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Hybridisation, introductions and partial genetic incompatibility shape the genomic landscape of expanding brown hares in northern Europe

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Abstract

The mountain hare (*Lepus timidus*) and brown hare (*Lepus europaeus*) coexist and hybridize across parts of northern Europe, providing an opportunity to investigate the genomic consequences of secondary admixture between closely related species. We analysed 210 Finnish hares using ddRAD sequencing and compared them with reference populations of brown hares from Western Europe and mountain hares from Russia. The analysis of 143,635 informative genome-wide markers showed genetic structure for both species on a broader geographical scale, although mountain hares showed substantially weaker differentiation than brown hares along its continuous distribution range. Finnish mountain hares formed part of a broad east–west genetic continuum, whereas Finnish brown hares were clearly differentiated from Western European populations, while retaining a detectable western ancestry component in southern Finland. Hybridisation was widespread, with evidence of introgression detected in 36 brown hares and 18 mountain hares. Introgression was strongly asymmetric, with substantially higher levels of mountain hare ancestry in brown hares than vice versa. In contrast to the autosomes, both X- and Y-chromosomal genotypes remained largely species-specific and no recombinant X chromosomes were detected, suggesting that sex chromosomes contribute disproportionately to the maintenance of the species barrier.

Analysis of an advanced-generation hybrid revealed extensive chromosomal mosaicism resulting from multiple generations of recombination following interspecific hybridisation. Several genomic regions showed elevated introgression while simultaneously displaying strong differentiation between Finnish and Western European brown hare populations. Candidate genes within these regions included loci associated with immune function, development, and metabolism.

Our results demonstrate multiple ancestral origins and hybridisation among Finnish hares. While reproductive barriers continue to limit gene flow, especially on the sex chromosomes, introgression may have contributed locally adapted genetic variation that may have facilitated the successful expansion of brown hares into northern boreal environments.

Introduction

Environmental changes in climate, landscape and habitats are major drivers of range shifts among animals [1]. In addition to natural expansions, human-mediated introductions can lead to rapid and long-distance colonisation beyond the species' native range, as exemplified by the history of many invasive species [2]. These range shifts can bring previously isolated populations – or even closely related species – into secondary contact, where the extent of genetic compatibility can allow introgressive hybridisation [3–5]. Consequently, the biogeographic history of a species can reflect multiple transient or more persistent genetic interactions between both conspecific and heterospecific lineages. This can leave a lasting imprint on the genomes of extant populations, consisting of multiple ancestries, shaped by its colonisation dynamics, secondary contacts with other populations and the degree of reproductive isolation.

In Europe, hares (*Lepus* spp.) provide a well-known example of frequent range shifts and genetic exchanges at different stages of their evolutionary history [3]. The situation is particularly well documented in the Iberian Peninsula, where the genomic composition of three extant hare species – the Iberian hare (*Lepus granatensis*), the broom hare (*L. castroviejoi*) and the brown hare (*L. europaeus*) – has been shaped not only by recurrent gene flow among them, but also by introgression from the mountain hare (*L. timidus*), which became locally extinct following the last glacial maximum [6–10]. Until relatively recently, the mountain hare was the only hare species in Fennoscandia, having colonised the region soon after the retreat of the Scandinavian Ice Sheet [11, 12]. In the absence of other hare species, this arctic-alpine adapted species also gave rise to a regionally differentiated subspecies [12, 13], the heath hare (*L. timidus sylvaticus*) in southern Scandinavia – adapted to more temperate conditions – much like it differentiated into the Irish hare (*L. timidus hibernicus*) in Ireland [14].

The mountain hare is a widespread species with its distribution spanning throughout the northern Palearctic from Ireland to Japan, reflecting the species' continuous distribution during the last ice age [14, 15]. In contrast, the brown hare – the only other hare species reaching northern Europe – was likely restricted to a few glacial refugia in southern Europe and the Near East [16], including several isolated refugia in the Balkans [17]. While the phylogeographic differentiation arising from these refugia is evident in the diversity of brown hare mitochondrial DNA (mtDNA) lineages [16, 18–20], the extent of this differentiation at the genomic level has not been thoroughly studied. In Europe, the ancestral makeup of local brown hare populations can be complicated by both ancient translocations as well as more recent restocking programmes [16].

Brown hares are not endemic to the Nordic countries, but they were either introduced or arrived naturally in the late 19th century [14, 21–23]. As a species better adapted to temperate climates, the brown hare has benefited from the anthropogenic climate change, often at the expense of the mountain hare, which is specialized to snowy and harsh winter conditions [7, 11, 15]. Consequently, brown hares are currently thriving in the north and expanding into the regions previously dominated by mountain hares [21, 22, 24]. The expansion of the brown hare distribution range in northeastern Europe is curious, as it seems to have begun already in the early 1800s [25]. The exact cause of this expansion is unknown, but anecdotal evidence suggests that a fashion of breeding and restocking brown hares for hunting purposes among the Russian nobility may have played a role. For example, there is a detailed account of a hare farm on the Karelian isthmus [26, 27], coinciding with the timing of brown hare expansion into Finland from the southeast in the late 1800s.

Unlike in Sweden, where the brown hare was deliberately introduced [21, 23], the species reached Finland largely on its own from the southeast, establishing a relatively stable distribution area by the 1930s [28]. Nevertheless, some restocking efforts occurred in southwestern Finland, particularly in the 1910s [26, 29]. Contemporary reports suggest that these efforts were very successful, with brown hares becoming locally highly abundant [26, 30]. Most of the animals in these restocking efforts originated from commercial providers in Russia, though imports from other countries, including Sweden, cannot be ruled

out. Since the 1990s, the brown hare has experienced an impressive range shift northward [24] and has already been reported north of the Arctic Circle in Finland.

The arrival of brown hares in Fennoscandia has brought them into contact with the native mountain hare, leading to frequent hybridisation, particularly in areas where mountain hares are abundant. This has resulted in asymmetric gene flow from the mountain hare to the brown hare [8, 24, 31–34]. Such a pattern is consistent with a scenario in which hybridisation within small founding populations at the expansion front leads to the stochastic increase of introgressed allele frequencies by genetic drift—beyond what would be expected based on hybridisation rates alone [4, 35]. In addition, there is evidence that brown hares may benefit from acquiring locally adapted alleles from mountain hares [36], similarly to what has been described for some other hare species in the context of winter pelage polymorphisms [37, 38]. This adaptive introgression could enable brown hares to effectively accelerate adaptation, potentially facilitating their continued expansion northward.

In this study, we investigated the genomic composition of Finnish brown hares and the processes that have shaped it, including contributions from historical introductions, natural range expansion and introgressive hybridisation with the mountain hare. We characterised genome-wide patterns of ancestry and assessed the genomic consequences of hybridisation. Specifically, we asked (i) whether Finnish brown hares derive from multiple ancestral source populations, reflecting both natural colonisation and historical translocations, (ii) to what extent mountain hare ancestry has become incorporated into the brown hare genome during its northward expansion, and (iii) whether introgression is distributed uniformly across the genome or shows signatures of selection or genetic incompatibilities.

Because reduced hybrid male fertility is a common outcome of speciation, we further tested whether introgression differs among autosomes and sex chromosomes. If hybrid incompatibilities limit gene flow, we predicted reduced introgression on the X chromosome and little or no exchange of Y chromosomes between species, consistent with Haldane's rule [39–42] and the large X-effect described in many hybridising taxa [43–45]. Conversely, genomic regions showing elevated mountain hare ancestry in brown hares may represent candidates for adaptive introgression associated with local adaptation to northern environments.

Together, these analyses provide a comprehensive view of how demographic history, human-mediated translocations and hybridisation have shaped the contemporary genome of the Finnish brown hare and offer broader insights into the genomic consequences of biological invasions and range expansions.

Materials and Methods

Sampling

A total of 282 hare samples were included in the study, comprising mountain hares (*Lepus timidus*) from Finland and Siberia and brown hares (*Lepus europaeus*) from Finland and western Europe (Supplementary Table 1). Out of these, 147 were originally identified as brown hares, with 104 of them

being from Finland, and 135 as mountain hares, with 106 of them being Finnish samples. For Finnish samples, species identification was initially made in the field by hunters based on external morphology and was subsequently re-evaluated using genetic data.

DNA isolation and sequencing

Total DNA was isolated using Omega Bio-Tek E.Z.N.A.® Tissue DNA Kit (#D3396) and following manufacturer's recommendations. For the double-digest Restriction-site Associated DNA (ddRAD) dataset, DNA was extracted using the EZ-10 Spin Column Animal Genomic DNA Miniprep Kit (Bio Basic Inc.) and quantified with Epoch Microplate Spectrophotometer (BioTek). Following Patterson et al. (2012) and Brelsford et al. (2016) with modification, individual libraries were prepared for each sample. DNA extractions were digested with two restriction enzymes (MspI and SbfI-HF, New England BioLabs), and fragments were ligated to barcoded P5 and P7 adapters with compatible overhangs. Library purification was performed using Sera-Mag SpeedBeads (Ge Healthcare), followed by amplification with indexed primers. Double-sided size selection with Kapa HyperPure beads (Roche) was used to recover libraries ranging from 520-770bp. Then, libraries were quantified and pooled equimolarly, and the final pool was analysed on 2200 TapeStation (Agilent Technologies) and quantified by qPCR with the CFX96 Touch System (Bio-Rad Laboratories, 16 Inc). Finally, libraries were sequenced on Illumina Novaseq 6000 at Novogene Co. Ltd. (Yuen Long, NT, Hong Kong).

Bioinformatics

Demultiplexing and Genotyping

Demultiplexing and adapter trimming was done using the process_radtags program of the Stacks v2.65 package [46]. FastQC [47] was used for quality control before and after demultiplexing, and the results were summarized with MultiQC [48]. Alignment to the reference genome was done with bwa-mem2 [49]. The reference used was the brown hare (*Lepus europaeus*) reference genome (NCBI RefSeq, GCA_033115175.1) [50].

Genotyping followed a protocol based on a modified GATK best practices workflow [51], with the main difference being that the deduplication step was omitted. In ddRAD sequencing, reads originate from restriction enzyme cut sites and therefore typically start and end at identical genomic positions. Consequently, most reads would be incorrectly flagged as duplicates and removed if this standard step were applied. Variant calling was done using the GATK HaplotypeCaller, then combined and jointly genotyped the files with the CombineGVCFs, and GenotypeVCFs programs. Variant filtering was carried out in several stages. First, hard quality filtering was applied separately to SNPs and short indels using VariantFiltration. All analyses were performed using GATK v4.5.0.0. For analyses requiring biallelic SNPs only, indels and multiallelic SNPs were subsequently removed. The combined SNP and indel dataset was then further filtered based on genotyping completeness, retaining only sites and samples with no more

than 25% missing genotypes, and to keep one of these SNPs per RADtag locus (with stacks' population –write-single-snp option).

Population genetics

We first confirmed species identity through PCA, using PLINK v2.00a5 [52], and re-labelled samples where needed. The sample ID was left intact for consistency, but we added the correct species to the metadata and moved these samples to their appropriate species groups for any further tests.

We calculated and plotted hybrid indices and interclass heterozygosity using the triangulaR R package [53]. For this test, we considered SNPs based on complete differentiation between the parental populations. Then, we ran ADMIXTURE tests assuming K = 2 and K = 4 ancestral populations [54]. The first run (K = 2) separating the two species, the second run (K = 4) also separating the Finnish and non-Finnish samples within species. Tests for autosomes and allosomes were run separately. For the X chromosome, male and female individuals were analysed separately to account for differences in ploidy (haploid and diploid, respectively). All other analysis parameters were kept constant across runs. For the Y chromosome, the number of markers successfully genotyped in both species was too low to support meaningful population genetic analyses, although the available markers were sufficient to distinguish between the species.

Overall differentiation between all populations and subpopulations was calculated with the Cockerham-Weir Fst test integrated in vcftools [55], in overlapping windows of 500kbp and window step size of 100 kbp. Following this, we also ran Tajima's D test at the same window sizes to help detect potential signals of selection between the parental and Finnish populations.

Localisation of interspecies chromosomal introgression was analysed with ELAI [56], which infers local ancestry from patterns of linkage disequilibrium. Because the dataset was generated using ddRAD sequencing, resulting in a reduced and uneven distribution of markers, ELAI was used primarily to identify broad patterns of local ancestry rather than to resolve individual introgression breakpoints or candidate genes. The analysis was therefore interpreted with caution, given that the marker density and distribution are suboptimal for local ancestry inference. For the ELAI analysis, individuals were classified as presumed non-admixed or potentially admixed based on the ADMIXTURE results. Individuals with ≥ 0.9999 ancestry from their attributed primary species were assigned to the corresponding parental group, whereas individuals with lower ancestry proportions were considered potentially admixed. All presumed non-admixed individuals were included in the parental groups. ELAI analyses were performed separately for each chromosome and independently for the two focal admixed groups, comprising 36 brown hares and 18 mountain hares, respectively. For each analysis, the corresponding focal species was treated as the recipient of introgressed ancestry, with the other species representing the potential donor. All individuals presumed non-admixed were included in the parental groups. X chromosomes in this analysis case were all treated as diploid, with males considered 100% homozygous.

Results

To characterize the genetic composition and population structure of hares in Finland within a broader Eurasian context, a comprehensive sampling effort was undertaken across the species' ranges. The dataset comprised 210 Finnish hares, including 104 brown hares (*Lepus europaeus*) and 106 mountain hares (*Lepus timidus*). To provide geographic context for the Finnish populations, an additional 43 brown hares from four Western European populations and 29 mountain hares from seven Russian populations were included, five of which originated from the Russian Far East (Fig. 1A). This sampling design enabled comparisons both between species and among populations across a broad geographic gradient, encompassing the native range of the mountain hare and the expanding range of the brown hare in northern Europe. In addition, the Finnish samples were divided into six geographical regions from south to north to survey the effects of distribution on genetic structure.

ddRAD sequencing and subsequent genotyping yielded a total of 4,235,422 variant sites across the mountain and brown hare samples. Following quality filtering and genotype completeness filtering, 143,635 high-quality, highly genotyped, and informative variant sites were retained for downstream analyses. The retained markers were required to have a genotyping rate of at least 75% across all individuals, irrespective of species, and a minor allele frequency of at least 5%, ensuring that only shared and informative polymorphisms were included.

Reads from both species aligned well to the brown hare reference genome, with a mean alignment rate of 93.95%, indicating a high degree of genomic similarity and data quality. Levels of genetic diversity differed among populations and species. Finnish brown hares exhibited the highest mean observed heterozygosity (0.1759), exceeding that observed in Western European brown hares (0.1462). Similarly, Finnish mountain hares showed higher observed heterozygosity (0.1477) than Russian mountain hares (0.1225).

All mountain hare samples clustered relatively close in the PCA, although they exhibited a west-to-east geographic gradient, and overall larger differentiation, likely due to isolation by distance, than the brown hares (Fig. 1B). Notably, some Finnish specimens were embedded within the mountain hares from the Russian Far East. Even though PC1 explains most of the overall variance (29.25%), the inspection of subsequent principal components showed the separation of the major population clusters in our dataset (Supplementary Fig. 1).

The brown hare specimens formed close, but distinct clusters representing Western European and Finnish populations, with some Western European individuals extending toward the Finnish cluster in the PCA, but not *vice versa*. This pattern was not explained by geographic proximity, as the most extended sample was from a population in the Pyrenees. By contrast, two Finnish brown hare samples differed markedly from the rest, extending toward the mountain hare cluster. However, unlike a first-generation hybrid reported in a previous study [34], these individuals did not fall midway between the mountain hare and brown hare clusters.

Finnish mountain hares exhibited a subtle north-to-south genetic gradient, with a few outlier individuals in the PCA (Fig. 2A). The ADMIXTURE analysis revealed that mountain hares from the Russian Far East are genetically distinct from the main Fennoscandian lineage, with populations from Central and Western Russia forming a geographical transition between these groups (Fig. 2B; see sampling locations in Fig. 1A). This Siberian ancestry extended into Finland, where it was particularly evident in mountain hares from the eastern part of the country (Supplementary Fig. 2A), resulting in a corresponding east-to-west gradient in ancestry proportions at the population level (Fig. 2B).

In addition, mountain hares from southeastern Finland exhibited detectable brown hare introgression, although at substantially lower levels than the reciprocal introgression observed in brown hares. At the individual level, brown hare admixture was largely confined to southeastern Finland, whereas Siberian ancestry was observed in mountain hares across much of eastern Finland.

Like the mountain hares, brown hare genotypes also exhibited a weak geographical gradient in the PCA (Fig. 3A; the hybrid individual LE-Joe_584 was omitted for better resolution). Northern and eastern brown hares showed the highest levels of mountain hare introgression, whereas individuals from southern Finland contained a substantially larger proportion of Western/Central European brown hare ancestry (Fig. 3B). At the population level, this resulted in a south-to-north decline in Western European ancestry accompanied by increasing mountain hare ancestry (Fig. 3B). Individual-level ADMIXTURE analysis further showed that the Western European component was widespread among southern Finnish brown hares but occurred sporadically elsewhere, whereas mountain hare introgression was most pronounced in eastern and northern individuals (Supplementary Fig. 2B).

As mitochondrial DNA introgression is widespread among hares, including in our dataset (Supplementary Table 1), we investigated whether similar patterns could be detected in the sex chromosomes. Interestingly, both Y- and X-chromosomal genotypes clustered strictly according to species assignment in the PCA (Fig. 4A, B). While the X-chromosome differentiated between Western European and Finnish brown hare populations (Fig. 4A), as observed in the genome-wide data (Fig. 1B), we did not observe a similar structure in the Y chromosome genotypes (Fig. 4B). Overall, the mountain hare Y-chromosomes showed more genetic variation than the brown hare ones. The X-chromosome of the brown hare individual LE-Joe_584, which occupied an intermediate position in the genome-wide PCA (Fig. 1B), clustered with brown hares (Fig. 4A), while its Y-chromosome with the mountain hares (Fig. 4B).

The chromosome-level reference genome assemblies of both species enabled a detailed examination of the genomic composition of the outlier brown hare individuals identified in the PCA (Fig. 1B). Specimen LE-Joe_584, which was morphologically identified as a brown hare but occupied an intermediate position between species in the genome-wide analyses, exhibiting a highly mosaic genomic composition (Fig. 5A). Several chromosome pairs were entirely heterozygous for mountain hare and brown hare alleles, whereas others appeared to be of exclusively brown hare origin or contained extensive homozygous tracts derived from the mountain hare. Similarly, individual LE-Oulu_777 carried long

homozygous segments of mountain hare ancestry on chromosomes 18 and 22 (Fig. 5B). In contrast to the autosomes, no evidence of recombinant X-chromosomal genotypes was observed in either individual.

To further characterize the ancestry of LE-Joe_584, a triangular plot analysis of hybrid index and interclass heterozygosity was performed (Fig. 6). The individual clustered closer to the brown hare group, with an estimated hybrid index of approximately 0.26 toward mountain hare ancestry and an interclass heterozygosity of approximately 0.36. These values are inconsistent with a first-generation (F1) hybrid and instead indicate a more complex hybrid ancestry. The chromosomal ancestry patterns were likewise incompatible with a simple backcross to brown hare, as several chromosomes contained long homozygous mountain hare-derived regions in addition to extensive heterozygous segments. Taken together, the results suggest that LE-Joe_584 represents a later-generation hybrid, likely originating from one or more generations of recombination following an initial interspecific cross and a possible backcross. As the X-chromosome of LE-Joe_584 clustered with brown hares and the Y-chromosome with the mountain hares (Fig. 4), the ancestral cross must have involved a mountain hare male and a brown hare female.

Overall, evidence of introgression, defined as individuals for which ADMIXTURE, ELAI and hybrid-index analyses consistently indicated introgression, was detected in 36 Finnish brown hares and 18 Finnish mountain hares. Despite only two-fold difference in the specimen number, the mean proportion of introgressed loci was 3.13% among admixed individuals of brown hares and 0.7% among mountain hares. As brown hares showed markedly higher levels of introgression, we next sought to identify genomic regions that may have been preferentially retained following hybridisation, by calculating their average ancestry dosage values. Regions showing elevated levels of introgression were subsequently compared with patterns of genetic differentiation between the Western European and Finnish brown hare populations. We reasoned those genomic regions exhibiting both high levels of introgression and strong differentiation from more southern populations could represent candidate loci involved in local adaptation. To further assess this possibility, Tajima's D was calculated across the genome and compared with the identified candidate regions. However, departures of Tajima's D from equilibrium expectations must be interpreted cautiously in introgressed regions, because gene flow itself can alter the local site-frequency spectrum and generate non-equilibrium patterns that are not necessarily attributable to selection.

Table 1
List of genes with the highest dosage of LT allelic variants in potential LE hybrid individuals. *Values are averaged from all overlapping windows, where relevant.

Gene	Genomic position	Tajima D values (LE)*	LT Dosage (mean across potentially hybrid LE)*
AGPAT3	NC_084828.1:2438949–2510888	1.768435	0.239
BTBD9	NC_084829.1:38542923–38996715	0.984217	0.3896
EPS15L1	NC_084846.1:7539165–7609913	-0.353233	0.17
GATA3	NC_084840.1:73573195–73590598	1.29777	0.17
ITIH5	NC_084840.1:74019189–74128398	0.280567	0.17
LOC133749851 (zinc finger protein 709-like)	NC_084846.1:7961135–8010024	-0.3748685	0.16
LOC133749916 (olfactory receptor 2Z1-like)	NC_084846.1:7761967–7762911	-0.353233	0.16
LOC133750072 (lysosomal alpha-mannosidase-like)	NC_084846.1:8087412–8103204	-0.396504	0.16
RRP1	NC_084828.1:2526922–2540921	1.74247	0.219
RRP1B	NC_084828.1:2600347–2616637	1.74247	0.219
SFMBT2	NC_084840.1:74291024–74550983	0.280567	0.17
ZFAND3	NC_084829.1:38206790–38530411	1.49307	0.357

Several highly introgressed regions coincided with regions showing elevated differentiation between the Western European and Finnish brown hare populations and contained several annotated genes (Table 1). Among these, regions harbouring GATA3 and ITIH5 also exhibited strongly negative Tajima's D values. Although this is compatible with recent positive selection, they can also arise from introgression alone, as stated above. These loci therefore represent promising candidates for further investigation into the potential adaptive consequences of introgression in Finnish brown hare populations.

Discussion

Our analyses reveal a complex history of population differentiation, range expansion and interspecific gene flow in Finnish hares. By combining extensive sampling across Finland with reference populations from Western Europe and Russia (Fig. 1), we demonstrate that both species exhibit genetic structure at longer geographical distances, with this pattern shaped locally by population connectivity and introgression.

The ddRAD approach employed in this study generated a large number of informative markers distributed throughout the genome. Nevertheless, some genomic regions remained inaccessible because they lacked suitable restriction sites or failed to produce reliable genotypes in one or both species. Such gaps are an inherent limitation of reduced-representation sequencing approaches. While it remains possible that mapping to the brown hare reference genome contributed to local differences in marker recovery, this explanation appears unlikely to be a major source of bias given the high sequence similarity between the mountain hare and brown hare genomes (93.09%) [57] and the uniformly high alignment rates (mean 93.95%) observed for both species.

Population structure and connectivity

Despite the vast geographical range encompassed by our sampling (Fig. 1), mountain hares in the examined continuous range have displayed large, but more gradual differentiation compared to the close, but distinct clusters of the brown hares (Figs. 2 and 3). The gradual west-to-east genetic continuum within mountain hares observed across Eurasia is consistent with extensive historical and contemporary gene flow. Particularly notable was the presence of Finnish mountain hare individuals showing partial ancestry from far eastern Russian populations (Fig. 2). While this pattern is not unexpected, as Finland lies at the western edge of the Siberian biogeographic realm and shares numerous faunal elements with northern Eurasia, including species such as the Siberian flying squirrel (*Pteromys volans*), wood lemming (*Myopus schisticolor*) and red-flanked bluetail (*Tarsiger cyanurus*), the extent of contemporary genetic connectivity between Fennoscandian and Siberian populations is likely to vary considerably among taxa. For example, population genomic studies of the flying squirrel and wood lemming have revealed substantial genetic differentiation between western and eastern populations, with the Ural Mountains acting as an important barrier to gene flow [58, 59]. Therefore the genetic affinity observed between Finnish and eastern mountain hare populations is unlikely to result from a long-distance dispersal of hares but rather represents distinct post-glacial colonisation events, as has been suggested based on the mtDNA haplotype variation [12]. Similarly, the relatively high genetic diversity observed in Finnish mountain hares compared with the Russian populations may partly reflect Finland's position as a contact zone where different postglacial lineages meet. However, within Finland the weak population differentiation observed in mountain hares is consistent with the high mobility of hares and their ability to disperse over long distances (Fig. 2). Such movements reduce the effects of isolation by distance and facilitate gene flow across large geographical areas.

In contrast to mountain hares, Finnish brown hares formed a distinct genetic cluster separated from the Western European populations, with southern Finland showing a small but detectable Western European

genetic component (Fig. 3). This pattern is consistent with the known history of brown hare expansion into Finland from the east, with smaller scale introductions in southwestern parts of the country in the early twentieth century [60]. Although the origin of the brown hares introduced into Finland is not known with certainty, Swedish brown hare populations are believed to derive largely from imported Central European stock [61, 62]. Given the close historical connections between Finnish and Swedish hunting circles, it is conceivable that the individuals responsible for introducing brown hares to Finland obtained animals through similar game animal trade networks. The admixture signal detected in southern Finnish populations could therefore indicate a lasting genetic legacy from these introductions. At the same time, northern and eastern Finnish brown hares showed stronger introgression from mountain hares, indicating that contact and hybridisation are most pronounced in regions where the two species coexist most extensively, as already known [24, 34]. Overall, genetic diversity was consistently higher in the Finnish populations of both species than in their respective reference populations, suggesting either admixture, larger effective population sizes, or contributions from multiple source populations in Finland.

Hybridisation and asymmetric introgression

Our results also confirmed the known asymmetry of introgression between the two species [3, 6, 8, 24, 31, 34, 36, 38]. Although hybridisation was detected in both directions, introgressed individuals were much more common among brown hares than mountain hares and the average proportion of introgressed loci was substantially higher in brown hares (ELAI: 3.13% vs 0.7%, hybrid index: 3.10% vs 1.11%). While this pattern can be driven by demographic processes at the leading edge of brown hare range expansion [6, 8, 36], it may also be amplified if purifying selection removes brown hare alleles more effectively from mountain hare genomes than *vice versa*. Furthermore, the generally low genome-wide introgression levels despite widespread hybridisation support the idea that many introgressed alleles are selected against also in brown hares, allowing only a limited subset to persist.

The ELAI-based estimates should nevertheless be interpreted with caution. ELAI infers local ancestry from linkage disequilibrium, whereas our ddRAD dataset provides a relatively sparse and uneven distribution of markers compared with whole-genome sequencing data. This limits the resolution of local ancestry inference and makes the precise boundaries and sizes of individual introgressed tracts uncertain. In addition, the analyses assumed a single generation of admixture, although our data likely include specimens with more complex hybrid history (e.g. Joe_584). Sensitivity analyses using 10 and 100 generations produced increasingly fragmented ancestry tracts and greater variation in ancestry dosage, while retaining broadly similar overall patterns (example in Supplementary Fig. 3). Thus, the ELAI results are most appropriately interpreted as evidence for broad genomic patterns of introgression rather than precise estimates of tract boundaries or gene-level introgression. The consistency between the ELAI and hybrid-index estimates nevertheless supports the conclusion that genome-wide introgression is substantially greater in brown hares than in mountain hares, as well as generally low hybrid index levels suggest fewer admixture events and more generations of breeding and selection within species.

Here, the mosaic ancestry observed in the advanced-generation hybrid LE-Joe_584 (Figs. 1B, 5A, 6) shows a rare snapshot of early stages of genomic introgression and genetic compatibility. This hybrid individual demonstrates that contemporary hybridisation occurs and that there are no major barriers for the interspecies gene flow at this stage, indicating that possible purging of introgressed alleles occurs in the population over an extended period of time.

Limited sex chromosome introgression and possible incompatibilities

In contrast to the autosomes, both the X and Y chromosomes clustered strictly by species (Fig. 4) and no recombinant X-chromosomal genotypes were detected. Interestingly, a similar lack of sex chromosome introgression has been reported also for Iberian hares [7]. This strong barrier to sex chromosome introgression is striking given the evidence for autosomal admixture and suggests the presence of reproductive incompatibilities affecting the sex chromosomes. Similar phenomenon has been reported in other hybridizing mammals [40, 63–65] and are often interpreted in the context of Haldane's rule [39, 41, 42], where hybrid incompatibilities disproportionately affect the heterogametic sex. Notably, such incompatibilities can restrict the introgression of both sex chromosomes, as has been suggested to have occurred also in Neanderthal to modern human gene flow [45]. While the exact mechanism remains uncertain, our results suggest that the sex chromosomes play a central role in maintaining species boundaries between brown hares and mountain hares. However, this hypothesis is somewhat challenged by the advanced-generation hybrid LE-Joe_584, which carried a mountain hare Y chromosome and a brown hare X chromosome. This observation suggests that sex chromosome incompatibilities may not prevent the initial transfer of the Y chromosome but instead act primarily in later generations, progressively removing incompatible sex chromosome combinations from the population.

An alternative explanation is that incompatibilities are concentrated in specific regions of the sex chromosomes rather than affecting the chromosomes as a whole. Under such a scenario, the persistence of an intact mountain hare Y-chromosome would be possible, while recombinant X-chromosomes and other sex-linked combinations are selectively removed from the population. The absence of detectable X-chromosome introgression elsewhere in the dataset nevertheless indicates that successful transmission of sex-linked variation across species boundaries is rare compared with the extensive recombination observed in the autosomes.

The lack of population structure in the Y-chromosome compared to both the autosomes and the X-chromosome may further indicate a smaller effective population size and stronger effects of genetic drift on the male-specific lineage in hares. Alternatively, male-mediated dispersal may contribute to homogenisation of Y-chromosomal variation across populations, although additional sampling would be required to distinguish between these possibilities.

Potential adaptive introgression

Although most introgression appears limited, some genomic regions were repeatedly introgressed and simultaneously showed high differentiation between non-hybrid Finnish and Western European brown hares. Such regions are strong candidates for adaptive introgression, where alleles acquired from mountain hares may provide a selective advantage in the northern environment [7, 36]. Among the candidate genes identified, *GATA3* and *ITIH5* stood out because they were both frequently introgressed and associated with strongly negative Tajima's D values, consistent with recent positive selection [66]. *GATA3* is a zinc-finger transcription factor involved in immune regulation [67, 68] and development [69–71], while *ITIH5* is a tumor suppressor gene [72, 73] with little experimental evidence of its biological functions. Besides *GATA3*, relatively many of the candidate genes are likely involved in transcription regulation or chromatin structure, including *Zinc Finger Protein 709-like*, *SFMTB2* and *ZFAND3*, which could have a number of roles at various stages of cell differentiation and development. Interestingly, *BTBD9* has been linked with circadian regulation and behaviour alterations in humans and mice [74, 75]. Energy metabolism associated *AGPAT3*, *Lysosomal alpha-mannosidase-like* and *Olfactory Receptor 2Z1-like* could represent adaptations to local food sources or nutritional stresses. Although AGPAT3, a 1-acylglycerol-3 phosphate-O-acyltransferase producing phosphatidic acid from lysophosphatidic acid – a key step in the synthesis of glycerophospholipids, including phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol – has also been linked with neuronal function and intellectual disability [76]. Ribosome biogenesis associated RRP1 and RRP1B have also specific functions in immunity [77] and cell proliferation [78], including tumor suppression. The association of these genes with metabolism, cell cycle regulation (i.e. tumor suppression) and by inference with ageing, reproduction and life-history traits makes them plausible targets of selection in hares, given the substantial ecological and physiological differences between the species, including differences in body size, reproductive timing, habitat use and adaptation to northern climates [11, 50, 57, 79].

The timing of brown hare expansion into Finland provides a particularly interesting context for these findings. Brown hares colonized much of Finland only recently, both through natural range expansion and documented human-assisted introductions. Consequently, introgressed mountain hare alleles may have provided a source of locally adapted genetic variation that facilitated establishment in northern environments, reducing the need for *de novo* adaptation.

While adaptive introgression has been documented for winter coat polymorphism, climate adaptation and immunity in hares [36–38], whether any of the candidate loci identified here have contributed to the remarkable success of brown hares in Finland remains to be tested. However, the observed genomic signatures might be consistent with this possibility.

The local ancestry analyses should nevertheless be interpreted in light of the reduced and uneven marker density inherent to ddRAD sequencing. Although the approach can still detect broad ancestry tracts, particularly in recent or later-generation hybrids, the precise boundaries of introgressed regions cannot be resolved with the same confidence as with whole-genome data. A comparable caveat applies to the single admixing generation assumed in ELAI, retained because the true admixture timing could not

be independently estimated for these samples. Comparative runs using higher generation numbers yielded more fragmented tracks and more variable dosages, although the broader ancestry patterns remained consistent. Consequently, the candidate regions and associated genes identified here should be considered as broad genomic targets for further investigation rather than as precisely mapped introgressed loci. Future whole-genome sequencing will be required to refine these regions and assess the robustness of the local ancestry patterns.

Conclusions

This study provides the most comprehensive genomic assessment of Finnish brown hares and mountain hares to date. We show that Finnish mountain hares carry small amounts of ancestry typical for the Russian Far east, possibly reflecting different routes of post-glacial colonisation. Finnish brown hares differ markedly from the Central European populations, although small amount of shared ancestry in Southern Finland could represent a signature of early 20th century introductions. Northern parts of the brown hare range show frequent and strongly asymmetric interspecific gene flow, with mountain hare alleles occurring substantially more often in brown hares than *vice versa*. At the same time, the near-complete absence of sex chromosome introgression suggests that reproductive barriers remain effective and that the sex chromosomes play a disproportionate role in maintaining species boundaries, although the detection of a mountain hare Y chromosome in an advanced-generation hybrid indicates that such barriers may act primarily by removing incompatible sex chromosome combinations over successive generations rather than preventing their initial introgression.

The genomic composition of admixed individuals demonstrates that hybridisation is not restricted to rare contemporary events but has contributed to the genetic makeup of Finnish hare populations over multiple generations. However, the low overall levels of introgression indicate that most introgressed variation may have been removed by selection. Against this background, the identification of repeatedly introgressed genomic regions containing candidate genes involved in immunity, development, metabolism and cellular regulation raises the possibility that a subset of mountain hare alleles has been selectively retained in Finnish brown hares. Future whole-genome studies combined with functional analyses will be needed to determine the adaptive significance of the candidate loci identified here and to further resolve the genetic mechanisms underlying species persistence and adaptation in this dynamic hybrid zone.

Declarations

Ethics approval and consent to participate

Hare specimens were collected by hunters during the legally established hunting seasons, in accordance with local hunting legislation and regional hunting regulations. No animals were killed specifically for research purposes, and no experimental procedures or animal interventions were conducted as part of this study. Therefore, ethical approval for animal experimentation was not required.

Competing interests

The authors declare no competing interest.

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Author Contribution

JM-F and JLOP conceived and designed the study, coordinated sample collection and acquisition, obtained funding and supervised the research. ZF, JP, JPM, LF, PCA and SG contributed to data collection and data analysis. ZF performed the genomic and population genetic analyses. ZF and JLOP prepared the figures and tables. ZF, JP, JPM, JM-F and JLOP contributed to interpretation of the results. ZF, JM-F and JLOP drafted the manuscript. All authors contributed to the interpretation of the results, critically reviewed and revised the manuscript, and approved the final version.

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Data Availability

The datasets supporting the conclusions of this article are available in the European Nucleotide Archive (ENA) under project accession PRJEB101756. Sample-specific accession numbers are provided in Supplementary Tables 2–3.

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Figures

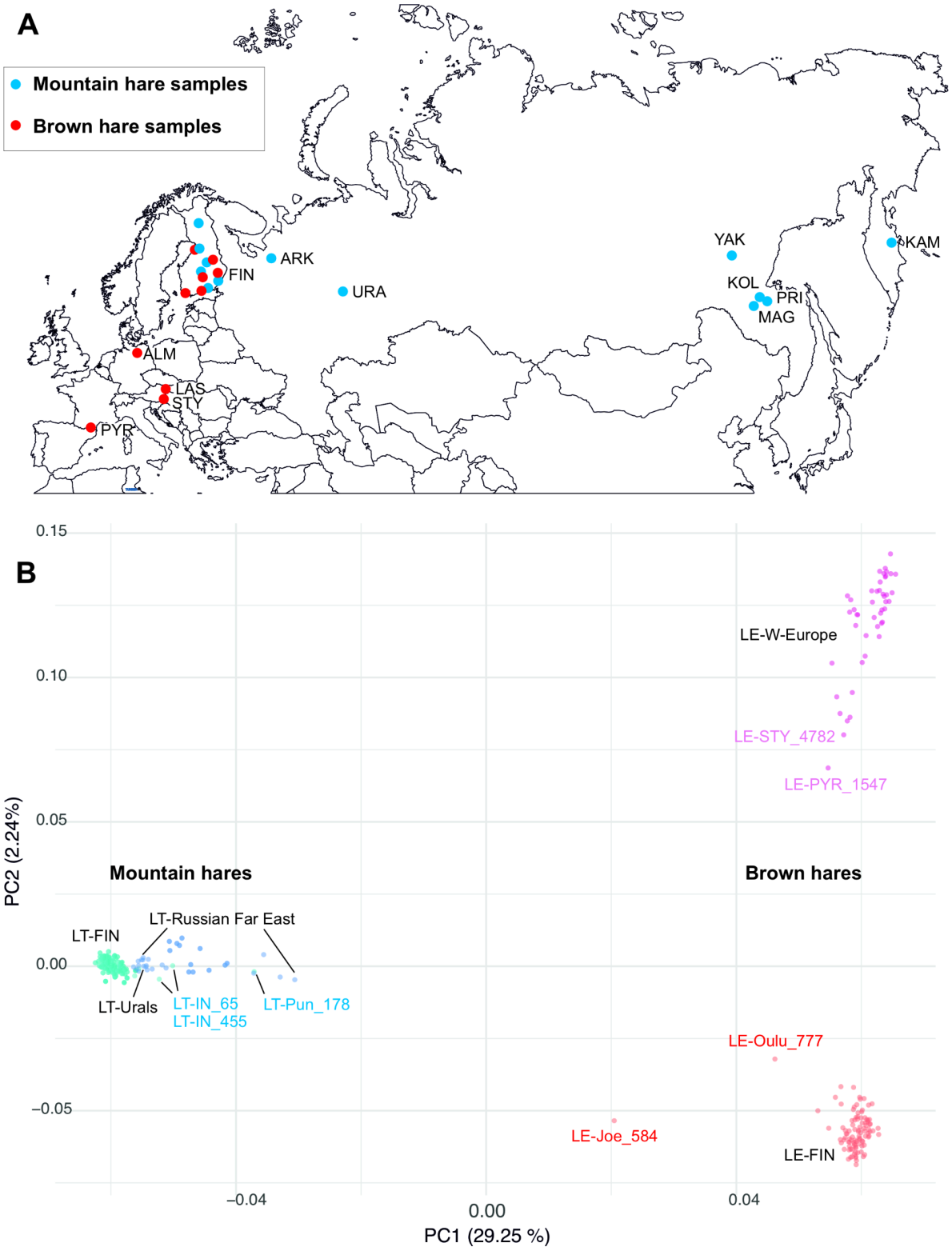


Figure 1

Sampling locations and genetic structure of hare populations included in the study. (A) A map of sampled populations. Abbreviations: FIN – Finland; PYR – Pyrenees; LAS and STY – East Austria; ALM – North Germany; ARK – Arkhangelsk; URA – Ural Mountains; YAK – Yakutia; KOL – Kolyma; MAG –

Magadan; PRI – Primorsky Krai; KAM –Kamchatka. (B) Principal component analysis (PCA) of hare genotypes. Finnish specimens clustering with individuals from the Russian Far East and the two outlier brown hare individuals are highlighted. LE – Brown hare (*Lepus europaeus*); LT – Mountain hare (*Lepus timidus*).

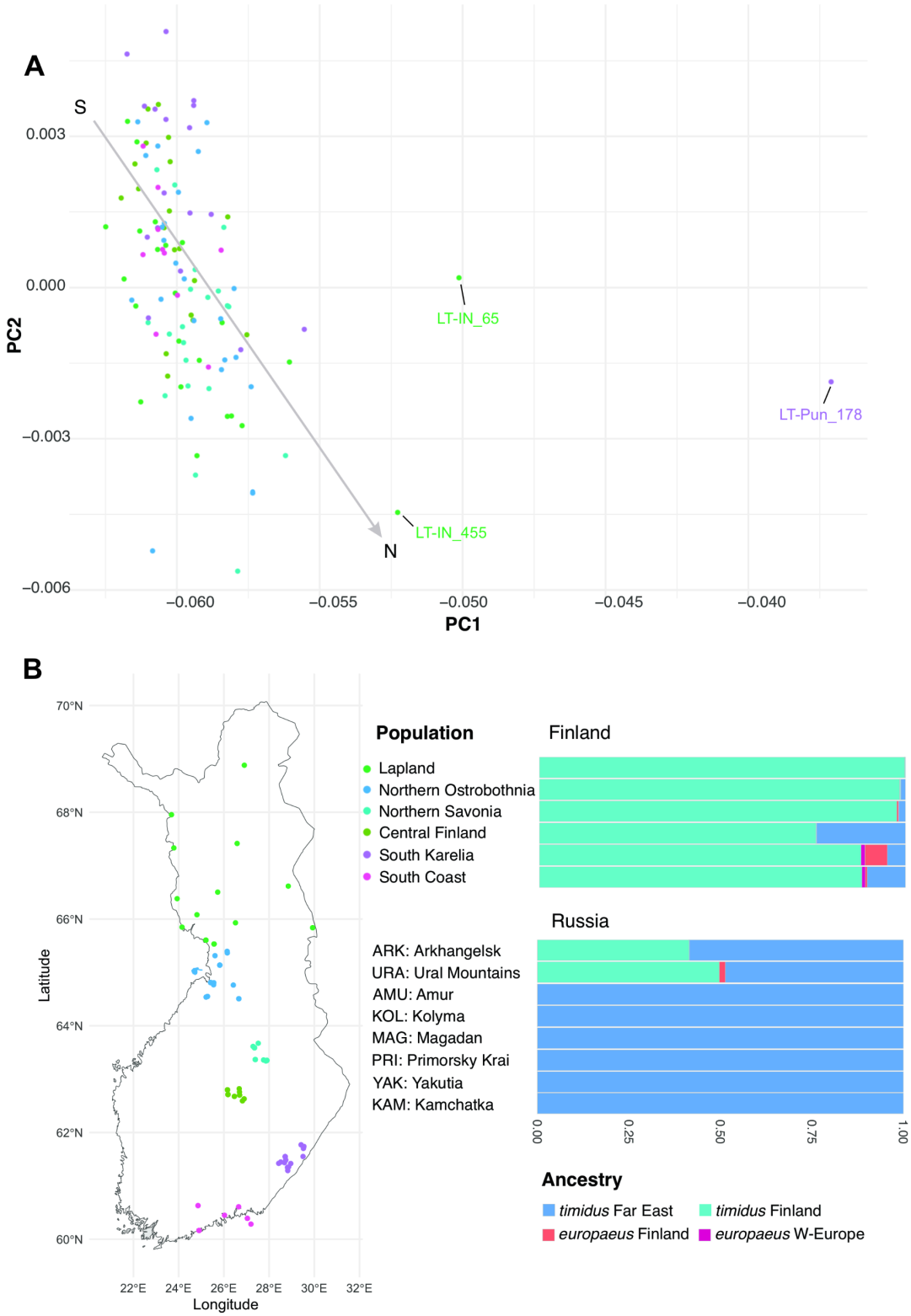


Figure 2

Population differentiation and admixture among Finnish mountain hares. (A) PCA of Finnish mountain hare genotypes show a subtle north-to-east gradient (colours indicate geographic origin, see B). Three outlier specimens are highlighted. (B) While the Finnish population is genetically distinct from the mountain hares in Russian Far East, they also exhibit admixture with the latter (Detailed in Supplementary Figure 2). Note also the intermediate ancestry observed in mountain hare populations from Western and Central Russia (see the map in Figure 1A).

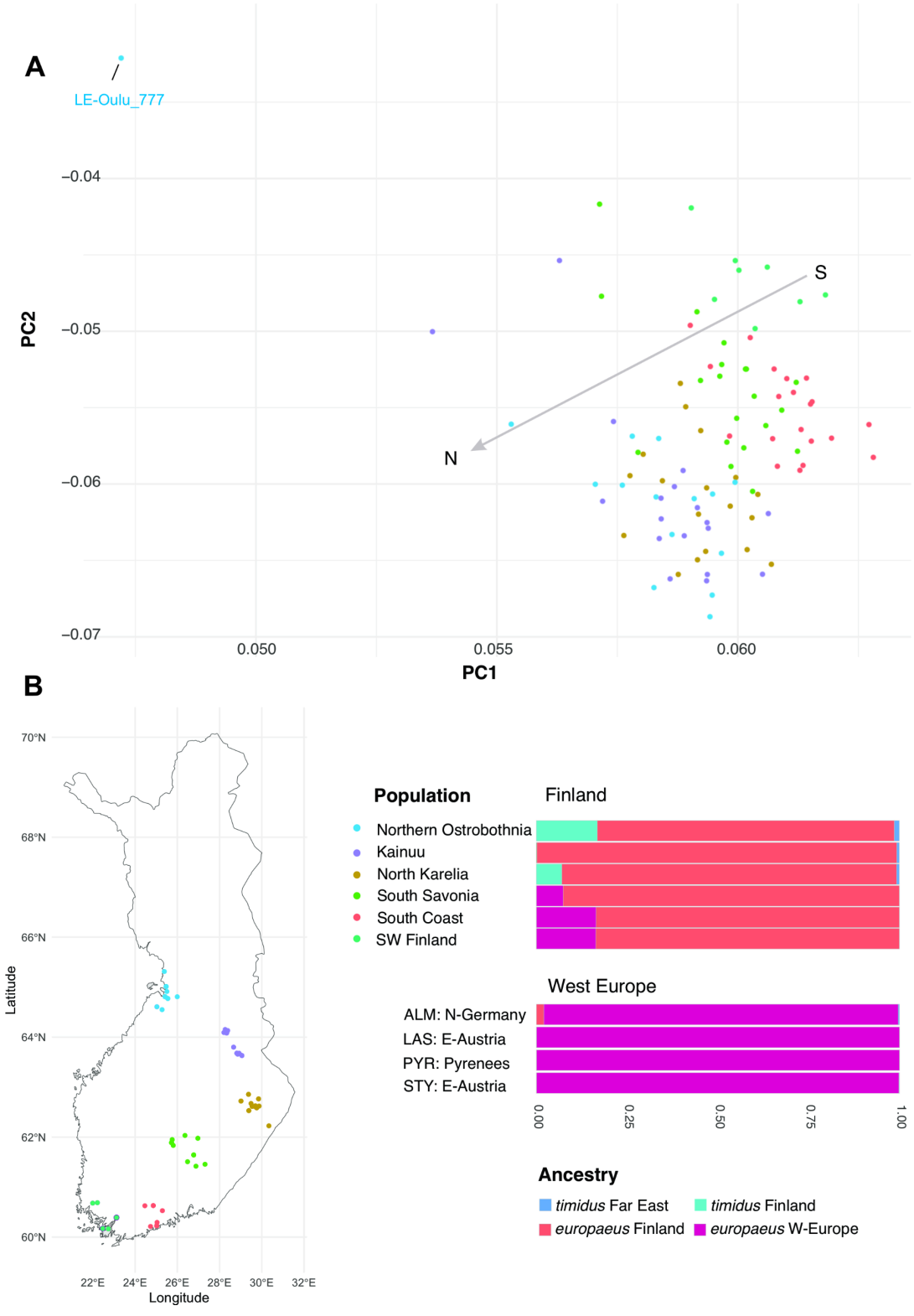


Figure 3

Population differentiation and admixture among Finnish brown hares. (A) Brown hares exhibit a similar subtle geographic genotype gradient as the mountain hares. One outlier sample (LE-Oulu_777) is highlighted. The back-crossed individual LE-Joe_584 has been omitted to improve resolution for the other samples (see Figure 1B). (B) Whereas the southern populations of brown hares derive a part of their ancestry from the Western European brown hare genotypes, the more northern brown hares exhibit admixture with mountain hares.

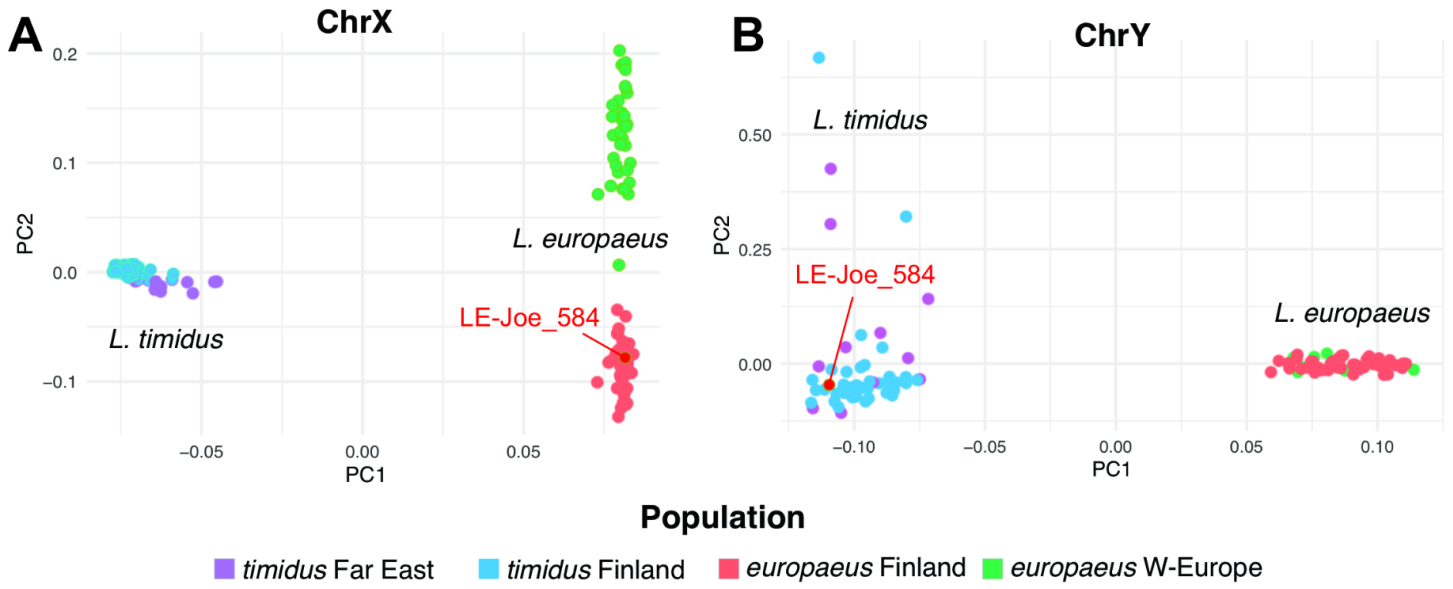


Figure 4

Sex chromosome diversity and population structure among mountain hares and brown hares. (A) PCA based on X-chromosomal genotypes. All individuals cluster according to species assignment, while the Finnish and Western European brown hare populations remain differentiated, consistent with the patterns observed in the autosomal data. (B) PCA based on Y-chromosomal genotypes. As with the X chromosome, mountain hare and brown hare individuals form distinct species-specific clusters. In contrast to the X chromosome and autosomal markers, no notable population structure is visible within brown hares. Mountain hare Y chromosomes exhibit greater genetic diversity than those of brown hares.

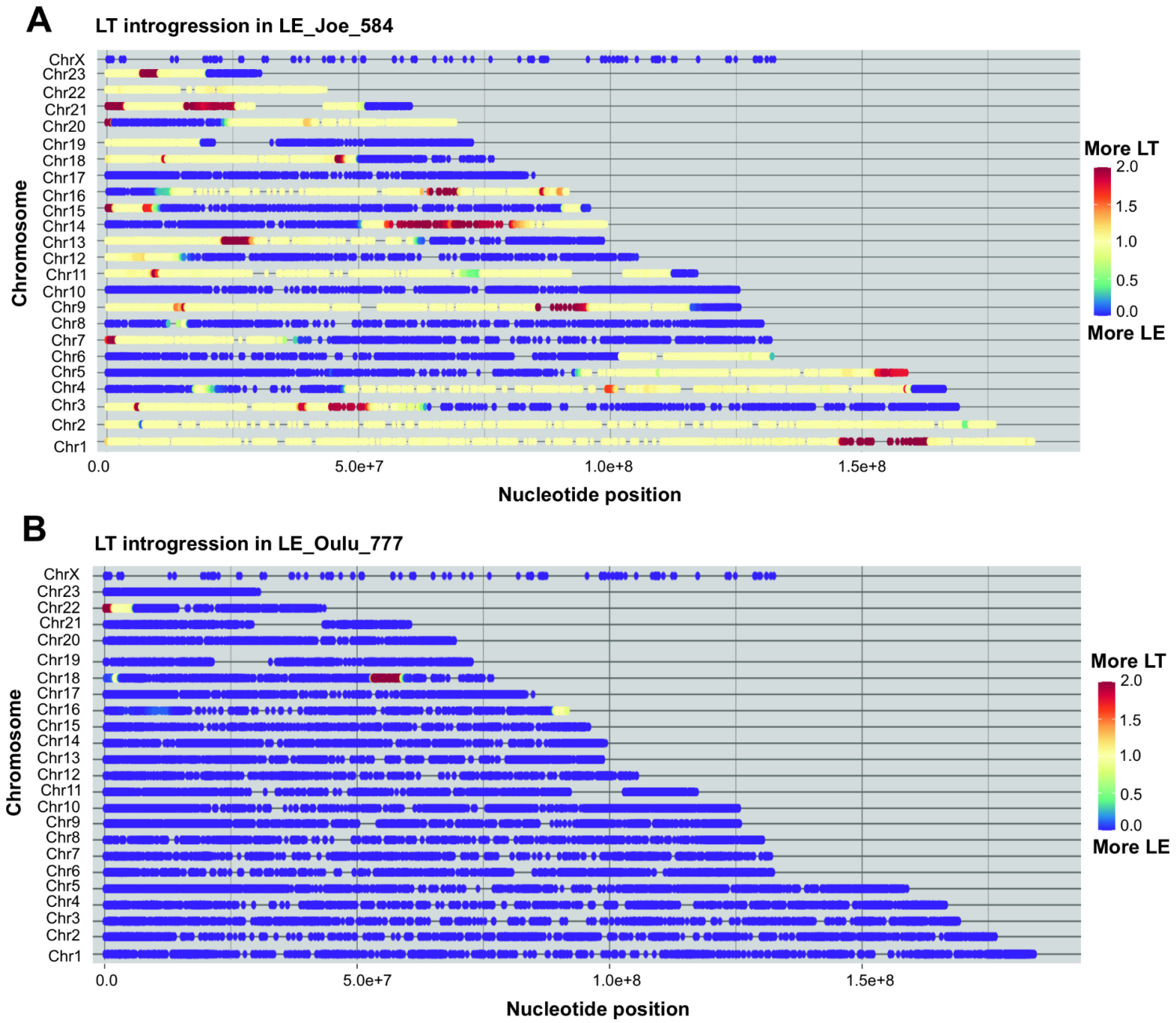


Figure 5

Chromosomal ancestry composition of two brown hare individuals exhibiting introgression from mountain hare. (A) Individual LE-Joe_584, morphologically identified as a brown hare, displays extensive interspecific heterozygosity across much of the genome. Several chromosomes contain long homozygous tracts of either mountain hare (*Lepus timidus*, LT) or brown hare (*Lepus europaeus*, LE) ancestry, whereas chromosome 17 and the X chromosome are entirely of brown hare origin. (B) Individual LE-Oulu_777, identified as a brown hare, contains extensive homozygous mountain hare-derived segments on chromosomes 18 and 22, together with heterozygous regions on chromosomes 16 and 22. Colours indicate genotype states relative to mountain hare-specific diagnostic SNPs (0 none; 1 haploid; 2 diploid).

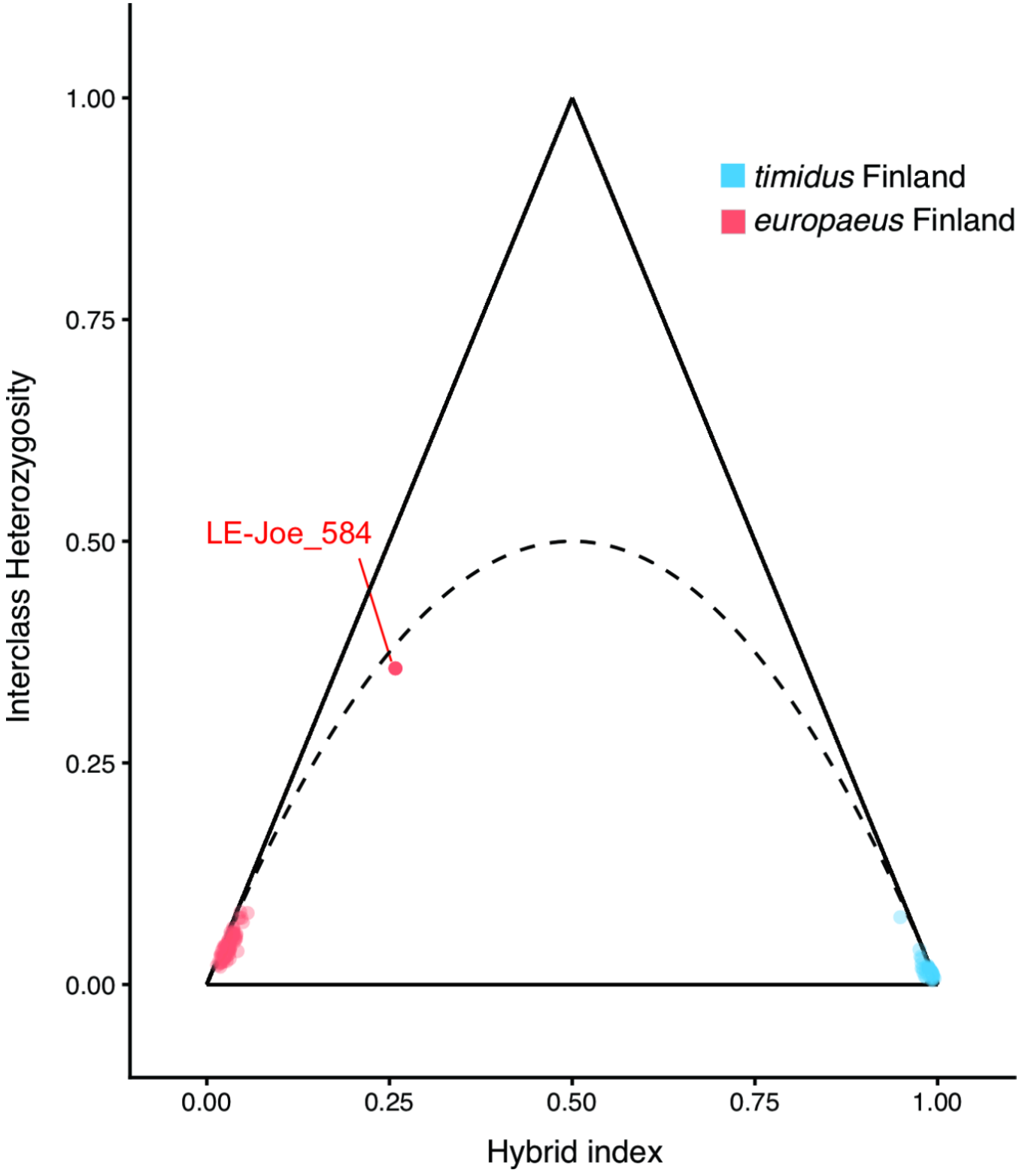


Figure 6

Hybrid ancestry of the brown hare individual LE-Joe_584. TriangulaR plot showing the hybrid index and interclass heterozygosity of Finnish mountain hare and brown hare individuals included in this study (total of 210 individuals). A first-generation (F1) hybrid would be expected to exhibit both a hybrid index and interclass heterozygosity of 0.5. The position of LE-Joe_584 indicates a later-generation hybrid ancestry with a stronger genomic affinity to brown hare. All other individuals cluster close to the parental

species, reflecting low levels of genome-wide admixture. The mean hybrid index of Finnish brown hares was 0.031, while the minimum was 0.014. A table of all hybrid indices of individuals included in the figure can be found in Supplementary Table 4.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SIFile1.0.docx
- STable1.xlsx
- STable2.xlsx
- STable3.csv
- STable4.xlsx