Genomics 113 (2021) 3430–3438

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Novel genome reveals susceptibility of popular gamebird, the red-legged partridge (*Alectoris rufa*, Phasianidae), to climate change

Balaji Chattopadhyay [a,b,1], Giovanni Forcina [a,c,d,1,1], Kritika M. Garg [a,e], Martin Irestedt [f], Monica Guerrini [c], Filippo Barbanera [c,g,**,2], Frank E. Rheindt [a,*,2]

[a] Department of Biological Sciences, National University of Singapore, 16 Science Drive 4, Singapore 117543, Republic of Singapore
[b] Trivedi School of Biosciences, Ashoka University, Sonipat 131029, Haryana, India
[c] Department of Biology, University of Pisa, Via A. Volta 4, I-56126 Pisa, Italy
[d] CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal
[e] Department of Biology, Ashoka University, Sonipat 131029, Haryana, India
[f] Department of Bioinformatics and Genetics, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden
[g] CIRSEC, Centre for Climatic Change Impact, University of Pisa, Via del Borghetto 80, I-56124 Pisa, Italy

ARTICLE INFO

Keywords:
Alectoris
Game species
Gene ontology
Genome assembly
PSMC
Run of homozygosity

ABSTRACT

We produced a high-quality *de novo* genome assembly of the red-legged partridge *A. rufa*, the first reference genome of its genus, by utilising novel 10× Chromium technology. The estimated genome size was 1.19 Gb with an overall genome heterozygosity of 0.0022; no runs of homozygosity were observed. In total, 21,589 protein coding genes were identified and assigned to 16,772 orthologs. Of these, 201 emerged as unique to *Alectoris* and were enriched for positive regulation of epithelial cell migration, viral genome integration and maturation. Using PSMC analysis, we inferred a major demographic decline commencing ~140,000 years ago, consistent with forest expansion and reduction of open habitats during the Eemian interglacial. Present-day populations exhibit the historically lowest genetic diversity. Besides implications for management and conservation, this genome also promises key insights into the physiology of these birds with a view to improving poultry husbandry practices.

1. Introduction

The genus *Alectoris* Kaup, 1829 (Phasianidae) consists of seven closely related polytypic game species exhibiting either allopatric or parapatric distributions across the Palaearctic. The phylogeny of this genus is not fully resolved as previous studies have been constrained by the use of only single mitochondrial DNA markers and limited sample sizes [1,2]. The relatively recent inception of this radiation (6 to 2 MYA: [1]) may account for the porous nature of interspecific reproductive barriers [3–5]. This lack of complete reproductive isolation (which is a quite common phenomenon in birds: [6]) is evident from the capability of hybrids to produce not only viable but also fertile offspring, both in the narrow zones between parapatric *Alectoris* species in the wild [7] as well as during human-mediated hybridisation associated with wildlife relocation for hunting purposes (e.g., [8–11]). These circumstances render the genus an ideal model to address micro- and macro-evolutionary questions spanning adaptive radiation, species diversification and ecological adaptation. Although *Alectoris* partridges have been the focus of a plethora of evolutionary studies since the 1990s (e.g., [1,2,12,13]), much of this work has been tentative as neither their phylogenetic relationships nor their adaptive radiation have been comprehensively addressed.

Recent scientific attention to *Alectoris* has been mainly propelled by commercial interests. The red-legged partridge *A. rufa* (Linnaeus, 1758), in particular, is a game species of considerable socio-economic importance in southwestern Europe and one of the most iconic birds in the traditional heritage of hunters and gourmets of the Old World. It may be the most valuable among Europe's small game species [14–16], with

* Correspondence to: F. E. Rheindt, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore.
** Correspondence to: F. Barbanera, Department of Biology, University of Pisa, Via A. Volta 4, I-56126 Pisa, Italy.
E-mail addresses: filippo.barbanera@unipi.it (F. Barbanera), dbsrfe@nus.edu.sg (F.E. Rheindt).
[1] Joint first authorship.
[2] Joint senior authorship

https://doi.org/10.1016/j.ygeno.2021.08.010
Received 15 December 2020; Received in revised form 2 August 2021; Accepted 11 August 2021
Available online 13 August 2021

more than four million farm-reared birds/year released in Spain for hunting over the last four decades [17,18]. Commonly found among the archaeozoological remains in human settlements dating back to the Palaeolithic [19–22], the red-legged partridge has been renowned since the times of the Roman Empire as a prized delicacy and was later portrayed in a number of still life paintings in 17th century Europe typically alongside other valuable game species (e.g., Supplementary Fig. 1).

The red-legged partridge currently occurs in scrublands and other open and well-drained terrestrial habitats [23,24] from the Iberian Peninsula across central and southern France to northwestern and central Italy, including Mediterranean islands (the Balearics, Corsica and the Tuscan Archipelago). It was introduced into Macaronesia (Azores, Madeira and the Canaries) over the last centuries [25] and into the United Kingdom in the second half of the 18th century as a valuable courtly gamebird [26–28], while attempts to establish self-sustaining populations occurred in Greece, Algeria, United States of America and New Zealand during the 20th century [29]. Nowadays, the red-legged partridge is harvested and reared by the millions every year [30,31] and its management has attracted massive funding by governmental agencies. Molecular investigations with a conservation management perspective began in the early 2000s [9,32–35] and were followed by numerous studies dealing with the species' ecology (e.g., [36–41] and husbandry [42,43]. More recently, the analysis of individual-based overall immune response [44] and transcriptome characterisation of genes expressed in different immune tissues [45] marked the advent of comparative genomics in this popular game species.

Since the second half of the 20th century, the red-legged partridge has experienced a sharp global demographic decline across 95% of its distribution range [46] due to the synergistic effects of overhunting, agricultural mechanisation, use of pesticides and rural abandonment [47–52]. These factors have warranted the inclusion of the red-legged partridge in the list of threatened species under European Union legislation (79/409 CEE Ap.2/1, 3/I; BERN Ap.3) and the status of Species of European Conservation Concern category 2 (“Vulnerable”: [53]). Moreover, the International Union for the Conservation of Nature and Natural Resources has recently upgraded the species' conservation status from Least Concern to Near Threatened [54]. More recently, an increasing and possibly more treacherous threat has been posed by human-mediated introgressive hybridisation with the congeneric but geographically disjunct chukar partridge (*A. chukar*) through captive breeding and illegal release of hybrid birds for restocking purposes [9,16,17,32,33,55–62]. This practice became widespread to supplement local populations and mitigate the effects of unsustainable “put-and-take” hunting [63]. On the other hand, chukar releases have often been accompanied by disease outbreaks [64–68] through parasite [69–71] and pathogen (e.g., [72,73]) transmission. Moreover, by spreading maladaptive traits selected for in captivity [16], the release of farm-reared birds can impair the evolutionary potential of wild populations to cope with present and future changes through the erosion of local gene pools [74]. In the easternmost part of the red-legged partridge's mainland distribution, drastic declines of native self-sustaining populations [11,34] have promoted the expansion of *A. rufa* captive-breeding and related restocking. As such, the species has been exposed to intense introgressive hybridisation and to the infiltration of non-native alleles through translocations from hetero-subspecific stocks of uncertain origin and admixed genetic identity [10,75]. In this context, the virtual extinction (sensu [74]) of the nominate *A. r. rufa* subspecies native to France and Italy has been mooted in the literature [9,34,56], while a further progression of biotic [76] and genetic [77] homogenisation also seems to compromise the integrity of the two Iberian subspecies [16,17,60,78]. Overall, this adulteration of native populations may have occurred to such an extent that the molecular identification of traditional subspecies on a geographic basis seems no longer possible ([11,59,79] but see [80,81]).

In this study, we produced a novel high-quality genome assembly (96× coverage) of the red-legged partridge, representing the first complete genome for the genus *Alectoris*. We then used publicly available and fully annotated avian genomes for comparative purposes including genome size estimation, gene annotation and functional assignment. Moreover, we reconstructed the demographic history of the red-legged partridge using the pairwise sequentially Markovian coalescent (PSMC) model to estimate changes in effective population size, thus inferring declines or expansions in the context of palaeoclimatic events which may have acted as drivers of these processes. Overall, our results demonstrate the utility of the first *Alectoris* genome in advancing our knowledge of the ecology and evolution of this genus in general and the red-legged partridge in particular. We also present important genomic conclusions regarding the physiology of this species that may have a positive bearing on poultry husbandry practices.

2. Materials and methods

2.1. Biological sampling

We captured a male red-legged partridge (nominate subspecies *A. r. rufa*) on the eastern slopes of Monte Maolo (730 m a. s. l.: 42°46′24.34″N, 10°11′19.01″E), located in the western part of Elba Island (Tuscan Archipelago National Park, Tuscany, central Italy), on 7 January 2004. The local partridge population is of undisputed national value because of its comparatively long persistence (i.e., since it was officially recorded it has never gone extinct unlike other Italian populations), self-sustainability and lack of restocking in the last decades [75]. During trapping efforts, which were managed by personnel of the former “Corpo Forestale dello Stato” (Italian Forest Service: Marciana Marina and Lucca), we used a wooden box covered with evergreen foliage and baited with commercial poultry feed (Supplementary Fig. 2). We collected a few drops of blood (ca. 0.5 ml) via puncture of the brachial vein and preserved them in a citrate-dextrose solution (ACD, 44 mM Citric Acid trisodium salt, 25 mM Citric Acid, 71 mM Glucose) before the bird was released less than 48 h later (Supplementary Video 1). The biological sample was stored at −40 °C upon arrival at the laboratory of the University of Pisa. Blood collection was carried out by former Italian Forest Service staff members (Lucca) in accordance with institutional ethics and upon issuance of due permits (Commissioner Resolution n. 307, 31 December 2003, Tuscan Archipelago National Park).

2.2. DNA isolation and library preparation

We extracted total genomic DNA using the King-Fisher™ Duo Prime Magnetic Particle Processor (Thermo Fisher Scientific, Waltham, MA, USA) and the King-Fisher Cell and Tissue DNA Kit (Thermo Fisher Scientific) following the manufacturer's protocol. 10× Chromium library preparation (adding location barcodes to fragments originating from single long DNA molecules by means of bead-in-emulsion barcoding, thus allowing post sequencing re-assembly of short reads into pseudo long-reads: [82]), sequencing on one Illumina HiseqX lane and subsequent de novo genome assembly were carried out by Science for Life Laboratory (SciLifeLab) in Stockholm.

2.3. Quality control, reference genome sequencing and assembly

We demultiplexed raw sequencing data and converted them from Bcl to FastQ files using bcl2fastq v.2.19.1.403 implemented in the CASAVA suite before transfer to the Uppsala Multidisciplinary Center for Advanced Computational Science [UPPMAX] (http://www.uppmax.uu.se/) for delivery. To ensure that all sequenced data met the guaranteed basic quality and quantity, we performed standardised bioinformatic control checks including assessments of yield, sequence read quality and cross-sample contamination before delivery. The quality scale used was Sanger/phred33/Illumina 1.8 + .

We used 10× Genomics Chromium Supernova v.2.1.1 [83] for read

processing, including the removal of low quality and clonally duplicated reads as well as adaptor trimming and genome assembly. This latter task relied on the statistics implemented in BUSCO (Benchmarking Universal Single-Copy Orthologs) v.3.0.2 [84] and QUAST v.5.0.2 [85] in the nf-core/neutronstar pipeline (https://github.com/nf-core/neutronstar). We obtained a 96× coverage of paired-end sequence data for de novo scaffold assembly of the red-legged partridge genome from the genomic DNA libraries. Genome redundancy was further reduced following [86]: we first pinpointed identical scaffolds using the sequniq function in GenomeTools v. 1.6.1 [87], which led to the removal of 479 elements. Next, we identified scaffolds <2 Mb in size and with ≥99% identity using CD-HIT v. 4.8.1 [88,89], discarding 925 additional elements. Finally, we used LAST v.1111 [90] to pinpoint scaffolds with ≥99% identity and ≥ 95% coverage, corroborating that prior steps had already removed highly identical scaffolds falling under this criterion. After all these clean-up steps we retained 10,598 scaffolds for downstream analyses. We assessed quality and completeness of the new genome assembly by checking for read pair coverage information and the associated standard contiguity metrics as inferred with BUSCO v.4.1.4. We used avian OrthoDB v.10.1 [91] for identifying single copy orthologs in BUSCO.

2.4. Genome size estimation

We used kmer analysis to estimate genome size by generating a frequency distribution of 17-mers with JELLYFISH v.2.2.6 [92]. Genome size was estimated as the ratio of k_num/k_depth, wherein k_num is the total number of k-mers and k_depth is the frequency of the most common k-mer. We estimated average autosomal coverage from clean reads using SAMtools 0.1.19-96b5f2294a [93] and overall genome coverage from the total number of reads obtained by following [94].

2.5. Genome-wide heterozygosity estimation and runs of homozygosity

We quantitatively assessed global genome-wide heterozygosity as well as runs of homozygosity (ROH) using the Bayesian framework implemented in ROHan [95]. We performed two different runs using ROHan assuming a transition to transversion rate of 2.1 (default estimate) and 2.5 (as estimated for the domestic chicken, *Gallus gallus*: [96]). We used default settings to define a ROH and considered a region as such if the average heterozygosity was below 0.00001 across a window size of 1 Mb.

2.6. Repeat masking

We used RepeatMasker v.4.1.0 [97] to identify and mask repeats across the genome. We employed the domestic chicken repeat library and performed a hard mask for further downstream processing.

2.7. Gene annotation and functional assignment

Unless otherwise stated, default parameters were applied for the different software used for downstream analyses. The ab initio gene prediction tool AUGUSTUS v.3.2.2 [98] was employed to identify genes using the repeat-masked partridge genome. We allowed recourse to hints from the chicken genome for gene predictions on both strands. Moreover, we performed functional annotations for the predicted protein-coding genes using eggNOG-mapper v.2.0.0 [99,100] along with the KEGG (Kyoto Encyclopedia of Genes and Genomes) automatic annotation server 2.1 (KAAS: [101]). The tool eggNOG-mapper enables a fast annotation of novel protein-coding genes based on orthology assignment as inferred from precomputed clusters and phylogenies [99], while KAAS performs gene annotation by using BLAST and by comparing the protein-coding genes to the manually curated KEGG database [101]. We also performed Gene Ontology (GO) functional enrichment analysis using the Pannzer2 web-based server, which carries out annotation using SANSparallel for homology search [102]. Finally, we compared the predicted protein coding genes across domestic chicken, zebra finch (*Taeniopygia guttata*) and Japanese quail (*Coturnix japonica*) proteomes using OrthoVenn2 [103]. We used a 0.00001 *E*-value cutoff in protein similarity comparisons and set the inflation value to 1.5 for generating orthologous clusters.

2.8. Demographic analysis

The demographic history of the red-legged partridge was reconstructed by means of the PSMC method [104], which is becoming increasingly popular by virtue of its power to deliver insights into the temporal fluctuations of a species in terms of effective population size (N_e) based on recombination and heterozygosity information from a single genome [94,105–107]. For details about this analytical pipeline, see [94]. In brief, we cleaned raw reads by removing adapter contamination with Trimmomatic 0.38 [108] and excluding any read that mapped to the chicken sex chromosomes (GenBank Accession ID: CM000121.5, CM000122.5) as well as to the mitogenome of the Japanese quail (GenBank accession code: AP003195.2). We mapped clean reads to the partridge genome using BWA-MEM 0.7.7-r441 [109], retaining only those with a high mapping score (> 20). We used samtools mpileup and bcftools to identify variable sites using the following parameters: -C 50, −d 20 and —D 130. The PSMC analyses consisted of the following parameters: -t 15 -r 5 -p 4 + 25*2 + 4 + 6 and comprised 30 iterations for parameter optimisation and 100 bootstraps to obtain a measure of uncertainty around parameter estimates. Effective population size was calculated using a mutation rate of 1.91×10^{-9} substitutions per site per year from the chicken genome [110]. We allowed for a generation time of 1 year (the time needed to reach sexual maturity in the red-legged partridge) and 2 years, which is conventionally used for many avian species [111,112].

3. Results

3.1. Sequencing results

A total of 378.59 million reads (Mreads) was generated, with 83.54% of bases exhibiting a quality score > Q30 (Illumina Q-value ≥ Q30). The average fragment size of the library was 614.57 bp.

3.2. Basic genome statistics

The estimated *A. rufa* genome size as inferred from k-mer analysis was 1.19 Gb. The overall genome coverage based on the sequencing effort was 96×, while the estimated coverage for autosomes was 67×. For the final assembly, the N50 was 11.577 Mb and total genome length 1.028 Gb with a largest contig length of 47.55 Mb. Based on BUSCO analysis, we identified 94.9% of single copy avian orthologs (7913 single copy orthologs out of 8338 proteins) in our genome, thus providing evidence for the good quality of our assembly. Overall genome heterozygosity was 0.0022 and no runs of homozygosity were detected on the basis of different transition to transversion ratios. We found few repetitive elements (5.58%: Table 1), while the overall GC content was

Table 1
Percentage of various repetitive elements detected within the red-legged partridge genome.

Repetitive element	Percentage of the genome
Short-interspersed nuclear elements	0.04%
Long- interspersed nuclear elements	3.96%
DNA transposons	0.11%
Simple repeats	1.17%
Small RNA	0.02%
Low complexity regions	0.26%
Unclassified	0.03%

41.44%.

3.3. Genome annotation

We detected 21,589 protein coding genes within the *A. rufa* genome. For 77.69% of these (n = 16,772), we identified orthologs using eggNOG-mappers. We further assigned GO terms to 12,013 protein coding genes and KEGG identifiers to 9049 proteins. When we compared the genome of the red-legged partridge to that of domestic chicken, zebra finch and Japanese quail, we observed 9171 common orthologous gene families and 201 gene families unique to the red-legged partridge, including a total of 1166 genes (Fig. 1). Finally, GO enrichment analysis across the unique gene families suggested the involvement of some of them in the positive regulation of processes such as epithelial cell migration, DNA recombination, viral genome integration and procapsid maturation (Table 2).

3.4. Demographic history

PSMC analyses revealed the demographic history of the red-legged partridge over the Quaternary. Results were consistent across both reconstructions (using a 1 and 2 year generation time). We observed signs of major cycles of population fluctuation over the last million years (Fig. 2). However, we restricted our interpretations to more recent events as deeper historical estimates are deemed to be less reliable with PSMC [113]. Most importantly, ~140,000 years ago marked the beginning of a steep decline in red-legged partridge effective population size (N_e) that led to a long spell characterised by unusually low levels of population size during subsequent years into the present Holocene. A

Table 2
Gene ontology (GO) enrichment for the 201 gene families (consisting of 1166 genes) unique to the red-legged partridge genome and isolated from an initial dataset of 21,589 protein coding genes. For the sake of clarity, GO terms were assigned to 54 gene families (out of 201) encompassing a total of 561 genes, with enrichment analyses pointing to 6 significant GO terms comprising 16 genes.

GO ID	GO term - number of genes	Ontology aspect	*p*-value
GO:0046797	Viral procapsid maturation - 3	Biological process	5.1E-08
GO:0010634	Positive regulation of epithelial cell migration - 3	Biological process	5.1E-08
GO:0006310	DNA recombination - 2	Biological process	8.34E-05
GO:0044826	Viral genome integration into host DNA - 2	Biological process	2.07E-04
GO:0006369	Termination of RNA polymerase II transcription - 2	Biological process	1.6E-03
GO:0007165	Signal transduction - 4	Biological process	1.7E-02

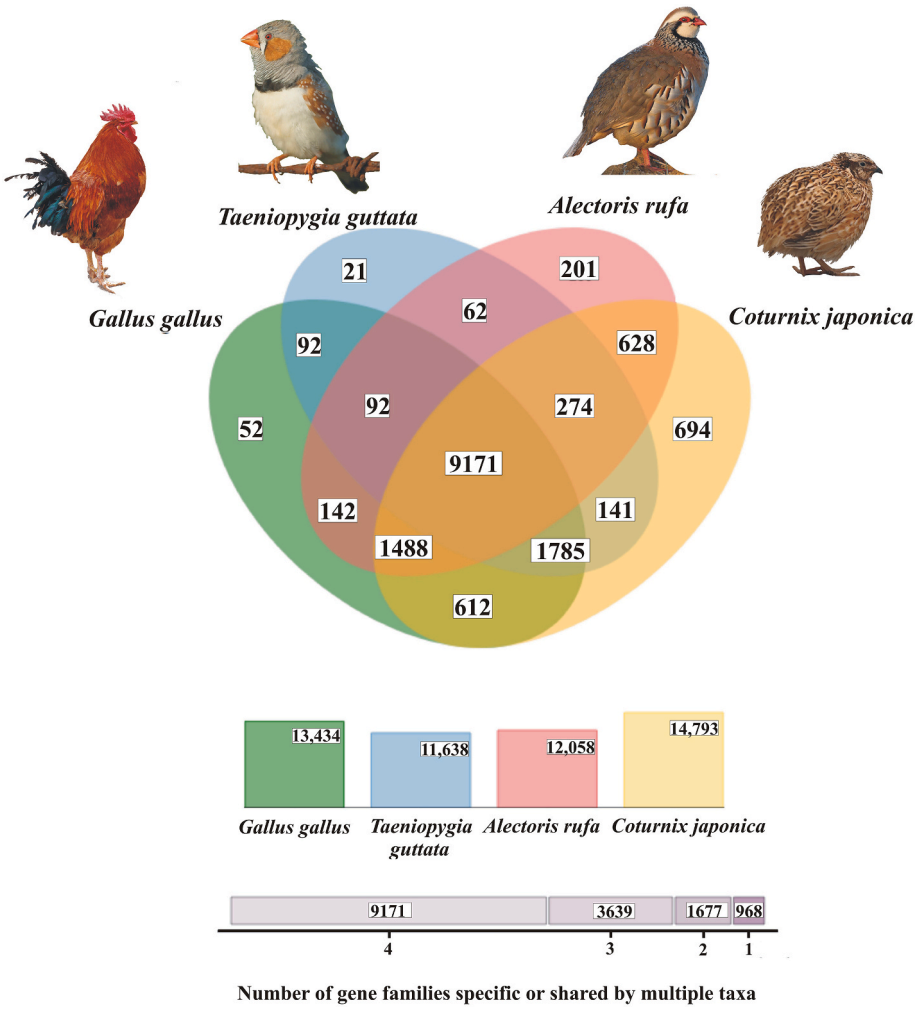

Fig. 1. Venn diagram of orthologous gene families either unique to or shared across select avian taxa (left to right: domestic chicken, zebra finch, red-legged partridge and Japanese quail) compared in this study. Pictures are not to scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

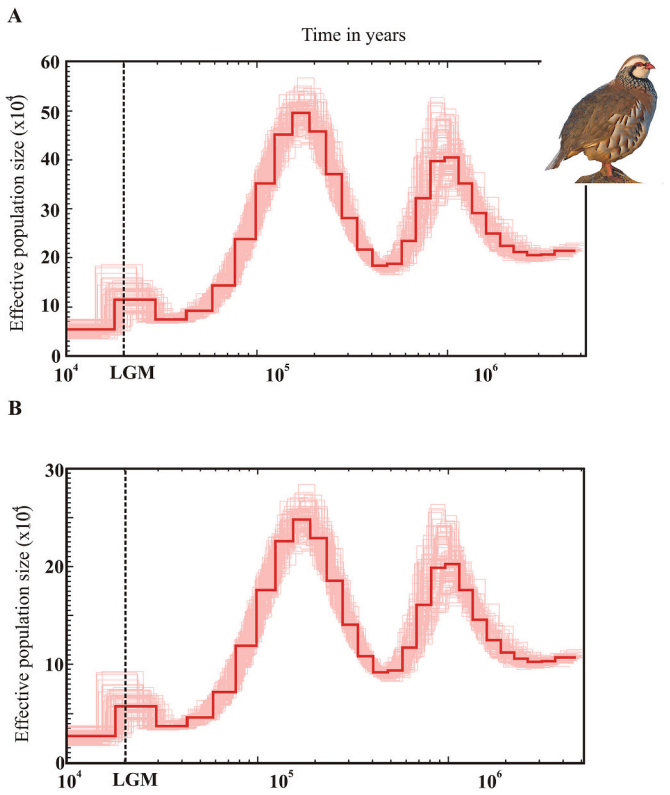

Fig. 2. Fluctuations in the effective population size of the red-legged partridge based on complete sequence (dark red) and bootstrap data (light red). The Last Glacial Maximum (LGM) is denoted with a black dotted line (~22,000 years ago). (A) Based on a generation time of 1 year, corresponding to the time to reach sexual maturity in this species [137]. (B) Based on a generation time of 2 years, corresponding to a widely-applied figure across birds [111,112]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

minor recovery of effective population size around the Last Glacial Maximum (~20,000 years ago) notwithstanding, this trend has continued into the most recent times, with an inference of the historically lowest-ever levels of effective population size during the transition into the Holocene (Fig. 2). PSMC bootstrap values were close to the parameter estimates, corroborating the robustness of our inferences especially during the last ~150,000 years (Fig. 2).

4. Discussion

In spite of the socioeconomic and conservation importance of *Alectoris* partridges, the genomic infrastructure for this genus has so far been limited to transcriptomic approaches [44,45]. We here sequenced the first high-quality and complete genome assembly of the red-legged partridge at 96× coverage on the basis of an individual from a population of high conservation value inhabiting Elba Island in central Italy [114].

4.1. Genomic features

The estimated genome size of the red-legged partridge (1.19 Gb) is within the range typically expected for birds in general [115] and for Galliformes in particular [116]. Equally, GC content (41.45%) was similar to that found in *G. gallus* [117], which is consistent with the lack of inter-macrochromosomal rearrangements between the two genera [118], both of which belong to the same family (Phasianidae). Considering that phylogenetic distance is a key factor affecting assembly completeness using homology-based approaches [119], we greatly benefited from the availability of the complete domestic chicken reference genome GCA_000002315.5. Overall genome heterozygosity (2.2 SNPs/kbp) was quite high as compared with that of other avian species [120]. This outcome, along with the total absence of runs of homozygosity, is surprising when considering that this genome belongs to a resident gamebird from a small insular population (i.e., Elba) which underwent a strong demographic decline recently [75], as the occurrence of such genomic features would be rather expected in inbred populations which have experienced a bottleneck [e.g., 105,121,122]. However, extensive restocking activities for hunting purposes carried out in some sectors of Elba Island from the 1950s to the 1990s may explain this seeming contradiction. Restocking reached its peak during the 1960s, when up to >4000 captive individuals were released annually in the southeastern sector of the island, whereas releases over the following decades involved fewer birds but were conducted at an increased frequency and across the entire island [75]. This sustained release of farm-reared hybrids with *A. chukar* and of hetero-subspecific stocks of Spanish descent has previously been invoked as the likely underlying cause of signals of genetic admixture in the Elba population [10,57,75; but see 114]. Hence, it is plausible that continual gene flow with released partridges has counterbalanced the effects of bottlenecks, preventing the local population from genetic depletion and inbreeding. Finally, the low incidence of repetitive elements (5.58%: Table 1) is consistent with values from the highly streamlined genomes of other birds, which typically display lower levels when compared with other tetrapod vertebrates [123–125].

4.2. Functional assignment

Concerning the 201 gene families identified as unique to *Alectoris* (Fig. 1), we observed an enrichment of genes involved in immune response and pathogen resistance among other functions (Table 2), consistent with previous findings pointing to a fairly strong ability of partridges to cope with environmental stressors and diseases [44]. Enrichment in genes involved in epithelial cell migration, a function associated with wound healing (e.g., [126]), can be interpreted in light of the morphological traits and behavioural ecology of *Alectoris* partridges, which comprise poor fliers. More specifically, wound repair might be particularly important in view of the intense physical stress faced by partridges when running on rough terrain, skulking in thorny vegetation, as well as the explosive muscle performance of their short-burst flights to escape predators.

4.3. Demographic analyses

For successful population management of economically important animals, it is vital to understand their adaptive biology in response to past climate change. Our analyses have revealed the signature of a massive historic decline in effective population size in the red-legged partridge in response to the onset of the previous interglacial (the 'Eemian' warm phase at ~140,000–110,000 years ago; Fig. 2: [127]). Against the backdrop of a warming Europe, this demographic decline is consistent with a dramatic expansion of forest habitat beyond the boundaries of today's forest belt, thereby greatly reducing the amount of natural open scrub and bushy grassland preferred by partridges. Among the three most recent interglacials before the present, the Eemian was the first one sufficiently warm and humid to match present-day conditions [128]. It marked the mild termination of a roughly 200,000 year-long European cold and dry spell characterised by glacial periods interrupted by two weaker interglacials. Our data confirm that *Alectoris* partridges – as open-land inhabitants – thrive demographically during periods of global cooling when drier conditions lead to a contraction of forest habitat. After the end of the Eemian interglacial (~110,000 years ago), the demographic decline of the red-legged partridge did not reverse, with continuing drops in effective population size until reaching a low plateau at ~70,000 years ago (Fig. 2). This trend is consistent with

vegetation reconstructions from pollen cores that indicate a persistence of forest habitat across Europe despite cooling temperatures [127]. Even after the onset of the first major ice advance between ~74,000 and 59,000 years ago, steady expansions of open steppe and grassland were frequently thwarted by re-expansions of conifers or juniper woodlands [127]. It was probably only during the build-up towards the very end of the last ice age, near its maximum (~20,000 years ago), that open habitat again became a steadier feature of Europe's landscape, as reflected in the slight recovery of red-legged partridges' effective population size between ~30,000 and ~ 18,000 years ago (Fig. 2). After the abrupt end of the Last Glacial Maximum and the precipitous increase of global temperatures and expansion of European forests post-15,000 BP, effective population size in this species again dropped markedly (Fig. 2), this time to the lowest documented level throughout its natural history.

PSMC analysis provided strong evidence for the susceptibility of *Alectoris* partridges to climatic fluctuations. Since effective population size is a powerful proxy for genetic diversity, conservationists and managers must consider that Europe's present forest-dominated landscape is the historically least favourable vegetation regime that red-legged partridge populations have experienced, leading to their fragmentation across relatively small scrub-dominated Mediterranean habitat patches that have remained suitable. While early human forest conversion and crop cultivation in the Mediterranean may have created novel habitats and favoured a secondary expansion of red-legged partridges, the effects of modern hunting and agricultural mechanisation would have quickly counteracted any such positive impact and generated further genetic bottlenecks.

4.4. Perspectives

This novel high-quality genome will be essential for future investigations into the evolutionary history of the genus *Alectoris*, which has been a model taxon in studies about avian adaptive radiations for more than 20 years [1,2]. Moreover, this genome will also aid in better understanding the adaptive responses of *Alectoris* partridges to global change. The availability of this resource in combination with the genomic characterisation of museum specimens pre-dating the spread of restocking practices will be invaluable for conservation management, allowing us to reconstruct the progression of a likely genomic erosion using a time series [129]. As a reference genome, it will also enhance the power of admixture analyses aimed at identifying the most preserved wild stock to be used for restocking purposes [130]. This genome will facilitate studies into the adaptive responses of admixed populations in increasingly changing environments, and the partial retention or loss of non-native genomic features based on local selective pressures and demographic trends. In addition, the release of the first *Alectoris* genome promises to represent a major contribution also to poultry husbandry, health and production studies on the red-legged partridge and related species, thus delivering key insights into the mechanisms underlying meat [131–133] and egg [134,135] production as well as disease resistance [136].

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2021.08.010.

Funding

This research was supported by the Erasmus Mundus project EURASIACAT (ref. no. 552067) coordinated by the University of Málaga and a fellowship from the Portuguese Foundation for Science and Technology (FCT PTDC/BAA-AGR/28866/2017) to G.F. M.I acknowledges support from the Swedish Research Council (621-2014- 5113), B.C. acknowledges SEABIG funding (R-154-000-648-733) from the National University of Singapore and startup funding from Trivedi School of Biosciences, Ashoka University, India. K.M.G. acknowledges support from the DBT-Ramalingaswami Fellowship (No. BT/HRD/35/02/2006). F.E.R acknowledges funding from a Singapore Ministry of Education Tier 2 grant (WBS R-154-000-C41-112).

Author contributions

F.B. and F.E.R. conceived the study; F.B. and M.G. collected the biological sample; M.G. carried out laboratory work; M.I. sequenced the sample; B.C. and K.M.G. analysed the molecular data and performed the genomic analyses; B.C., G.F., K.G. and F.E.R. interpreted the data. G.F. and B.C. prepared the figures; G.F. wrote the first draft of the manuscript; all authors contributed to discussions, review and editing.

Data archiving

The dataset used for this study has been deposited in GenBank under BioProject PRJNA662415/BioSample accession SAMN16083939. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JADBKV000000000. The version described in this paper is version JADBKV010000000.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

The authors are thankful to Renato Giombini, Federico Parri (former Corpo Forestale dello Stato, Marciana Marina), Fabio Cappelli, Franco Bertoncini (former Corpo Forestale dello Stato, Lucca) and Francesca Giannini (Parco Nazionale Arcipelago Toscano) for assisting in the sampling of the red-legged partridge on Elba Island. The pictures of zebra finch (https://commons.wikimedia.org/wiki/File:Taeniopygia_guttata_-_Bushell%27s_Lagoon.jpg, CC BY-SA 4.0), red-legged partridge (https://commons.wikimedia.org/wiki/File:Perdrix_rouge.jpg, CC BY-SA 3.0), domestic chicken (https://commons.wikimedia.org/wiki/File:Gallus_gallus_domesticus_-_Lanzarote.jpg#filelinks, CC-BY-SA-3.0) and Japanese quail (https://commons.wikimedia.org/wiki/File:Japanese_Quail.jpg, CC BY 2.0) used in Fig. 1 were freely available in Wikimedia Commons and authored by John J. Harrison (https://www.jjharrison.com.au/), Pierre Dalous, H. Zell, and Ingrid Taylar, respectively. The authors acknowledge support from the National Genomics Infrastructure in Stockholm funded by Science for Life Laboratory, the Knut and Alice Wallenberg Foundation, the Swedish Research Council and SNIC/Uppsala Multidisciplinary Center for Advanced Computational Science for assistance with massively parallel sequencing and access to the UPPMAX computational infrastructure.

Author statement

F.B. and F.E.R. conceived the study; F.B. and M.G. collected the biological sample; M.G. carried out laboratory work; M.I. sequenced the sample; B.C. and K.M.G. analysed the molecular data and performed the statistical genomic analyses; B.C., G.F., K.G. and F.E.R. interpreted the data. G.F. and B.C. prepared the figures; G.F. wrote the first draft of the manuscript; all authors contributed to discussions, review and editing.

References

[1] E. Randi, A mitochondrial cytochrome b phylogeny of the *Alectoris* partridges, Mol. Phylogenet. Evol. 6 (1996) 214–227, https://doi.org/10.1006/mpev.1996.0072.
[2] E. Randi, V. Lucchini, Organization and evolution of the mitochondrial DNA control region in the avian genus *Alectoris*, J. Mol. Evol. 47 (1998) 449–462, https://doi.org/10.1007/pl00006402.
[3] G.E. Watson, Sympatry in Palearctic *Alectoris* partridges, Evolution 16 (1962) 11–19.
[4] G.E. Watson, Three sibling species of *Alectoris* partridges, Ibis 104 (1962) 353–367, https://doi.org/10.1111/j.1474-919X.1962.tb08663.x.

[5] J. Blondel, Biogéographie évolutive à differentes échelles: L'histoire des avifaunes méditerranéennes, Acta XIX Congressus Internationalis Ornithologici 1 (1988) 1658–1664.

[6] E.M. McCarthy, Handbook of Avian Hybrids of the World, Oxford University Press, Oxford, New York, NY, 2006.

[7] E. Randi, C. Tabarroni, S. Rimondi, V. Lucchini, A. Sfougaris, Phylogeography of the rock partridge (*Alectoris graeca*), Mol. Ecol. 12 (2003) 2201–2214, https://doi.org/10.1046/j.1365-294X.2003.01899.x.

[8] M. Barilani, A. Sfougaris, A. Giannakopoulos, N. Mucci, C. Tabarroni, E. Randi, Detecting introgressive hybridization in rock partridge populations (*Alectoris graeca*) in Greece through Bayesian admixture analyses of multilocus genotypes, Conserv. Genet. 8 (2007) 343–354, https://doi.org/10.1007/s10592-006-9174-1.

[9] M. Barilani, A. Bernard-Laurent, N. Mucci, C. Tabarroni, S. Kark, J.P. Garrido, E. Randi, Hybridisation with introduced chukars (*Alectoris chukar*) threatens the gene pool integrity of native rock (*A. graeca*) and red-legged (*A. rufa*) partridge populations, Biol. Conserv. 137 (2007) 57–69, https://doi.org/10.1016/J.BIOCON.2007.01.014.

[10] F. Barbanera, M. Guerrini, A.A. Khan, P. Panayides, P. Hadjigerou, C. Sokos, et al., Human-mediated introgression of exotic chukar (*Alectoris chukar*, Galliformes) genes from East Asia into native Mediterranean partridges, Biol. Invasions 11 (2009) 333–348, https://doi.org/10.1007/s10530-008-9251-0.

[11] F. Barbanera, O.R.W. Pergams, M. Guerrini, G. Forcina, P. Panayides, F. Dini, Genetic consequences of intensive management in game birds, Biol. Conserv. 143 (2010) 1259–1268, https://doi.org/10.1016/j.biocon.2010.02.035.

[12] V. Lucchini, E. Randi, Mitochondrial DNA sequence variation and phylogeographical structure of rock partridge (*Alectoris graeca*) populations, Heredity 81 (1998) 528–536, https://doi.org/10.1046/j.1365-2540.1998.00413.x.

[13] E. Randi, A. Bernard-Laurent, Population genetics of a hybrid zone between the red-legged partridge and rock partridge, Auk 116 (1999) 324–337, https://doi.org/10.2307/4089367.

[14] R.L. Bernabeu, Evaluación económica de la caza en Castilla-La Mancha. PhD thesis, Universidad de Castilla-La Mancha, Spain, 2000.

[15] C. Gortázar, R. Villafuerte, M.A. Escudero, J. Marco, Post-breeding densities of the red-legged partridge (*Alectoris rufa*) in agrosystems: a large-scale study in Aragón, northeastern Spain, Z. Jagdwiss. 48 (2002) 94–101, https://doi.org/10.1007/BF02193547.

[16] M. Martínez-Fresno, N. Henriques-Gil, P. Arana, Mitochondrial DNA sequence variability in red-legged partridge, *Alectoris rufa,* Spanish populations and the origins of genetic contamination from *A. chukar*, Conserv. Genet. 9 (2008) 1223–1231, https://doi.org/10.1007/s10592-007-9449-1.

[17] J.A. Blanco-Aguiar, P. Gonzalez-Jara, M.E. Ferrero, I. Sánchez-Barbudo, E. Virgós, R. Villafuerte, J.A. Dávila, Assessment of game restocking contributions to anthropogenic hybridization: the case of the Iberian red-legged partridge, Anim. Conserv. 11 (2008) 535–545, https://doi.org/10.1111/j.1469-1795.2008.00212.x.

[18] F. Casas, F. Mougeot, J. Viñuela, Double-nesting behaviour and sexual differences in breeding success in wild red-legged partridges *Alectoris rufa*, Ibis 151 (2009) 743–751, https://doi.org/10.1111/j.1474-919X.2009.00958.x.

[19] S.J.M. Davis, The mammals and birds from the Gruta do Caldeirão, Portugal, Rev. Arq. Port. 5 (2002) 29–98.

[20] M. Moreno García, C. Pimienta, Arqueozoología cultural: o aerofone de Conímbriga, Rev. Port. Arqueol. 7 (2004) 407–425.

[21] M. Moreno-Garcìa, C. Detry, The dietary role of hens, chickens and eggs among a 17th-century monastic order: the Clarisse of Santa Clara-a-Velha, Coimbra (Portugal). Birds in Archaeology, Proceedings of the 6th Meeting of the ICAZ Bird Working Group in Groningen (23.8–27.8.2008), Groningen Archaeological Studies 12 (2010) 45–55.

[22] J. Haws, Paleolithic socionatural relationships during MIS 3 and 2 in Central Portugal, Quat. Int. 264 (2012) 61–77, https://doi.org/10.1016/j.quaint.2011.10.003.

[23] J. del Hoyo, A. Elliott, J. Sargatal, Handbook of the Birds of the World Volume 2, New World Vultures to Guineafowl. Barcelona, Lynx Edicions, 1994.

[24] F.J. Buenestado, P. Ferreras, M. Delibes-Mateos, F.S. Tortosa, J.A. Blanco-Aguiar, R. Villafuerte, Habitat selection and home range size of red-legged partridges in Spain, Agric. Ecosyst. Environ. 126 (2008) 158–162, https://doi.org/10.1016/j.agee.2008.01.020.

[25] S. Madge, P. McGowan, Pheasants, Partridges and Grouse, A and C Black Ltd., London, 2002.

[26] C. Lever, The Naturalized Animals of the British Isles, Hutchinson, London, 1977.

[27] D.W. Snow, C.M. Perrins, The Birds of the Western Palearctic, Oxford University Press, London, 1998.

[28] G.R. Potts, Partridges, HarperCollins Publishers, London, 2012.

[29] C.M. Miskelly, Red-legged partridge, in: C.M. Miskelly (Ed.), New Zealand Birds, 2015. www.nzbirdsonline.org.nz (Accessed: 06 Jul 2020).

[30] F. Ponce-Boutin, La perdrix rouge, Gibier Faune Sauvage 251 (2000) 46–53.

[31] J. Millán, C. Gortázar, R. Villafuerte, Does supplementary feeding affect organ and gut size of wild red-legged partridges *Alectoris rufa*? Wildl. Biol. 9 (2003) 229–233, https://doi.org/10.2981/wlb.2003.055.

[32] J.J. Negro, M.J. Torres, J.A. Godoy, RAPD analysis for detection and eradication of hybrid partridges (*Alectoris rufa* x *A. graeca*) in Spain, Biol. Conserv. 9 (2001) 19–24, https://doi.org/10.1016/S0006-3207(00)00129-4.

[33] M. Baratti, M. Ammannati, C. Magnelli, F. Dessì-Fulgheri, Introgression of chukar genes into a reintroduced red-legged partridge (*Alectoris rufa*) population in Central Italy, Anim. Genet. 36 (2005) 29–35, https://doi.org/10.1111/j.1365-2052.2004.01219.x.

[34] F. Barbanera, J.J. Negro, G. Di Giuseppe, F. Bertoncini, F. Cappelli, F. Dini, Analysis of the genetic structure of red-legged partridge (*Alectoris rufa*, Galliformes) populations by means of mitochondrial DNA and RAPD markers: a study from Central Italy, Biol. Conserv. 122 (2005) 275–287, https://doi.org/10.1016/j.biocon.2004.07.017.

[35] F. Barbanera, M. Guerrini, P. Hadjigerou, P. Panayides, C. Sokos, P. Wilkinson, et al., Genetic insight into Mediterranean chukar (*Alectoris chukar*, Galliformes) populations inferred from mitochondrial DNA and RAPD markers, Genetica 131 (2007) 287–298, https://doi.org/10.1007/s10709-006-9138-x.

[36] R. Borralho, C. Stoate, M. Araujo, Factors affecting the distribution of red-legged partridges *Alectoris rufa* in an agricultural landscape of southern Portugal, Bird Study 47 (2000) 304–310, https://doi.org/10.1080/00063650009461190.

[37] G.R. Potts, Some differences in the breeding ecology of *Alectoris* and *Perdix* partridges and implications for the conservation of *Alectoris*: a review, Avocetta 37 (2013) 107–110.

[38] A.J. Carpio, J. Guerrero-Casado, F.S. Tortosa, J. Vicente, Predation of simulated red-legged partridge nests in big game estates from South Central Spain, Eur. J. Wildl. Res. 60 (2014) 391–394, https://doi.org/10.1007/s10344-013-0786-8.

[39] A.J. Carpio, J. Oteros, J. Vicente, F.S. Tortosa, J. Guerrero-Casado, Factors affecting red-legged partridge *Alectoris rufa* abundance on big-game hunting estates: Implications for management and conservation, Ardeola 62 (2015) 283–297, https://doi.org/10.13157/arla.62.2.2015.283.

[40] J. Nadal, C. Ponz, A. Margalida, Age and sex ratios in a high-density wild red-legged partridge population, PLoS One 11 (2016), e0159765, https://doi.org/10.1371/journal.pone.0159765.

[41] J. Nadal, C. Ponz, A. Margalida, The effects of scaling on age, sex and size relationships in red-legged partridges, Sci. Rep. 8 (2018) 2174, https://doi.org/10.1038/s41598-018-20576-x.

[42] V.R. Gaudioso, C. Sánchez-García, J.A. Pérez, P.L. Rodríguez, J.A. Armenteros, M. E. Alonso, Does early antipredator training increase the suitability of captive red-legged partridges (*Alectoris rufa*) for releasing? Poult. Sci. 90 (2011) 1900–1908, https://doi.org/10.3382/ps.2011-01430.

[43] J.A. Pérez, C. Sánchez-García, C. Díez, D.J. Bartolomé, M.E. Alonso, V. R. Gaudioso, Are parent-reared red-legged partridges (*Alectoris rufa*) better candidates for re-establishment purposes? Poult. Sci. J. 94 (2015) 2330–2338, https://doi.org/10.3382/ps/pev210.

[44] N. Sevane, J. Cañon, I. Gil, S. Dunner, Transcriptomic characterization of innate and acquired immune responses in red-legged partridges (*Alectoris rufa*): a resource for immunoecology and robustness selection, PLoS One 10 (2015), e0136776, https://doi.org/10.1371/journal.pone.0136776.

[45] N. Sevane, J. Cañon, P.G. Eusebi, I. Gil, S. Dunner, Red-legged partridge (*Alectoris rufa*) de-novo transcriptome assembly and identification of gene-related markers, Genom. Data 11 (2017) 132–134, https://doi.org/10.1016/j.gdata.2017.02.003.

[46] N.J. Aebischer, G.R. Potts, Red-legged partridge *Alectoris rufa*, in: G.M. Tucker, M. F. Heath (Eds.), Birds in Europe, their Conservation Status. Birdlife Conservation Series no 3, BirdLife International, Cambridge, 1994, pp. 214–215.

[47] M.R.W. Rands, Effects of hedgerow characteristics on partridge breeding densities, J. Appl. Ecol. 23 (1986) 479–487, https://doi.org/10.2307/2404030.

[48] A. Lucio, F.J. Purroy, Red legged partridge (*Alectoris rufa*) habitat selection in Northwest Spain, Gibier Faune Sauvage 9 (1992) 417–429.

[49] J.A. Blanco-Aguiar, E. Virgós, R. Villafuerte, La perdiz roja (*Alectoris rufa*), in: R. Martí, J.C. Del Moral (Eds.), Atlas de las Aves Reproductoras de España, Madrid: Dirección General de Conservacion de la Naturaleza-Sociedad Española de Ornitología, 2003, pp. 212–213.

[50] J.M. Vargas, J.C. Guerrero, M.A. Farfán, A. Marcia Barbosa, R. Real, Land use and environmental factors affecting red-legged partridge (*Alectoris rufa*) hunting yields in southern Spain, Eur. J. Wildl. Res. 52 (2006) 188–195, https://doi.org/10.1007/s10344-006-0028-4.

[51] F. Casas, J. Viñuela, Agricultural practices or game management: which is the key to improve red-legged partridge nesting success in agricultural landscapes? Environ. Conserv. 37 (2010) 177–186, https://doi.org/10.1017/S0376892910000299.

[52] A. López-Antia, M.E. Ortiz-Santaliestra, E. García-de Blas, P.R. Camarero, F. Mougeot, R. Mateo, Adverse effects of thiram-treated seed ingestion on the reproductive performance and the offspring immune function of the red-legged partridge, Environ. Toxicol. Chem. 34 (2015) 1320–1329, https://doi.org/10.1002/etc.2925.

[53] BirdLife International, Birds in Europe: Population Estimates, Trends and Conservation Status. Bird Life Conservation Series *volume* 12, BirdLife International, Wageningen, The Netherlands, 2004, pp. 1–374.

[54] https://www.iucnredlist.org/species/22678711/183481909#conservation-actions (Accessed: 14 Dec 2020).

[55] M. Vallance, G. Queney, D. Soyez, J.C. Ricci, *Mise au point et validation d'un système de marqueurs génétiques pour les perdrix hybrids* (pp. 76-81), Rapport scientifique, ONCFS, 2006.

[56] M.T. Tejedor, L.V. Monteagudo, S. Mautner, E. Hadjisterkotis, M.V. Arruga, Introgression of *Alectoris chukar* genes into a Spanish wild *Alectoris rufa* population, J. Hered. 98 (2007) 179–182, https://doi.org/10.1093/jhered/esm001.

[57] M. Guerrini, F. Barbanera, Noninvasive genotyping of the red-legged partridge (*Alectoris rufa*, Phasianidae): semi-nested PCR of mitochondrial DNA from feces, Biochem. Genet. 47 (2009) 873–883, https://doi.org/10.1007/s10528-009-9288-5.

[58] N. Sevane, O. Cortés, D. García, J. Cañón, S. Dunner, New single nucleotide polymorphisms in *Alectoris* identified using chicken genome information allow *Alectoris* introgression detection, Mol. Ecol. Resour. 10 (2010) 205–213, https://doi.org/10.1111/j.1755-0998.2009.02738.x.

[59] F. Barbanera, G. Forcina, M. Guerrini, F. Dini, Molecular phylogeny and diversity of Corsican red-legged partridge: hybridization and management issues, J. Zool. 285 (2011) 56–65, https://doi.org/10.1111/j.1469-7998.2011.00813.x.

[60] F. Casas, F. Mougeot, I. Sánchez-Barbudo, J.A. Dávila, J. Viñuela, Fitness consequences of anthropogenic hybridization in wild red-legged partridge (*Alectoris rufa*, Phasianidae) populations, Biol. Invasions 14 (2012) 295–305, https://doi.org/10.1007/s10530-011-0062-3.

[61] A. Negri, I. Pellegrino, N. Mucci, E. Randi, P. Tizzani, P.G. Meneguz, G. Malacarne, Mitochondrial DNA and microsatellite markers evidence a different pattern of hybridization in red-legged partridge (*Alectoris rufa*) populations from NW Italy, Eur. J. Wildl. Res. 59 (2013) 407–419, https://doi.org/10.1007/s10344-012-0686-3.

[62] F. Barbanera, G. Forcina, A. Cappello, M. Guerrini, H. van Grouw, N.J. Aebischer, Introductions over introductions: the genomic adulteration of an early genetically valuable alien species in the United Kingdom, Biol. Invasions 17 (2015) 409–422, https://doi.org/10.1007/s10530-014-0739-5.

[63] S.M. Byers, G.V. Burger, Evaluation of three partridge species for put and take hunting, Wildl. Soc. Bull. 7 (1979) 17–20.

[64] C. Gortázar, R. Villafuerte, M. Martín, Success of traditional restocking of red-legged partridge for hunting purposes in areas of low density of Northeast Spain Aragón, Z. Jagdwiss. 46 (2000) 23–30, https://doi.org/10.1007/BF02240661.

[65] J. Millán, C. Gortázar, M.P. Martin-Mateo, R. Villafuerte, Comparative survey of the ectoparasite fauna of wild and farm-reared red-legged partridges (*Alectoris rufa*), with an ecological study in wild populations, Parasitol. Res. 93 (2004) 605–611, https://doi.org/10.1007/s00436-004-1113-9.

[66] C. Gortázar, P. Acevedo, F. Ruis-Fons, J. Vicente, Disease risks and overabundance of game species, Eur. J. Wildl. Res. 52 (2006) 81–87, https://doi.org/10.1007/s10344-005-0022-2.

[67] F.J. Buenestado, P. Ferreras, J.A. Blanco-Aguiar, F.R. Tortosa, R. Villafuerte, Survival and causes of mortality among wild red-legged partridges *Alectoris rufa* in southern Spain: implications for conservation, Ibis 154 (2009) 720–730, https://doi.org/10.1111/j.1474-919X.2009.00952.x.

[68] J. Millán, Diseases of the red-legged partridge (*Alectoris rufa* L.): a review, Wildl. Biol. Pract. 5 (2009) 70–88, https://doi.org/10.2461/wbp.2009.5.2.

[69] J. Millán, C. Gortázar, R. Villafuerte, A comparison of the helminth faunas of wild and farm-reared red-legged partridges, J. Wildl. Manag. 68 (2004) 701–707, https://doi.org/10.2193/0022-541X(2004)068[0701:ACOTHF]2.0.CO;2.

[70] J. Millán, C. Gortázar, R. Villafuerte, Ecology of nematode parasitism in red-legged partridges (*Alectoris rufa*) in Spain, Helminthologia 41 (2004) 33–37.

[71] D. Villanúa, L. Pérez-Rodríguez, F. Casas, V. Alzaga, P. Acevedo, J. Viñuela, C. Gortázar, Sanitary risks of red-legged partridge releases: introduction of parasites, Eur. J. Wildl. Res. 54 (2008) 199–204, https://doi.org/10.1007/s10344-007-0130-2.

[72] K. Bertran, E. Pérez-Ramírez, N. Busquets, R. Dolz, A. Ramis, A. Darji, et al., Pathogenesis and transmissibility of highly (H7N1) and low (H7N9) pathogenic avian influenza virus infection in red-legged partridge (*Alectoris rufa*), Vet. Res. 42 (2011) 24, https://doi.org/10.1186/1297-9716-42-24.

[73] S. Díaz-Sánchez, A. Moriones Mateo, F. Casas, U. Höfle, Prevalence of *Escherichia coli*, *Salmonella* sp. and *Campylobacter* sp. in the intestinal flora of farm reared, restocked and wild red-legged partridges (*Alectoris rufa*): is restocking using farm-reared birds a risk? Eur. J. Wildl. Res. 58 (2012) 99–105, https://doi.org/10.1007/s10344-011-0547-5.

[74] J. Rhymer, D. Simberloff, Extinction by hybridization and introgression, Annu. Rev. Ecol. Evol. Syst. 27 (1996) 83–109, https://doi.org/10.1146/annurev.ecolsys.27.1.83.

[75] G. Forcina, M. Guerrini, F. Barbanera, Non-native and hybrid in a changing environment: conservation perspectives for the last Italian red-legged partridge (*Alectoris rufa*) population with long natural history, Zoology 138 (2020) 125740, https://doi.org/10.1016/j.zool.2019.125740.

[76] J.D. Olden, N.L. Poff, M.R. Douglas, E.M. Douglas, K.D. Fausch, Ecological and evolutionary consequences of biotic homogenization, Trends Ecol. Evol. 19 (2004) 18–24, https://doi.org/10.1016/j.tree.2003.09.010.

[77] J.D. Olden, L. Comte, G. Xingli, Biotic Homogenisation. In *eLS*, John Wiley & Sons, Ltd, Chichester, 2016, https://doi.org/10.1002/9780470015902.a0020471.pub2.

[78] D. Dias, Rock (*Alectoris graeca*) and chukar (*A. chukar*) partridge introductions in Portugal and their possible hybridization with red-legged partridges (*A. rufa*): a research project, Gibier Faune Sauvage 9 (1992) 781–784.

[79] M.J. Rodríguez-García, J. Galián, Lack of mitochondrial genetic structure in the red-legged partridge *Alectoris rufa* (Phasianidae), J. Zool. Syst. Evol. Res. 52 (2014) 59–64, https://doi.org/10.1111/jzs.12039.

[80] M.E. Ferrero, J.A. Blanco-Aguiar, S.C. Lougheed, I. Sánchez-Barbudo, P.J.G. de Nova, R. Villafuerte, J.A. Dávila, Phylogeography and genetic structure of the red-legged partridge (*Alectoris rufa*): more evidence for refugia within Iberian glacial refugium, Mol. Ecol. 20 (2011) 2628–2642, https://doi.org/10.1111/j.1365-294X.2011.05111.x.

[81] M.E. Ferrero, J.A. Blanco-Aguiar, J.A. Davila, Climatic and geographic effects on the spatial genetic pattern of a landbird species (*Alectoris rufa*) on the Iberian Peninsula, Popul. Ecol. 58 (2016) 429–440, https://doi.org/10.1007/s10144-016-0553-2.

[82] A. Ott, J.C. Schnable, C.-T. Yeh, L. Wu, C. Liu, H.C. Hu, Linked read technology for assembling large complex and polyploid genomes, BMC Genomics 19 (2018) 651, https://doi.org/10.1186/s12864-018-5040-z.

[83] N.I. Weisenfeld, V. Kumar, P. Shah, D.M. Church, D.B. Jaffe, Direct determination of diploid genome sequences, Genome Res. 27 (2017) 757–767, https://doi.org/10.1101/gr.214874.116.

[84] R.M. Waterhouse, M. Seppey, F.A. Simão, M. Manni, P. Ioannidis, G. Klioutchnikov, et al., BUSCO applications from quality assessments to gene prediction and phylogenomics, Mol. Biol. Evol. 35 (2017) 543–548, https://doi.org/10.1093/molbev/msx319.

[85] A. Gurevich, V. Saveliev, N. Vyahhi, G. Tesler, G. QUAST: quality assessment tool for genome assemblies, Bioinformatics 29 (2013) 1072–1075.

[86] M.Y. Ozerov, F. Ahmad, R. Gross, L. Pukk, S. Kahar, V. Kisand, A. Vasemägi, Highly Continuous Genome Assembly of Eurasian Perch (*Perca fluviatilis*) Using Linked-Read Sequencing, G3 (Bethesda) 8, 2018, pp. 3737–3743, https://doi.org/10.1534/g3.

[87] G. Gremme, S. Steinbiss, S. Kurtz, GenomeTools: a comprehensive software library for efficient processing of structured genome annotations, IEEE/ACM Trans. Comput. Biol. Bioinform. 10 (2013) 645–656, https://doi.org/10.1109/TCBB.2013.68.

[88] W. Li, A. Godzik, Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences, Bioinformatics 22 (2006) 1658–1659, https://doi.org/10.1093/bioinformatics/btl158.

[89] L. Fu, B. Niu, Z. Zhu, S. Wu, W. Li, CD-HIT: accelerated for clustering the next-generation sequencing data, Bioinformatics 28 (2012) 3150–3152, https://doi.org/10.1093/bioinformatics/bts565.

[90] S.M. Kiełbasa, R. Wan, K. Sato, P. Horton, M.C. Frith, Adaptive seeds tame genomic sequence comparison, Genome Res. 21 (2011) 487–493, https://doi.org/10.1101/gr.113985.110.

[91] E.V. Kriventseva, D. Kuznetsov, F. Tegenfeldt, M. Manni, R. Dias, F.A. Simão, E. M. Zdobnov, OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs, Nucleic Acids Res. 47 (2019) D807–D811.

[92] G. Marçais, C. Kingsford, A fast, lock-free approach for efficient parallel counting of occurrences of k-mers, Bioinformatics 27 (2011) 764–770, https://doi.org/10.1093/bioinformatics/btr011.

[93] H. Li, B. Handsaker, A. Wysoker, The sequence alignment/map format and SAMtools, Bioinformatics 25 (2009) 2078–2079, https://doi.org/10.1093/bioinformatics/btp352.

[94] B. Chattopadhyay, K.M. Garg, R. Ray, I.H. Mendenhall, F.E. Rheindt, Novel de novo genome of *Cynopterus brachyotis* reveals evolutionarily abrupt shifts in gene family composition across fruit bats, Genome Biol. Evol. 12 (2020) 259–272, https://doi.org/10.1093/gbe/evaa030.

[95] G. Renaud, K. Hanghøj, T.S. Korneliussen, E. Willerslev, L. Orlando, Joint estimates of heterozygosity and runs of homozygosity for modern and ancient samples, Genetics 212 (2019) 587–614, https://doi.org/10.1534/genetics.119.302057.

[96] M.F.L. Derks, H.J. Megens, M. Bosse, J. Visscher, K. Peeters, M. Bink, et al., A survey of functional genomic variation in domesticated chickens, Genet. Sel. Evol. 50 (2018) 17, https://doi.org/10.1186/s12711-018-0390-1.

[97] A. Smit, R. Hubley, P. Green, RepeatMasker Open-4.0. [Internet]. 2013–2015. http://www.repeatmasker.org/, 2015.

[98] M. Stanke, O. Keller, I. Gunduz, A. Hayes, S. Waack, B. Morgenstern, AUGUSTUS: ab initio prediction of alternative transcripts, Nucleic Acids Res. 34 (2006) W435–W439, https://doi.org/10.1093/nar/gkl200.

[99] J. Huerta-Cepas, D. Szklarczyk, K. Forslund, H. Cook, D. Heller, M.C. Walter, et al., eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences, Nucleic Acids Res. 44 (2016), https://doi.org/10.1093/nar/gkv1248. D286-D293.

[100] J. Huerta-Cepas, D. Szklarczyk, D. Heller, A. Hernández-Plaza, S.K. Forslund, H. Cook, et al., eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses, Nucleic Acids Res. 47 (2019), https://doi.org/10.1093/nar/gky1085. D309-D314.

[101] Y. Moriya, M. Itoh, S. Okuda, A.C. Yoshizawa, M. Kanehisa, KAAS: an automatic genome annotation and pathway reconstruction server, Nucleic Acids Res. 35 (2007) W182–W185, https://doi.org/10.1093/nar/gkm321.

[102] P. Törönen, A. Medlar, L. Holm, PANNZER2: a rapid functional annotation web server, Nucleic Acids Res. 46 (2018) W84–W88, https://doi.org/10.1093/nar/gky350.

[103] L. Xu, Z. Dong, L. Fang, Y. Luo, Z. Wei, H. Guo, et al., OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species, Nucleic Acids Res. 47 (2019) W52–W58, https://doi.org/10.1093/nar/gkz333.

[104] H. Li, R. Durbin, Inference of human population history from individual whole-genome sequences, Nature 475 (2011) 493–496, https://doi.org/10.1038/nature10231.

[105] F. Abascal, A. Corvelo, F. Cruz, J.L. Villanueva-Cañas, A. Vlasova, M. Marcet-Houben, et al., Extreme genomic erosion after recurrent demographic bottlenecks in the highly endangered Iberian lynx, Genome Biol. 17 (2016) 251, https://doi.org/10.1186/s13059-016-1090-1.

[106] B. Chattopadhyay, K.M. Garg, R. Ray, F.E. Rheindt, Fluctuating fortunes: genomes and habitat reconstructions reveal global climate-mediated changes in bats' genetic diversity, Proc. R. Soc. B 286 (2019) 20190304, https://doi.org/10.1098/rspb.2019.0304.

[107] Y. Sato, R. Ogden, T. Kishida, N. Nakajima, T. Maeda, M. Inoue-Murayama, Population history of the golden eagle inferred from whole-genome sequencing of

three of its subspecies, Biol. J. Linn. Soc. 130 (2020) 826–838, https://doi.org/10.1093/biolinnean/blaa068.
[108] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.
[109] H. Li, R. Durbin, Fast and accurate long-read alignment with burrows-wheeler transform, Bioinformatics 26 (2010) 589–595, https://doi.org/10.1093/bioinformatics/btp698.
[110] K. Nam, C. Mugal, B. Nabholz, H. Schielzeth, J.B. Wolf, N. Backström, Molecular evolution of genes in avian genomes, Genome Biol. 11 (2010) R68, https://doi.org/10.1186/gb-2010-11-6-r68.
[111] P.D. Keightley, A. Eyre-Walker, Deleterious mutations and the evolution of sex, Science 290 (2000) 331–333, https://doi.org/10.1126/science.290.5490.331.
[112] L. Smeds, A. Qvarnström, H. Ellegren, Direct estimate of the rate of germline mutation in a bird, Genome Res. 26 (2016) 1211–1218, https://doi.org/10.1101/gr.204669.116.
[113] N. Mather, S.M. Traves, S.Y. Ho, A practical introduction to sequentially Markovian coalescent methods for estimating demographic history from genomic data, Ecol. Evol. 10 (2020) 579–589, https://doi.org/10.1002/ece3.5888.
[114] G. Forcina, Q. Tang, E. Cros, M. Guerrini, F.E. Rheindt, F. Barbanera, Genome-wide markers redeem the identity of a heavily managed and doomed-too-early gamebird, Proc. R. Soc. B 288 (2021) 20210285, https://doi.org/10.1098/rspb.2021.0285.
[115] A. Kapusta, A. Suh, C. Feschotte, Dynamics of genome size evolution in birds and mammals, Proc. Natl. Acad. Sci. U.S.A 114 (8) (2017) E1460–E1469, https://doi.org/10.1073/pnas.1616702114.
[116] G. Venturini, R. D'Ambrogi, E. Capanna, Size and structure of the bird genome–I. DNA content of 48 species of Neognathae, Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 85 (1986) 61–65, https://doi.org/10.1016/0305-0491(86)90221-x.
[117] M.K. Fujita, S.V. Edwards, C.P. Ponting, The *Anolis* lizard genome: an amniote genome without isochores, Genome Biol. Evol. 3 (2011) 974–984, https://doi.org/10.1093/gbe/evr072.
[118] F. Kasai, C. Garcia, M.V. Arruga, M.A. Ferguson-Smith, Chromosome homology between chicken (*Gallus gallus domesticus*) and the red-legged partridge (*Alectoris rufa*); evidence of the occurrence of a neocentromere during evolution, Cytogenet. Genome Res. 102 (2003) 326–330, https://doi.org/10.1159/000075770.
[119] V. Sharma, P. Schwede, M. Hiller, CESAR 2.0 substantially improves speed and accuracy of comparative gene annotation, Bioinformatics 33 (2017) 3985–3987, https://doi.org/10.1093/bioinformatics/btx527.
[120] S. Kolchanova, S. Kliver, A. Komissarov, P. Dobrinin, G. Tamazian, K. Grigorev, et al., Genomes of three closely related Caribbean amazons provide insight for species history and conservation, Genes 10 (2019) 54, https://doi.org/10.3390/genes10010054.
[121] A. Benazzo, E. Trucchi, J.A. Cahill, P. Maisano Delser, S. Mona, M. Fumagalli, et al., Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers, Proc. Natl. Acad. Sci. U. S. A. 114 (2017), https://doi.org/10.1073/pnas.1707279114. E9589-E9597.
[122] Y. Yang, T. Ma, Z. Wang, Z. Lu, Y. Li, C. Fu, et al., Genomic effects of population collapse in a critically endangered ironwood tree *Ostrya rehderiana*, Nat. Commun. 9 (2018) 5449, https://doi.org/10.1038/s41467-018-07913-4.
[123] L.W. Hillier, W. Miller, E. Birney, W. Warren, R.C. Hardison, C.P. Ponting, et al., Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution, Nature 432 (2004) 695–716, https://doi.org/10.1038/nature03154.
[124] T. Wicker, The repetitive landscape of the chicken genome, Genome Res. 15 (2004) 126–136, https://doi.org/10.1101/gr.2438004.
[125] G. Zhang, C. Li, Q. Li, B. Li, D.M. Larkin, C. Lee, et al., Comparative genomics reveals insights into avian genome evolution and adaptation, Science 346 (2014) 1311–1320, https://doi.org/10.1126/science.1251385.
[126] C. Velez-delValle, M. Marsch-Moreno, F. Castro-Muñozledo, I.J. Galván-Mendoza, W. Kuri-Harcuch, Epithelial cell migration requires the interaction between the vimentin and keratin intermediate filaments, Sci. Rep. 6 (2016) 24389, https://doi.org/10.1038/srep24389.
[127] T.H. van Andel, P.C. Tzedakis, Palaeolithic landscapes of Europe and environs: 150,000-25,000 years ago: an overview, Quat. Sci. Rev. 15 (1996) 481–500, https://doi.org/10.1016/0277-3791(96)00028-5.
[128] L.E. Lisiecki, M.E. Raymo, A Pliocene-Pleistocene stack of 57 globally distributed benthic δ 18O records, Paleoceanography 20 (2005), PA1003, https://doi.org/10.1029/2004PA001071.
[129] D. Díez-Del-Molino, F. Sánchez-Barreiro, I. Barnes, M.T.P. Gilbert, L. Dalén, Quantifying temporal genomic erosion in endangered species, Trends Ecol. Evol. 33 (2018) 176–185, https://doi.org/10.1016/j.tree.2017.12.002.
[130] IUCN, Guidelines for re-introductions. International Union for the Conservation of nature and natural resources. IUCN/SSC, in: Gland, and Cambridge: Re-introduction Specialist Group, 1998.
[131] R. Jůzl, P. Suchý, E. Straková, L. Rusníková, M. Macháček, P. Marada, Nutritional value of breast and thigh muscle of chukar partridge (*Alectoris chukar*) under intensive fattening conditions, Acta Vet. Brno 81 (2012) 251–255, https://doi.org/10.2754/avb201281030251.
[132] I.C. Antunes, M.C.P. Coimbra, A.P. Ribeiro, J.D. Ferreira, A.F. Santos, S.P. Alves, et al., Nutritional value of meat lipid fraction from red-legged partridge (*Alectoris rufa*) obtained from wild and farmed specimens, Poult. Sci. J. 98 (2019) 1037–1046, https://doi.org/10.3382/ps/pey367.
[133] Y. Wen, H. Liu, K. Liu, H. Cao, H. Mao, X. Dong, Z. Yin, Analysis of the physical meat quality in partridge (*Alectoris chukar*) and its relationship with intramuscular fat, Poult. Sci. J. 99 (2020) 1225–1231, https://doi.org/10.1016/j.psj.2019.09.009.
[134] J.L. Mourão, A.C. Barbosa, D. Outor-Monteiro, V.M. Pinheiro, Age affects the laying performance and egg hatchability of red-legged partridges (*Alectoris rufa*) in captivity, Poult. Sci. 89 (2010) 2494–2498, https://doi.org/10.3382/ps.2009-00377.
[135] Z. Liu, N. Yang, Y. Yan, G. Li, A. Liu, G. Wu, C. Sun, Genome-wide association analysis of egg production performance in chickens across the whole laying period, BMC Genet. 20 (2019) 67, https://doi.org/10.1186/s12863-019-0771-7.
[136] P. Kaiser, J. Howell, M. Fife, J.R. Sadeyan, N. Salmon, L. Rothwell, et al., Integrated immunogenomics in the chicken: deciphering the immune response to identify disease resistance genes, in: M.-H. Pinard, C. Gay, P.-P. Pastoret, B. Dodet (Eds.), *Animal Genomics for Animal Health. vol. 132, Developments in Biologicals* (pp. 57-66), Basel: S. Karger. Animal Genomics for Animal Health International symposium, Paris, France, 2008, https://doi.org/10.1159/000317144, 25/10/07.
[137] S. Spanò, La pernice rossa, Edizioni Il Piviere, Gavi (AL), 2010.