* output). These columns were called scan_start and scan_end, and correspond to 100,000 less than and 100,000 greater than the start and end of the window respectively. In R, we scanned for genes in the gff that fell within our f_d scanning windows. We repeated this for F_{ST} outlier clusters.

Phylogenetics

We converted the VCF of 871,968 SNPs into a file in the PHYLIP format using Stacks –phylip (Catchen et al., 2013). This file was used to infer a maximum likelihood phylogenetic tree using variable sites with IQ-TREE 2, rooted using *Columba rupestris* (Minh et al., 2020). The best fitting model for this data matrix was selected automatically by IQ-TREE 2, enforcing (-m) ascertainment bias correction (+ASC) to correct for branch lengths in the absence of constant sites (Kalyaanamoorthy et al., 2017) and using the Bayesian information criterion, and was determined to be TVM + F + ASC + R3 (where TVM is the transversion model with unequal base frequency, F means that empirical base frequencies were used and R3 means that a FreeRate model of rate heterogeneity across sites with three categories was employed). A 1000 non-parametric bootstrapped re-sampling approach was taken to infer support values at internal nodes in the tree (Hoang et al., 2018). The tree was visualized using Dendroscope (Huson et al., 2007). The most basal node shows the split between the Rock Doves and the group containing both contemporary feral and domestic pigeons and captive 'Rock Doves' from private and public avicultural collections. Each of these clades has robust bootstrap support (Figure S2).

Phenotypic analysis

The six univariate morphological traits were standardized and log-transformed to conform to assumptions of normality and homogeneity of variance, and population differences between birds caught in putative Rock Dove regions and feral pigeons compared with t-tests (Table S3). A Principal Component Analysis (PCA) including all six morphometric traits and 149 adult birds was conducted with the function prcomp in the base R package (R Development Core Team, 2010). The first two Principal Components (PC's) collectively explained 59.42% of the variance (PC1 38.58% and PC2 20.84%). PC1 described increasing cere width, tarsus length, wing length and head angle, with feral pigeons having larger PC1 scores than Rock Doves. PC2 largely described bill length, with higher PC2 values corresponding to larger bills (Table S2).

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Supplemental information

Limited domestic introgression in a final refuge of the wild pigeon

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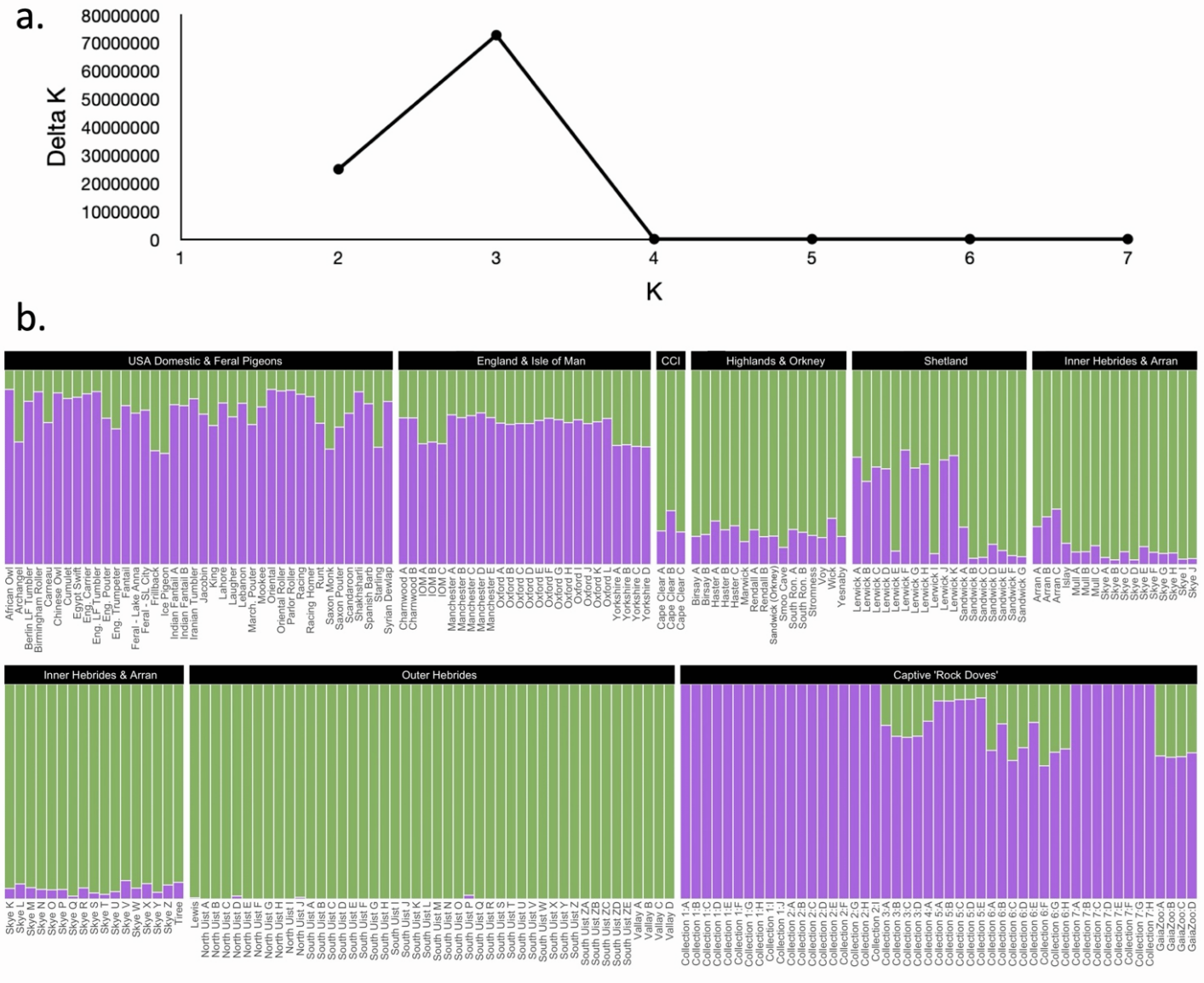


Figure S1. Population structure of wild, captive, domestic and feral *Columba livia*, assuming two population genetic groupings, based on a linkage disequilibrium-filtered dataset of genotype likelihoods containing 2,583,745 polymorphic nuclear sites, and 232 individual birds.

(A) CLUMPAK identified *K*=2 (with the first group being the wild Rock Doves and the second group including both the feral/domestic pigeons and captive 'Rock Doves') as being the second most likely value after *K*=3, using the NGSadmix results and the method of Evanno *et al.* [37].

(B) Ancestry estimates for individual birds show varying levels of admixture between the wild Scottish/Irish Rock Doves (primarily green) and the feral/domestic/captive 'Rock Doves' (primarily purple). Bars depict maximum likelihood estimation of individual ancestries using NGSadmix (K=2).

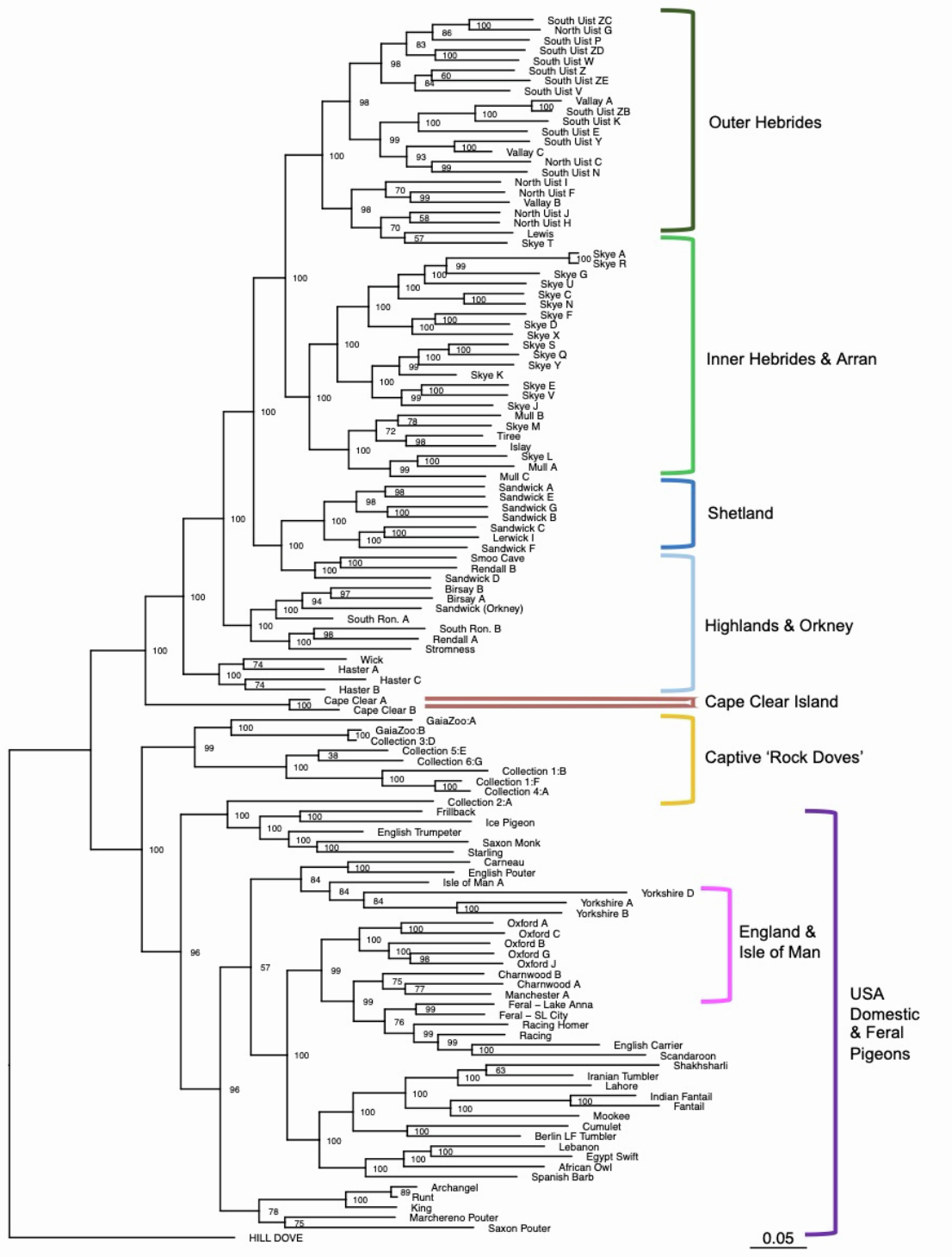


Figure S2. The maximum-likelihood topology from a VCF of 871,968 SNPs and 121 birds, using IQTREE-2, shows a split between the wild Rock Doves and both the feral/domestic pigeons and captive 'Rock Doves'. This split is supported by ultrafast bootstrap support values of 100%. The Hill Dove *Columba rupestris* was used as an outgroup. Within the Rock Dove clade there is well-supported geographic structure.

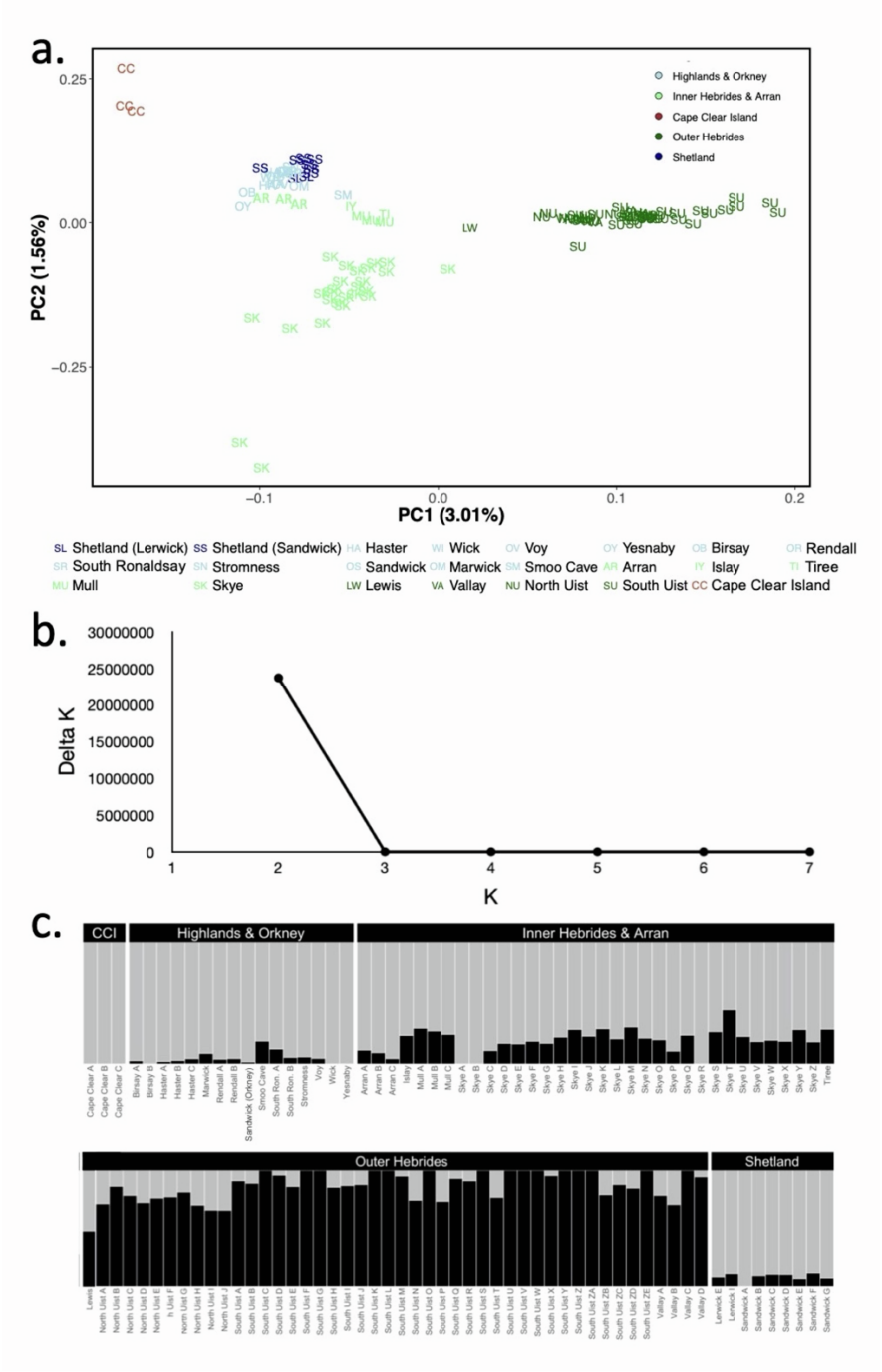


Figure S3. Population structure of Rock Doves from the British Isles based on a linkage disequilibrium-filtered dataset of genotype likelihoods containing 2,554,226 polymorphic nuclear sites, and 108 individual birds.

(A) Principal Component Analysis conducted with PCAngsd showed strong geographic structure of wild Rock Doves, highlighting the Outer Hebridean and Cape Clear Island populations as being different from other Rock Doves in the British Isles according to PC1. Variance explained by PC1 and PC2 is shown in parentheses. Site names (two letter code) are grouped by color representing geographic region (as per inset legend.

(B) CLUMPAK identified *K*=2 as being the most likely value, using the NGSadmix results and the method of Evanno et al. [37].

(C) Maximum likelihood estimation of individual ancestries from NGSadmix, with K=2 showed separation between Outer Hebridean Rock doves (primarily composed of the black cluster) and other Rock dove populations in Scotland and Ireland (primarily composed of the grey cluster but with varying levels of admixture).

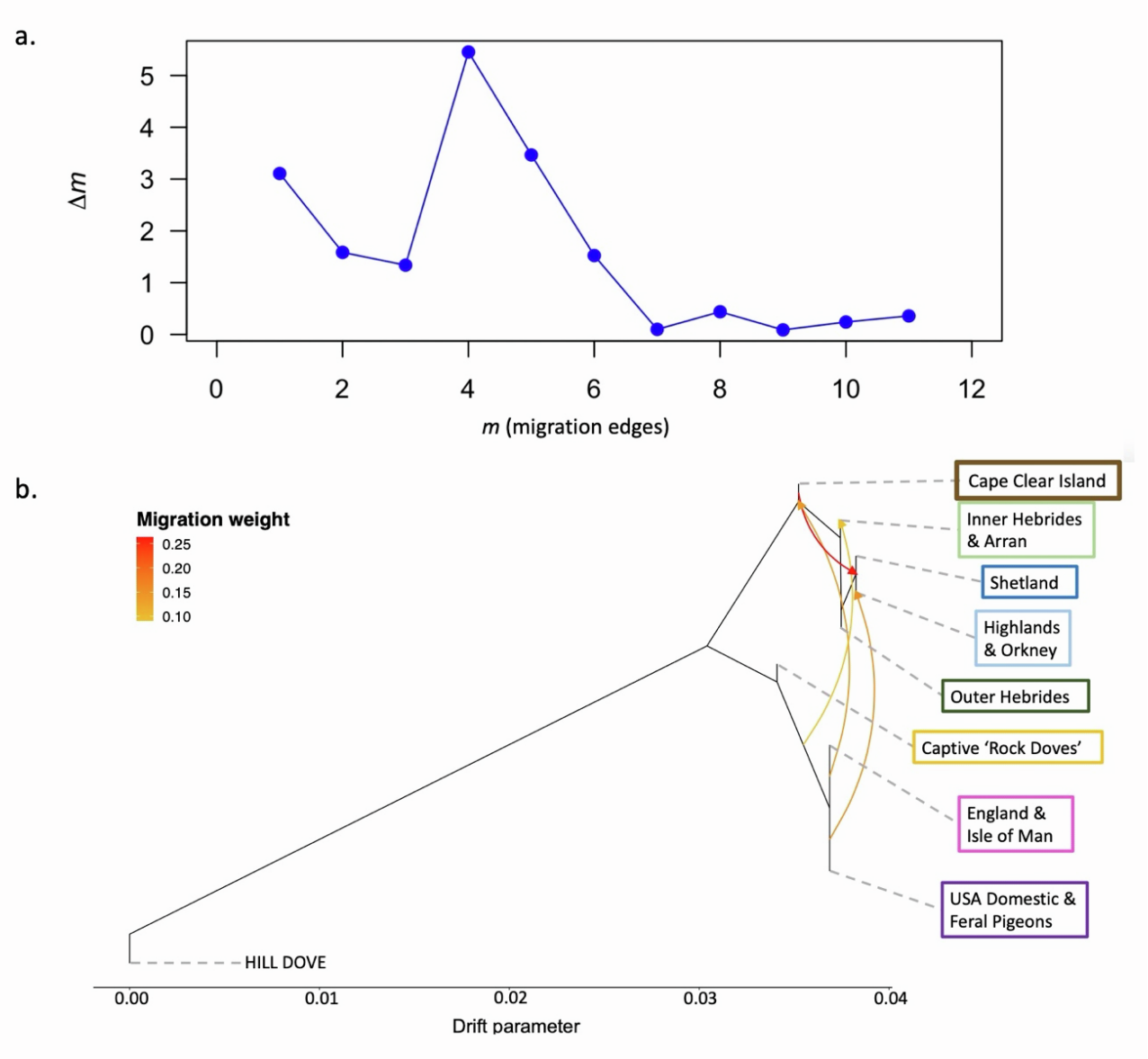


Figure S4. TreeMix estimation of relationships and migration among populations using a VCF thinned to 675,581 SNPs to account for linkage disequilibrium.

A) The optimum number of migration edges was four (highest Δm), as calculated by OptM

B) TreeMix identified gene flow between wild Rock Doves and feral/domestic pigeons, with three migration edges from the feral/domestic group to different Rock Dove populations, and one migration edge from the Cape Clear Island Rock Dove population to Shetland/Highlands & Orkney. The color of migration edges corresponds to the proportion of the genome being exchanged.

Table S1. Sampling information

Region	Subsite names (if more than one site within region)	(Sub)site GPS coordinates (latitude/longitude in decimal degrees)	Sampling method	Adults measured	Sequenced	Region category (as seen in figures/analyses)
Oxford	N/A	51.75, -1.26	Hand caught	22	12	England & Isle of Man
Leicestershire	Loughborough Charnwood (Thrussington) Leicester	52.77, -1.21 52.73, -1.04 52.64, -1.14	Hand caught Hand caught Hand caught	44	2	England & Isle of Man
Yorkshire	N/A	54.13, -1.22	Pest control	0	4	England & Isle of Man
Manchester	N/A	53.42, -2.23	Pest control	0	5	England & Isle of Man
Isle of Man	N/A	54.31, -4.52	Pest control	0	3	England & Isle of Man
Highlands	Smoo Cave (Sutherland) Tongue (Sutherland) Wick (Caithness) Haster (Caithness) Scrabster	58.56, -4.72 58.49, -4.42 58.43, -3.09 58.44, -3.16 58.61, -3.55	Hand net Hand net Carcass Hand net Hand net	21	5	Highlands & Orkney
Orkney	Birsay Marwick Rendall Sandwick (Orkney) South Ronaldsay Stromness Voy Yesnaby	59.13, -3.23 59.10, -3.34 59.07, -3.01 59.06, -3.28 58.80, -2.97 58.97, -3.30 59.02, -3.29 59.02, -3.35	Hand net Carcass Hand net Carcass Hand net Carcass Carcass Carcass	15	11	Highlands & Orkney
Shetland	Sandwick (Rock Doves) Lerwick (feral pigeon/Rock Dove mix)	60.00, -1.23 60.15, -1.15	Walk-in trap Walk-in trap	18	18	Shetland
Arran	N/A	55.44, -5.25	Mist net	0	3	Inner Hebrides & Arran
Inner Hebrides	Skye Mull Tiree Islay	57.41, -6.20 56.57, -6.00 56.47, -6.94 55.63, -6.25	Hand net Hand net Carcass Carcass	9	31	Inner Hebrides & Arran
Outer Hebrides	North Uist South Uist Vallay Lewis	57.67, -7.23 57.2, -7.39 57.66, -7.41 58.33, -6.63	Hand net Hand net Hand net Mist/Whoosh nets	18	46	Outer Hebrides
Cape Clear Island	N/A	51.44, -9.50	Walk-in trap/ Carcasses	2	3	Cape Clear Island (CCI)
Captive birds	GaiaZOO and 7 private collections, Netherlands	N/A	N/A	0	49	Captive ‘Rock Doves’
USA Domestic & Feral Pigeons	NA	N/A	N/A	0	40	USA Domestic & Feral Pigeons
Columba rupestris	NA	N/A	N/A	0	1	N/A
TOTALS				149	233	

Table S2. Principal Component Analysis of six phenotypic traits, showing the variance explained by each principal component in parentheses, as well as the factor loadings of each trait. Principal component one represents a composite of multiple traits, with increasing head angle, cere width and tarsus length making strong contributions. Principal component two predominantly represents increasing bill length.

Trait	PC1 (38.58%)	PC2 (20.84%)
Wing length	0.388	0.159
Bill length	-0.097	0.745
Head angle	0.420	-0.403
Cere width	0.492	-0.081
Tarsus length	0.511	0.039
Mass	0.400	0.500

Table S3. Summary of data comparing morphological traits in Rock Doves and feral pigeons. All traits were significantly different except for mass (each t-test had 147 degrees of freedom and used a critical value of $\alpha < 0.01$ when a Bonferroni correction for multiple comparisons was applied). N denotes sample size.

Trait	Rock Dove mean (N = 83)	Feral pigeon mean (N = 66)	t	p	95% CI of difference
Wing length	228.2	234.4	-3.7	<0.001	2.9 to 9.5 mm
Bill length	19.6	18.7	4.6	<0.001	-0.1 to 1.9 mm
Head angle	138.0	148.6	-10.3	<0.00001	8.6 to 12.6 °
Cere width	8.9	12.1	-13.3	<0.00001	2.7 to 3.7 mm
Tarsus length	73.8	76.3	-5.4	<0.00001	1.6 to 3.5 mm
Mass	361.2	373.1	-1.7	0.0923	-25.7 to 1.9 g

Table S4. Genes identified as being within ±100,000bp from clustered outlier windows of high introgression (*fd*) and differentiation (F_{ST}). *fd* reflects introgression between the focal population and the 'England & Isle of Man' population, relative to Outer Hebridean Rock Doves, using *C. rupestris* as an outgroup. F_{ST} reflects differentiation between the focal population and the 'England & Isle of Man' population. Genes starting with 'LOC' are those where a published symbol is not available and orthologs have not been determined yet.

Population	Genes associated with *fd* outlier regions		Genes associated with F_{ST} outlier regions	
Outer Hebrides	N/A		CUNH3orf52 LOC102086396 TMPRSS7 TAGLN3 ABHD10 PHLDB2 PTPN13 LOC102091166 CFAP46 TCTN3 ALDH18A1 LOC106145704	CLUH PAFAH1B1 MNT SGSM2 TNFAIP1 IFT20 TMEM97 NLK LYRM9 NOS2 KSR1
Inner Hebrides & Arran	HJURP LOC102084577 KCTD12 CUNH1orf131 GNPAT INTU NDST4 ARSJ LOC102091784 DLX1 METAP1D HAT1 DYNC1I2 LOC102091411 PLPPR4 PALMD	AGL LOC102088687 LOC110359454 LOC102087400 LOC106145714 LOC102084653 LOC102097754 LOC102097580 LOC102097395 LOC102097214 LOC110363535 PCGF3 MFSD7 LOC102095407 LOC102096265 LOC102089226	TRPV5 KEL PAQR3 BMP2K G3BP2 USO1 ANKRD22 LOC102087031 LOC102087219 LOC102083961 STAMBPL1 SPHKAP AGFG1 MRPL44 MFF LOC102086994 CBFB BRD3	WDR5 WBP2 TRIM47 TRIM65 ANKFN1 NOG CUNH17orf67 DGKE DHX40 YPEL2 SLC45A1 ERRFI1 PARK7 TNFRSF9 UTS2 PER3 VAMP3
Highlands & Orkney	LOC110355335 LOC110356666 CUNH12orf50 CUNH12orf29 LOC102098963 ARFGAP3 HEPACAM2 TMEM106B LOC102086317 RWDD1 TRAPPC3L FAM26D OVCH2 FAR1 PTH BBOF1 ABCD4 IFT43 LOC110359455 LOC110359454 LOC110359572 LOC102087577 LOC102087220 LOC102097754 LOC102097580 LOC102097214 LOC102096854	ZMAT2 LOC102091308 DND1 WDR55 TMCO6 LOC110359571 KCND3 FAM212B ADORA3 WDR77 GUCA1A IRF6 TRAF3IP3 HSD11B1 LAMB3 LOC102088076 PFKFB2 CUNH1orf116 LOC102085460 LOC102086616 MAPKAPK2 EIF2D SUB1 SHB CLTA LOC110363534	XKR4 RHPN1 LOC110356776 C1QTNF4 FAM180B NDUFS3 KBTBD4 PTPMT1 CELF1 RAPSN PSMC3 WDFY4	GIGYF2 KCNJ13 CUNH2orf82 NGEF NEU2 LOC102086994 B3GNT9 CUNH16orf70 CBFB WSCD1 RAP1GAP2
Shetland	LOC110355335 LOC110356666 CUNH12orf50 CUNH12orf29 HJURP LOC102084577 SH2D1B MED10 CLVS1 KCNG3 LOC102090179 ST3GAL5 MRPL35 LMLN LOC102097467 PARP9 FAIM LOC102092924 STOX1 LOC106146130 ERCC6 SLC35G1 LGI1 CLRN3 PTPRE FCHSD1 LOC102089251 LOC102089056 LOC102088687 LOC110359454 LOC110359572 LOC102087943 LOC102087400 LOC106145714 LOC102097754	LOC102096854 HARS NRG2 HBEGF MTM1 MTMR1 GPR50 TBC1D88 RNF128 RHBDL3 CUNH17orf75 LOC106145832 LOC102098485 ERN1 TEX2 DLX4 DLX3 ITGA3 PDK2 PPP1R9B COL1A1 LOC110355437 LOC102091836 LOC102091659 LOC102091287 CDC27 MAPT RUNDC3A UBTF CUNH17orf53 LNPEP SHB NANS PLIN2 LOC110363071	NDP EFHC2 CDH10 NKX2-1 PAX9 PTBP2 LOC102093731 AIRE EFHD1 GIGYF2 KCNJ13 CUNH2orf82 CBFB MMAB	UBE3B KCTD10 MYO1H FOXN4 ACACB UNG ALKBH2 USP30 LOC102098809 WSCD1 RICTOR LOC102086673 PIK3C3
Cape Clear Island	LOC11035335 LOC110356666 CUNH12orf50 PARP1 MMS22L NKX2-1 PAX9 TNKS2 LOC102088684 TBC1D12 NOC3L ASPM LOC102095930 RPL39 TAF7L TMEM35A TRMT2B F8 VPS13D	FUNDC2 GCNA OGT ITGB1BP2 NONO NLGN3 AGTRAP MTHFR BNP LOC102089527 NPPC LOC102088956 LOC102089151 KIAA2013 PLOD1 MFN2 MIIP TNFRSF8 LOC102093466	FAM84A CHRFAM7A FAM81A	LOC11036877 CER1 FREM1

5

Gene flow and genomic diversity in British and Irish pigeons *Columba livia*

Title page – Conservation Genetics – Short Communication

Gene flow and genomic diversity in British and Irish pigeons *Columba livia*

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Abstract:

Human transportation of animals and plants around the world has caused a diverse array of conservation problems, including extinction by hybridisation. This results from interbreeding of non-native and native populations, causing genomic homogenisation. However, hybridisation can also have a constructive evolutionary role via an influx of genetic variation, and this is a potential benefit of anthropogenic hybridisation, especially when it is limited in extent. The Rock Dove *Columba livia* has been genetically replaced across much of its range by the feral pigeon. Relict populations in the British Isles have experienced varying levels of wild-feral gene flow. Here we show that feral contributions to these relict populations are generally modest in comparison to another British example, the Scottish Wildcat. Introgression correlates with increased genomic diversity, potentially improving chances of persistence of small, relict Rock Dove populations. However, the beneficial impacts of interbreeding may be short-lived if hybridisation levels continue to increase.

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Data availability statement: The datasets analysed during the current study are available at ENA: PRJEB52260 and GenBank: PRJNA167554.

Author contributions: WJS conceived of the study with input from SMC, and carried out the analyses. WJS wrote the first draft, and both authors edited the manuscript.

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Keywords: hybridisation, introgression, birds, genomic diversity, gene flow

Gene flow and genomic diversity in British and Irish pigeons *Columba livia*

Abstract

Human transportation of animals and plants around the world has caused a diverse array of conservation problems, including extinction by hybridisation. This results from interbreeding of non-native and native populations, causing genomic homogenisation. However, hybridisation can also have a constructive evolutionary role via an influx of genetic variation, and this is a potential benefit of anthropogenic hybridisation, especially when it is limited in extent. The Rock Dove *Columba livia* has been genetically replaced across much of its range by the feral pigeon. Relict populations in the British Isles have experienced varying levels of wild-feral gene flow. Here we show that feral contributions to these relict populations are generally modest in comparison to another British example, the Scottish Wildcat. Introgression correlates with increased genomic diversity, potentially improving chances of persistence of small, relict Rock Dove populations. However, the beneficial impacts of interbreeding may be short-lived if hybridisation levels continue to increase.

Introduction

Increasing anthropogenic movement has led to a commensurate increase in introductions of non-native organisms (Pyšek et al., 2020). A genomic-level process associated with such introductions is ‘extinction by hybridisation’, where introgression leads to the replacement of a native form with its relative, or the formation of a hybrid population

(Rhymer and Simberloff, 1996). Extinction by hybridisation threatens birds (Wu et al., 2020), reptiles (Vuillaume et al., 2015), amphibians (Fitzpatrick and Shaffer, 2007), fish (Glover et al., 2020), insects (Muñoz et al., 2015), mammals (Senn et al., 2019), and plants (Kron and Husband, 2009). Natural hybridisation can play a diversifying evolutionary role (Quilodrán et al., 2020) via hybrid speciation (e.g. Lamichhaney et al., 2018) or the sharing of genomic diversity among populations (e.g. Mallet et al., 2007). 'Genetic rescue', where translocated individuals interbreed with a threatened native population, can increase heterozygosity in small populations, reducing the expression of deleterious alleles (Frankham, 2015). These examples raise the possibility that even anthropogenically-induced hybridisation might have positive impacts, if it occurs to a restricted extent (Smith et al., 2022a).

Following interbreeding with feral pigeons *Columba livia*, the Rock Dove (also *Columba livia*) has been replaced by hybrids in many regions (Smith, 2023). In the British Isles, gene flow varies with proximity to feral pigeons (which are common in England, Wales, southern Scotland, and Ireland). Whilst Outer Hebridean Rock Doves experience negligible introgression, pigeons from Orkney have mixed ancestry (Smith et al., 2022b). In Yorkshire and the Isle of Man, feral pigeons have replaced Rock Doves (Morris and Sharpe, 2021, Brown and Grice, 2005). The regional mosaic of wild-feral pigeon introgression provides a tractable system to compare differing levels of admixture. The remaining, peripherally distributed Rock Doves in the British Isles persist as small populations and therefore may face the risks associated with low genetic diversity. Paradoxically, whilst hybridisation with feral pigeons poses the major global risk to Rock

Dove conservation, maintaining low levels of hybridisation at regional scales might bolster genetic diversity in relict populations.

Here, we use Rock Doves with known introgression levels to characterise the relationship between wild-feral admixture and genomic diversity, placing the amount of hybridisation in context by comparing it to that experienced by the well-studied Scottish Wildcat *Felis silvestris*, where almost all individuals have extensively admixed ancestry (Senn et al., 2019). We assess whether barriers to gene flow act to prevent complete genomic homogenisation, but allow sufficient gene flow to augment genetic diversity. We outline the potential positive impacts of anthropogenically-mediated gene flow in maintaining global biodiversity.

Materials and Methods

We used an available genomic dataset (Smith et al., 2022b), consisting of whole-genome sequencing data from 232 pigeons (depth 4-10x). These include free-flying Rock Doves ('Outer Hebrides', 'Inner Hebrides & Arran', 'Highlands & Orkney', 'Shetland', 'Cape Clear Island') plus 'domestic-type' birds, including free-flying feral pigeons ('England & Isle of Man' and 'Shetland Feral') (Fig. 1a) as well as 'captive Rock Doves' (genetically similar to domestic breeds) (ENA: PRJEB52260), and various domestic breeds (GenBank: PRJNA167554) (Shapiro et al., 2013). We used the 'colLiv2' genome (NCBI Assembly GCA_001887795.1) as the reference (Damas et al., 2017) and carried out mapping and data preparation as described in Smith et al., 2022b.

To place the scale of hybridisation in feral pigeons and Rock Doves in the British Isles in context, we compared it with a well characterised hybrid population – the Scottish wild/feral cat. We ranked ancestry proportions ('Q') available for pigeons (Smith et al., 2022b) calculated using NGSadmix v32 (Skotte et al. 2013), and those available for wildcats (Senn et al., 2019) calculated using STRUCTURE 2.3.4 (Falush et al., 2003). Both papers split ancestry into K=2, separating 'wild' and 'feral' ancestry. The Q values included for birds were those from within Rock Dove habitat (Outer Hebrides, Inner Hebrides & Arran, Highlands & Orkney, Shetland, and Cape Clear Island), and those for cats for individuals within Scottish Wildcat habitat, from Smith et al., (2022b) and Senn et al., (2019) respectively.

To examine how population structure and introgression interact, we calculated F_{ST} for each pigeon population pair, using ANGSD v0.933 to identify variable sites for all individuals in both populations by: inferring major and minor alleles from GL data (-doMajorMinor 1), calculating allele frequency assuming major and minor are known (-doMaf 1), using sites with minor allele frequency above 0.01 (-minmaf 0.01), setting base and mapping quality thresholds of 20 and 30 (-minQ 20 -minMapQ 30), discarding 'bad' reads (-remove_bads 1), estimating posterior genotype probability (-doPost 2), estimating genotype likelihoods using the GATK model (-GL 2), using qscore recalibration (-baq 1), and keeping sites with data from ≥10 individuals (-minInd 10). We extracted columns 1 and 2 from the resulting "mafs.gz" file, before indexing the output with 'angsd index'. Then, for each population, for these sites we calculated allele frequency likelihoods with folded 2D site frequency spectra: setting thresholds of base and mapping quality of 20 and 30, discarding 'bad' reads, estimating genotype likelihoods using the

GATK model, using qscore recalibration, and calculating site allele frequency likelihood based on individual genotype likelihoods assuming Hardy-Weinberg Equilibrium (-dosaf 1). We calculated genome-wide F_{ST} using realSFS fst index, and estimated global population pairwise weighted F_{ST} using 'realSFS fst stats'.

We used EEMS v0.0.0.9 to characterise gene flow (Petkova et al., 2016). We generated a genetic distance matrix in ANGSD for 132 free-flying individuals (excluding Shetland's feral pigeons, which fall within the same EEMS geographic mapping unit of Shetland Rock Doves and would artificially inflate Rock Dove genetic diversity and migration estimates at that location). ANGSD was run as follows: estimating genotype likelihoods with the SAMtools model, inferring major and minor from GL data, calculating allele frequency assuming known major and minor alleles, recording base frequency (-doCounts 1), printing the consensus base (-doIBS 2), and outputting a pairwise IBS matrix (-makeMatrix 1). We used qscore recalibration (-baq 2), set thresholds of base and mapping quality of 30, and discarded 'bad' reads and those not mapping uniquely (-uniqueonly 1). We kept sites with data from ≥71 individuals and skipped triallelic sites (-skipTriallelic 1). We carried out five EEMS runs, each with an MCMC chain of 10,000,000 iterations with a 20,000-iteration burn-in and 1800 thinning iterations. The number of geographic demes was set to 100, 150, 200, 250, and 300 (Petkova et al., 2016). Runs were combined using rEEMSplots in R v4.0.2 (R Development Core Team, 2010).

To quantify heterozygosity, we used ANGSD: estimating genotype likelihoods using the SAMtools model, setting minimum base and mapping quality of 20 and 30, and calculating site allele frequency likelihood (-dosaf 1), before estimating site frequency

spectrum using realSFS (Nielsen et al., 2012). Heterozygosity was outputted as outlined at http://www.popgen.dk/angsd/index.php/Heterozygosity. We calculated inbreeding with default ngsF settings (Vieira et al., 2013), generating the input via ANGSD: estimating genotype likelihood with the SAMtools model, inferring major and minor, calculating allele frequency assuming known major and minor alleles, including only proper pairs (only_proper_pairs 1) and sites with minor allele frequency >0.05. We excluded 'bad' reads and those not mapping uniquely. We used qscore recalibration, set minimum base and mapping quality of 30 and 25, and kept sites with data from ≥115 birds and with a p-value of 1e-6 (-SNP_pval 1e-6), outputting a genotype likelihood file (-doGlf 3). We tested for differences in heterozygosity and inbreeding between populations and types (Rock Doves, feral, domestic, or 'captive Rock Dove') with a Kruskal-Wallis test. We plotted heterozygosity and inbreeding against individual Q scores. To quantify the difference between the slopes of the relationship between Inbreeding/Heterozygosity and Introgression Score between domestic-type pigeons and Rock Doves, we ran a linear regression in R using function lm() with formula *Introgression/Heterozygosity ~ Introgression_score*Type*.

Results

Rock Dove Q scores do not drop below 0.75, contrasting with wildcat Q scores which form a continuum from individuals with mostly domestic ancestry to those with mostly wild ancestry (Fig. 1b). As expected, pairwise F_{ST} shows that, where there is known introgression (e.g. Highlands & Orkney), individuals become more genetically similar to feral pigeons (e.g. England & Isle of Man). However genetic structure is also influenced by

geographic distance, as seen for Cape Clear Island birds that are highly divergent from feral pigeons despite known introgression (Fig. 1c). EEMS shows there is reduced gene flow across seas, moorland, and mountains (Fig. 1d).

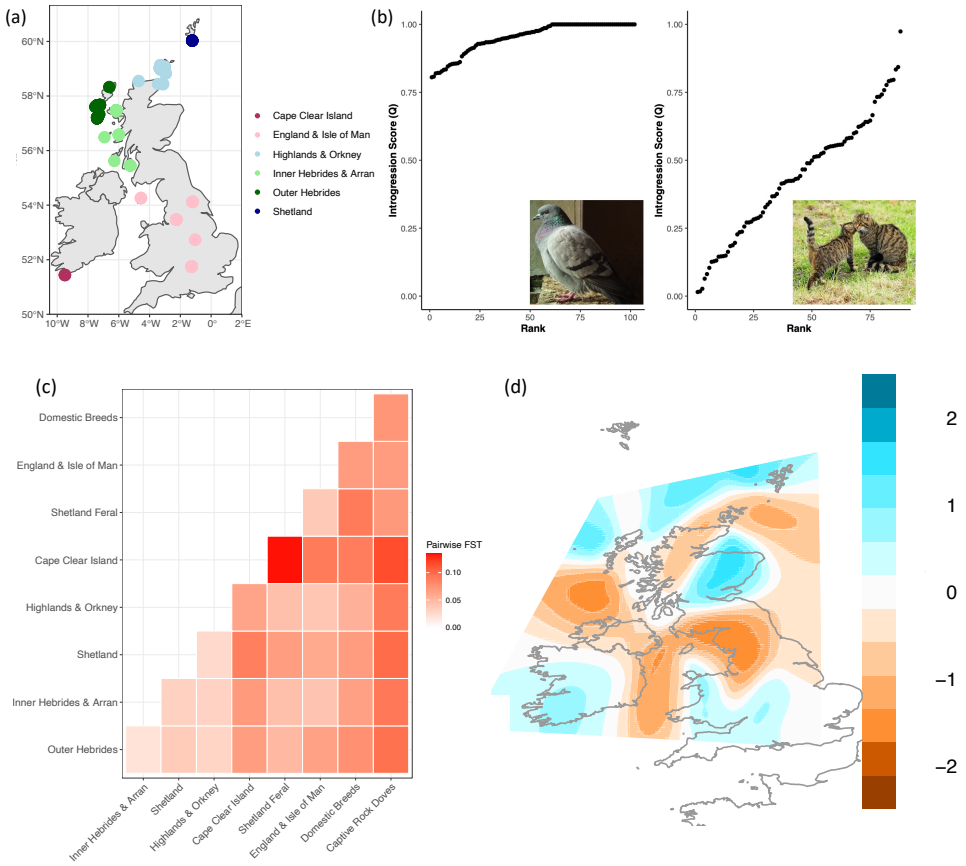


Figure 1: (a) Locations of the populations of free-flying pigeons sampled from the British Isles which are included in this study. (b) Ranked admixture Q proportions for British Isles Rock Doves from Smith et al., 2022b (photograph: author) versus Scottish Wildcats from Senn et al., 2019, (photograph: Peter Trimming CC BY 2.0) showing individuals ranked from lowest to highest 'wild' ancestry proportion, assuming K=2 (ancestry split into wild and feral components). Analyses included only those individuals from within the putative range and habitat of the wild form. (c) Heatmap of pairwise F_{ST} values among the

study populations, with brighter reds indicating higher levels of divergence. (d) Effective migration surfaces inferred by EEMS using a pairwise genetic difference matrix generated by ANGSD. Darker orange regions have reduced gene flow (migration barriers) with respect to the global mean and darker blue regions, higher gene flow (migration corridors).

Populations at different locations differ in level of inbreeding (Kruskal–Wallis test, $\chi 2 = 125.68$, $n = 9$, df = 8, $P < 0.001$, Fig. 2a) and heterozygosity (Kruskal–Wallis test, $\chi 2 = 111.16$, $n = 9$, df = 8, $P < 0.001$; Fig. 2b). Pigeon types also differ in inbreeding (Kruskal–Wallis test, $\chi 2 = 111.62$, $n = 4$, df = 3, $P < 0.001$) and heterozygosity (Kruskal–Wallis test, $\chi 2 = 95.946$, $n = 4$, df = 3, $P < 0.001$). The domestic breeds and 'captive Rock Doves' have lower genetic diversity than free-flying populations. Within free-flying populations, those that experience hybridisation have higher genetic diversity than unadmixed populations (whether feral pigeons or Outer Hebridean Rock Doves). The impact of increasing domestic ancestry on inbreeding (Fig. 2c) and heterozygosity (Fig. 2d) differed between Rock Doves and 'domestic-type' (feral, domestic, and 'captive Rock Dove') birds. The difference between the slopes between domestic-type birds and Rock Doves is significant for both inbreeding (estimate = -0.791, $P < 0.001$) and heterozygosity (estimate = 0.004, $P < 0.001$).

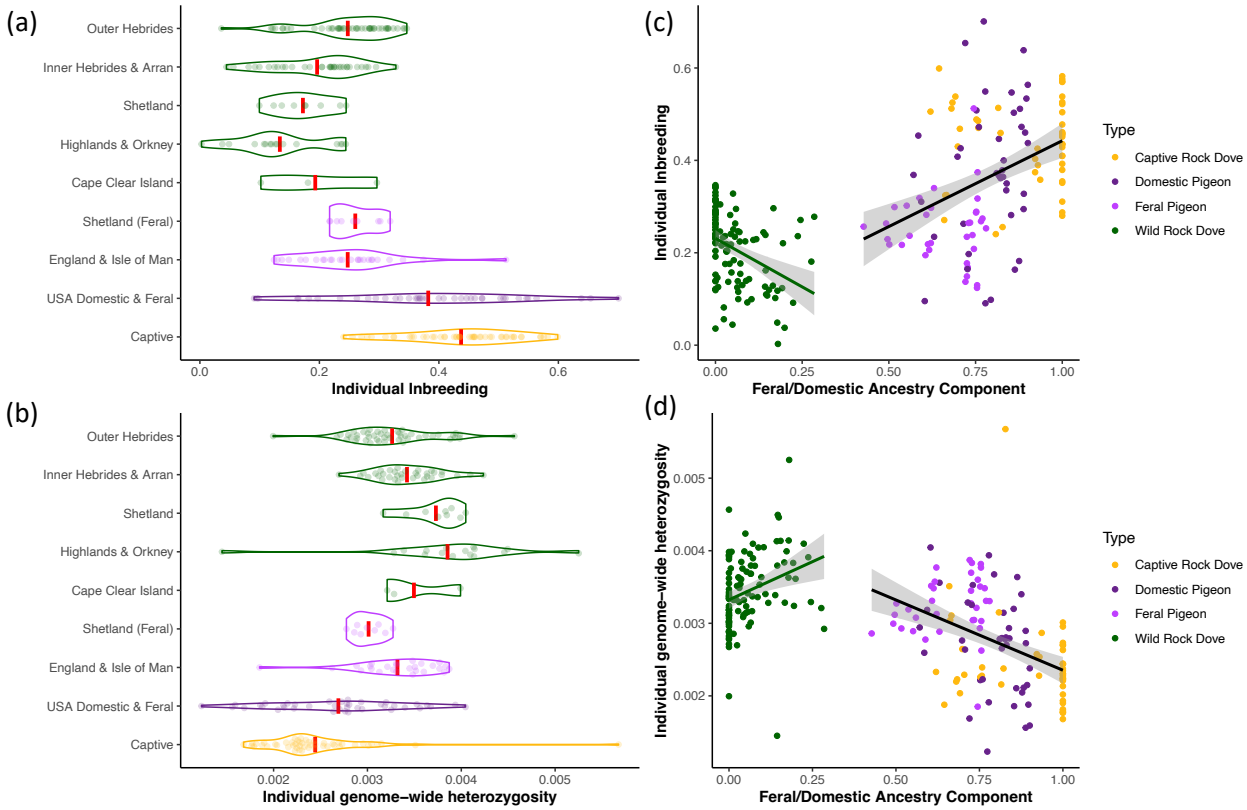


Figure 2: (a) Individual inbreeding varies with region and type of pigeon, with wild Rock Doves having the lowest levels of inbreeding and domestic-type pigeons highest. Red lines indicate the mean values. (b) Individual genome-wide heterozygosity varies with region and type of pigeon, with wild Rock Doves having the highest levels of heterozygosity and domestic-type pigeons lowest. Red lines indicate the mean values. (c) As the domestic-type ancestry component increases, individual inbreeding decreases in Rock Doves but increases in domestic-type pigeons. (d) As the domestic-type ancestry component increases, individual genome-wide heterozygosity increases in Rock Doves but decreases in domestic-type pigeons.

Discussion

Many Rock Dove populations have suffered from genetic extinction (Johnston and Janiga, 1995), yet in the British Isles, relict populations have persisted though with some level of introgression. Here we show that wild-feral gene flow is not as extensive in British Isles Rock Doves as it is in the Scottish Wildcat. This may be due to less extensive introduction of the feral pigeon form within the remaining wild range than experienced by feral cats (Smith, 2023). Nevertheless, the difference in pattern is surprising given that flight might be expected to provide feral pigeons with higher natural dispersal capacity across the region. Analyses of gene flow and genetic differentiation patterns align with observations that feral pigeons are mostly unwilling to cross seas, and are rare in mountains and moorlands (Lack, 2010). Hence the Scottish Highlands and Hebridean Sea act as natural barriers to feral pigeon dispersal, protecting the least introgressed Rock Dove populations on the Outer Hebrides (Smith et al. 2022b). This geographic protection does not eliminate the threat of genetic swamping because of possibility of escapees from captive domestic collections, or arrival of feral pigeons that hitch a ride on ferries (Smith, 2023).

Introgression has increased genomic diversity (notably in the admixed Highlands & Orkney region). This could alleviate the genomic impacts of small population size, domestication, and feralisation. The domestic breeds and 'captive Rock Doves', which consist of small gene pools and are selectively bred, have lower genetic diversity than free-flying populations. One 'captive Rock Dove' with high heterozygosity is suspected to have mixed Rock Dove x Stock Dove *Columba oenas* ancestry. Among free-flying populations, both feral pigeons (having undergone genetic bottlenecks during

domestication and feralisation) and Outer Hebridean Rock Doves (an unadmixed remnant wild population) have lower genetic diversity than hybridising Rock Dove populations. Among these mixing populations, regional variation in genomic diversity is explained by variation in wild-feral gene flow. Such introgression might facilitate adaptation to novel environments. For example, it has been hypothesised that hybridisation of domestic dogs with wild relatives has enhanced the latter's abilities to survive in anthropogenic environments (Monzón et al., 2014).

Despite the potential positive impact of hybridisation, it may be a temporary effect. Feral pigeons replaced Rock Doves in Yorkshire and the Isle of Man in recent decades (Smith, 2023). These birds have the high inbreeding and low heterozygosity associated with long-standing feral populations. These events suggest that the demographic imbalance between Rock Doves and feral pigeons will lead to the eventual replacement of genomically admixed populations with feral pigeons. The complete replacement of Rock Doves with feral pigeons would be undesirable if we are to maintain genomic diversity within the species *Columba livia* (Smith, 2023, Smith et al., 2022a, Smith et al., 2022b).

Conclusion

Whilst hybridisation of the Rock Dove with its invasive feral conspecific has led to the loss of many genetically unadulterated wild populations, in remnant British populations, individual birds with wild-feral ancestry have higher genetic diversity than unadmixed individuals. This could facilitate persistence of relictual wild populations, but only if hybridisation is kept at low levels. Further research characterising bird longevity, ecology,

and reproductive success would reveal links between individual introgression status, genomic diversity, and fitness in populations of feral, wild and hybrid origin.

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6

Rapid assessment of the extent of feral introgression in British and Irish Rock Dove (*Columba livia*) populations

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Short Communication

Rapid assessment of the extent of feral introgression in British and Irish Rock Dove (*Columba livia*) populations

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Interbreeding of related forms following anthropogenic activity can lead to genomic homogenization, contributing to decreasing biodiversity. Assessing spatiotemporal variation in the extent of introgression is important for conservation but can be expensive and logistically complex. The Rock Dove *Columba livia* is threatened by interbreeding with its feral conspecific and it is difficult to identify wild birds when wild, feral and mixed populations exist. Here, using British and Irish populations, I assess whether the proportion of birds with wild type plumage corresponds to distinctive head morphotypes differing between Rock Doves and feral pigeons, and whether plumage can be used to identify Rock Dove flocks and infer the extent of hybridization. I also determine whether plumage proportions have changed since a prior study was carried out in the 1960s. I show that flocks in which > 75% of individuals have wild type plumage consist of Rock Doves, as determined by head morphotype. Plumage proportions vary geographically, corroborating known genomic assessments of Scottish and Irish Rock Doves. Finally, the number of birds with aberrant plumages has increased since the 1960s, even in regions where no such birds were identified previously. This highlights the value of the Rock Dove as a current case study with which to explore the process of extinction by hybridization.

Keywords: citizen science, extinction, feralization, hybridization, gene flow.

Hybridization can be both a diversifying and homogenizing force in evolution (Abbott *et al.* 2013, Quilodrán *et al.* 2020). Homogenization can cause extinction by hybridization, where introgression from an introduced population leads to the genomic replacement of a native form (Rhymer & Simberloff 1996). Extinction by hybridization is common where anthropogenic activity establishes contact between previously allopatric populations (Todesco *et al.* 2016). Despite this, our understanding of its spatiotemporal dynamics is limited and this reduces our ability to conserve at-risk populations (Smith *et al.* 2022a). Despite developments in genomic technologies that allow determination of hybrid ancestry with increasing precision, the challenges of data generation mean that it remains difficult to quantify hybridization, especially over large geographical scales (Senn *et al.* 2019). DNA sampling of many species in numbers, and across a wide area, can be logistically and financially challenging. An alternative option is to identify phenotypic traits that allow rapid assessment of patterns of introgression. This has the advantage of being fast, inexpensive and able to guide more tailored genomic sampling.

Extinction by hybridization is a particular threat to the undomesticated forms of domestic animals which have feral populations (Smith *et al.* 2022a). The wild–domestic interface is of significant economic importance given the frequent global use of domestic species (Zeder 1982, Bähr & Wolf 2012). Long-standing wild-feral hybridization has, in many cases, rendered the genetic status of the undomesticated form uncertain (Smith *et al.* 2022a). This represents the loss of a source of genetic diversity, as well as a loss of opportunity for scientific advancement by studying undomesticated populations in a natural setting to explore the evolution of model organisms (Taberlet *et al.* 2008, Bähr & Wolf 2012).

The persistence of the wild Rock Dove *Columba livia* across most of its native range is uncertain following hybridization with feral pigeons (Baldaccini 2020). Genomic analysis has revealed that Scotland and Ireland harbour relict Rock Dove populations that have experienced variable levels of gene flow from feral pigeons, with Outer Hebridean birds experiencing little introgression (Smith *et al.* 2022b). This corroborates the view of the range of the Rock Dove in the British Isles as encompassing northern Scotland and the western fringe of Ireland (Hewson 1967, Goodwin & Gillmor 1970). Further work to characterize the dynamics of introgression is required to inform conservation efforts focusing on this important reservoir of ancient genetic diversity.

A main hurdle to evaluating the status of the Rock Dove is the challenge of discriminating them from feral pigeons without genomic analysis (Blasco *et al.* 2014). One phenotypic difference suggested to differentiate them is head shape (Johnston *et al.* 1988, Michaelsen & Refvik 2003). A rounded head, thinner bill and smaller cere distinguish the Rock Dove from the feral pigeon

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(Fig. 1a). While single traits are not diagnostic, overall head morphotype is an effective multivariate trait reflecting genomic differences between Rock Doves and feral pigeons. This has been shown by plotting the first two principal components of craniofacial morphology, which has highlighted that the distribution of individuals according to their head shape corresponds to their genomic classification as a Rock Dove or feral pigeon (Smith *et al.* 2022b). Notably, birds sampled from actively hybridizing populations had intermediate head morphology. Those not familiar with feral pigeons and Rock Doves might find it hard to assess head morphotype from a distance, especially in currently hybridizing populations where intermediates predominate. It would therefore be useful to explore methods more compatible with a citizen science approach, which would be valuable in maximizing the geographical coverage of Rock Dove monitoring.

Rock Doves have a wild type plumage only, contrasting with feral pigeons that display plumage polymorphism, although many feral individuals have wild type plumage (Fig. 1b; Fr *et al.* 1949, Johnston & Janiga 1995). Wild type plumage consists of a blue-grey colour with (in the subspecies *livia*, the primary ancestor of the domestic pigeon, and the subspecies found in Europe) a large white rump patch and two black wing bars (Johnston & Janiga 1995). The variable plumage of feral and captive domestic pigeons results from selective breeding and introgression. For example, the common 'chequer' pattern was introduced from the Speckled Pigeon *Columba guinea* (Vickrey *et al.* 2018). Although plumage variation cannot be used to categorize individuals (some feral pigeons have wild type plumage, and Rock Doves with domestic introgression can have aberrant plumage features) it could be used at the level of a flock, on the basis that Rock Dove flocks will have a very high proportion of wild type individuals, whereas feral pigeon flocks will be dominated by plumage types originating under domestication, and mixed wild–feral flocks will have intermediate plumage proportions. For example, Hewson (1967) sought to categorize Rock Dove populations in Scotland; flocks composed of < 75% wild type individuals were designated as mixed or feral/domestic flocks, and those that surpassed this threshold were identified as flocks consisting of 'mainly Rock Doves'. This plumage threshold appears to have been determined fairly arbitrarily (another suggested threshold for identifying a Rock Dove flock was 80% wild type plumage) and the authors did not suggest a threshold to separate mixing flocks from unadmixed feral flocks. Plumage proportions could also allow us to infer the extent of feral introgression. Such an inference would be based on the premise that a flock of Rock Doves with solely wild type birds is likely to have experienced minimal gene flow from feral pigeons, and increasing numbers of birds with aberrant plumage indicates increasing levels of feral–wild admixture.

In this paper I combine head morphotype and flock-level plumage data to assess the validity of Hewson's 75% plumage threshold as a way of distinguishing Rock Doves and feral pigeons. I also infer the extent of feral hybridization among Rock Dove populations by using plumage variation as a proxy for domestic introgression, with the aim of providing a better description of the status of Rock Doves in the British Isles than has previously been possible. Finally, I compare records of plumage proportions collected by Hewson in the 1960s with those collected at the same locations as part of this study, in order to highlight the temporal progression of wild–feral hybridization in Rock Doves in Scotland.

METHODS

I examined flock plumage counts and head morphotype in birds from locations across the British Isles. Citizen scientist involvement in either counting birds of wild type and non-wild type plumage in a flock, or submitting a photograph for the author (W.J.S.) to count, was solicited through social media and local ornithological newsletters. Head morphotype was categorized by the author for birds in all 124 sites. Birds were categorized into the Rock Dove or feral pigeon head morphotype based on direct observations and photographic evidence. Photographs were supplied by citizen scientists, found on eBird and, in order to specifically target Rock Dove regions of interest, by searching for 'Rock Dove (s) + Scotland/Ireland (as well as individual island, region and location names) with Google, Facebook and Twitter. If head morphotypes varied within flocks, or individuals had a combination of traits of both forms, then they were categorized as 'intermediate/mixed' (Fig. 1a). To make the Rock Dove and feral pigeon morphotype classifications more objective, four traits were assessed (see also Fig. S1 for further comparisons of Rock Dove and feral pigeon head morphotypes).

A feral pigeon has:

- A swollen, fleshy cere, with the white part running up to, or past, the end of the nares.
- A flattened head shape with the top line of the forehead rising roughly in line with the top line of the bill.
- A thick, 'triangle-shaped', bill
- A pale/fleshy orbital ring surrounding the eye.

A Rock Dove has:

- A small cere, with the white part not running up to, or past, the end of the nares.
- A rounded head shape with the top line of the forehead rising well above the line of the top of the bill.
- A thin and long bill.

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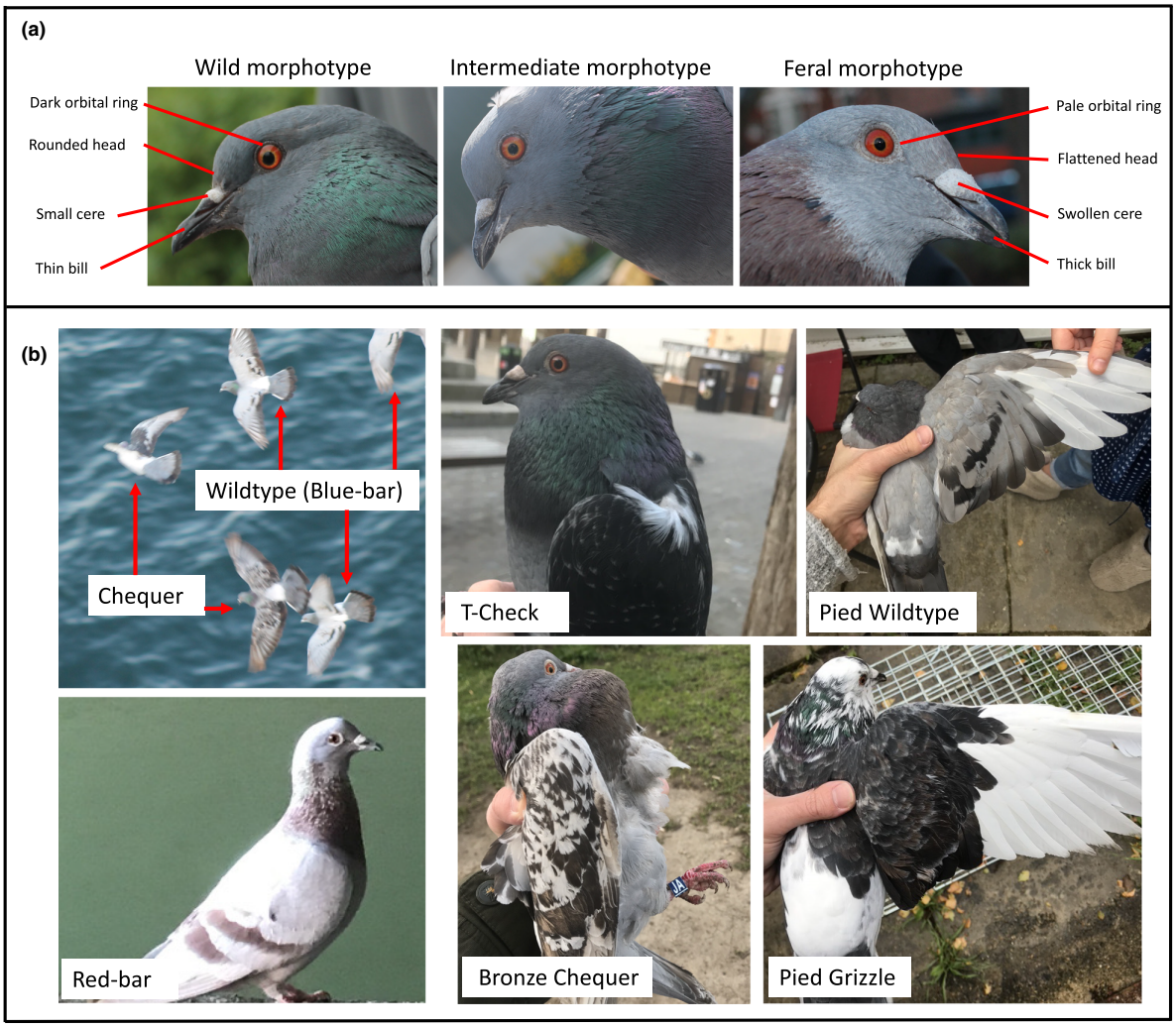


Figure 1. (a) Head morphology of *Columba livia* with key diagnostic features labelled. Wild bird is from Mull, Inner Hebrides, Scotland. Intermediate morphotype is from Mainland, Orkney, Scotland. Feral morphotype is from Leicester, England. (b) Common plumage morphs found in feral domestic pigeons, including those exhibiting the wild type plumage commonly found in wild Rock Dove populations. All photographs are from English feral pigeons.

- A dark grey/thin orbital ring surrounding the eye.

For a bird to be categorized as belonging to the Rock Dove or feral pigeon morphotype, it must have all four traits. A minimum of three birds needed to be visible to determine head morphotype for a site. In practice, many more birds were usually visible and images or observations of as few as three birds' head morphotypes were only used when the overall count involved fewer than 10 individuals. Overall, site morphotype was based on assessing the head morphology of > 75% of individuals in 41% of flocks, 25–75% of individuals in 44% of flocks and < 25% of individuals in 15% of flocks.

At 100 of 124 sites (Figs 2 and 3), flocks of birds were observed to record plumage proportions. To prevent repeat counting of the same birds, multiple counts from the same site were not used. In cases where more than one flock was observed at the same site, the flock with the highest number of birds was used. To reduce the risk of counting the same birds at different sites (only a risk in the Outer Hebrides, Caithness and Orkney due to the higher density of counts in these

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regions) I only used counts made within an hour of each other if the sites in question were > 10 km apart. The number of birds in each flock with wild type and non-wild type plumage ('flock plumage counts') was recorded (Fig. 1b). Flock plumage counts were collected by a combination of direct observations during fieldwork expeditions, photographs (collected as for head morphotype) and citizen scientist counts. Birds were surveyed at 124 locations (Fig. 2) between 2015 and 2020 (76 sites within and 48 outside the putative Rock Dove range), and flocks ranged from seven to 118 birds (mean flock count was 30 birds). Full location information and observation type are detailed in Table S1.

To determine the validity of Hewson's 75% plumage threshold, I assessed whether populations where the percentage of birds with wild type plumage was > 75% consisted of birds with the Rock Dove head morphotype rather than the feral pigeon or intermediate/mixed head morphotypes. I then mapped the presence of the Rock Dove head morphotype to depict the distribution of the Rock Dove. I also mapped plumage percentages to estimate variation in the level of feral introgression between different Rock Dove populations. Finally, I explored temporal change in plumage proportion to infer change in the level of introgression, by comparing the flock plumage proportions at sites where Hewson made a count in the 1960s with a repeat count made for this study between 2015 and 2020. These comparisons were made for either the same site or sites within 30 km of each other, given that 30 km is less than the normal diurnal movement between sites for this species (Baldaccini *et al.* 2000). For these historical comparisons, I excluded contemporary counts of feral pigeon populations in human settlements within the Rock Dove range (although such counts are included in the geographical assessment and the Hewson's threshold verification), as they were not noted by Hewson and so including them in temporal plumage proportion comparisons would have led to the inference of an exaggerated decline in the Rock Dove's genetic integrity. Using the raw counts, I used a two-proportion z-test with *prop.test* in R v4.0.2., and tested whether the proportion of birds with aberrant plumage types in the 1960s count for each site was less than the proportion of birds with aberrant plumage in the 2015–2020 count at the same site. I then plotted the percentages to visualize the changes.

RESULTS

Classifying flocks by Hewson's 75% threshold fully aligned with classifications based on head morphotype (Fig. 3). Where > 75% of birds in a flock had wild type plumage, individuals had the Rock Dove head morphotype (50 flocks). Below this threshold, populations were composed of individuals with only the feral head morphotype (43 flocks) or with an intermediate/mixed head morphotype (in seven flocks).

Within the Rock Dove range, there is considerable variation in the percentage of birds in flocks with wild type plumage (Fig. 2). In general, aberrant plumage morphs are less prevalent in populations further north and west, which are further from large feral pigeon populations. The only region where flocks of 100% wild type plumage were exclusively observed was the southern part of the Outer Hebrides (particularly in North Uist and South Uist). Outside the Rock Dove range, the only flock composed of > 50% wild type plumage was on Flamborough Head on the east coast of England, and this population was composed of birds with intermediate head morphology.

Of the 14 locations where a temporal comparison could be made between Hewson's and contemporary counts (2015–2020), nine had experienced an increase in the proportion of birds with non-wild type plumage. One region (Caithness) experienced a small (non-statistically significant) decrease from 17% to 13% non-wild type ($\chi^2 = 2.08$, $P = 0.07$, df = 1). Four regions, all islands with low feral pigeon presence, had no birds with aberrant plumage in either sample (Fig. 4). Only three of the nine sites where the proportion of birds with non-wild type plumage increased experienced a statistically significant change in the proportion of these birds present. These were North Ronaldsay (0–10%, $\chi^2 = 3.35$, $P = 0.03$, df = 1), Evie (7–16%, $\chi^2 = 2.90$, $P = 0.04$, df = 1) and north-west Sutherland (0–5%, $\chi^2 = 13.25$, $P = 0.0001$, df = 1). Despite this, at four of the nine sites, an increase from zero (in the 1960s) to a small handful of birds with aberrant plumage (in 2015–2020) is certainly biologically relevant.

DISCUSSION

In most species where extinction by hybridization has been studied, it is not possible to classify easily the different entities at a population level using phenotypic traits (Randi 2008, Senn *et al.* 2019). One reason for this is that the species are often not gregarious. Here, I show that it is possible to infer the presence of wild Rock Dove populations from field observations of flocks. This method does not require either the capture of birds or genomic analyses. It is amenable for citizen science and has the potential to be used over a wide area. I propose that it provides a useful tool to identify Rock Dove populations for more in-depth study, specifically where contemporary fronts of introgression occur.

Using flock assessments to infer geographical variation in the extent of introgression has allowed a more comprehensive and fine-scale coverage of the Rock Dove range than was possible in the recent genomic assessments. Genomic work shows that northern Scotland and

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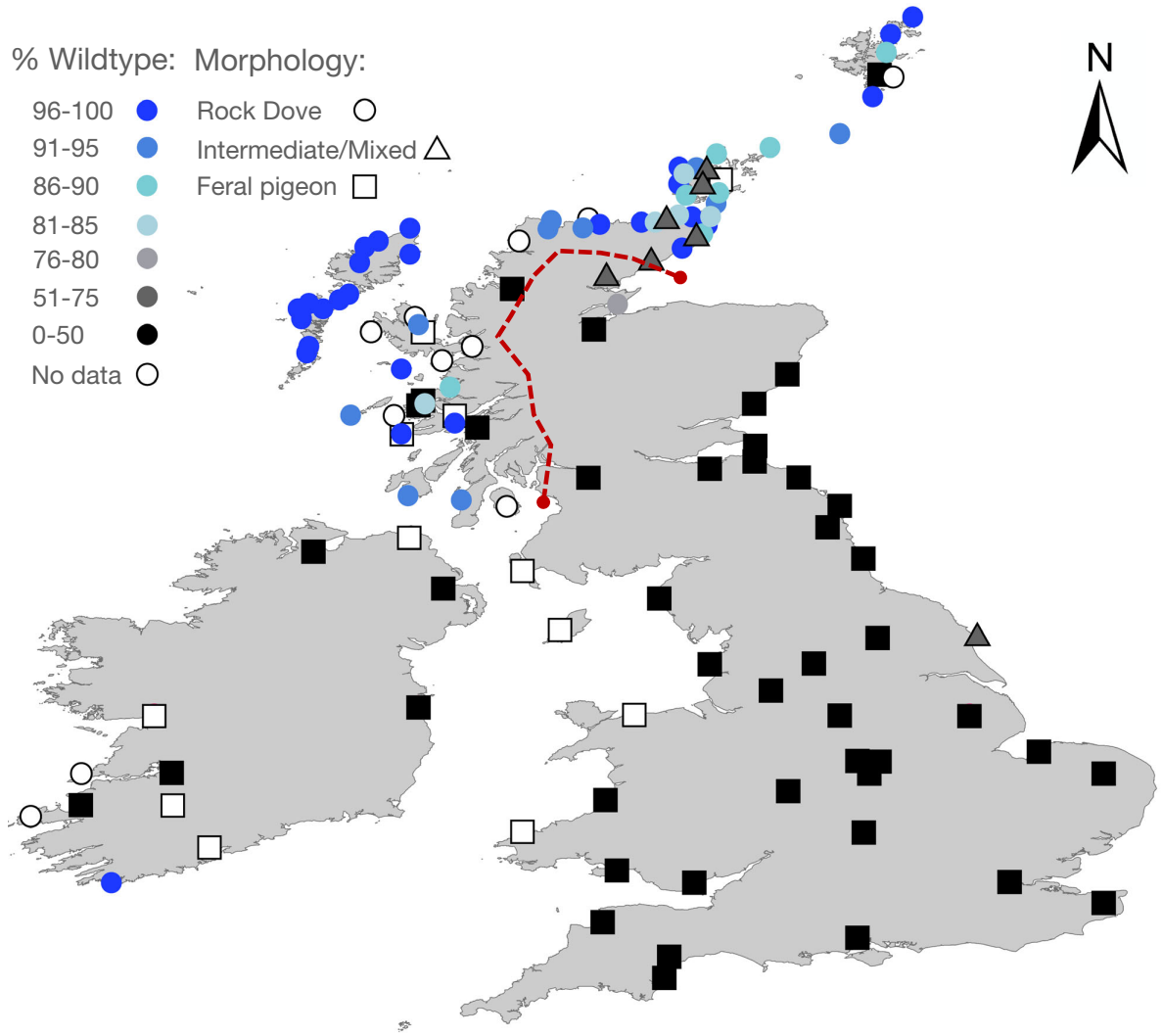


Figure 2. Locations of samples of Rock Dove and feral pigeon flocks that were assessed for this study, indicating both the percentage of birds with wild type plumage and head morphology. 'No data' indicates that the flock at that site was not large enough for the percentage of birds with wild type plumage to be useful. The putative limit of the Rock Dove's distribution in Scotland is indicated with a dashed line (Smith *et al.* 2022b). The putative range of the Rock Dove in Ireland is not well characterized but includes much of the western coastline.

Orkney are home to hybrid swarms (Smith *et al.* 2022b). Our phenotypic assessments corroborate this, as there is variable morphology and wild type population plumage percentages in these regions. Within England, the count with the highest wild type percentage was from the feral pigeon colony at Flamborough Head – where Rock Doves are known to have persisted for longer than in the rest of the country (Johnston *et al.* 1988, Brown & Grice 2005). Populations of Rock Doves with low levels of feral introgression still predominate in the Inner Hebrides and Shetland. Significant populations with negligible signs of hybridization appear to be limited to the Outer Hebrides.

The assessment of flock plumage proportions could be used throughout the Rock Dove range to make a global appraisal of its hybridization status. This is important, as the species is currently classed as of Least Concern by the International Union for the Conservation of

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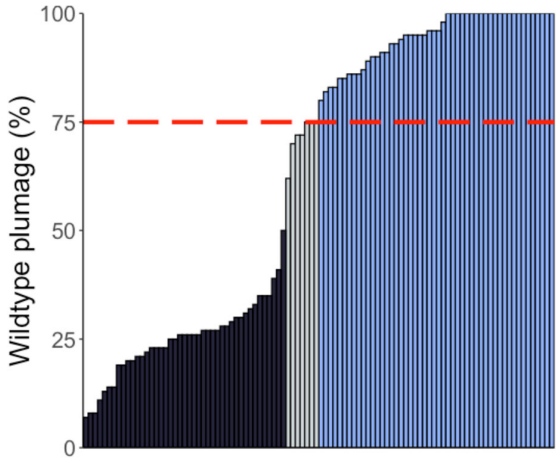


Figure 3. Percentage of birds with wild type plumage in individual flocks throughout the British Isles (n = 100). Each bar is an individual flock, colour-coded by head morphotype of birds within these flocks – from left to right: feral (black), intermediate/mixed (grey) and wild (blue). Hewson's 75% plumage threshold is indicated.

Nature (IUCN). As a comparison, the European Rabbit *Oryctolagus cuniculus* is currently classed as Endangered as it is assessed based on its wild status, excluding its introduced populations. If the Rock Dove is shown to be as affected by introgression in North Africa, the Middle East and Asia as it is in Europe, then revision of its conservation status would be justified. To do this we would need to resolve the origins of different populations and to assess whether plumage proportions are valid for assessing provenance and inferring genetic integrity outside Europe (i.e. in subspecies other than the nominate *livia*). In some urban 'feral' populations, especially in the Middle East, there could be synanthropic Rock Doves (Baldaccini 2020). In contrast, many 'Rock Dove' populations could be domestic pigeons, as was the case with the previously claimed subspecies from Macaronesia and central Asia, now redesignated as feral pigeons (Vaurie 1961, Goodwin & Gillmor 1970). Resolving both issues would require genetic sampling, but mapping populations using flock percentages would be useful in identifying locations of interest.

At most of the revisited Hewson sites, there has been a decrease in the percentage of birds with wild type

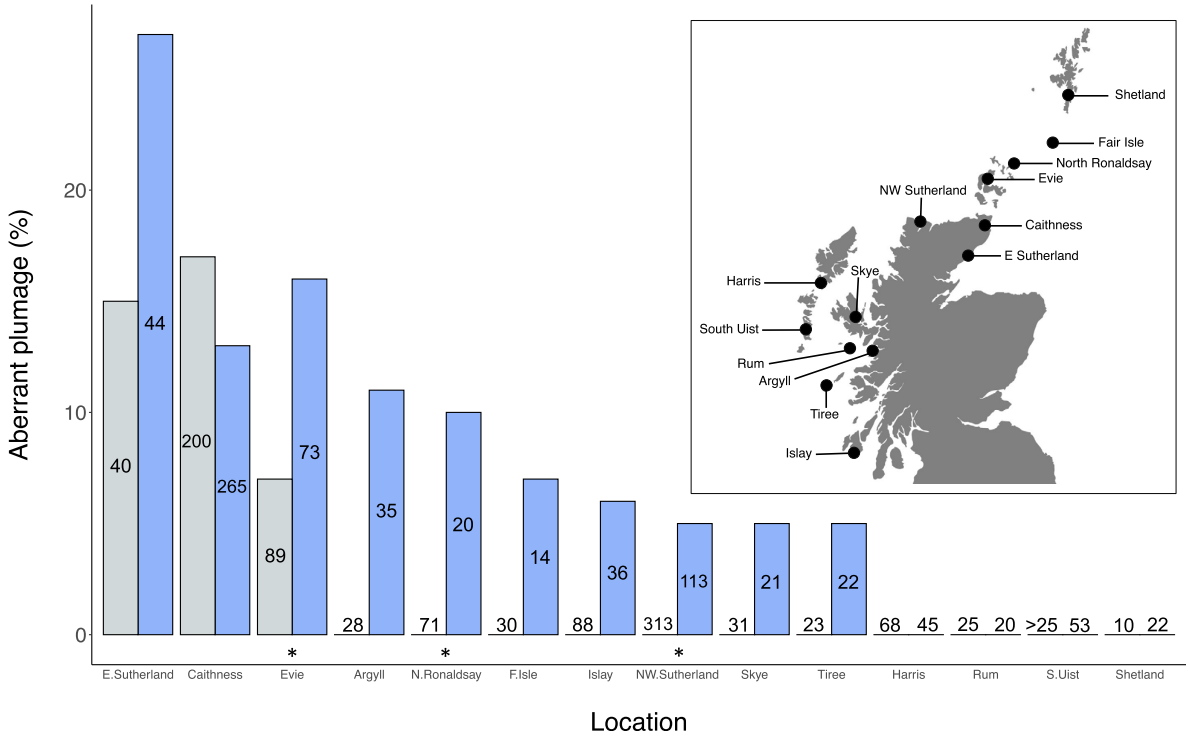


Figure 4. Change in the percentage of birds in flocks that exhibit aberrant (i.e. non-wild type) plumage. Grey bars represent Hewson's counts made in the 1960s. Blue bars represent counts in the present study, made between 2015 and 2020. The total number of birds used to estimate the percentage is recorded within the bars. The inset map shows the location of each region within Scotland, and an asterisk indicates statistical significance.

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plumage. This reflects high levels of domestic admixture, which will almost certainly, in the absence of conservation intervention, lead to the replacement of Rock Doves with birds of hybrid ancestry and eventually with feral populations, as predicted previously (Johnston *et al.* 1988) and observed in England, Wales (Brown & Grice 2010, Lovegrove *et al.* 2010) and, more recently, north-east Scotland and parts of Orkney (Smith *et al.* 2022b). The four individual sites where there was no evidence of introgression in either the 1960s or 2017–2020 are islands, but it is important to note that many islands, including Fair Isle, North Ronaldsay and Skye, held populations which showed evidence of admixture, indicating that although the relative isolation clearly delays contact, it is unlikely to prevent it in the long term.

CONCLUSIONS

Overall, I have shown that assessments of plumage are a valid method with which to identify Rock Dove populations and infer the level of domestic introgression that they have experienced. The Rock Dove in the British Isles experiences varying extents of genetic replacement, and in many locations, populations have been shown to have experienced declines in their genetic integrity. I have highlighted the generally unfavourable conservation status of these globally important populations. In making these assessments, I have highlighted the value of plumage polymorphism in making this system amenable to the study of extinction by hybridization.

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AUTHOR CONTRIBUTIONS

William J. Smith: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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ETHICAL NOTE

None.

Data Availability Statement

All data are available in the Supporting Information for this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Additional examples of Rock Dove/feral pigeon head morphotypes.

Table S1 Pigeon plumage data 2015–2020.

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Supplementary Fig. 1: Additional examples of Rock Dove/feral pigeon head morphotypes.

Rock Dove *Feral pigeon*



7

The interplay of parasite exchange and hybridisation at a wild-feral-domestic interface

The interplay of parasite exchange and hybridisation at a wild-feral-domestic interface

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Abstract

Interactions between wild, feral, and domestic animals are of economic and conservation significance. The pigeon *Columba livia* is a synanthropic species in a feral form, but it also includes the rare Rock Dove. *C. livia* is an important player at the wild-domestic interface; acting as a carrier of avian diseases, and the feral form threatens Rock Doves with extinction via hybridisation. Despite its abundance, little is known about drivers of disease prevalence in *C. livia*, or how disease and hybridisation represent synergistic threats to Rock Doves. We focused on infection by the parasite *Trichomonas*, first collating prevalence in domestic and free-flying populations from relevant studies of *C. livia*. We characterised variation in the diversity and prevalence of *Trichomonas* among three *C. livia* populations in the United Kingdom: a feral; a Rock Dove; and a feral-wild hybrid population. Across multiple continents, free-flying pigeons had lower *Trichomonas* infection than captive conspecifics, but the effect was weak. Environmental factors which could impact *Trichomonas* infection status did not explain variation in infection among populations. Among the British populations, strain diversity varied, and there was lower parasite prevalence in Rock Doves compared to feral pigeons. Individual infection status was not explained by the available covariates, including hybrid score and site. The drivers of *Trichomonas* prevalence are unclear, perhaps due to idiosyncratic local-scale drivers. However, given the population-level variation in both infection prevalence and introgressive hybridisation, the potential combined effects could accelerate the Rock Dove's extinction. Further study of the synergistic effects of multiple types of biotic interactions at the wild-feral-domestic interface is warranted – especially where vagile, globally distributed and superabundant animals are involved.

Keywords: *Trichomonas*; Birds; feralisation; introgression; non-native populations

1. Introduction

The wild-domestic interface is the sum of interactions between domesticated organisms and the wider environment (Clark et al., 2018). Processes that occur at this interface, including predation, competition, and habitat alteration, can have demographic and health repercussions for both domesticated and wild organisms. These impacts have broad importance, from the economic significance to domesticates such as livestock, through to the conservation implications for endangered wild populations (Gortazar et al., 2015; Rossi et al., 2019). One such interaction that has received significant levels of attention is disease transmission, particularly infection of domestic organisms via contact with wild relatives (Gortazar et al., 2015). For example, migratory waterfowl are known to transmit avian influenza to domestic poultry (Khan et al., 2018). Interactions at the wild-domestic interface can be mediated by feral (self-sustaining, free-living, naturalised domestic-origin) populations which, being commensal and tightly associated with anthropogenic environments, could come into contact with both wild and captive domestic populations (Doherty et al., 2017). For example, domestic and feral dogs *Canis familiaris* have spread canine distemper to the African wild dog *Lycaon pictus* (van de Bildt et al., 2002). Another type of interaction at the wild domestic interface is interbreeding (Smith, Quilodrán, et al., 2022). When interbreeding occurs extensively, it can result in genomic homogenisation, rendering a population or species extinct as an evolutionarily distinct lineage; a process known as extinction by hybridisation (Rhymer & Simberloff, 1996). Feral populations pose a particular threat to their wild conspecifics due to relatively recent evolutionary divergence and porous reproductive boundaries (Smith, Quilodrán, et al., 2022). For example, contemporary Singaporean Red Junglefowl *Gallus gallus* and Scottish wildcats

Felis silvestris each exist as part of mixed wild-feral-domestic hybrid populations rather than as entities which are distinct from their feral and domestic relatives (Senn et al., 2019; Wu et al., 2020).

Knowledge of specific types of wild-domestic interactions is variable, and some are better understood than others. For example, whilst disease transmission receives significant research funding and effort (Gortazar et al., 2015; Clark et al., 2018), the evolution of escaped domesticates is relatively poorly understood (Daniels & Bekoff, 1989). Additionally, there has been little attention paid to the synergistic impacts of different processes which occur upon contact between wild, feral and domestic entities. For example, disease and genetic homogenisation are rarely mentioned in the context of one another. Indeed, studies of extinction by hybridisation rarely explore factors that impact the temporal and spatial dynamics of introgression, and often can only highlight that it is in progress - in itself a challenging endeavour (Randi, 2008). In the well-studied wildcat population of Scotland, multiple papers have been published exploring feral-wild hybrid ancestry (Daniels et al., 1998; Beaumont et al., 2001; Witzenberger & Hochkirch, 2014; Senn et al., 2019; Tiesmeyer et al., 2020). There have also been papers discussing the transmission of diseases from feral cats to wildcats (Daniels et al., 1999; Meredith et al., 2018). Despite this, there has been no work assessing potential synergistic effects linking disease and hybridisation. If, for example, the severity of a disease is higher in an individual lacking hybrid ancestry (perhaps due to lower genetic diversity in unadmixed individuals), then hybrid individuals may be favoured by natural selection over their wild relatives, potentially accelerating the extinction by hybridisation process.

In birds, trichomoniasis, caused by *Trichomonas gallinae* and possibly related species such as *T. tenax* and *T. vaginalis* (Quillfeldt et al., 2018), can have variable effects on both wild and domestic forms. Many infections are apparently asymptomatic (Borji et al., 2011), but in other cases individuals exhibit ruffled feathers, weight loss, and the build-up of lesions and fluid in the crop and mouth, followed by death (Amin et al., 2014). Trichomoniasis can lead to localised population declines, as well as declines at a whole-species level. For example, the Type A strain of *T. gallinae* has been implicated in the decline of the British Greenfinch *Chloris chloris* (Lawson et al., 2012). In European Turtle Doves (*Streptopelia turtur*) in Britain, all individuals with clinical signs of trichomoniasis also had *T. gallinae* with an ITS gene sequence identical to that of the Type A strain (Stockdale et al., 2015). Transmission from wild birds into domestic populations can occur, and *T. gallinae* parasites have been identified in domestic poultry, especially where husbandry standards are low (Willoughby et al., 1995; Williams, 2005). Studies of *Trichomonas* suggest a wide spectrum of transmission capability and virulence, depending on the strain involved and the relative immune response of the host individual and species (Höfle et al., 2004). It is also known that birds can lose as well as gain detectable infections (Bunbury et al., 2008). Strain composition differs geographically and temporally, even within single species (Thomas et al., 2022). In particular, the disease is known to pose a threat to pigeon and dove species, the most affected bird family and the main global hosts (Borji et al., 2011). This includes the declining European Turtle Dove and the endemic Mauritian Pink Pigeon *Nesoenas mayeri* (Bunbury et al., 2008; Marx et al., 2017). Of the wild pigeons and doves in Europe, *T. gallinae* has been isolated from Woodpigeons *Columba palambus* (47% infection rate among sampled birds in the UK), Stock Doves *C. oenas* (40%), Turtle Doves (86%) and Collared Doves *S. decaocto* (86%) (Lennon et al., 2013). More recent work assessing Turtle

Doves in the UK reported prevalence of 90% and 100% in 2014 and 2015 respectively (Thomas et al., 2022).

Within the pigeon and dove family, the domestic pigeon *C. livia* is the main host facilitating the global spread of *T. gallinae* (Chi et al., 2013). This is borne out by studies showing that domestic and feral pigeons host *T. gallinae* (Greguric et al., 1986; Borji et al., 2011; Alkharigy et al., 2018) often at high prevalence (e.g. 68% prevalence across multiple sites in Spain and Portugal (Santos et al., 2019)). Indeed, the species has been proposed as the 'ultimate source' of this particular parasite (Richard et al., 2008) and the feral pigeon acts as an important bi-directional transmission link for disease between domestic poultry or other captive birds, and wild bird species in general (Johnston & Janiga, 1995; Capoccia et al., 2018)**.** Feral pigeons are an example of a superabundant and vagile commensal, whose association with anthropogenic environments allows dispersal across the globe. This category of species, which also includes the Brown Rat *Rattus norvegicus* and the House Sparrow *Passer domesticus,* inevitably play a disproportionate role at the wild-feral-domestic interface, including in terms of disease transmission. Their study is therefore of particular importance. Feral pigeons differ from both fully captive and free-flying contemporary domestic pigeons in that they are longstanding unmanaged populations which have undergone adaptation to anthropogenic environments (Johnston & Janiga, 1995). They are now genetically, morphologically, behaviourally and physiologically distinct from their domestic relatives. *C. livia* also exists in a wild, undomesticated form known as the Rock Dove, that persists in certain locations but is threatened with extinction by hybridisation via introgression with the feral pigeon (Smith, Sendell-Price, et al., 2022). This wild form is far less studied than its domestic, model-

species cousin (Shapiro & Domyan, 2013), including with respect to its epidemiology (Smith, 2023). Contact between the different types of pigeon at the wild-feral-domestic interface occurs in various different circumstances (Johnston & Janiga, 1995). For example, free-flying domestic pigeons can encounter feral pigeons or Rock Doves through different routes. Shared feeding locations are an obvious site of contact. Feral pigeons or Rock Doves often make use of food provided for domestic pigeons, and free-flying domestic pigeons can mix with Rock Doves or feral pigeons in agricultural land. Lost domestic pigeons can also directly join up with feral pigeon or Rock Dove colonies. Similarly, Rock Doves and feral pigeons can interact with one another through shared feeding sites on the edge of urban areas, or following the expansion of the feral pigeon distribution into areas with relict Rock Dove populations (e.g. sharing roosting and breeding sites within ruined buildings). Disease transmission between these three genetically, morphologically, physiologically and behaviourally different forms of *C. livia* is therefore multi-directional. The only epidemiological research that has been carried out to date on undomesticated 'truly wild' Rock Doves assessed a putative population near to Sinj in Croatia (though no genomic assessment was carried out to confirm Rock Dove status), and compared them with feral pigeons in urban Zagreb (Greguric et al., 1986). Whilst *Trichomonas* was detected in 146 of the 268 feral pigeons sampled (54.47%), only 2 of Sinj's 17 Rock Doves (11.76%) were found to be infected. The extent to which *Trichomonas* infection varies among *C. livia* populations with different genomic backgrounds is unknown, yet in combination with the hybridisation threat, parasitic disease is likely to be an important consideration for Rock Dove conservation (Smith, 2023).

Here we examine the drivers of *Trichomonas* infection in *C. livia*; its variation among the feral, hybrid and Rock Dove populations; its variation among individuals; and, for the Rock Dove in particular, how the joint threats of hybridisation and disease could interact. We collated information from published studies of *Trichomonas* infection in *C. livia* to ask i) if infection prevalence could be explained by captive domestic versus free-flying status, and a suite of environmental variables. Next, we sampled from three populations of *C. livia* in the U.K, that had previously been genomically confirmed to represent feral pigeons, Rock Doves and also birds from a feral-wild hybrid population where all birds have mixed ancestry, although they may not be first-generation hybrids (Smith, Sendell-Price, et al., 2022) to ask ii) if *Trichomonas* prevalence and diversity varied across these populations; and iii) if individual infection status was related to a morphological hybrid score or condition score. Combining these global, population, and individual-level perspectives, we build on our understanding of a globally important actor at the wild-domestic interface, and discuss potential synergies linking disease transmission with the process of extinction by hybridisation in the Rock Dove.

2. Materials and methods

Global analysis of prevalence

To assess factors important in explaining variation in the prevalence of *Trichomonas* in *C. livia* we collected data from all available literature on the prevalence of this infection in the species. We searched the Web of Science using: TS = (feral pigeon OR Rock Dove OR pigeon OR domestic pigeon OR dove OR *Columba livia*) AND TS = trichom*. We included papers

that conducted population screening of *Columba livia*, excluding veterinary papers that worked specifically on clinical cases of *Trichomonas*, and studies targeting individuals of known infection status or poor condition. This was done to ensure the dataset did not include works that could artificially increase prevalence. We collated data on the numbers of populations studied, type of pigeon population (domestic pigeons, feral pigeons, wild Rock Doves), identified species of *Trichomonas*, and its prevalence and strains present. We extracted the geographic coordinates of sites either directly from papers, or using GeoHack (https://geohack.toolforge.org/).

In recording prevalence, ideally we would have used a binomial term of number infected versus number uninfected. However some studies did not report actual numbers, therefore we were constrained in expressing prevalence as a proportion of infected birds in this analysis to maximise the number of observations in the dataset. We used the recommended approach of beta regression when the response variable is bound between 0 and 1 (Douma & Weedon, 2019), within the *glmmTMB* package in R v4.2.1 (Brooks et al., 2017). In addition to the explanatory variable of pigeon type, we included additional variables that have been shown to influence disease prevalence in other systems: urban or rural environment (Evans et al., 2009), temperature (Holand et al., 2019), and precipitation (Ferenczi et al., 2016). Using the geographic co-ordinates from each study, we categorised each site as urban or rural based on the land-type designation from NaturalEarthData (https://www.naturalearthdata.com/), and extracted average annual temperature and average annual precipitation from WorldClim at 10m resolution (Fick & Hijmans, 2017). Climate variables were centred (by subtracting column means) and scaled (by dividing the

centred columns by their standard deviation) using command *scale* from *stats* in R. We used the *dredge* function of *MuMIn* scored using BIC to discriminate among models.

Field data collection

Adult birds (n=120) were captured in three locations across the U.K. between the 21st of March and the 16th of May 2021 (Figure 1a) from populations previously identified as one of three types (Smith, Sendell-Price, et al., 2022): undomesticated Rock Doves (n = 40, Figure 1b) from Uist, Outer Hebrides, Scotland (57°12'00.0"N 7°24'00.0"W); a hybrid swarm population (n = 40, Figure 1c) from Orkney, Northern Isles, Scotland (59°03'00.0"N 3°01'12.0"W); and feral domestic pigeons (n = 40, Figure 1d) from Oxford in southern England (51°45'00.0"N 1°15'36.0"W). Feral pigeons were caught by hand during the daytime, baited using seed and bread, and ringed with individually coded plastic Darvic blue colour rings to prevent resampling of individuals. Rock Doves and hybrids were caught at night whilst roosting in caves, ruins or farm buildings, and ringed with individually numbered metal leg rings provided by the British Trust for Ornithology. Age was established based on moult progression and plumage traits, and juvenile birds (plus those of unknown age category) were not sampled to avoid age adding confounding morphological variation to our analyses (Baker, 2016). We recorded: mass using a digital Pesola scale (±1g); maximum wing chord and tarsus length to claw using a butted metal ruler (±1mm); and cere width, tarsus width, and bill length (to feather edge) using dial callipers (±0.1mm). For genetic sexing, we took a blood sample from each bird by pricking the brachial vein with a 26G (0.45 x 25 mm) hypodermic needle, collecting the resulting blood droplet with a capillary tube, and transferring to a Whatman® FTA card™ (Whatman,

Maidstone, United Kingdom) for storage at ambient temperature while in the field. We swabbed each birds' crop and buccal cavity with a sterile viscose swab. The swab was used to inoculate an individual InPouch® TV culture kit (Biomed Diagnostics, Oregon, USA). The kits were incubated in the field at 37°C for 4-7 days with an Octagon Egg Incubator (Brinsea, Weston-super-Mare, United Kingdom). Following incubation, the culture medium of each individual bird was stored in 100% ethanol at 1:1 volume of ethanol:culture medium (Stockdale et al., 2015). On return to the laboratory, the cultured crop swab samples were stored at -20°C and the blood samples on FTA cards stored at 4°C.

DNA extraction and PCR

Cultured crop/buccal cavity swab samples were transferred to one or two microcentrifuge tubes per individual. Samples were centrifuged for five minutes at 3200 rpm, and the ethanol poured off the resulting pellet. Pellets were then washed in 1 ml of phosphate buffered saline (PBS) using the same centrifugation process, and the PBS poured off. We then added 200 *µl* of PBS to each microcentrifuge tube and mixed by vortexing the tubes. Samples from the same individual were pooled for DNA extraction. Half of the volume of each pooled sample (which varied among individual birds) was used, with a two hour digestion phase (with 4 *µl* Proteinase K and 250 *µl* DIGSOL extraction buffer) and a standard phenol-chloroform extraction technique (described in Smith, Sendell-Price, et al., 2022). DNA from blood samples (a punch-out of 1x1cm of the area of the FTA card©) was extracted using the same phenol-chloroform extraction method but preceded by a six-hour digestion phase. Genomic DNA extracts from both crop/buccal samples and blood samples

were visualised via gel electrophoresis on a 1% agarose gel, to confirm the presence of high-molecular-weight DNA.

We carried out a polymerase chain reaction (PCR) protocol using Qiagen AllTaq™ MasterMix Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. We included a negative control in each PCR reaction as a check for potential contamination. The internal transcribed spacer ITS1/5.8S/ITS2 ribosomal region of rDNA (referred to as the ITS region) was used to determine positive infections and to identify *Trichomonas* strains ('ribotypes') (Grabensteiner et al., 2010; Alrefaei, Gerhold, et al., 2019). This region has a low mutation rate meaning that sequences not identical to known strains are assumed to be novel strains. We repeated ITS amplification twice to minimise false negatives. The PCR used the primers TFR1 (5'- TGCTTCAGTTCAGCGGGTCTTCC-3') and TFR2 (5'-CGGTAGGTGAACCTGCCGTTG G-3'), which give an expected product length of 400bp (Robinson et al., 2010). PCR reactions with ITS primers were carried out with thermal cycling of: 2 min denaturation at 94°C, then 40 cycles of 30s at 94°C, 30s at 67°C, and 2 min at 72°C, followed by final elongation for 15 min at 72°C (Robinson et al., 2010). We then carried out PCR amplification for the hydrogenosomal iron dehydrogenase (*Fe-hyd*) gene region (expected product length 900bp) to identify subtypes of *Trichomonas gallinae* (McBurney et al., 2015; Alrefaei, Gerhold, et al., 2019) on all individuals. PCR was repeated for any individuals that were positive for infection at the ITS region but failed to amplify in the first *Fe-hyd* PCR. We used the primers TrichhydFOR (5'-GTTTGGGATGGCCTCAGAAT-3') and TrichhydREV (5'-AGCCGAAGATGTTGTCGAAT-3') which had an expected product length of 900bp (McBurney et al., 2015). Thermal cycling was conducted as follows: 15 min at 94°C, followed by 35 cycles of 94°C for 1 minute, 66°C for 30s, 72°C for 1 min, and a final

extension at 72°C for 5 min. The *Trichomonas* PCR products were electrophoresed on a 1% agarose gel to confirm presence or absence of amplified DNA. For 50 randomly selected positive ITS and 12 *Fe-hyd* samples, the PCR products were purified at Eurofins Genomics (Eurofins, Luxembourg, Luxembourg), and the forward and reverse reactions sequenced using the TubeSeq service. For genetic sexing, we used the CHD1 pigeon sexing primers, which have the following sequences: forward: 5'-TTCTGAGGATGGAAATGAGT-3' and reverse: 5'-AGCAATGGTTACAACACTTC-3', and thermal cycling of: 94°c for 5 min, then 38 cycles of 94°C for 30s, 53.5°C for 30s and 72°C for 30s, followed by a final extension of 72°C for 5 min (Liang et al., 2019). A single band (of 474bp) on a 1% agarose gel signified a male, and two bands (of 319bp and 474bp) a female.

Phylogenetic analysis and strain diversity

Using MEGA11 (Tamura et al., 2021), we trimmed and manually aligned the forward sequence with the reverse sequence for each PCR product. We then assessed them for sequencing errors to generate a consensus sequence, by identifying unassigned ('wobble') bases on the forward strand that had been successfully determined to a specific base on the corresponding reverse strand. We identified the closest matching known sequence to each consensus sequence using NCBI's BLAST algorithm (Altschul et al., 1997). To construct phylogenetic trees for the ITS and *Fe-hyd* regions, we first generated an alignment using the MUSCLE algorithm (Edgar, 2004) including one example of each consensus sequence isolated in this study, along with single sequences of all known strains reported in a recent study (Thomas et al., 2022). These MEGA alignments were trimmed to 216bp for the ITS region and 578bp for the *Fe-hyd* gene region. For the tree generated for the ITS region, we

used identical publicly available GenBank sequences to those used in the phylogeny reported in Thomas et al. 2022, except for ITS strain A, where we used sequence A/N KX459445 rather than A/N MN587093 (which is 214bp long). We also included representative ITS region sequences of the related species *Trichomonas vaginalis* (A/N AY871048), *Trichomonas tenax* (A/N KX459486), and *Tetratrichomonas gallinarum* (A/N AY871048), the latter used as an outgroup. In the *Fe-hyd* alignment, we added the GenBank sequence of novel strain A3 (A/N MZ128143), and used a representative sequence of *Trichomonas vaginalis* (A/N XM001310179) as an outgroup (*Trichomonas tenax* and *Tetratrichomonas gallinarum* did not have any *Fe-hyd* gene sequences published on GenBank). Of the nucleotide substitution models available in the BEAUti graphical-user-interface (Drummond et al., 2012), we selected the optimal model for each alignment using ModelFinder (via the Bayesian Information Criterion) (Kalyaanamoorthy et al., 2017). This was Hasewaga-Kishino-Yano plus gamma with empirical base frequencies for both the ITS and *Fe-hyd* alignments. Using this model, the alignments (of 27 sequences for the ITS region, and 36 sequences for the *Fe-hyd* gene region) were used as inputs in BEAUti v1.10.4 and BEAST v1.10.4 using a strict clock and the Yule speciation process, and then Markov Chain Monte Carlo simulations with 100,000,000 generations sampled every 1000 generations after a 10% burn-in (Drummond et al., 2012; Suchard et al., 2018). The maximum clade credibility trees were created using TreeAnnotator v1.10.4 (part of the BEAST suite) and visualised in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

To test for nonrandom associations between location and the numbers of birds identified as being infected with each ITS strain, we carried out Fisher's exact test with the Freeman-Halton extension. We then carried out Fisher's exact tests to identify whether there were

nonrandom associations between sex and the number of birds identified as being infected with each ITS strain at either Oxford, Orkney, or Uist. Insufficient *Fe-hyd* sequences were obtained to repeat these tests for the *Fe-hyd* subtypes.

Prevalence and correlates of *Trichomonas* infection and condition

Prevalence was determined as the number of infected versus uninfected individuals in each population, determined by ITS region PCR. Using a generalised linear model (GLM) with a binomial error structure, we determined if variation in infection at the individual level (dependent variable; 0 if negative for infection, 1 if positive for infection) was explained by sample location, sex, and hybrid score. Because we did not have whole genome sequences available from the particular individuals used in this study, we calculated a proxy for individual introgression level using individual principal component 1 scores from a principal component analysis (PCA) using the logged values of wing, bill and tarsus length, cere width and mass. Morphology has been shown in prior work to represent a suitable proxy for extent of genomic admixture (Smith, Sendell-Price, et al., 2022). The PCA included individuals screened here for *Trichomonas* plus other Rock Dove and domestic/feral individuals measured in Smith, Sendell-Price, et al., 2022. Using all morphological data available improves reliability of hybrid status estimates for the samples from the three focal sites used here. Distance from the mean PC1 value was used to calculate a hybrid score for each individual (the absolute value of the difference from the mean PC1 value).

We next tested whether body condition of individual pigeons is influenced by *Trichomonas* infection. Condition of an individual was estimated as the residual value of a linear

regression of logged mass versus logged wing length (Santos et al., 2019). Using condition as the response variable, we applied a GLM with gaussian error structure, and *Trichomonas* infection status, site, sex, hybrid score and the interaction of hybrid score and infection status, as explanatory variables. Sex was included as males and females differ in their body shape which influences the condition metric (Johnston, 1990). Models were run within R 4.2.1 (R Core Team, 2022) using the base *stats* package. Within R, data organisation and visualisation were achieved using *tidyverse* (Wickham et al., 2019). For model selection purposes we used the package *MuMIn* (Bartoń, 2022). We used the *dredge* function within *MuMIn* to test all possible models, using BIC as our information criterion (Schwarz, 1978), and using ΔBIC>2 to indicate significant difference between models (Raftery, 1995).

Data availability

Parasite sequence data is available at GenBank under the accession numbers reported throughout this manuscript. Alignments, data, and R code required to repeat analyses are publicly available at https://data.mendeley.com/10.17632/nzmpnf9r5m.1.

3. Results

Trichomonas prevalence in pigeons varies widely among published studies

We identified a total of 27 published studies that produced data fitting our selection criteria, covering 47 geographically different populations of *Columba livia* including those assessed as part of this study. Due to low sample size (two publications, three populations)

undomesticated wild Rock Doves were lumped with feral pigeons as a category of 'free living' *C. livia* for statistical analyses (Figure 2 shows the Rock Dove and feral pigeon components of 'free living' separately). *Trichomonas* prevalence varied from 1.6% to 78% across studies. Only seven previously published studies reported the *Trichomonas* strain: in 5/7 strain A was reported; in 6/7 strain B was reported. Two studies reported novel strains (Mohammed & Abdulwahed, 2019; Santos et al., 2019). Most studies come from Europe or Asia (39/47 populations) with sparse representation on other continents.

In Table 1, the best performing model suggested that variation in prevalence was primarily explained by pigeon type (free-living or not), with free-living pigeons having decreased prevalence of *Trichomonas* infection (estimate = -0.567 $\pm$ 0.26, p-value = 0.029). However two other well-performing models (with ΔBIC<2 from the best model) were identified. These were the null model and the model that included temperature and type of pigeon. Temperature negatively correlated with prevalence, but not significantly (estimate = -0.199 $\pm$ 0.13, p-value = 0.136), while free-living pigeons again had lower infection prevalence (estimate = -0.6105 $\pm$ 0.26, p-value = 0.0175).

<u>Correlates of infection status and impact of disease on condition are unclear.</u>

These analyses included only adult individuals with complete data on all morphological traits measured (113/120 individuals across all three sites). Two top models for infection status were identified (with ΔBIC < 2). One was a model with sex as the explanatory variable where males had higher odds of being infected relative to females (estimate = 0.9357 $\pm$

0.394, p-value = 0.0174). However this model was not clearly better than the null model (Table 2).

Bird condition varied by site and sex (Table 3). Specifically, males had better condition than females; and relative to Uist birds, Oxford birds had significantly worse condition, and Orkney birds trended in the same direction but not significantly so (Table 4). Body condition was not explained by *Trichomonas* infection, hybrid score, or their interaction, however birds with infection do consistently show lower average condition across all three populations in the data (Figure S1).

Parasite prevalence and diversity differs between pigeon types

From the 120 birds caught in the UK, we obtained 59 positive PCR reactions for the *Trichomonas* ITS gene, giving an overall prevalence of 49.2%. Fifty of these (randomly selected) were sequenced, with 45 producing good quality, useable DNA sequences. We also gained good quality *Fe-hyd* gene sequences from all of the 12 individuals that were successfully amplified. Prevalence of *Trichomonas* infection (based on the proportion of samples with successful ITS gene amplification) was significantly lower in the Uist Rock Dove population (32.5%, n = 13/40) than in the Oxford feral pigeons (62.5%, n = 25/40, p = 0.007, χ^2 = 6.065, df = 1) but not in the Orkney hybrid population (52.5%, n = 21/40, p = 0.0567, χ^2 = 2.506, df = 1). The Orkney and Oxford populations did not differ from one another in terms of proportion of positive samples (p = 0.498, χ^2 = 0.4604, df = 1). All birds except three from Uist were molecularly sexed successfully. There was lower prevalence of *Trichomonas* infection in females compared to males in the Uist (3/18 females vs 10/19

males) and Orkney (3/13 vs 18/27) populations (p = 0.0004, χ^2= 11.315, df = 1) but not in Oxford (13/19 vs 12/21, p = 0.6586, χ^2= 0.167, df = 1) (Figure 1E). Only one bird (a male from Orkney) showed clinical signs of infection (lesions around the oral area). We identified three distinct ITS sequences, two of which were identical to previously known strains (Table 5). These were Type C, found in Oxford feral pigeons (in 15 individuals), and Type A, found in all three populations at varying proportions (68.75% of infections for which a DNA sequence was generated in Orkney, 25% in Oxford and 44.44% in Uist). A novel ITS strain, named Ttx-RD (A/N OQ925392), was found in 31.25% (5/16) of the sequences from infected Orkney hybrids, 56% (5/9) of Uist Rock Dove sequences, and none of the Oxford feral pigeons. This novel strain had 99.71% identity to the 'GEO' *T. tenax*-like strain previously isolated from German Woodpigeons (A/N KX459459), and also Turtle Doves from various locations in Europe (A/N MN587090). Phylogenetic analysis (Figure 3a) showed that, in the alignment of 216 base pairs, the novel ITS strain Ttx-RD was most similar to the known strain 'GEO-TD', falling within the 'tenax-like' clade of strains that is composed of the widespread GEO sequence as well as the apparently less common GEO-TD and Ttl-TD sequences. Strain diversity differed by location (Uist, Orkney, Oxford) (p < 0.001), but not by sex at any of the three locations (Supplementary Table 1), although it is important to note that sample sizes were small (see Supplementary Figure 2).

We identified five *Fe-hyd* gene variants (Table 6), three of which were previously known. The strain A1.2 was isolated in one Uist and three Orkney birds. The strains C4 and A3 were isolated in four and two of the Oxford feral pigeons, respectively. The two new *Fe-hyd* region strains isolated were related to the known strain C1, and are hereafter known as C1-FP.1 (identified in one Oxford feral pigeon – 99.39% similar to *Fe-hyd* strain C1, A/N

OQ927984) and C1-FP.2 (identified in one Oxford feral pigeon – 99.38% similar to *Fe-hyd* strain C1, A/N OQ911502). The phylogeny constructed for the *Fe-hyd* region (578 bp alignment) showed that the novel strains C1-FP.1 and C1-FP.2 were most closely related to C10/C11 and C8 respectively, falling within the clade containing Type A and Type C variants, and more specifically in the weakly-supported Type C clade (Figure 3b).

4. Discussion

Trichomonas infection appears to be less prevalent in free-flying pigeons compared to captive animals, but nevertheless represents a diverse parasite that occurs at variable frequency, even at relatively small regional scales. Lineage diversity is currently underappreciated given the number of new lineages found here. Among the three populations that we sampled, we did not find significant predictors of individual infection status, and found that condition was not significantly affected by infection status. With this rather surprising result, we have performed the first characterisation of *Trichomonas* prevalence and strain diversity in Rock Doves that have been genomically confirmed to be undomesticated.

Trichomonas infection varies substantially in populations of *C. livia* spread across Europe, North America, Asia and Africa. While no clear best model explaining this variation was identified, captive versus free-flying (both feral and Rock Dove forms) emerged as an important variable, with higher prevalence in captive birds. This is perhaps not surprising given that domestic pigeons are often kept at very high densities (Johnston & Janiga, 1995). Urbanisation, temperature and precipitation did not explain infection prevalence, unlike in

other avian host-parasite systems where these factors play a role (Evans et al., 2009; Holand et al., 2019). Many examples come from studies of avian malaria, a disease which requires an invertebrate vector for its transmission. Because abiotic variables strongly impact vector biology, e.g. the larval stage of mosquitos proliferate where rainwater accumulates (Trewin et al., 2019), and temperature-related delays in sporogonic development of malaria parasites can negatively affect their transmission (Platonova & Palinauskas, 2021), such relationships are expected. In contrast, *Trichomonas* has direct transmission among individuals, and therefore variables that mediate proximity among individuals at a local level, e.g. suitable feeding and drinking sites, may be more important in explaining variation.

Variation in parasite prevalence among feral and wild, undomesticated pigeons is supported by our more regional analysis of three populations within the UK. The prevalence of *Trichomonas* infection in Oxford and Orkney falls within the expected range found in prior studies of domestic and feral pigeons, whereas prevalence in the Uist population of wild Rock Doves is lower than is usual in feral or domestic conspecifics (Alkharigy et al., 2018; Santos et al., 2019). The only other study of *Trichomonas* infection in wild (but not genomically verified) Rock Doves was Greguric et al.'s (1986) study in the Mediterranean that reported a prevalence half that of Uist's Rock Doves. Several decades separate that study from ours, and it relied on microscopy alone, a method that has reduced parasite detectability compared to the genetic methods available today (Nabweyambo et al., 2017). Nevertheless, the low prevalence in both Mediterranean and Scottish Rock Dove populations provides support for undomesticated Rock Doves having lower *Trichomonas* prevalence than feral/domestic pigeons. Three non-mutually exclusive

factors could explain lower prevalence in the Uist population, and potentially Rock Doves in general; geographical isolation, behavioural variation reducing opportunities for individuals to mingle, and genetically-based resistance to infection. Geographical isolation reduces opportunities for the spread of parasites and disease. Whilst both are insular, Uist is further away from large feral pigeon colonies than Orkney, and prevailing winds make feral pigeon colonisation from mainland Scotland more difficult. Reduced parasite infection on islands for vector-mediated parasites has been identified in some avian systems (Loiseau et al., 2017), but not others (Ishtiaq et al., 2010). With respect to directly transmitted parasites, there has been less research attention, but it has been shown that ectoparasite prevalence and diversity for Azorean Blackbirds *Turdus merula* is higher than on the mainland (Tomás et al., 2021).

Ecological and behavioural characteristics of Uist Rock Doves may contribute to their lower prevalence of *Trichomonas* infection. *Trichomonas* is transmitted through drinking water or at shared feeding sites, or through courtship behaviour or the feeding of offspring by adults (Marx et al., 2017). In urban and village contexts favoured by feral pigeons and hybrids, deliberate or inadvertent provision of food and water by humans acts to concentrate those essential sources, resulting in more opportunities for close proximity among individuals (Lawson et al., 2018). Feral pigeons in particular form dense feeding flocks in streets, town squares, and pavements (Johnston & Janiga, 1995). Rock Doves, at least in Britain, have lower direct dependence on humans for food and water and form comparatively dispersed groups feeding in open land (Murton & Westwood, 1966). Opportunities for direct transfer of *Trichomonas* are therefore likely reduced in Rock Doves in a foraging context. Roosting behaviour also results in differences in local densities. Some

individuals within the hybrid population we sampled in Orkney make use of an ancient 'doocot' (dovecote) along with modern farm buildings, which have limited airflow and can house up to 100 individuals in close proximity to one another. Such an environment would provide increased opportunity for disease transmission when compared to the relatively well-ventilated caves and ruined buildings favoured by Rock Doves in Uist. Support for the role of transmission at concentrated roost sites also comes from the higher rates of *Trichomonas* infection seen in free-living domestic pigeons which roost in enclosed buildings, and captive pigeons sampled in pigeon lofts, compared to those roosting in lower density situations (Sansano-Maestre et al., 2009).

Rock Doves may be more resistant to infection due to genetic factors, and such resistance has been much-discussed in various wild relatives of different domestic animal species (Taberlet et al., 2008; Redford & Dudley, 2018; Smith, Quilodrán, et al., 2022). For such an argument to hold true, this resistance would need to have been lost in the hybrid swarm in Orkney, which has prevalence similar to that of Oxford's feral pigeons. Further research is needed to identify the mechanism responsible. Disease resistance in birds can be due to functional changes in gene expression (Bonneaud et al., 2011), which could happen during domestication or feralisation, and therefore explain different immunity or disease tolerance among the types of *Columba livia*. One notable result is that, in the Uist and Orkney populations, females had significantly lower prevalence of infection than males. This is not the case in Oxford's feral pigeons and has not been seen in comparable studies of feral or domestic pigeons (Santos et al., 2019). The biology of the wild Rock Dove is poorly understood (Johnston et al., 1988) so it is unknown if ecological or immuno-genetic factors could underlie differences seen, or indeed the impact of domestication on sexual

selection, and subsequent variation in resource allocation between males and females. More broadly, there is mixed evidence for both male- and female-biased parasite infection among birds (van Oers et al., 2010). Further work across multiple populations, including genomic analysis of immune genes in each sex, could help to explain the observed patterns in our study populations. It is worth noting that, because sampling at each site was not possible simultaneously, sampling date may have impacted parasite prevalence and diversity. Although all of our sampling occurred within a short period, studies of blood parasites have shown that drastic changes in infection status can occur throughout the breeding season (Szöllősi et al., 2016). In *Columba livia*, where the breeding season is especially prolonged and where there is extensive individual variation in both time of breeding and number of broods hatched per year, it is likely that the birds we sampled were at various stages of breeding, reducing the impact of breeding status on our results. Nevertheless, it would be interesting to characterise temporal variation in parasite diversity and prevalence in the future, especially within individual birds.

Unlike Santos et al., 2019, we did not find a significant decrease in condition in *Trichomonas* infected birds, although our data shows they tended to have lower mean conditions. This could be due to sample size limitations. The apparent lack of impact of infection on condition might also be due to a lack of nestling or recently-fledged juvenile birds in the sample; sampling in the summer or autumn would be required to assess this. It is known that adult birds are often less impacted by disease than younger individuals, such as Turkeys *Meleagris gallopavo* infected with *Mycoplasma* (Efsa Panel on Animal Health et al., 2017). Very strong impacts of *Trichomonas* infection on body condition were observed in a prior study of free-flying domestic pigeons in Spain and Portugal (Santos et al., 2019)

but not in our results in either feral, wild or hybrid populations. This might be because the pigeons in the previous study might be relatively immunologically naïve as they were sourced from captive domestic stock that could have been kept almost parasite-free for multiple generations. Whilst the strain we identified may differ to the epidemic strain A at other regions, the identical ITS region is worrying and further research is needed to assess its risk, especially with respect to the endangered wild Rock Doves' chicks. The remaining Rock Dove populations are likely to come into increasing levels of contact with the wider global metapopulation of feral pigeons in the coming decades (Johnston et al., 1988). The relative susceptibility of wild Rock Doves to diseases introduced by their feral domestic conspecifics might contribute to their prospects of extinction via hybridisation in the future (Johnston & Janiga, 1995).

Despite similar prevalence of *Trichomonas* between Orkney and Oxford when compared to the Uist population, strain diversity at both sequence targets was much more similar between Orkney and Uist compared to Oxford and there was a significant nonrandom association between site and numbers of birds identified as being infected with different ITS strains. ITS strain Type C, common in Oxford pigeons but not recorded in the other two populations, is present across Europe and is found in various pigeons, doves and birds of prey (Thomas et al., 2022). It has also been isolated from the domestic Budgerigar *Melopsittacus undulatus* in Iran (A/N KT869151). Type A, found in all three populations but especially prevalent in the hybridising population in Orkney, has been implicated as a causative factor in the declines of British Greenfinches (Alrefaei, Low, et al., 2019). ITS strains that are more similar to *T. tenax* than *T. gallinae*, such as the novel ITS strain Ttx-RD, have been identified in Europe, Asia and Africa before (in Woodpigeons, Turtle Doves

and Stock Doves – but apparently not in *C. livia* until now) (Marx et al., 2017; Thomas et al., 2022). The *Fe-hyd* strains that we identified follow a similar pattern to the ITS strains; of those samples successfully sequenced, the Orcadian and Uist birds had the same sequence (A1.2). This strain is commonly isolated in avian epidemiological studies and has been found in birds of prey, pigeons and doves, and passerines (Thomas et al., 2022). Of the strains identified in Oxford pigeons, C4 is commonly found in raptors and pigeons (Thomas et al., 2022) and strain A3 has been previously identified in a Brahminy Kite *Haliastur indus* and domestic Canary *Serinus canaria* sampled in Japan (Chou et al., 2022). The other two Oxford individuals each had a novel *Fe-hyd* strain which was closely-related to the known C1 strain – which has been isolated in *C. livia* in the USA. It is clear that much of the diversity of these parasites has not so far been characterised. As we only sequenced the ITS and *Fe-hyd* gene regions, there may have been differences at other functional genes. Such differences may underlie the differences in prevalence between Uist and Orkney, despite the two populations apparently having similar *Trichomonas* strains. Of course, the difference could also be explained by behavioural or environmental differences discussed previously, and by geographical/latitudinal differences in the presence of the particular strains involved. Whilst sex-differences in parasite lineages detected have been seen in certain case studies (Reimchen & Nosil, 2001; Krasnov et al., 2005), we did not detect such differences within the subset of our birds for which an ITS sequence could be generated. This may be because of small sample size, and more work on this topic would be valuable.

The UK Rock Dove – feral pigeon case study offers an excellent opportunity to explore the dynamics of hybridisation and disease transfer, with clear variation in *Trichomonas*

infection prevalence and diversity among the different types of *Columba livia*. To further develop the system, sampling from multiple populations across the range of the feral pigeon and Rock Dove, including their hybrid zones in e.g. Caithness, Orkney and Shetland, would allow us to tease apart the different factors of geography, environmental variables, and type of pigeon. This will be vital in characterising the interaction between hybrid status and parasite infection. It would also be beneficial to sample the full range of age categories of the birds. Sampling juveniles, which may be more impacted by *Trichomonas* infection than older birds, might also allow us to better quantify the impacts of infection on individuals. Coupled with this, it will be possible to assess individual variation in reproductive success, behaviour, longevity and gain/loss of infections through mark-recapture studies and GPS tracking. This will facilitate a thorough characterisation of fine-scale variation in the role *Trichomonas* plays in populations of differing wild-feral ancestry proportions.

Conclusions

This study is the first to genetically characterise *Trichomonas* infection in the wild form of *Columba livia*. The wild Rock Dove and wild-feral hybrid populations that we have sampled exhibit different *Trichomonas* strain diversity to their more abundant feral conspecifics. The Rock Doves also have lower infection prevalence. Increasing contact of feral and wild pigeons will increase the transmission of parasitic diseases among populations, likely contributing to the decline of wild Rock Doves. Overall, we have increased knowledge of an important actor at the wild-domestic interface. *Columba livia* has immense economic and ecological importance through disease transmission to poultry and other captive and

wild birds – and is perhaps the most capable wild avian species in terms of facilitating disease transmission between wild and captive populations. Much of the work studying *Trichomonas* infection that happens in birds today focusses on declining native species (Greenfinches, Turtle Doves, Pink Pigeons). Improved focus on the most globally important transmitter of the parasite would be of utility in understanding its history and potential future impacts on both declining native species and captive birds. More broadly, the study of globally superabundant synanthropic commensal species (e.g. Brown Rats *Rattus norvegicus*, feral cats *Felis catus*, and feral dogs *Canis familiaris*) and their diseases is becoming more and more important in an increasingly homogenised global ecosystem.

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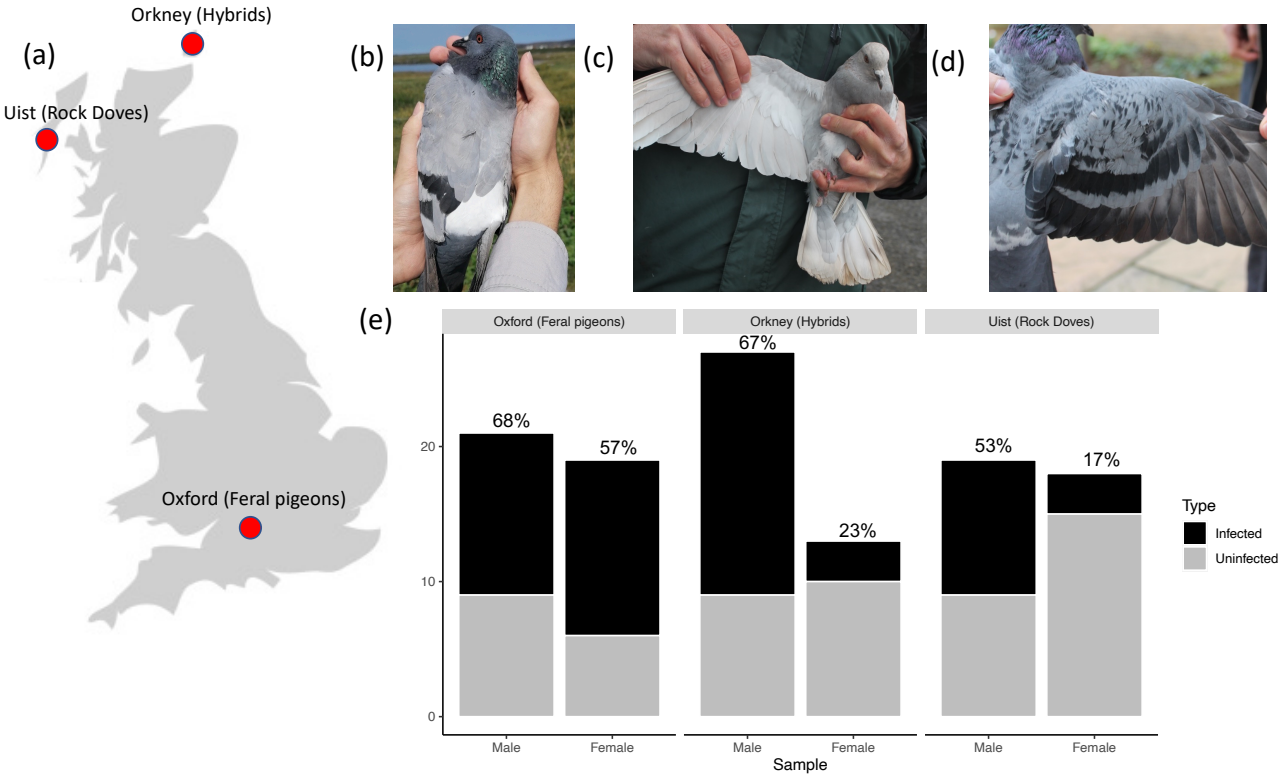


Figure 1 (a) Sampling locations in England and Scotland. (b) Rock Doves caught in Uist all showed the distinctive plumage of the undomesticated form, including a white rump patch. (c) Hybrid pigeons from Orkney mostly have the 'Rock Dove' plumage seen in Uist, but many display feral pigeon plumage traits including the 'red-white' colouration displayed by this individual. (d) In Oxford, most feral pigeons display black chequering on the wings (above the black wing bar) – a trait known to have been introduced during the domestication process. (e) Sex differences in infection prevalence at each site.

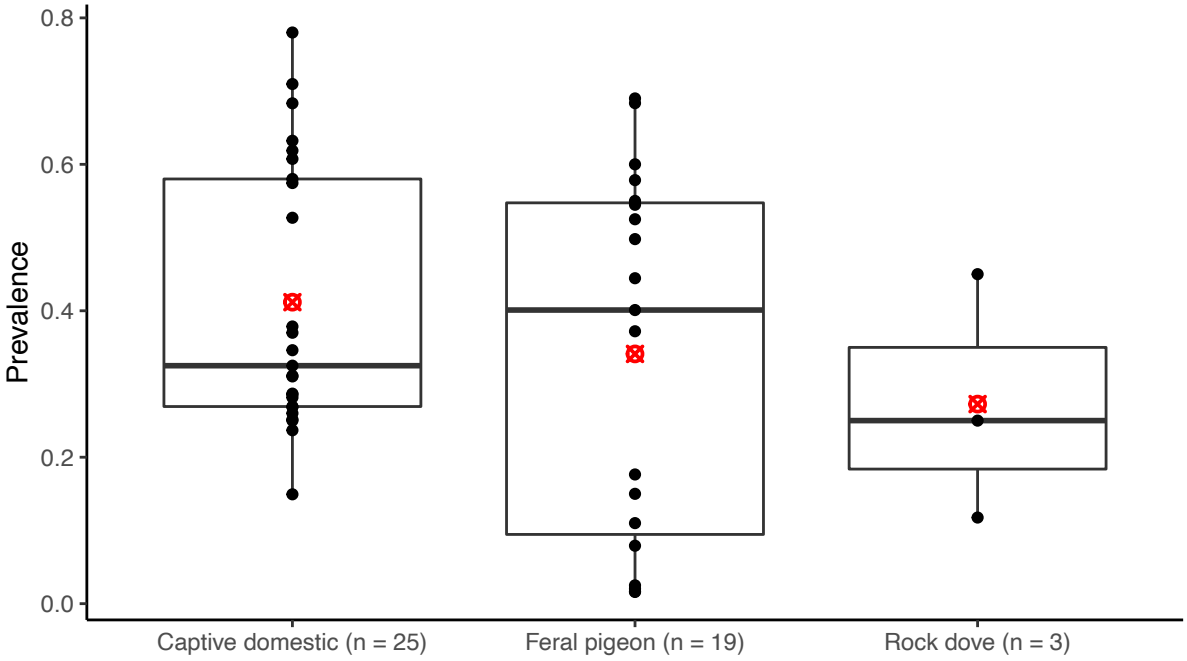


Figure 2: Prevalence values reported for individual studies of captive domestic pigeon, feral pigeon, and Rock Dove populations. Mean prevalence indicated with red crosses. The highest 'Rock Dove' point represents the Orkney feral-wild hybrid population.

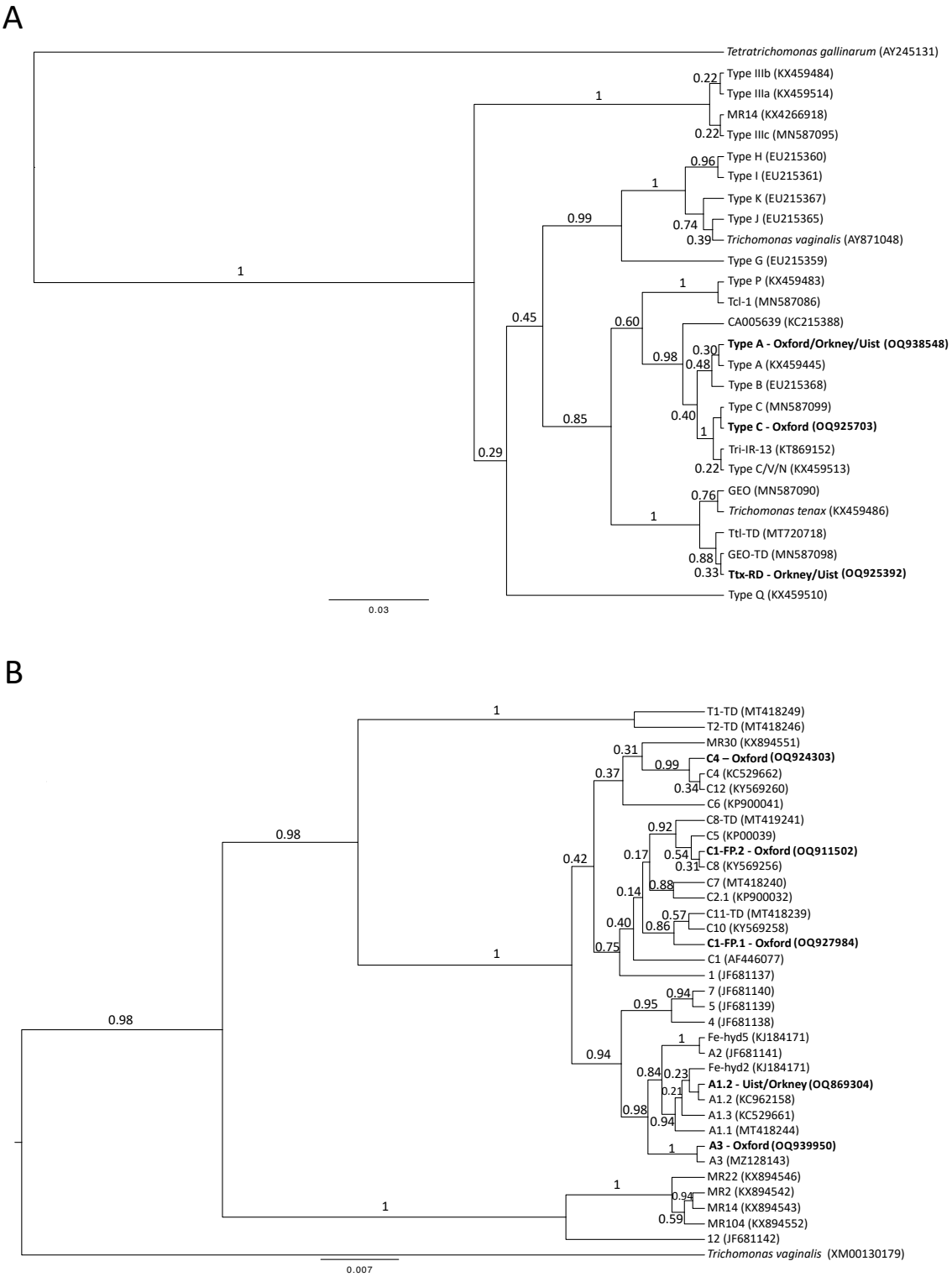


Figure 3: Maximum clade credibility tree of the (a) ITS and (b) *Fe-hyd* gene regions of known sequences/strains for *Trichomonas gallinae*, as inferred by BEAST. Sequences isolated from birds during our study are presented in bold. All sequences are given an example GenBank accession number. Branch support is indicated by Bayesian posterior probabilities.

Table 1. Model selection table showing the models best explaining observed variation in disease prevalence among populations included in the global study. BIC stands for Bayesian Information Criterion, and ΔBIC shows the difference from the best model (top). Only the top seven models and the null model are shown for brevity.

Model	BIC	ΔBIC	Akaike's weight	Log-likelihood	df
prevalence ~ pigeon type	-11.116	0.000	0.268	11.333	3
prevalence ~ 1 (null model)	-10.365	0.751	0.184	9.033	2
prevalence ~ temperature + pigeon type	-9.479	1.637	0.118	12.440	4
prevalence ~ urban location	-8.726	2.390	0.081	10.138	3
prevalence ~ pigeon type + urban location	-8.206	2.911	0.063	11.803	4
prevalence ~ temperature	-7.908	3.208	0.054	9.729	3
prevalence ~ precipitation	-7.884	3.233	0.053	9.717	3
prevalence ~ precipitation + pigeon type	-7.689	3.428	0.048	11.545	4

Table 2. Model selection table showing models best explaining observed variation in infection status among individuals from the three British populations. BIC stands for Bayesian Information Criterion, and ΔBIC shows the difference from the best model (top). df – degrees of freedom.

Model	BIC	ΔBIC	Akaike's weight	Log-likelihood	df
Infection status ~ sex	160.269	0.000	0.470	-75.407	2
infection status ~ 1 (null model)	161.370	1.101	0.271	-78.321	1
infection status ~ sex + site	163.873	3.604	0.078	-72.482	4
infection status ~ site	164.445	4.176	0.058	-75.131	3
Infection status ~ hybrid score + sex	164.537	4.268	0.056	-75.177	4
Infection status ~ hybrid score	165.283	5.014	0.038	-77.914	3
infection status ~ hybrid score + sex + site	167.157	6.888	0.015	-71.760	5
infection status ~ hybrid score + site	167.213	6.944	0.015	-74.152	4

Table 3. Model selection table showing five models best explaining observed variation in condition among individuals from the three British populations. BIC stands for Bayesian Information Criterion, and ΔBIC shows the difference from the best model (top). df – degrees of freedom.

Model	BIC	ΔBIC	Akaike's weight	Log-likelihood	df
condition ~ sex + site	-238.317	0.000	0.754	130.977	5
condition ~ sex + site + infection status	-234.519	3.798	0.113	131.442	6
condition ~ hybrid score + sex + site	-233.611	4.706	0.072	130.988	6
condition ~ sex	-230.602	7.715	0.016	122.392	3
condition ~ site	-230.193	8.124	0.013	124.551	4

Table 4. Results as presented by summary() function of R of the best linear model explaining bird condition; condition ~ sex + site. R^2 denotes marginal value as applied in MuMIn.

Predictors	Estimates	Standard error	P-value
Intercept - Uist	0.002	0.015	0.892
Site – Orkney	-0.025	0.018	0.168
Site – Oxford	-0.075	0.018	<0.001
Sex - Male	0.054	0.015	<0.001
Sample size	113		
R^2	0.218		

Table 5: ITS region strains identified from Sanger sequencing of 400bp fragment. Ttx-RD is a novel strain, and strains A and C are previously known.

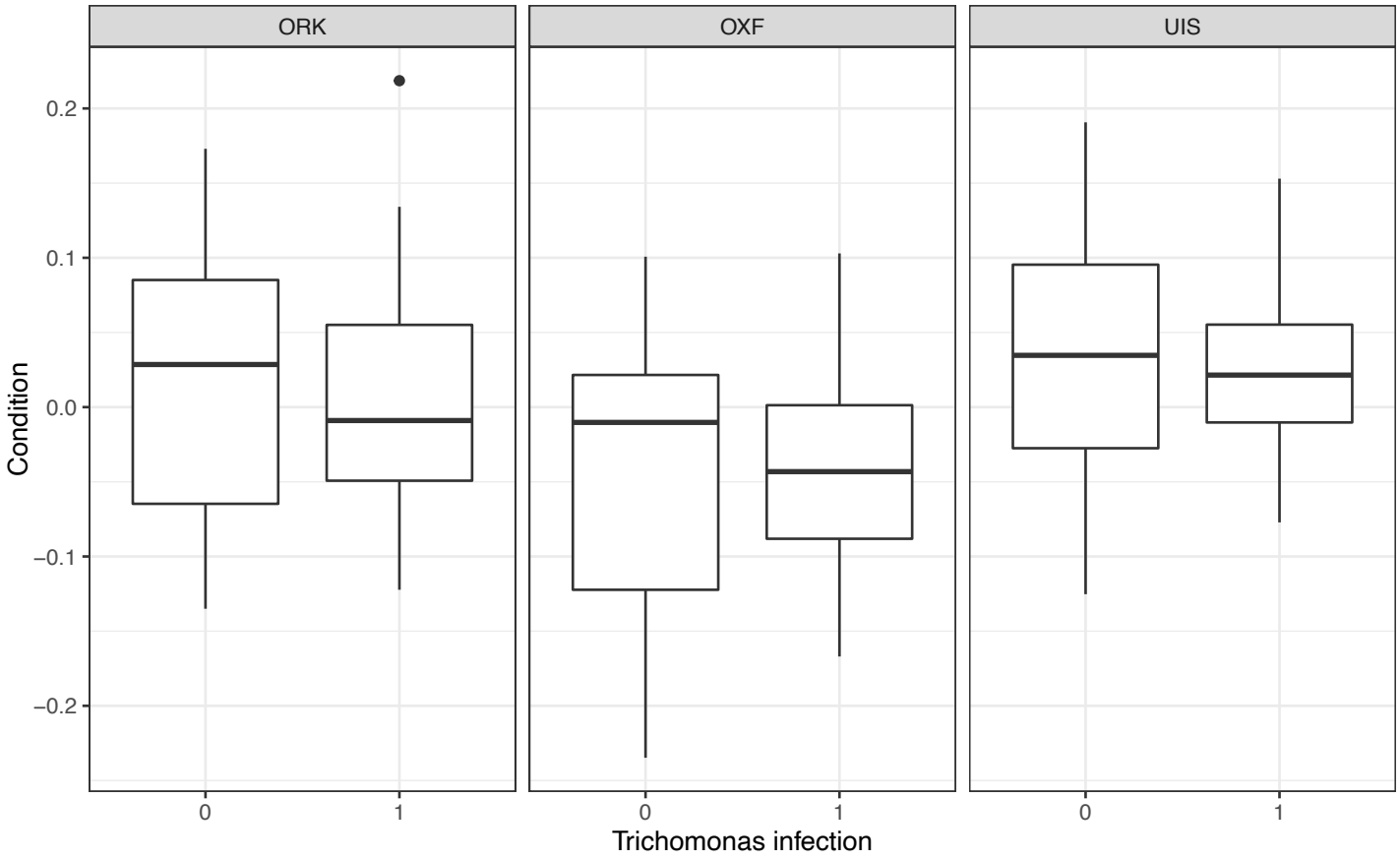
Strain	Oxford	Orkney	Uist	TOTAL
Type C	15	0	0	15
Type A	5	11	4	20
Ttx-RD (novel strain)	0	5	5	10
TOTAL	**20**	**16**	**9**	**45**

Table 6: *Fe-hyd* gene region strains identified from Sanger sequencing of 900bp fragment. C1-FP.1 and C1-FP.2 are novel strains. A1.2, A3 and C4 are previously known.

Strain	Oxford	Orkney	Uist	TOTAL
A1.2	0	3	1	4
A3	2	0	0	2
C4	4	0	0	4
C1-FP.1 (novel strain)	1	0	0	1
C1-FP.2 (novel strain)	1	0	0	1
TOTAL	**8**	**3**	**1**	**12**

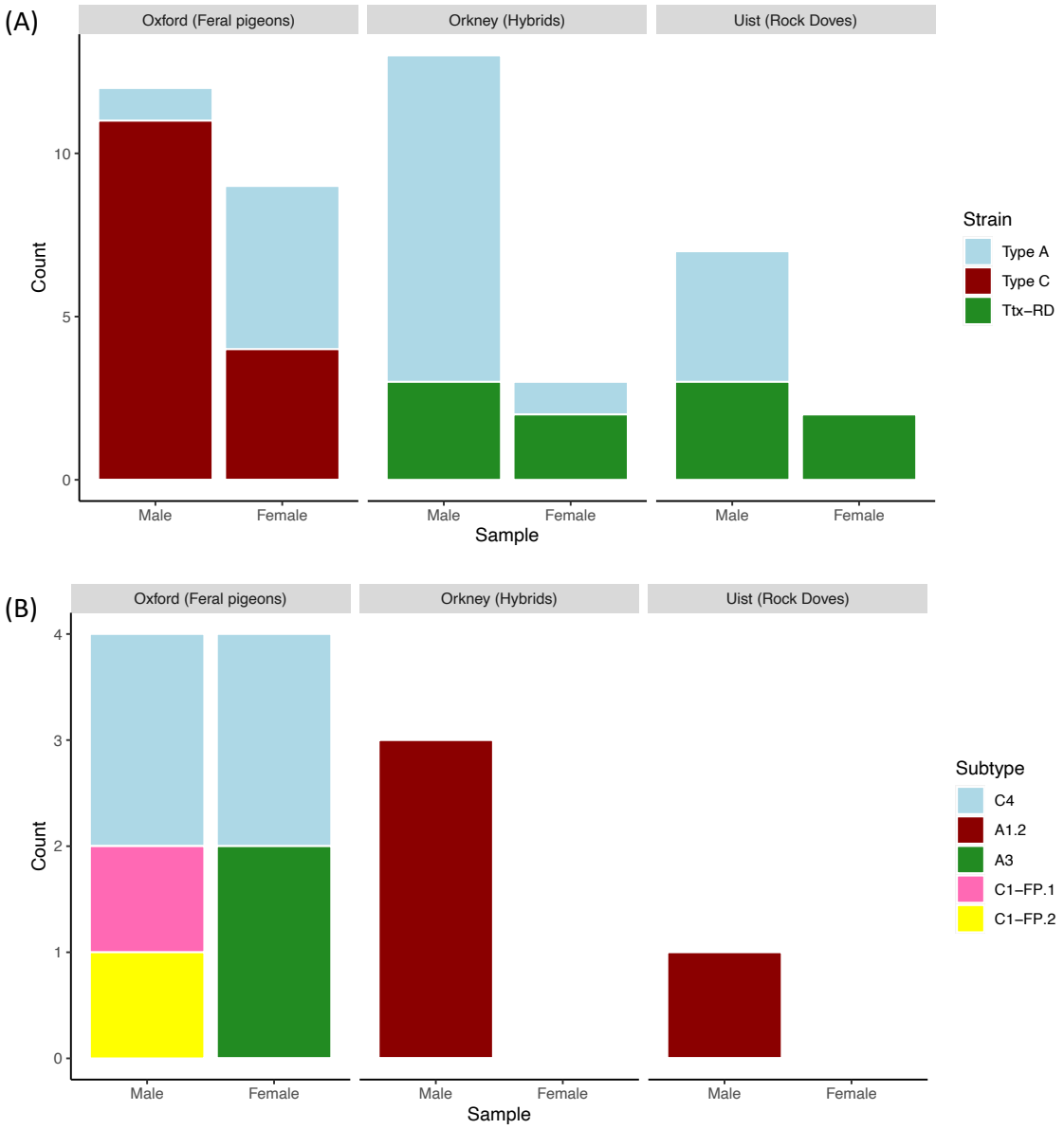
Supplementary Material: The interplay of parasite exchange and hybridisation at a wild-feral-domestic interface

Supplementary Figure 1



Average condition score is lower in birds which are infected with *Trichomonas* than those which are not, in each of the three British populations sampled.

Supplementary Figure 2



Trichomonas diversity of samples collected from different *Columba livia* populations and sexes, for both (A) ITS and (B) *Fe-hyd* sequences.

Supplementary Table 1: Numbers of birds in which each *Trichomonas* ITS strain was detected in each of the three sampling locations. Fisher's exact tests suggest that there are no statistically significant relationships between sex and strain at any of the three locations (although note that sample sizes are small).

LOCATION	**Number of infected birds (within each sex)**		**Fisher's exact test: p-value**
OXFORD	**Males (n = 21)**	**Females (n = 19)**	0.1089
Type A	1	4	
Type C	11	4	
ORKNEY	**Males (n = 27)**	**Females (n = 13)**	0.2143
Type A	10	1	
Ttx-RD	3	2	
UIST	**Males (n = 19)**	**Females (n = 18)**	0.4444
Type A	4	0	
Ttx-RD	3	2	

8

Discussion

Chapter 8: Discussion

Hybridisation is becoming increasingly common in nature, as human activity results in increased contact of previously isolated forms (Rhymer & Simberloff, 1996). This will inevitably lead to an increase in cases of extinction by hybridisation, involving plants, invertebrates (Muñoz et al., 2015), fish (Wringe et al., 2018), reptiles (Vuillaume et al., 2015), mammals (Biedrzycka et al., 2012; Smith et al., 2018), and birds (Heikkinen et al., 2020). Extinction by hybridisation poses a particular risk to the wild forms of domestic animals, where relatively recent divergence of lineages coupled with ongoing interbreeding had led to porous reproductive boundaries. This increased risk is a major issue given that the wild forms of domestic animals are of scientific, economic, and cultural importance (Redford & Dudley, 2018). This thesis combines fieldwork, citizen science, laboratory work, genomics, and epidemiology in order to characterise the contemporary status of the wild Rock Dove (*Columba livia*), which is threatened by hybridisation with its feral domestic conspecifics.

Main findings

In genomic terms, the Rock Dove has gone from one of the least well-studied birds in Europe to one of the most. Indeed, it is the only avian species to date to have received detailed population genomic characterisation across the British Isles. Here I briefly

summarise each chapter making up the main body of this thesis, its contribution to our understanding, and the way each chapter builds on the previous one:

2) Chapter 2 reviewed the status of the undomesticated forms of domestic animals (Smith, Quilodrán, et al., 2022). It highlighted that these types of animals are of economic, scientific and cultural importance, and are threatened by extinction via hybridisation. Despite the many papers outlining the conservation importance of the wild forms of domestic plants, this is the first such piece of work afforded to the wild forms of domestic animals. The resulting peer-reviewed publication will hopefully encourage further research focussing on this varied selection of species.

3) Following on from a comprehensive review of the wild forms of domestic animals, this chapter focusses, in more depth, solely on the undomesticated Rock Dove, the wild conspecific of the domestic (and feral) pigeon (Smith, 2023b). Here, I highlight the history of the species in the British Isles, outlining its decline and contemporary distribution, before discussing its prospects given ongoing gene flow from feral domestic pigeons.

4) This chapter presents the first population genomic study of wild Rock Doves, and identifies phylogenetically and phenotypically differentiated Rock Doves and feral pigeons in the British Isles (it had previously been suggested that no such Rock Doves persist) (Smith, Sendell-Price, et al., 2022). It also quantifies hybridisation between the two, and revealed the Outer Hebridean populations as having experienced negligible hybridisation. This provides a valuable case study as most

contemporary wild-domestic systems are extensively introgressed. It also shows that Rock Doves are endangered by ongoing wild-feral hybridisation, and that they might be completely replaced by feral pigeons in the future.

5) Building on the previous chapter, here we present a genomic study highlighting the impact of wild-feral introgression on genomic diversity. We show that, in the British Isles' Rock Doves, higher introgression reduces inbreeding and increases heterozygosity. This raises the idea of positive, though perhaps transient, impacts of anthropogenic-induced hybridisation.

6) After the previous two chapters focussing on the genomics of introgression, this chapter highlights the impact of hybridisation on the phenotype (Smith, 2023a). I show that variations in plumage polymorphism at a population level can be used to distinguish Rock Doves and feral pigeons, and to estimate the extent of past hybridisation among the two forms across multiple locations. These results provide additional tools to expand the geographic coverage of Rock Dove studies, and allow a citizen science approach to documenting variation in introgression.

7) Finally, we carried out a study of the prevalence and diversity of the disease-causing parasite *Trichomonas*, in wild (i.e. Rock Dove), hybrid and feral pigeon populations. We showed that the different forms of pigeon vary in their parasite prevalence and diversity. This allowed us to discuss factors affecting the dynamics of hybridisation at the wild-feral interface.

The importance of the Rock Dove to studies of extinction by hybridisation

As has been touched upon in several places throughout this thesis, the Rock Dove system occupies a unique position amongst the contemporary wild forms of domestic animals. Despite the importance of such animals, and the potential value of researching them, studying most of these species is challenging. Either the wild, 'ancestral-type', form is now extinct as is the case for modern domestic dogs (*Canis lupus*), cattle (*Bos taurus*), or horses (*Equus caballus*) (Freedman et al., 2014; Park et al., 2015; Gaunitz et al., 2018); critically endangered and range-restricted like the African Wild Ass (*Equus africanus*) (Tesfai et al., 2019), or exists in hybrid swarms where it is difficult to study interacting populations because the two forms have formed a single population of mixed ancestry, as with Scottish Wildcats (*Felis silvestris*) or Hawaiian Junglefowl (*Gallus gallus*) (Johnsson et al., 2016; Senn et al., 2019). We have shown that Rock Doves in the British Isles persist in populations with varying extents of domestic ancestry, ranging from the Outer Hebrides, where negligible domestic introgression can be detected, to Orkney, where all individuals sampled have experienced pervasive introgression throughout their entire genomes. These populations are distributed across a relatively large area, and it is likely that other relict populations across Europe, Asia and Africa will eventually be identified. We also know that we can use both genomic and phenotypic cues to distinguish the wild and feral forms, and to estimate the extent of hybridisation that has occurred within a population. This helps to make this system particularly tractable, and could facilitate a citizen science

approach. We also identify variation in the prevalence and diversity of disease-causing parasites, meaning that the system will also allow studies assessing the impact of both biological and environmental factors on patterns of hybridisation.

The contribution of the Rock Dove to research using 'model' domestic pigeons

As well as providing a tractable case study that can be used to explore questions regarding hybridisation and introgression, the biology of the Rock Dove represents an interesting and valuable comparison with that of the domestic pigeon. Much like other domestic animals including mice (*Mus musculus*) (Justice & Dhillon, 2016), rats (*Rattus norvegicus*) (Szpirer, 2020), chickens (Burt, 2007), guinea pigs (*Cavia porcellus*) (Padilla-Carlin et al., 2008), dogs (Karlsson & Lindblad-Toh, 2008), cats (*Felis catus*) (Griffin & Baker, 2002), ferrets (*Mustela furo*) (Belser et al., 2020), and sheep (*Ovis aries*) (Ribitsch et al., 2020), the domestic pigeon is an important model organism. Although it is particularly well-known for its significance in behavioural studies – including cognition (Güntürkün et al., 2014) and flight behaviour (Portugal et al., 2017) – it has in recent years proven to have utility in research projects looking at developmental and functional genetics (Stringham et al., 2012; Holt et al., 2018; Vickrey et al., 2018; Pacheco et al., 2020). As a result of the hundreds of different scientific papers published on captive domestic pigeons every year, our understanding of their behaviour, physiology, development, health, breeding biology, and genetics is well-developed (certainly for a

bird species) (Shapiro & Domyan, 2013; Shapiro et al., 2013; Pacheco et al., 2020). Feral pigeon populations are also subject to extensive research, and are of increasing importance as models of urban evolution and persistence in anthropogenic environments (Johnston & Janiga, 1995; Capoccia et al., 2018; Carlen & Munshi-South, 2021). This contrasts with the state of our understanding of Rock Dove biology which, to date, is restricted to a handful of papers and books (Fr et al., 1949; Hewson, 1967; Johnston et al., 1988; Baldaccini et al., 2000; Baldaccini, 2020).

Now that we have identified wild populations of Rock Doves that have experienced limited feral-wild genomic admixture (and it is likely that more such populations will be identified in the future, perhaps in Africa or Asia), we can study these birds in the wild. Interesting facets of research include characterising their movement ecology (with GPS tracking), annual cycle (through assessing moult progression and carrying out mark-recapture studies), diet (through isotope analysis) or social behaviour (through observation of marked birds, as well as PIT-tagging studies at feeding or roosting sites). The biology of the wild Rock Dove will provide a proxy for baseline data on the pre-domestication biology of the species *Columba livia*. In turn, this will provide context for the myriad studies on their domestic conspecifics. For example, movement ecology research in domestic pigeons has been used to inform ideas about leadership (Biro et al., 2006), homing capability (Portugal et al., 2017), intelligence (Sasaki & Biro, 2017), and self-recognition (Wittek et al., 2021). GPS-tracking of wild Rock Doves would allow us to characterise flocking and diurnal migration behaviours in a wild context, enabling us to understand how traits of interest evolved and what their fitness benefits in the wild are. Understanding the biology of the wild Rock Dove will provide a rare opportunity to

bolster the utility of a major model organism by allowing us to make more sensible conclusions regarding the functions of various behaviours and traits. Increased study of the wild populations of model organisms, including mice (Phifer-Rixey & Nachman, 2015) and rats (Modlinska & Pisula, 2020), has often been suggested – but limited efforts have been made to remedy these research gaps. The Rock Dove system has the additional dimension of including wild, domestic and feralised populations. Improving our knowledge of feral domestic pigeons could allow us to study feralisation, and whether 'domestic traits' are retained following escape from captivity, or whether some of the traits of wild Rock Doves are reconstituted. It has been suggested in the past that, to an extent, feral pigeons revert to Rock Dove morphology (and often lose the more dramatic domestic features such as feathered legs, crests, or drastically shortened bills) (Johnston & Janiga, 1995). Our work has however shown that morphological differences between wild and feral populations remain (Smith, Sendell-Price, et al., 2022). The extent to which this is true with respect to other traits (e.g. behavioural and physiological) is unknown.

Research priorities and future directions

The potential value of the Rock Dove system, both as a case study with which to explore extinction by hybridisation, and as a comparison with domestic pigeons used as model organisms, could be further unlocked. There are several focal areas that could do with increased scientific attention. Firstly, further work would be beneficial to establish the history of Rock Dove populations and patterns of wild-feral hybridisation among various pigeon populations. Sampling of both museum specimens (to explore wild-feral

hybridisation 50-200 years ago) and, using ancient DNA techniques, subfossils (to explore the origin of various Rock Dove populations and subspecies, and to determine whether any putative Rock Dove populations actually descend from ancient feral or domestic pigeons) would enable us to develop a fuller picture of the origin and evolution of today's wild Rock Doves, as well as feral domestic populations. Such work should be carried out both at regional and global scales, for a more developed understanding of genetic distinctiveness and adaptation in the various putative subspecies is likely to be of conservation importance. Whilst the genomic status, and therefore the extent of wild-feral hybridisation, has now been well-characterised in the British Isles, the global status of the Rock Dove remains poorly resolved. Future sampling from other regions within its range would be required to assess the conservation status of the species. With respect to the nominate subspecies *livia*, this would require sampling around the Mediterranean (especially on islands such as Crete, Sardinia, and Corsica), the Faroe Islands, and Iceland. Other subspecies are also of interest. It should be possible to include both the widely distributed subspecies such as the South Asian *intermedia*, which has a conservation status that is potentially more favourable than that of ancestral-type *livia*, and more geographically restricted (and phenotypically distinct) forms including Senegal's *gymnocyclus* and Egypt's *dakhlae*. A global appraisal of the Rock Dove and the extent of wild-feral hybridisation would provide a more complete understanding of the species' genomic status, and allow us to study multiple contact zones, resulting from independent introductions of feral pigeons in vastly different environments. This would require international collaboration, but would be a unique study system, providing multiple opportunities to explore questions regarding reproductive boundaries, biotic homogenisation, and adaptation of subspecies to different environments. A global and

historical exploration of Rock Dove genomics would also allow us to determine both the extent to which contemporary Outer Hebridean Rock Doves represent a useful baseline for an unadmixed wild Rock Dove, and also to quantify the progression of the Rock Dove's extinction by hybridisation through time. A recent preprint, using historical/museum samples, provided the first outline of global Rock Dove population genetics (Hernández-Alonso et al., 2023). Further work, including that which makes use of contemporary samples, is needed to further develop this system.

At a more fine-scale level, now that the Rock Dove-feral pigeon hybridisation mosaic has been characterised, it is possible to carry out more in-depth studies exploring the dynamics of introgression. Compared to well-studied systems involving wild-feral-domestic species interbreeding, such as wildcats in Scotland (Senn et al., 2019) or junglefowl in Hawaii (Johnsson et al., 2016), Rock Doves in the British Isles, and around their entire global range, provide multiple populations of varying levels of wild-feral admixture, which can be easily sampled and worked with. This provides the potential to move hybridisation and introgression research past brief studies centred around simple estimation of ancestry contributions, at a single snapshot in time. For example, more in-depth genomics and fieldwork will now be possible. This could include studies linking the extent of hybrid ancestry to fitness (and therefore evolutionary) impacts. Individual-based studies of breeding success, longevity, disease and parasite status, and behaviour in multiple populations will allow us to explore the ways in which extinction by hybridisation progresses, and to assess the impacts of hybridisation. Studies exploring how genetic load (the decrease in fitness due to deleterious genes) interacts with introgression will also be possible.

Finally, as previously mentioned, the Rock Dove holds promise in acting as a wild comparison to domestic pigeons used as model organisms in a laboratory context. It should be a priority to carry out comparative research to establish similarities and differences between wild and domestic forms of the species. This could include behavioural assays, estimates of cognitive ability, characterising movement ecology, and studying the different ways in which their physiologies may vary. The more well-understood these differences are, the more useful the Rock Dove will be in providing baseline knowledge about the wild life and evolutionary context of the domestic pigeon.

Conclusion

The species *Columba livia*, in a domestic, feral, and wild form; is among the most culturally, economically, and ecologically significant animal species. Its symbolic use pervades multiple mythologies, and its role as a superabundant urban pest makes it one of the most familiar animals on the planet. From its origin as a food source to its contemporary role as a model organism in the sciences and a species of major ecological importance, the domestic/feral pigeon has played, and continues to play, a major role in human history. Up until now, our understanding of the wild, undomesticated strain of the species, the Rock Dove, has been a major gap in our knowledge of this species. This DPhil thesis provides basic information about wild Rock Doves. It establishes the genomic status of populations in the British Isles, includes studies of plumage and epidemiology, and highlights areas of promise for future research. It contains extensive discussion

highlighting the scientific and conservation significance of the wild Rock Dove. More generally, the process of extinction by hybridisation impacts multiple animal and plant species (and subspecies) all over the world, and the rate at which introgression among evolutionarily different lineages is happening has increased with increased anthropogenic impact on the natural world. Studying feral-wild hybridisation among pigeons will provide a potent tool to understand this phenomenon.

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