Range-wide phylogeography of the golden jackals (*Canis aureus*) reveals multiple sources of recent spatial expansion and admixture with dogs at the expansion front

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Abstract

The current rapid climate change and human-induced alteration of landscapes and animal communities have led to range expansions in numerous species, raising concerns about potential negative impacts on genetic diversity, biotic interactions and hybridization with related species in newly colonized areas, and the need to adjust management plans. The recent explosive range expansion of golden jackals in Europe, now extending to the Arctic Circle, provides an opportunity to assess the consequences of this process at a broad geographical scale. We analysed the genomewide diversity of golden jackals from nearly the entire species range, including recently colonized areas. Grey wolves and free-ranging dogs sympatric with golden jackals were also analysed to test for introgression among these canids. Our results showed that golden jackals expanded from Asia to south-eastern Europe at the end of the Pleistocene, while in more recent times they naturally broadened their range in central and northern Europe from at least two distinct southern populations. At the northern edge of the range and in the recently expanded area, golden jackals showed evidence of admixture with dogs. Further monitoring of introgression rates and phenotypic effects of this process is crucial to ensure they do not facilitate the synurbization process. No reduction in genetic diversity was observed at the expansion front, most likely due to complex expansion routes, involving multiple waves and source populations, and introgression of genetic variants from dogs. We propose the development of management and legal plans that focus on transboundary cooperation considering the observed genetic diversity and structuring.

Keywords: colonization, free-ranging dogs, genetic diversity, introgression, hybridization, mesocarnivore, range expansion

1 Introduction

Range shifts have been a common feature in evolutionary histories of many species, but in recent decades anthropogenic habitat alterations and climate change have led to their increased occurrence and rate (Diamond, 2018). Many species have reduced their range due to population declines or local extinctions, while others have followed changing environmental or biotic conditions by colonizing new geographic areas (Pacifici et al., 2020). Although species ranges are usually highly plastic, fluctuating to match spatial shifts of their ecological niches, the species ability to track changing environmental conditions affects not only its own survival, but also the population dynamics and ecosystem services of other species in the newly colonized areas (Miller et al., 2020). Expanding species can alter ecosystem dynamics of native species through biotic interactions, pathogen transmission, and hybridization, as well as affect human health and impose economic costs, ultimately leading to policy-relevant issues and management challenges (Pecl et al., 2017).

In order to develop appropriate management strategies for expanding species, it is important to distinguish between natural expansions and invasions. Invasive species are considered as a threat to the native ecosystems and are actively prosecuted. In contrast, naturally-expanding species may be recovering from earlier range contractions or adjusting their ranges to changing environmental conditions, and are therefore often protected. An invasion can be distinguished from a natural expansion by the geographic distance between the native range of the species and the region of expansion, and by the need for human intervention (e.g. physical translocation, substantial habitat modifications) for the species to appear in the expansion region (own definition, consistent with the definition of invasion as human-mediated extra-range dispersal in Wilson et al. 2016). Besides obvious cases of inter-continental translocations, e.g. American mink Neogale vison and racoon *Procyon lotor* in Europe, there are many less obvious cases where expansions occur via natural dispersal from native ranges and expanding populations occupy natural habitats similar to those within their established ranges, e.g. expanding stone marten Martes foina populations in northeastern Europe (Wereszczuk et al., 2017). Knowledge of the geographic extent of the native range is therefore essential for the correct identification of species as invasive or naturally-expanding. The definition of a native range frequently assumes a long-term temporal stability of species distributions, but some species experienced considerable range shifts throughout the Holocene, and for many of them historical and sub-fossil record is insufficient to establish the extent of their ranges beyond contemporary times.

The golden jackal (*Canis aureus*) is one of such cases. This mesocarnivore is widespread in southern Eurasia (from Europe through the Middle East to Central and Southeast Asia), and represents one of the fastest recent expansions among mammals in Europe (Trouwborst et al., 2015). The European population of this species was restricted to coastal regions of the Mediterranean and Black Sea until the 19th century (Krofel et al., 2017; Spassov and Acosta-

Pankov, 2019). After recovering from population declines and local extinctions in the middle of the 20th century (Spassov and Acosta-Pankov, 2019), golden jackals started spreading throughout central and northern Europe in the late 1960s. The expansion accelerated over the past two decades, reaching as far north as Norway, Finland and the Russian European subarctic and as far west as France (Trouwborst et al., 2015; Krofel et al., 2017; Hatlauf et al., 2021; Sørensen and Lindsø, 2021; Rykov et al., 2022), with reproduction confirmed as far north as in Poland and the Baltic countries (Kowalczyk et al., 2020; Männil and Ranc, 2022). A single individual has recently been recorded in north-eastern Spain (A. Caro and M. J. Madeira, unpublished information). The spread of jackals has led to various conservation and management issues, including negative impacts on wildlife and livestock through predation, kleptoparasitism and pathogen transmission, but also positive consequences such as ecosystem services (e.g. rodent control, carcass removal) provided by the species (e.g. Ćirović et al. 2016; Hatlauf et al., 2021; Krofel et al., 2022a).

In European countries, the legal status of golden jackal varies from "fully protected" through "hunted" (i.e. killing is regulated by hunting legislation) to "unprotected" (killing is not restricted or regulated), and in some countries the status varies in different parts of the country (for details, see Trouwborst et al. 2015). In Austria, for example, five provinces list the golden jackal in their hunting laws (some of which have an open hunting season without any quotas) and four provinces list it in the conservation law with full protection (Hatlauf et al. 2021). Even in cases where the legal status is consistent across the country, the regulations may be unclear or subject to change over a short period. In Lithuania, for example, the golden jackal was initially classified as an invasive alien species, but this status was later changed and the current status is "hunted" (Stratford 2015; Hatlauf et al. 2021).

The lack of legal regulations for the species may have different consequences in different countries. For example, in Latvia where no specific regulations exist, the status of the golden jackal is regulated by hunting legislation in the same way as the grey wolf. In Belarus, on the other hand, the lack of regulations results in no restrictions on its killing. In Georgia, killing of any species of wild animals is prohibited without proper permits or licenses, but the lack of species-specific regulations can lead to ambiguity that facilitates illegal hunting. A very similar situation occurs in Iran, where the golden jackal is unprotected, but hunting wild animals is illegal. In contrast, in India the status of the golden jackal is strictly regulated, and recently the species has been elevated to Schedule 1 of the Wildlife (Protection) Act 1972 (2022 Amendment), giving it the highest level of protection.

In European Union member countries, the golden jackal is subject to the 1992 Directive 92/43 on the Conservation of Natural Habitats and of Wild Fauna and Flora (Habitats Directive), being listed in its Annex V which covers "Animal and plant species of community interest whose taking in the wild and exploitation may be subject to management measures" (Trouwborst et al. 2015). Obligations resulting from the Habitats Directive apply to species in their 'natural range', which is problematic in countries of northern and western Europe where there are no historic records of the golden jackal's presence (Trouwborst et al. 2015). As such, a key question in establishing the

legal status of golden jackal is whether to consider its ongoing expansion as an extension of its natural range or whether it should have a status of an alien species, potentially subject to prevention of further spread, control and eradication (Somsen & Trouwborst 2019).

Defining native range of the golden jackal is problematic, as fossil data are scarce and records of past presence/absence may be questionable, given that the skeletal remains may be misassigned as wolves or domestic dogs. One of the oldest records attributed to the golden jackal is from a Palaeolithic cave in Lebanon (Moehlman and Hayssen, 2018). There is no agreement regarding the time of the first appearance of the golden jackal in Europe. Some studies suggest the Neolithic, based on records from two sites in Greece (Sommer and Benecke, 2005). Other authors point out that the earliest reliable record of the golden jackal in Europe (in Bulgaria, near Sofia) comes from a 14th-century Turkish chronicle (Spassov and Acosta-Pankov 2019). The earliest record of the golden jackal in the Caucasus is dated to the Bronze Age (Vereshchagin, 1959). Mitochondrial DNA analysis of modern specimens suggests that India is the dispersal centre of the species and its expansion from that region occurred about 37 ky ago (Yumnam et al., 2015). This hypothesis, however, is based on the analysis that did not include samples from large areas of the Middle East, which represents one of the major knowledge gaps for this species (Krofel et al., 2022b). Given the limited information on the species past distribution and evolutionary history, it is unclear how its native range should be defined. However, based on the genetic data available so far (Rutkowski et al. 2015; Yumnam et al., 2015), it may be suggested that C. aureus experienced dynamic changes throughout the Late Pleistocene and Holocene, expanding westwards from south Asia through the Caucasus to Europe. Given these dynamic changes, it is only possible to define a geographic area in Europe with fixed boundaries that constitutes a long-term native range of the species; it is only possible to define the recent historical distribution. From the evolutionary perspective, the recent species expansion to northern and western Europe can be considered as a continuation of the long-term process of the species expansion from Asia.

Another important question is whether the expansion may be enhanced by any anthropogenic factors. Several factors favouring the spread of golden jackals have been proposed, including climate and land use changes, as well as changes in grey wolf (*Canis lupus*) distribution and abundance (reviewed in Spassov and Acosta-Pankov, 2019). Wolf extermination was proposed as a key driver of jackal dispersal, given that socially-stable packs of grey wolves may exert strong "top-down" effects and therefore human-induced declines in wolf populations could have enabled the rapid spread of jackals (Krofel et al., 2017; Newsome et al., 2017). Although there are relatively few studies that assess the contribution of current climate change to the spread of jackals, projections of future climatic suitability for golden jackals predict the existence of a stable population in central Europe and further expansion to areas with favourable climatic conditions similar to those in the current core distribution range (Cunze and Klimpel, 2022; Lanszki et al., 2022). Moreover, golden jackals prefer agricultural lands and mixed landscapes than woodlands (Šálek et al., 2014). The combination of climate change and expansion of human-modified landscapes may have contributed to the recent expansion of this species.

A similar expansion pattern has been observed in a North American mesocarnivore representing the same genus, the coyote (*Canis latrans*). Until the late 19th century, this canid was restricted to western and central parts of North America (Nowak, 2002), and during the 20th century it underwent a rapid expansion and currently occupies almost the entire North American continent (Hody & Kays, 2018). The colonisation of new regions occurred via multiple expansion routes across distinct environments (Heppenheimer et al. 2018) and was driven by climate change and/or extirpation of wolves by humans (Koblmüller et al. 2012, Newsome et al. 2017). Similarity of the spatial expansion patterns of the coyote and the golden jackal following the population size reduction of an apex predator has been pointed out by Newsome et al. (2017), and we hypothesise that it has led to the similarity in the population genetic patterns of these two species.

In this study, we focus on the Eurasian golden jackal across its distribution range. The African canid previously considered as a subspecies of the golden jackal has been recognised as a distinct species, African golden wolf (*Canis lupaster*) (Koepfli et al. 2015, Krofel et al. 2022b) and therefore is not included in this study. Admixture between African golden wolves and golden jackals, most likely resulting from ancient introgressive hybridisation, has been reported in the Arabian Peninsula, at the edges of the respective distribution areas of both species (Koepfli et al. 2015, Barash et al. 2023). It is unknown whether this admixture is restricted to the Arabian Peninsula only or has a broader geographic extent, but this question is beyond the scope of this study.

Hybridisation with closely related native species is an important process that may facilitate the range expansion of golden jackals (Pfenning et al., 2016). The regions of recent jackal expansion in Europe is inhabited by two other representatives of the genus *Canis*, the grey wolf and the domestic dog *C. lupus familiaris*. Several studies reported cases of recent admixture between golden jackals and both free-ranging dogs and grey wolves (Moura et al., 2014; Galov et al., 2015) as well as ancient jackal-wolf admixture (Freedman et al., 2014). Golden jackals developed thriving populations in human-dominated landscapes across south-eastern and central Europe (Fenton et al., 2021) and therefore cross-breeding with free-ranging dogs may be more frequent there due to the higher likelihood of encounter. Introgression of genetic variants from domestic dogs may enhance golden jackals' expansion by facilitating their adaptation to human-modified landscapes, including urban areas. This may change the perception of the jackal's colonisation of northern and western Europe from an entirely natural range expansion of a wild species to an expansion facilitated by genetic variants from a domesticated species. Therefore, assessing the character and frequency of jackal hybridisation with other canids is important for establishing appropriate management strategies for the expanding populations.

Golden jackals show further similarities to coyotes in terms of hybridisation with other representatives of the genus *Canis*. In the northeastern parts of their expanded range, coyotes where shown to cross-breed with grey wolves and eastern wolves (e.g. Kays et al., 2010), while in the southeastern parts, they cross-breed with the remnant population of red wolves (e.g. Bohling et al., 2016). Moreover, these coyote populations also interbred with domestic dogs (e.g. Monzõn et al.,

2014). Hybridisation is mostly reported from the areas of recent coyote expansion rather than from the core region the species range was confined to until the late 19th century. Therefore, we hypothesise that golden jackals will show higher admixture proportions with wolves and domestic dogs in the areas of their recent expansion.

In this study, we aim to reconstruct the golden jackal's range-wide phylogeography, past demographic trends, expansion routes, and admixture patterns in order to demonstrate the importance of species' evolutionary history for determining its status as a naturally-expanding versus invasive species. We analyse genome-wide Single Nucleotide Polymorphism (SNP) data to reconstruct population genetic structure in the area covering nearly the entire range of the species, reconstruct the routes of range expansion, estimate recent and past changes in effective population sizes, and assess current and past levels of admixture with grey wolves, and free-ranging dogs. We also assess whether the patterns of recent range expansion are consistent with natural dispersal, and whether this expansion could have been enhanced by interspecific hybridisation. Our results provide recommendations for conservation and management of the species on a global scale.

2 Material and methods

All methods used in this study are briefly presented below; detailed descriptions of the analyses performed and the parameters used can be found in the Appendix - Supplementary Methods.

2.1 Sample collection

Tissue samples from golden jackals used in this study were collected from dead, road killed or legally hunted animals; archived samples from earlier studies were also used. No animals were killed specifically for this research, and all tissue samples from hunted animals were obtained by collaborators in compliance with national laws and regulations of the respective country.

2.2 SNP genotyping and filtering

Overall, 328 individuals were genotyped using the Axiom Canine HD Array (Thermo Fischer Scientific, USA), and an additional 101 individuals were genotyped using the Axiom Canine Genotyping Array Set A (Thermo Fischer Scientific, USA). Genotype calling was performed using Axiom Analysis Suite version 5.1.1.1, followed by quality control, filtering, linkage disequilibrium pruning, and removal of related individuals.

2.3 Population structure and genetic diversity

Genetic structuring was ascertained using both fastStructure (Raj et al., 2014) and Admixture (Alexander et al., 2009). Principal Component Analysis (PCA) was performed in PLINK (Chang et al., 2015), whereas the discriminant analysis of principal components (DAPC) was performed in the R package *adegenet* (Jombart et al., 2011). Spatially explicit individual-based clustering analysis was carried out in R package *TESS3* (Caye et al., 2016). Isolation by distance (IBD)

among individuals was tested using the Mantel test between a matrix of Euclidean genetic distances and geographic distances in *adegenet*. Sex-biased dispersal was tested using the function *sexbias.test* implemented in the R package *hierfstat*. Spatial autocorrelation between males and females was calculated using the function *gl.spatial.autoCorr* in the R package *dartR* (Gruber et al., 2018).

For each genetic cluster revealed from genetic structuring analyses, the genetic diversity parameters were calculated using *poppr* (Kamvar et al., 2014) and *hierfstat* (Goudet et al., 2005) R packages. Genetic differentiation between the identified clusters was tested by the Analysis of Molecular Variance (AMOVA).

2.4 Admixture with other canids

To identify dog and/or grey wolf ancestry signatures in jackals from Europe and Asia, genotypes of additional 227 wolves and 241 free-ranging dogs (that cannot be assigned to a specific breed and are unrestrained in their choice of mate) from the same geographic area where the golden jackal samples were collected were analysed using Axiom Array A and Axiom HD Chip, and merged with golden jackal data. To detect the possible hybridization events, a clustering approach implemented in Admixture was used, as well as three-way admixture local ancestry block analysis implemented in the software ELAI (Guan, 2014). Ancestry relationships among jackal's populations and gene flow from free-ranging dogs and wolves were assessed using the software TreeMix (Pickrell and Pritchard, 2012).

2.5 Demographic analyses

Estimates of contemporary effective population size (Ne) were calculated using NeEstimator v2.1 (Do et al., 2014) (for details see the Supplementary Methods). To estimate demographic history over longer time periods (> 500 generations), a coalescent method based on the frequency spectrum was used as implemented in Stairway Plot 2 package (Liu and Fu, 2020). Recent changes in effective population size (over the last 500 generations) were estimated using the linkage disequilibrium-based method implemented in SNeP (Barbato et al., 2015) and GONE (Santiago et al., 2020).

The decay of linkage disequilibrium (LD) with physical distance between SNPs was calculated and visualized using PopLDdecay software (Zhang et al., 2019).

3. Results

3.1 Data set description

After quality control, filtering, and removal of related individuals, genotyping of 328 golden jackals with the Axiom Canine HD array resulted in a dataset of 256 individuals and either 68,937 or 153,022 variants (depending on the assumptions of the analyses performed), hereafter referred to as the 150 K dataset. An additional dataset created by merging individuals genotyped with

Axiom Canine HD and Array A (429 in total) comprised the final dataset containing 363 individuals and 19,670 SNPs, hereafter referred to as the 20 K dataset (see Appendix Supplementary Methods for further details). We used the 20 K dataset only to analyse the genetic structure by applying all the methods described below, as well as to calculate genetic diversity within the revealed genetic groups, while the 150 K dataset was used for all demographic analyses, as described below.

3.2 Patterns of genetic differentiation

In the 20 K dataset, the first three axes of the PCA accounted for 57.4 % of the variance and indicated the presence of three broad-scale genetic clusters (Figure 1C): one encompassing individuals from India, second with individuals from Iran, the Caucasus, East Europe and the Baltic countries, and the third cluster comprising individuals from Central and South-eastern Europe. Similar pattern was also supported by DAPC analyses (Appendix Figure S2). Further structuring within the three main clusters was detected, with eight genetic clusters that included individuals from southern India (SIND), northern India and Iran (NIND), the Caucasus and Eastern Europe (CAU), the island of Samos (SAM), the Baltic countries – mainly Estonia (BAL), mainland Greece and the Adriatic coast (GRE), Southern and Central Europe (SCE), and an additional cluster with individuals originating mainly from Hungary (HUN) (Figure 1E, Figure S3). Individuals were assigned to the appropriate clusters only if they had at least 60 % of ancestry from that cluster, while the remaining individuals were considered admixed, and therefore were not included in any of the clusters so as not to bias the calculated genetic diversity indices. The structuring pattern observed using Admixture was also similar to the results shown by TESS (when accounting for spatial coordinates; Figure 1A, Figure S4) and fastStructure analyses, although the latter did not support the partitioning of the jackals from the south of India (Figure S5). A significant support for isolation by distance was detected (r = 0.86, p < 0.01). No sign of sexbiased dispersal among pre-defined genetic clusters based on the 20 K dataset was observed (ttest, t = -0.745, p = 0.45). Accordingly, the similarity of spatial autocorrelation patterns in males and females throughout the study area (Figure S6) did not provide support for sex-biased dispersal.

Highest genetic diversity was found in golden jackals from India, with a noticeable decrease in genetic diversity indices in Iran, the Caucasus, and onward to Europe (Figure 1A, Table S1). Within Europe, highest heterozygosity was observed in the Baltic region and the lowest on the island of Samos (Table S1). Low genetic differentiation was observed between India, the Middle East, the Caucasus, and the Baltic countries (Table S2), while the highest genetic differentiation was observed between India and Central Europe. AMOVA revealed that 13.31 % of variation occurred among genetic clusters, 83.49 % occurred within individuals, and 3.2 % of genetic variation occurred among individuals within genetic clusters.

Structuring patterns based on the 150 K dataset with a smaller number of individuals (n = 256) generally confirmed the results of the 20 K dataset (Figure 1D, Figures S7-S11), albeit at a lower resolution, suggesting six genetic clusters that included individuals from India (IND), Iran (IRN),

the Caucasus and Eastern Europe (CAU), the island of Samos (SAM), the Baltic countries, primarily Estonia (BAL), and Southern and Central Europe (SCE) (Tables S3-S5) (see details in Appendix Supplementary results). Because the individuals from Iran showed admixture between the CAU and IND clusters, all genetic diversity parameters were calculated to treat the samples from Iran as a separate genetic cluster to avoid possible bias in assignment to one of these clusters.

3.3. Admixture with grey wolves and domestic dogs

The results of the Admixture analysis at K = 3, including free-ranging dogs and wolves, showed that 61 jackals have an ancestry coefficient of <99 %. Most of these individuals were found in India (47.54 %), the Middle East (22.95 %), and the Caucasus (11.47 %) (Table S6). However, after additional admixture runs conducted on regional datasets, only 18 individuals had jackal ancestry lower than 99 %. Of these 18 individuals, three turned out to be dog-jackal F1 hybrids, while five had jackal ancestry between 76.6 % and 89.0 %, which may correspond to the F2 or F3 backcrosses (e.g., F1 hybrid backcross 75 %, F2 offspring backcross 87.5 %). Ten individuals had ancestry coefficients > 90 %, indicating more distant admixture events. Although some individuals had similar values of wolf and dog ancestry coefficients, the dog ancestry proportion dominated in the remaining individuals (Table S6, Figure 2). The results of the Admixture analyses were confirmed by the local ancestry blocks analysis carried out in ELAI (Table S6).

Tree topology reconstructed with TreeMix shows the east-west differentiation of golden jackals from India through the Middle East to Europe (Figure S12). Two admixture events were detected: one between Indian golden jackals and wolves and the other between golden jackals from Europe and black-backed jackals (*Lupulella mesomelas*) from Africa. Given the large geographic distance, the latter event is likely the result of a sampling bias due to TreeMix detecting divergent ancestry that is not represented in the outgroup (since black backed jackal was the only outgroup) or simply because only three black-backed jackals were used. The remaining admixture events reflect mixing between dogs and wolves.

3.4. Demography and runs of homozygosity

Contemporary effective population sizes were estimated to be highest in the Middle East and Southern and Central Europe, while the lowest values were recorded in the Baltic countries and India (Table S7). However, given the limited sample size for some of the populations examined (Table S7), and since the estimates may be influenced by the sample size, it is important to note that reported values may be underestimated. Indeed, N_e estimates for the island of Samos were found to be equal to infinity, which is usually the consequence of limited sample size, when the mean correlation coefficient of allele frequencies at different loci is smaller than the sample size correction by chance, resulting in a negative value reported as infinity. A continuous linear decline in the effective population size was noticed over the past 1000 generations for all populations according to SNeP results, with the most recent estimates going back 13 generations being highest in the CAU and SCE (Figure 1F), and lowest in the island of Samos. However, contrary to SNeP,

the GONE results indicated nonlinear changes in the effective populations sizes over the last 500 generations, suggesting the population expansion starting around 200 generations ago for CAU and SCE group (Figure S13), followed by the reduction in effective population sizes starting around 50 generations ago. GONE seems to outperform other current methods for estimating very recent changes in Ne, as the other methods perform poorly in the presence of complex demographic scenarios involving multiple bottleneck episodes, population declines and expansions, as is the case with golden jackals. Observed changes in effective population size are broadly consistent with LD decay analyses, as the lowest average r² values were found for ME, IND and CAU populations (Figure 1G), while the highest average r² values and slowest LD decay were observed in SAM and BAL populations. The slower LD decay in SAM populations might be a consequence of lower within-population diversity and consequent inbreeding, as the recombination rate is reduced and genetic polymorphism remains correlated over longer distances.

Stairway plot analyses indicated that the expansion signal started around 120,000 generations ago for CAU, and around 20,000 generations ago for SCE, while other locations exhibit more or less constant effective population sizes over time (Figure 1B, Figure S14). We excluded the BAL population from this analysis, because golden jackals have only recently appeared there, as well as from the calculations of effective population changes over last 500 generations (see above).

4. Discussion

Over the past decades golden jackals have been steadily expanding northward and westward in Europe, providing a suitable model for ecological studies aimed at elucidating the possible mechanisms and causes of range expansion, as well as implicating new challenges for conservation of this species. Here we provide a deeper temporal perspective on the golden jackal expansion by reconstructing the patterns of demographic and phylogeographic changes. We found that based on the molecular data this species experienced periods of range expansion during the Late Pleistocene and the Holocene, possibly driven by climate changes. The recent expansion can therefore be considered as a continuation of this natural process. However, the expansion is facilitated by anthropogenic habitat changes, as jackals show preference for such modified habitats (Šálek et al., 2014) and areas where people reduced grey wolf populations (Krofel et al., 2017, Newsome et al., 2017). Therefore, the ongoing expansion of golden jackals can be considered as a natural response to environmental changes driven by humans.

4.1. Historical range expansion from Asia to Europe

A clear gradual decline of genetic diversity and marked increase in linkage disequilibrium in the east-west gradient, suggest that golden jackals diversified in Southeast Asia and spread from there through south-west Asia to the Caucasus and on to Europe, the latter most likely occurring during the Pleistocene – Holocene transition. Throughout the jackal's range, the large-scale genomic structuring indicates a split into three clusters: first in eastern Iran and India, second in the Caucasus and Eastern Europe, and third in southern and central Europe, largely corresponding to

the historical range development. The population structure with genetic clusters encompassing large geographic areas and consistent with distinct expansion episodes shows close resemblance to the pattern observed in coyotes throughout their distribution range (Heppenheimer et al., 2018).

As proposed in a recent study based on mitogenomes, the species could originate from Southeast Asia rather than India (Sosale et al., 2023). We could not confirm this suggestion as the easternmost samples in our dataset were from India. Expansion from Asia to Europe was also inferred for several other carnivores with a wide distribution in Eurasia, such as grey wolves, red foxes (Vulpes vulpes) and brown bears (Ursus arctos), as glacial and interglacial periods during the Pleistocene impacted their distribution in a similar way (Korsten et al., 2009; Loog et al., 2020; Bergström et al., 2022; McDevitt et al., 2022). Therefore, multiple species follow east to west expansion trajectories across Eurasia, with populations from northern regions being of more recent origin, while those from southern regions retain greater ancestral diversity (e.g., McDevitt et al., 2022; Wang et al., 2022). Unlike other carnivores that are widespread in the Holarctic Region, golden jackals did not move northward after the Last Glacial Maximum and remained restricted to southern regions of Asia (and possibly Europe, despite the lack of species presence records), most likely due to adaptations to warmer climate and more open habitats (Šálek et al., 2014; Spassov & Acosta-Pankov, 2019; Kamler et al., 2021). Expansion from Southeast Asia via the Transcaucasian route or from northeast Asia westward via the northern hemisphere seems to be a common response of multiple mammalian species to climatic fluctuations during the Late Pleistocene and Holocene (e.g., Korsten et al., 2009; McDevitt et al., 2022), with some species even dispersing through distinctly different colonisation waves across southern and northern latitudes of Eurasia (e.g., wild boars; Choi et al., 2020). The possible additional routes of historical expansion of golden jackals, such as the expansion from Anatolia via Bosphorus to south-eastern Europe remain unclear, given the lack of samples from this region.

Our coalescence analysis suggests two waves of demographic expansion in south-eastern European jackals, with the first wave beginning as early as 120 kya, at a similar time to the expansion of jackals from the Caucasus, and the second wave beginning around 20 kya. The latter expansion wave is supporting previous results for the time of expansion of golden jackals from Europe and Israel based on the sequences of mitochondrial control region (Yumnam et al., 2015). Since the demographic expansion suggested by molecular data analyses does not necessarily represent the expansion from the current geographic range of the source population, but instead may show the signal of expansion traced to the region from which this population originated, the first inferred expansion from the Caucasus, thus suggesting that the Pleistocene-Holocene transition could be the time when jackals expanded into south-eastern Europe from the east. European populations from Greece and Adriatic coasts may represent the lineages originating from the earlier expansion (as proposed by Rutkowski et al., 2015) that survived to present day and merged with the second expansion wave from the Caucasian region into Europe during the 20th century. Recent genomic studies pointed to the end of the Pleistocene as the time for the expansion of grey

wolves from north-eastern Asia to Europe (Loog et al., 2020), which was preceded by several earlier expansion waves from that region towards Europe, with incomplete ancestry replacements (Bergström et al., 2022). Despite the lack of reliable subfossil data for golden jackals in Europe and the Caucasus, the existence of two expansion waves could explain the genetic distinctiveness of the Dalmatian-Adriatic population, which might represent the remnants of the diversified gene pool from the earlier expansion wave, while the populations that have appeared in the western region of the Black Sea more recently derive from the Transcaucasian population that arrived there during the most recent expansion wave. Similar conclusions were reached for the Eurasian lynx (*Lynx lynx*), where mitochondrial data indicated divergence of the Balkans and the Caucasus population already about 50 kya, although autosomal data indicated that they may be closely related, explaining the discordance between mitogenomic and nuclear data as a result of male-biased admixture following episodes of isolation (Bazzicalupo et al., 2022).

4.2. Recent range expansion to central and northern Europe

Due to the preference to more open habitats, the historical range expansions of the golden jackal during the Late Quaternary were likely associated with periods of expansion of such habitats and reduction of dense forests, similar as in the case of coyotes (Koblmüller et al. 2012). The recent expansion of both species during the 20th and 21st centuries could have resulted from anthropogenic habitat changes resulting in the increase of open agricultural areas and reduction of forest cover (Spassov and Acosta-Pankov, 2019), combined with the population declines and extirpations of an apex predator, the grey wolf (Newsome et al., 2017).

Although the reasons for the recent spread of the golden jackal towards central and northern Europe are not fully elucidated, our data indicate that this species naturally expands its range from at least two source populations, one originating in the Balkans and the other in the Transcaucasian region, with the north-eastern part of the Black Sea, and the regions of north-eastern Poland and southern Lithuania forming the contact zones between them. The dispersal patterns of the golden jackal seem to be following a long-distance dispersal model, given the timing of the emergence of the Baltic population (Männil and Ranc, 2022) and its origin from the Caucasus, similar to historic records of jackal colonisations (Rutkowski et al., 2015). Long-distance dispersal from the Caucasus may be related to the lack of strong geographic barriers to the north, the presence of migratory corridors formed by river valleys (Kowalczyk et al., 2020; Frangini et al., 2022), and lower human densities in Eastern Europe compared to central Europe, where the mountain ranges of the Alps and Carpathians and heavily populated, strongly modified environment with a dense road network may reduce the pace of dispersal (Frangini et al., 2022). Therefore, dispersal in central Europe may follow instead the stepping stone model (Fabbri et al., 2014). However, golden jackals can disperse rapidly and over long distances, even in human-dominated landscapes (Lanszki et al., 2018). Genetic substructuring within golden jackals in Europe and the presence of a genetically distinct subpopulation in Hungary and Austria suggests that golden jackal colonisation on a larger scale involves smaller groups of individuals dispersing in several distinct,

repeated waves, possibly from different populations of origin. In contrast to the male-biased dispersal observed in several other carnivores (e.g., brown bears (Matosiuk et al., 2019), Eurasian lynx (Herrero et al., 2021)), our results indicate no difference in dispersal rate between sexes in golden jackals.

Range expansion and shifts usually lead to a reduction in genetic diversity with increasing distance from the origin of expansion, as a series of founder events during expansion leads to a progressive loss of genetic variability, resulting in a rapid fixation of alleles through allele surfing in extreme cases (Excoffier et al., 2009). However, range expansion from different directions along a broader expansion front may lead to smaller reduction in genetic diversity, as founders may come from different core populations. Here we found only minor differences when comparing observed heterozygosity values between jackals from southern Europe and the regions where they are currently expanding. However, the number of private alleles is highest in south-eastern Europe, suggesting that this population acted as the expansion source, and very low in central and northern Europe, consistent with the founder effect scenario, and almost mimicking the patterns observed for dispersing coyote populations in North America (Heppenheimer et al., 2018). This could be an example of the dual role of dispersal, where on the one hand migrants create genetic differentiation through the spatial analogue of drift (Slatkin and Excoffier, 2012), while gene flow between established populations reduces genetic differentiation and promotes diversity. Recent genomic studies demonstrated that contact and mixing of lineages colonizing from different source regions reduce the influence of repeated founder events otherwise thought to lead to low genetic diversity outside the core areas (Marková et al., 2020). Indeed, a variety of population genetic processes affect evolution in spatially-expanding populations, which can lead to reduced genetic diversity, the presence of allele frequency gradients, the spread of rare or even deleterious variants at population edges, introgression from local species, and selection for locally adaptive or expansionpromoting traits (Excoffier et al., 2009; Heppenheimer et al., 2018). The inconsistency of range expansion consequences with theoretical predictions indicates that realistic dispersal scenarios tend to be more complex and may be shaped by the interplay of several distinct evolutionary mechanisms.

4.3. Changes in the effective population size of the golden jackal throughout its range

In contrast to slower recovery of apex predators, generalist patterns of habitat use by mesocarnivores have often led to more rapid recovery of the latter, with some species exhibiting rapid expansion across large geographic areas (e.g. coyotes; Heppenheimer et al., 2018). Compared to grey wolf population expansion, which is known to be a recolonization given the reliable records of historical presence, the range expansions of coyotes and golden jackals are examples of rapid colonisation of new areas possibly facilitated by human-mediated climate, habitat and animal-community changes.

Apart from the rapid spread of the golden jackal into regions where it was not found in the past, the species is also recovering and showing expansion in some parts of its historic range, where it

went through temporal declines (Krofel et al., 2017). Our results suggest contrasting patterns of demographic trends throughout the range of the golden jackal. The highest contemporary estimates of effective population size are observed in the Middle East, which is also the region that historically had a long-term stable population, as suggested by the very low average LD decay. A similar pattern is also observed in jackals from India, although current estimates of effective population size appear to be much lower. In the Caucasus, demographic expansion began about 80 years ago, probably as a result of declines in wolf density and abundance, while the recent population decline, which began about 40 years ago, is probably due to greater hunting pressure on jackals in this region (Shakarashvili et al., 2020). A similar pattern was observed in southern and central European populations, with an inferred bottleneck dating back about 50 years ago. This bottleneck could have been the consequence of habitat loss and persecution, including poisoning campaigns, especially in Bulgaria and Greece, after which new expansion waves started (Krofel et al., 2017; Spassov and Acosta-Pankov, 2019).

4.4. Hybridisation with dogs at the edge of the golden jackal's range

Our results suggest that the recent hybridization of golden jackals with other canid species, mainly with free-ranging dogs and possibly also with grey wolves, occurs almost exclusively at the northern edges of the species' range and in regions where jackals have only recently appeared. During range expansion, hybridization between closely related species can occur as a result of low population density of an expanding species coming into regions where a closely related species is already established at a demographic equilibrium. Therefore, when hybrids are found close to the edge of the species' expanding range, hybridization may be the result of range expansion (Nussberger et al., 2018). Although wolf populations show an increasing trend in several countries where jackals have recently expanded (Chapron et al., 2014), our results show that most hybridization events involved free-ranging dogs, which may indicate spatial segregation between wolves and golden jackals, with the latter moving into more open and human-modified habitats where encounters with free-ranging dogs are more likely (Cunze and Klimpel, 2022). Our results also suggest that admixture between golden jackals and grey wolves occurred in the more distant past, consistent with genomic analyses indicating an ancient golden jackal ancestry in wolves from the Middle East (Freedman et al., 2014, Koepfli et al., 2015).

Golden jackals also show signatures of admixture with African golden wolves in the region where the geographic ranges of the two species meet in the Arabian Peninsula (Koepfli et al. 2015, Barash et al. 2023). The geographic range of this introgression could be broader, but we cannot assess it because our study does not include reference samples from African golden wolves. This potential undetected admixture may contribute to the genetic differentiation between golden jackals from the Middle East and those from the neighbouring geographic regions, which should be assessed in future studies.

Introgressive hybridization can threaten the genetic integrity of a species, especially when domesticated counterparts are involved (Allendorf et al., 2001). However, gene flow with a

closely-related species can also be beneficial by increasing genetic diversity, including the transfer of adaptive alleles from one species to another, and therefore it may facilitate range expansion (Pfennig et al., 2016). In general, genetic diversity of edge populations is a limiting factor for dispersal, as adaptability sets the limits for a species' range (Excoffier et al., 2009). Apart from increasing genetic diversity in impoverished populations following founder effects, hybridization may provide alleles for key traits from closely related species already adapted to the new environment (Kirkpatrick and Peischl, 2013). For example, hybridization of coyotes with wolves in North America contributed to increase in coyotes body mass providing them with better hunting abilities, which facilitated their northward expansion (Kays et al., 2010), but also caused the shifts in trophic interactions (Jensen et al., 2022).

The geographic distribution of admixed individuals in our study showed a similar pattern as in the case of coyotes, where hybridisation is mainly reported from the areas of recent (20th century) expansion (Kays et al., 2010; Monzõn et al., 2014; Bohling et al., 2016). However, in contrast to golden jackals, in coyotes the admixture with wolves (grey, eastern and red) outweighs that with domestic dogs (Monzõn et al., 2014). Possible reasons may include the probability of encounters (with free-ranging dogs being more numerous in Eurasia than North America), more intense avoidance of wolves by golden jackals as compared to coyotes, and/or differences in the relative adaptive benefits of cross-breeding with either wolves or domestic dogs. For example, Monzõn et al., (2014) showed that coyotes in areas of high deer density have higher wolf admixture proportions, suggesting natural selection for wolf-like traits leading to local adaptation. Very little is known about the fitness consequences of introgressive hybridisation between golden jackals and other canids, so further research is needed to understand the reason for the contrasting admixture patterns between jackals and coyotes.

It is unknown whether golden jackals' preference towards human-modified landscapes results from the avoidance of wolves or preference towards open habitats, but the resulting admixture with free-ranging dogs may help jackals to acquire adaptations to living in human-modified landscapes, which in turn may accelerate their range expansion. Our study shows that hybridisation with dogs in recently colonized areas does not affect the genetic integrity of the expanding populations. However, studies on other canids show that even rare hybridisation events may have a major effect on phenotypic variation of affected populations via adaptive introgression (e.g. Schweizer et al. 2018). Although gene flow from domestic dogs to coyotes is low throughout North America, coyotes in urbanized areas were shown to carry transposable elements insertions associated with human-directed canine hypersociability (Caragiulo et al., 2022). Accordingly, golden jackals may acquire from dogs domestication-related traits which could enhance their synurbisation. Therefore, further studies are needed to assess the effect of hybridization with dogs on golden jackals' phenotypic traits and their expansion abilities. It is also important to carry out genetic monitoring of golden jackals across the areas of their recent expansion to identify admixed individuals and assess the introgression rates. Moreover, the monitoring should also include the occurrence of atypical phenotypic traits to identify potential introgression of domestication-related traits (morphological, physiological as well as behavioural) that may facilitate the synurbisation process. For example, reduced fear of humans may facilitate colonisation of areas with high human densities and improved ability to digest starch may facilitate using anthropogenic food sources.

4.5. Conclusions

Our results indicate a historical expansion of the golden jackal from Asia to south-eastern Europe at the end of Pleistocene, while its recent dispersal occurred via at least two spatially and temporally separate expansion routes from south-eastern Europe and the Caucasus. From an evolutionary perspective, the current expansion can be considered as a continuation of the longterm expansion process of this species from Asia towards Europe. Surprisingly, we noted that individuals from central and northern Europe, where jackals have only recently emerged, had comparable genetic diversity to individuals from the southern source regions. This finding suggests that genetic diversity may be maintained on the dispersal front due to secondary contact of multiple expansion routes and possibly hybridization with closely related local species, contradicting the usual signatures of reduced diversity following the founder event.

Long-distance dispersal, regional population expansion, and introgression from closely-related species played important roles in the observed patterns of genetic diversity of golden jackals across Eurasia. Although in the recent past the golden jackal has been considered an invasive species in some parts of its expanded range (Trouwborst et al., 2015), our analyses of long-term dispersal patterns and demographic trends suggest its natural expansion across Eurasia (see also Rutkowski et al. 2015) and do not support the classification of the golden jackal as an invasive alien species in northern Europe. Regardless of the factors favouring the expansion of the golden jackal in Europe, this process is occurring remarkably rapidly and raises important questions for wildlife management agencies and legal regulations (Trouwborst et al., 2015; Hatlauf et al., 2021). Golden jackals play an important role in the ecosystem and their expansion can lead to significant changes in predator-prey, scavenger-prey and host-parasite relationships in expanding areas (Gherman and Mihalca, 2017), yet still, most studies to date have not provided scientific evidence of such negative ecological impacts (Hatlauf et al., 2021; but see Krofel et al. 2022a).

We found that the golden jackal's range expansion is associated with an increased hybridisation rate, and this can potentially lead to negative ecological impacts. Our study shows that jackals interbreed primarily with free-ranging dogs and rarer with wolves, which may suggest either their preference towards human-modified areas where the probability of dog encounter is higher, and/or avoidance of wolves.

4.6 Implications for conservation and management

Broad genetic structuring suggests that golden jackal management should focus on large regional units, which implies the need for transboundary cooperation. The development of trans-border legislative procedures has already been demonstrated beneficial for large carnivores (Trouwborst et al., 2015). Although transnational management practices in Europe may be to some extent

facilitated due to the Habitats Directive legislation, there is still considerable conflict regarding the status of the golden jackal not only in national laws of neighbouring countries, but also in regulations within the same country (Hatlauf et al., 2021). Our novel results on its Asian part of range suggest the necessity to develop transboundary cooperation beyond Europe. According to the IUCN, the golden jackal is considered a species of Least Concern (Hoffmann et al. 2018). However, in India the jackal is included in Appendix III of CITES and the 2022 amendment to the Wildlife (Protection) Act 1972 puts it in Schedule I, which gives it the highest level of protection. Our results also show that Indian and Middle Eastern populations of the golden jackal display the highest genetic diversity, thus it becomes important to extend the legal protection of the species to their historical range in the Middle East to conserve the diverse gene pool. Having in mind that our results suggest a low effective population size of golden jackals from India (although it may be underestimated given the sample size used for the calculations) and genetic differentiation between northern and southern India, further monitoring of this population is recommended. Our study, as well as several earlier studies (e.g. Rutkowski et al., 2015; Yumnam et al., 2015, Sosale et al., 2023) shows the importance of Asian golden jackal populations in shaping the phylogeographic and demographic patterns of the species, which warrants further research with more comprehensive sampling of these populations.

The results of our study offer an insight into the issue of the golden jackal's legal status in the areas of its recent expansion. The Conference of the Parties of the Convention on Biological Diversity has defined an "alien species" as a species "introduced outside its natural past or present distribution" (CBD COP Decision VI/23, 2002, Annex). Introduction is defined as the "movement by human agency, indirect or direct", of a species "outside of its natural range (past or present)" (Somsen & Trouwborst, 2019). Accordingly, the European Union's Invasive Alien Species Regulation defines alien species as "introduced outside its natural range" (Article 3(1). Both these legal acts consider an alien species invasive if it threatens native biodiversity (Somsen & Trouwborst, 2019). Our results show that the current expansion of the species northwards can be considered as a continuation of the long-term expansion process of this species driven by the emergence of new habitats suitable for the species, i.e. with limited winter snow cover and open or mosaic landscapes. While past expansion episodes during the Late Quaternary were driven by natural climate and habitat changes (with possible contribution of human-induced changes during the Holocene), the present expansion results from human-induced changes of climate, habitats and animal communities, such as transformation of natural forest habitats into agricultural lands and reduction of densities of apex predators by hunting. Nevertheless, the range expansion is a natural response to these anthropogenic environmental changes, and we found no evidence of any human intervention such as translocations or creation of migration corridors enhancing the species dispersal.

There is a concern that native species expanding their ranges due to climate change may be considered as alien given that the climate change is human induced, but this interpretation has been dismissed. According to the Recommendation by the Bern Convention's parties, the term "alien

species" does not include "native species naturally extending their range in response to climate change" (Recommendation No. 142, 2009, par. 1). Accordingly, the EU's Invasive Alien Species Regulation does not apply to "species changing their natural range without human intervention, in response to changing ecological conditions and climate change" (Article 2(1)-(2)) (Somsen & Trouwborst, 2019). Our findings therefore provide further support to the conclusion by Somsen & Trouwborst (2019) that golden jackals in regions of their recent expansion do not meet the legal definition of alien species and therefore should not be subject to any legal regulations concerning invasive alien species. This implies that no action preventing the spread of golden jackals into new regions of Eurasia is required. More generally, the opposite terms "natural" vs. "alien" species are not sufficient for describing complex range dynamics of taxa in the Anthropocene. For species expanding their range as a consequence of human-induced environmental change, a new term "neonative" has been coined (Essl et al., 2019), which can be applied to expanding golden jackals. In this respect, the focus of the policy on range changes alone is debatable, as human impact on biogeography of world's biotas is traceable since the Pleistocene (Thomas, 2020) and it is difficult to establish the time frame to which the term "native" refers.

The unambiguous conclusion regarding the conservation status of expanding golden jackals gains more complexity when we account for the fact of hybridisation between golden jackals and other canids in regions of the recent expansion. Hybrids and back-crosses do not have the same legal status as representatives of "pure" species. Hybridization is not explicitly addressed in the international laws on nature conservation and therefore the legal status of hybrids is not precisely defined (Trouwborst 2014). Moreover, the status of golden jackal - grey wolf hybrids, resulting from cross-breeding between two wild species, is different compared to the status of golden jackal - domestic dog hybrids. Hybridization with dogs can be considered anthropogenic hybridization, therefore taking preventive and mitigation measures to reduce its rate would be consistent with obligations under the Habitats Directive and the Bern Convention (Trouwborst 2014). This may involve removal or sterilisation of feral and stray dogs, as well as removal of hybrid animals from the wild. On the other hand, according to the Convention and the Directive, the prohibition on the killing and capturing of grey wolves (strictly protected in the European Union), also covers wildliving hybrids between wolves and other species, including jackals (Trouwborst 2014). In practice, golden jackal-wolf and golden jackal-dog hybrids may be difficult to identify and distinguish from each other based on morphological features. Therefore, a systematic monitoring of hybridization is needed, especially in areas where golden jackals have recently expanded. The occurrence of hybrids also stresses the need to increase stability of jackal social groups, which should reduce the frequency of their cross breeding with both dogs and wolves. This can be achieved by the reduction of hunting pressure on jackals, especially during the mating season, given that group stability is primarily linked to survival of the breeding pair (Csányi et al. 2023).

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Data availability

All information necessary to reproduce the results obtained in the manuscript are provided in the main manuscript text and supplementary materials. Raw SNP data obtained in this study are available upon request.

References

Alexander, D.H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19(9), 1655-1664.

Allendorf, F.W., Leary, R.F., Spruell, P., & Wenburg, J.K. (2001). The problems with hybrids: setting conservation guidelines. Trends Ecol. Evol. 16(11), 613-622.

Barash, A., Preiss-Bloom, S., Machluf, Y., Fabbri, E., Malkinson, D., Velli, E., ... & Dekel, Y. (2023). Possible origins and implications of atypical morphologies and domestication-like traits in wild golden jackals (*Canis aureus*). Sci. Rep. 13(1), 7388.

Barbato, M., Orozco-terWengel, P., Tapio, M., & Bruford, M.W. (2015). SNeP: a tool to estimate trends in recent effective population size trajectories using genome-wide SNP data. Front. Genet. 6, 109.

Bazzicalupo, E., Lucena-Perez, M., Kleinman-Ruiz, D., Pavlov, A., Trajçe, A., Hoxha, B., ... & Godoy, J.A. (2022). History, demography and genetic status of Balkan and Caucasian *Lynx lynx* (Linnaeus, 1758) populations revealed by genome-wide variation. Divers. Distrib. 28(1), 65-82.

Bergström, A., Stanton, D.W., Taron, U.H., Frantz, L., Sinding, M.H.S., Ersmark, E., ... & Skoglund, P. (2022). Grey wolf genomic history reveals a dual ancestry of dogs. Nature 607(7918), 313-320.

Bohling, J.H., Dellinger, J., McVey, J.M., Cobb, D.T., Moorman, C.E., & Waits, L.P. (2016). Describing a developing hybrid zone between red wolves and coyotes in eastern North Carolina, USA. Evol. App. 9(6), 791–804.

Caragiulo, A., Gaughran, S.J., Duncan, N., Nagy, C., Weckel, M., & VonHoldt, B. M. (2022). Coyotes in New York City carry variable genomic dog ancestry and influence their interactions with humans. Genes 13(9), 1661.

Caye, K., Deist, T.M., Martins, H., Michel, O., & François, O. (2016). TESS3: fast inference of spatial population structure and genome scans for selection. Mol. Ecol. Resour. 16(2), 540-548.

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., & Lee, J.J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 2015; 4: 7.

Chapron, G., Kaczensky, P., Linnell, J.D., von Arx, M., Huber, D., Andrén, H., ... & Boitani, L. (2014). Recovery of large carnivores in Europe's modern human-dominated landscapes. Science 346(6216), 1517-1519.

Choi, S.K., Kim, K.S., Ranyuk, M., Babaev, E., Voloshina, I., Bayarlkhagva, D., ... & Markov, N. (2020). Asia-wide phylogeography of wild boar (*Sus scrofa*) based on mitochondrial DNA and Y-chromosome: revising the migration routes of wild boar in Asia. PLoS One 15(8), e0238049.

Ćirović, D., Penezić, A., Krofel, M. 2016. Jackals as cleaners: ecosystem services provided by a mesocarnivore in human-dominated landscapes. Biol. Conserv. 199: 51-55.

Csányi, E., Lanszki, J., Heltai, M., Pölös, M., Schally, G., & Sándor, G. (2023). The first evidence of a monogamous golden jackal's adaptive response to partner loss. Appl. Anim. Behav. Sci. 106095.

Cunze, S., & Klimpel, S. (2022). From the Balkan towards Western Europe: range expansion of the golden jackal (*Canis aureus*) - a climatic niche modeling approach. Ecol. Evol. 12(7), e9141.

Diamond, S.E. (2018). Contemporary climate-driven range shifts: putting evolution back on the table. Funct. Ecol. 32(7), 1652-1665.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., & Ovenden, J.R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (ne) from genetic data. Mol. Ecol. Resour. 14(1), 209-214.

Essl, F., Dullinger, S., Genovesi, P., Hulme, P.E., Jeschke, J.M., Katsanevakis, S., Kühn, I., Lenzner, B., Pauchard, A., Pyšek, P., Rabitsch, W., Richardson, D.M., Seebens, H., van Kleunen, M., van der Putten, W.H., Vilà, M., Bacher, S., 2019. A conceptual framework for range-expanding species that track human-induced environmental change. BioScience 69, 908–919.

Excoffier, L., Foll, M., & Petit, R.J. (2009). Genetic consequences of range expansions. Annu. Rev. Ecol. Evol. Syst. 40, 481.

Fabbri, E., Caniglia, R., Galov, A., Arbanasić, H., Lapini, L., Bošković, I., ... & Randi, E. (2014). Genetic structure and expansion of golden jackals (*Canis aureus*) in the north-western distribution range (Croatia and eastern Italian Alps). Conserv. Genet. 15(1), 187-199.

Fenton, S., Moorcroft, P.R., Ćirović, D., Lanszki, J., Heltai, M., Cagnacci, F., ... & Ranc, N. (2021). Movement, space-use and resource preferences of European golden jackals in human-dominated landscapes: insights from a telemetry study. Mammalian Biol. 101, 619-630.

Frangini, L., Sterrer, U., Franchini, M., Pesaro, S., Rüdisser, J., & Filacorda, S. (2022). Stay home, stay safe? High habitat suitability and environmental connectivity increases road mortality in a colonizing mesocarnivore. Landsc. Ecol. 37, 2343-2361

Freedman, A.H., Gronau, I., Schweizer, R.M., Ortega-Del Vecchyo, D., Han, E., Silva, P.M., ... & Novembre, J. (2014). Genome sequencing highlights the dynamic early history of dogs. PLoS Genet.10(1), e1004016.

Galov, A., Fabbri, E., Caniglia, R., Arbanasić, H., Lapalombella, S., Florijančić, T., ... & Randi, E. (2015). First evidence of hybridization between golden jackal (*Canis aureus*) and domestic dog (*Canis familiaris*) as revealed by genetic markers. R. Soc. Open Sci. 2(12), 150450.

Gherman, C.M., & Mihalca, A.D. (2017). A synoptic overview of golden jackal parasites reveals high diversity of species. Parasit. Vectors 10(1), 1-40.

Goudet, J. (2005). Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5(1), 184-186.

Gruber, B., Peter, J.U., Oliver, F.B., & Arthur, G. (2018). dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Mol. Ecol. Resour. 18(3), 691-699.

Guan, Y. (2014). Detecting structure of haplotypes and local ancestry. Genetics 196(3), 625-642.

Hatlauf, J., Bayer, K., Trouwborst, A., & Hackländer, K. (2021). New rules or old concepts? The golden jackal (*Canis aureus*) and its legal status in Central Europe. Eur. J. Wild. Res. 67(2), 1-15.

Herrero, A., Klütsch, C.F., Holmala, K., Maduna, S.N., Kopatz, A., Eiken, H.G., & Hagen, S.B. (2021). Genetic analysis indicates spatial-dependent patterns of sex-biased dispersal in Eurasian lynx in Finland. PLoS One 16(2), e0246833.

Heppenheimer, E., Brzeski, K.E., Hinton, J.W., Patterson, B.R., Rutledge, L.Y., DeCandia, A.L., ... & vonHoldt, B.M. (2018). High genomic diversity and candidate genes under selection associated with range expansion in eastern coyote (*Canis latrans*) populations. Ecol. Evol. 8(24), 12641-12655.

Hody, J.W., & Kays, R. (2018). Mapping the expansion of coyotes (*Canis latrans*) across America. ZooKeys 97, 81–97.

Hoffmann, M., Arnold, J., Duckworth, J.W., Jhala, Y., Kamler, J.F. & Krofel, M. (2018). *Canis aureus* (errata version published in 2020). The IUCN Red List of Threatened Species 2018: e.T118264161A163507876. <u>https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS</u>. T118264161A163507876.en.

Jensen, A.J., Marneweck, C.J., Kilgo, J.C., & Jachowski, D.S. (2022). Coyote diet in North America: geographic and ecological patterns during range expansion. Mammal Rev. 52(4), 480-496.

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27(21), 3070-3071.

Kamler, J.F., Minge, C., Rostro-García, S., Gharajehdaghipour, T., Crouthers, R., In, V., ... & Macdonald, D.W. (2021). Home range, habitat selection, density, and diet of golden jackals in the Eastern Plains landscape, Cambodia. J. Mammalogy 102(2), 636-650.

Kamvar, Z.N., Tabima, J. F., & Grünwald, N.J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2, e281.

Kays, R., Curtis, A., & Kirchman, J.J. (2010). Rapid adaptive evolution of northeastern coyotes via hybridization with wolves. Biol. Lett. 6(1), 89-93.

Kirkpatrick, M., & Peischl, S. (2013). Evolutionary rescue by beneficial mutations in environments that change in space and time. Philos. Trans. R. Soc., B 368(1610), 20120082.

Koblmüller, S., Wayne, R.K., & Leonard, J.A. (2012). Impact of Quaternary climatic changes and interspecific competition on the demographic history of a highly mobile generalist carnivore, the coyote. Biol. Lett. 8(4), 644-647.

Koepfli, K.P., Pollinger, J., Godinho, R., Robinson, J., Lea, A., Hendricks, S., ... & Wayne, R.K. (2015). Genome-wide evidence reveals that African and Eurasian golden jackals are distinct species. Curr. Biol. 25(16), 2158-2165.

Korsten, M., Ho, S.Y., Davison, J., Pähn, B., Vulla, E., Roht, M., ... & Saarma, U. (2009). Sudden expansion of a single brown bear maternal lineage across northern continental Eurasia after the last ice age: a general demographic model for mammals? Mol. Ecol. 18(9), 1963-1979.

Kowalczyk, R., Wudarczyk, M., Wójcik, J.M., & Okarma, H. (2020). Northernmost record of reproduction of the expanding golden jackal population. Mammalian Biology 100(1), 107-111.

Krofel, M., Giannatos, G., Ćirovič, D., Stoyanov, S., & Newsome, T.M. (2017). Golden jackal expansion in Europe: a case of mesopredator release triggered by continent-wide wolf persecution? Hystrix: Italian J. Mammal. 28(1), 9-15.

Krofel, M., Hočevar, L., Fležar, U., Topličanec, I., Oliveira, T. 2022a. Golden jackal as a new kleptoparasite for Eurasian lynx in Europe. Global Ecology and Conservation 36: e02116.

Krofel, M., Hatlauf, J., Bogdanowicz, W., Campbell, L.A.D., Godinho, R., Jhala, Y.V., Kitchener, A.C., Koepfli, K.-P., Moehlman, P., Senn, H., Sillero-Zubiri, C., Viranta, S., Werhahn, G., Alvares, F. 2022b. Towards resolving taxonomic uncertainties in wolf, dog and jackal lineages of Africa, Eurasia and Australasia. J. Zool. 316: 155–168.

Lanszki, J., Schally, G., Heltai, M., & Ranc, N. (2018). Golden jackal expansion in Europe: first telemetry evidence of a natal dispersal. Mammalian Biology 88(1), 81-84.

Lanszki, J., Hayward, M.W., Ranc, N., & Zalewski, A. (2022). Dietary flexibility promotes range expansion: The case of golden jackals in Eurasia. J. Biogeogr. 49(6), 993-1005.

Liu, X., & Fu, Y.X. (2020). Stairway plot 2: demographic history inference with folded SNP frequency spectra. Genome Biol. 21(1), 1-9.

Loog, L., Thalmann, O., Sinding, M.H.S., Schuenemann, V.J., Perri, A., Germonpré, M., ... & Manica, A. (2020). Ancient DNA suggests modern wolves trace their origin to a late Pleistocene expansion from Beringia. Mol. Ecol. 29(9), 1596-1610.

Männil, P., & Ranc, N. (2022). Golden jackal (*Canis aureus*) in Estonia: development of a thriving population in the boreal ecoregion. Mammal Research 67(2), 245-250.

Marková, S., Horníková, M., Lanier, H.C., Henttonen, H., Searle, J.B., Weider, L.J., & Kotlík, P. (2020). High genomic diversity in the bank vole at the northern apex of a range expansion: the role of multiple colonizations and end-glacial refugia. Mol. Ecol. 29(9), 1730-1744.

Matosiuk, M., Śmietana, W., Czajkowska, M., Paule, L., Štofik, J., Krajmerová, D., ... & Ratkiewicz, M. (2019). Genetic differentiation and asymmetric gene flow among Carpathian brown bear (*Ursus arctos*) populations - implications for conservation of transboundary populations. Ecol. Evol. 9(3), 1501-1511.

McDevitt, A.D., Coscia, I., Browett, S.S., Ruiz-González, A., Statham, M.J., Ruczyńska, I., ... & Wójcik, J.M. (2022). Next-generation phylogeography resolves post-glacial colonization patterns in a widespread carnivore, the red fox (*Vulpes vulpes*), in Europe. Mol. Ecol. 31(3), 993-1006.

Miller, T.E., Angert, A.L., Brown, C.D., Lee-Yaw, J.A., Lewis, M., Lutscher, F., ... & Williams, J. L. (2020). Eco-evolutionary dynamics of range expansion. Ecology 101(10), e03139.

Moehlman, P.D., & Hayssen, V. (2018). *Canis aureus* (Carnivore: Canidae). Mamm. Species 50(957), 14-25.

Monzõn, J., Kays, R., & Dykhuizen, D.E. (2014). Assessment of coyote-wolf- dog admixture using ancestry-informative diagnostic SNPs. Mol. Ecol. 23(1), 182–197.

Moura, A.E., Tsingarska, E., Dąbrowski, M.J., Czarnomska, S.D., Jędrzejewska, B., & Pilot, M. (2014). Unregulated hunting and genetic recovery from a severe population decline: the cautionary case of Bulgarian wolves. Conservation Genetics 15(2), 405-417.

Newsome, T.M., Greenville, A.C., Ćirović, D., Dickman, C.R., Johnson, C.N., Krofel, M., ... & Wirsing, A.J. (2017). Top predators constrain mesopredator distributions. Nat. Commun. 8(1), 15469.

Nowak, R.M. (2002). The original status of wolves in eastern North America. Southeast. Nat. 1(2), 95–130.

Nussberger, B., Currat, M., Quilodran, C.S., Ponta, N., & Keller, L.F. (2018). Range expansion as an explanation for introgression in European wildcats. Biol. Conserv. 218, 49-56.

Pacifici, M., Rondinini, C., Rhodes, J.R., Burbidge, A.A., Cristiano, A., Watson, J.E., ... & Di Marco, M. (2020). Global correlates of range contractions and expansions in terrestrial mammals. Nat. Commun. 11(1), 2840.

Pecl, G.T., Araújo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Chen, I.C., ... & Williams, S.E. (2017). Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. Science 355(6332), eaai9214.

Pfennig, K.S., Kelly, A.L., & Pierce, A.A. (2016). Hybridization as a facilitator of species range expansion. Proc. R. Soc. B Biol. Sci. 283(1839), 20161329.

Pickrell, J., & Pritchard, J. (2012). Inference of population splits and mixtures from genome-wide allele frequency data. Nature Proceedings 1.

Raj, A., Stephens, M., & Pritchard, J.K. (2014). fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics 197(2), 573-589.

Rutkowski, R., Krofel, M., Giannatos, G., Ćirović, D., Männil, P., Volokh, A.M., ... & Bogdanowicz, W. (2015). A European concern? Genetic structure and expansion of golden jackals (*Canis aureus*) in Europe and the Caucasus. PLoS One 10(11), e0141236.

Rykov, A.M., Kuznetsova, A.S., & Tirronen, K.F. (2022). The first record of the golden jackal (*Canis aureus* Linnaeus, 1758) in the Russian subarctic. Polar Biol. 45(5), 965-970.

Šálek, M., Červinka, J., Banea, O.C., Krofel, M., Ćirović, D., Selanec, I., ... & Riegert, J. (2014). Population densities and habitat use of the golden jackal (Canis aureus) in farmlands across the Balkan Peninsula. Eur. J. Wildlife Res. 60, 193-200.

Santiago, E., Novo, I., Pardiñas, A.F., Saura, M., Wang, J., & Caballero, A. (2020). Recent demographic history inferred by high-resolution analysis of linkage disequilibrium. Mol. Biol. Evol. 37(12), 3642-3653.

Schweizer RM, Durvasula A, Smith J, Vohr SH, Stahler DR, Galaverni M, Thalmann O, Smith D, Randi E, Ostrander EA, Lohmueller K, Green RE, Novembre J, Wayne RK. (2018). Natural selection and origin of a melanistic allele in North American gray wolves. Mol. Biol. Evol. 35:1190-1209.

Shakarashvili, M., Kopaliani, N., Gurielidze, Z., Dekanoidze, D., Ninua, L., & Tarkhnishvili, D. (2020). Population genetic structure and dispersal patterns of grey wolfs (*Canis lupus*) and golden jackals (*Canis aureus*) in Georgia, the Caucasus. J. Zool. 312(4), 227-238.

Slatkin, M., & Excoffier, L. (2012). Serial founder effects during range expansion: a spatial analog of genetic drift. Genetics 191(1), 171-181.

Sommer, R., & Benecke, N. (2005). Late-Pleistocene and early Holocene history of the canid fauna of Europe (Canidae). Mamm. Biol. 70(4), 227-241.

Somsen, H., Trouwborst, A. (2019). Are pioneering coyotes, foxes and jackals alien species? Canid colonists in the changing conservation landscape of the Anthropocene. Oryx 54(3), 392–394

Sørensen, O., & Lindsø, L. (2021). The golden jackal Canis aureus detected in Norway– Management challenges with naturally dispersed species new to the country. Fauna 74(3–4), 74-87.

Sosale, M.S., Songsasen, N., İbiş, O., Edwards, C., Figueiró, H.V., & Koepfli, K.P. (2023). The complete mitochondrial genome and phylogenetic characterization of two subpopulations of golden jackal (*Canis aureus cruesemanni* and *Canis aureus moreotica*). Gene 147303.

Spassov, N., & Acosta-Pankov, I. (2019). Dispersal history of the golden jackal (*Canis aureus moreoticus* Geoffroy, 1835) in Europe and possible causes of its recent population explosion. Biodiversity Data Journal 7.

Stratford, J. (2015). Golden jackal in Lithuania, a consideration of its arrival, impact and status. Zool. Ecol. 25(4), 277–287

Thomas, C.D. (2020) The development of Anthropocene biotas. Philos. Trans. R. Soc. B 375, 20190113.

Trouwborst, A. (2014) Exploring the legal status of wolf-dog hybrids and other dubious animals. Review of European, Comparative & International Environmental Law 23, 111–124.

Trouwborst, A., Krofel, M., & Linnell, J. D. (2015). Legal implications of range expansions in a terrestrial carnivore: the case of the golden jackal (*Canis aureus*) in Europe. Biodivers. Conserv. 24(10), 2593-2610.

Vereshchagin, N.K. (1959). Mammals of the Caucasus: The History of the Fauna. Academy of Sciences of the USSR Institute of Zoology, Moscow, Leningrad.

Wang, M.S., Thakur, M., Jhala, Y., Wang, S., Srinivas, Y., Dai, S.S., ... & Shapiro, B. (2022). Genome sequencing of a gray wolf from peninsular India provides new insights into the evolution and hybridization of gray wolves. Genome Biol. Evol. 14(2), evac012.

Wereszczuk, A., Leblois, R., & Zalewski, A. (2017). Genetic diversity and structure related to expansion history and habitat isolation: stone marten populating rural–urban habitats. BMC Ecol. *17*, 1-16.

Wilson, J.R., García-Díaz, P., Cassey, P., Richardson, D.M., Pyšek, P., & Blackburn, T.M. (2016). Biological invasions and natural colonisations are different – the need for invasion science. NeoBiota 31, 87–98.

Yumnam, B., Negi, T., Maldonado, J.E., Fleischer, R.C., & Jhala, Y.V. (2015). Phylogeography of the golden jackal (*Canis aureus*) in India. PLoS One 10(9), e0138497.

Zhang, C., Dong, S.S., Xu, J.Y., He, W.M., & Yang, T.L. (2019). PopLDdecay: a fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. Bioinformatics 35(10), 1786-1788.

Figures



Figure 1. Genetic structure, diversity and changes in effective population size over time in golden jackals across Eurasia

A. Map showing genetic clusters derived from TESS analysis with K = 8 using the 20 K dataset. The dots represent individuals, while the interpolated values of the ancestry coefficients are shown for each cluster, with the colour gradient corresponding to the degree of ancestry. Genetic diversity indices for each cluster are showing mean allelic richness across all loci (Ar), expected heterozygosity (He), and observed heterozygosity (Ho) in boxes in font coloured according to the cluster memberships. Labels for 20 K dataset: SIND - southern India, NIND - northern India and Iran, CAU - the Caucasus and Eastern Europe, BAL - the Baltic countries, primarily Estonia, SAM - the island of Samos, Greece, SCE - Southern and Central Europe, GRE - mainland Greece and the Adriatic coast, HUN - mainly Hungary. B. Demographic histories for SCE (red) and CAU (blue) golden jackals genetic clusters inferred by Stairway plot for 150 K data set, with the effective population size (Ne) on the Y-axis and the time before present on the Xaxis. C. Plot of the first two principal components based on 20 K dataset, coloured as the cluster groups revealed in the TESS analysis. D. Admixture plot (top diagram) and fastStructure plot (bottom diagram) for the 150 K dataset. E. Admixture plot for K = 8 for the 20 K data set. F. The trend in modern effective population size (Ne) of golden jackals over last 1000 generations based on 150 K dataset. G. Decay in linkage disequilibrium (r2) over increasing intermarker distance for 150 K dataset. The designations for the 150 K dataset are the same as for the 20 K dataset, except that India is not subdivided into north and south, and IRN stands for Iran. In panels D and E, countries are labeled according to ISO 3166-1 alpha-3 codes, while NAK is used for Nagorno-Karabakh. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Figure 2. Hybridization of golden jackals with free-ranging dogs and wolves.

Map showing the distribution of admixed jackal individuals throughout the study area. The yellow shaded area represents the distribution of the golden jackal as indicated in the IUCN database. (Black dots – locations of golden jackals analysed). In the right upper corner, the studied area is enclosed in the world map. Only individuals showing < 95 % of jackal ancestry are plotted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)