Human activity has caused dramatic population declines in many wild species. The resulting bottlenecks have a profound impact on the genetic makeup of a species with unknown consequences for health. A key genetic factor for species survival is the evolution of deleterious mutation load, but how bottleneck strength and mutation load interact lacks empirical evidence. We analyze 60 complete genomes of six ibex species and the domestic goat. We show that historic bottlenecks rather than the current conservation status predict levels of genome-wide variation. By analyzing the exceptionally well-characterized population bottlenecks of the once nearly extinct Alpine ibex, we find genomic evidence of concurrent purging of highly deleterious mutations but accumulation of mildly deleterious mutations. This suggests that recolonization bottlenecks induced both relaxed selection and purging, thus reshaping the landscape of deleterious mutation load. Our findings highlight that even populations of ~1000 individuals can accumulate mildly deleterious mutations. Conservation efforts should focus on preventing population declines below such levels to ensure long-term survival of species.
Climatic change and pressure from human activities such as hunting caused profound changes in the population and demographic structure of many species. This is because extinction events and subsequent recolonization severely alter the genetic makeup. The demographic changes have important consequences for wildlife management and the conservation of endangered species including raising the risk of genetic disorders. However, nearly all plant and animal populations including humans suffered from temporary reductions in population size—so-called bottlenecks. Bottlenecks increase genetic drift and inbreeding, which leads to a loss of genetic variation, reduces the efficacy of natural selection, and increases the expression of deleterious recessive mutations. The expression of recessive mutations under inbreeding creates the potential for selection to act against these mutations. This process known as purging reduces the frequency of deleterious mutations depending on the degree of dominance and the magnitude of the deleterious effects. Because purging depends on levels of inbreeding, bottlenecks tend to purge highly deleterious, recessive mutations unless population sizes are extremely low. Bottlenecks also increase genetic drift and reduce the efficacy of selection. This allows mildly deleterious mutations to drift to substantially higher frequencies. Hence, bottlenecks generate complex dynamics of deleterious mutation frequencies due to the independent effects of purging and reduced selection efficacy.

A major gap in our understanding is how reduced selection efficacy and purging jointly determine the mutation load in wild populations. Theoretical predictions are well established but empirical evidence is conflicting including for humans. Previous research used changes in fitness to infer possible purging events, but changes in fitness can result from causes unrelated to purging. Direct evidence for purging exists only for isolated mountain gorilla populations that split off larger lowland populations ~20,000 years ago. However, it remains unknown how recent, dramatic bottleneck events on the scale caused by human activity impacts levels of deleterious mutations in the wild. Here, we take advantage of exceptionally well characterized repeated bottlenecks during the reintroduction of the once near-extinct Alpine ibex to retrace the fate of deleterious mutations. Alpine ibex were reduced to ~100 individuals in the 19th century in a single population in the Gran Paradiso region of Northern Italy. In less than a century, a census size of ca. 50,000 individuals has been re-established across the Alps. Thus, the population bottleneck of Alpine ibex is among the most dramatic recorded for any successfully restored species. The recolonization efforts focused on founding local populations across distinct mountain ranges with very limited opportunities for gene flow. Some successfully established populations were used to initiate secondary or tertiary populations elsewhere. As a consequence, most extant populations experienced two to four, well-recorded bottlenecks leaving strong footprints of low genetic diversity.

We find exceptionally low genome-wide variation and an accumulation of deleterious mutations in Alpine ibex compared to most closely related species. Over the course of population reintroductions, Alpine ibex populations that experienced the strongest bottlenecks have lower nucleotide diversity and higher individual inbreeding. In combination, our empirical analyses and individual-based simulations strongly suggest that the reintroductions led to the simultaneous accumulation of mildly deleterious mutations and purging of highly deleterious mutations.

Results
Genomic variation and inbreeding in ibex. We analyzed 60 genomes covering seven species including the Alpine ibex (C. ibex), five additional wild goats and the domestic goat. Siberian ibex have the largest population size (~200,000), followed by Alpine and Iberian ibex (both ~50,000), Bézoar (~25,000), Markhor (~6000) and Nubian ibex (~2500). We found that Alpine ibex together with Iberian ibex and Markhor (C. falconeri) have exceptionally low genome-wide variation compared to closely related species (Fig. 1). All three species either underwent severe bottlenecks in the past century or are currently threatened (Fig. 1). In contrast to Alpine ibex suffering a bottleneck of ~100 individuals, Iberian ibex are thought to have been reduced to a census of ~1000 individuals historically. Overall, there was clear genomic evidence that the near extinction and recovery of the Alpine ibex resulted in substantial genetic drift and inbreeding.
derived alleles $R_{xy}$\textsuperscript{28} for the different categories of mutations (Fig. 2d). We used a random set of intergenic SNPs for standardization, which makes $R_{xy}$ robust against sampling effects and population substructure\textsuperscript{28}. Low and moderate impact mutations (i.e. mildly deleterious mutations) showed a minor excess in Alpine ibex compared to Iberian ibex, indicating a higher load in Alpine ibex. In contrast, we found that highly deleterious mutations were strongly reduced in Alpine ibex compared to Iberian ibex (Tukey test, $p < 0.0001$, Fig. 2e). Individual allele counts at highly deleterious sites were also significantly lower in Alpine ibex compared to Iberian ibex ($t$ test, $p = 0.003$). We assessed the robustness of the $R_{xy}$ analyses using four additional mutation scoring methods (i.e. SIFT, REVEL, CADD, and VEST3). We found that the highest impact category had a consistent deficit in Alpine ibex compared to Iberian ibex ($t$ test, $p = 0.015$, Fig. 2e). Together, this shows that highly deleterious mutations were substantially purged in Alpine ibex. We also found evidence for the accumulation of mildly deleterious mutations through genetic drift in Alpine ibex.
Accumulation and purging of deleterious mutations. Consistent with the fact that all extant Alpine ibex originate from the Gran Paradiso, this population occupies the center of a principal component analysis (Fig. 3a, b, Supplementary Fig. 13a, b;38). The first populations re-established in the Alps were clearly differentiated from the Gran Paradiso source population and showed reduced nucleotide diversity due to reintroduction bottlenecks37 (Fig. 3a–c). These initial three reintroduced populations were used to establish additional populations raising the total number of experienced bottlenecks to 3 or 4. The additional bottlenecks led to further loss of nucleotide diversity and genetic drift, as indicated by the increasing spread in the principal component analysis (Fig. 3a–c). An exceptional case constitutes the Alpi Marittime population, which was established through the translocation of 25 Gran Paradiso individuals of which only six successfully reproduced44. As expected from such an extreme
bottleneck, Alpi Marittime showed strong genetic differentiation from all other Alpine ibex populations and highly reduced nucleotide diversity (Fig. 3b, c). To compare the strength of drift experienced by different populations, we estimated long-term effective population sizes. For this, we used detailed demographic records spanning the near century since the populations were established. We found that nucleotide diversity decreased with smaller long-term population size (Spearman’s rank correlation, rho = 0.93, p = 0.007, Fig. 3c). We found the same trend for the individual number of heterozygous sites per kilobase (Supplementary Fig. 14). Populations with the lowest harmonic mean population sizes also showed the highest levels of inbreeding. Genomes from the Gran Paradiso source population generally showed the lowest proportions of the genome affected by ROH, while reintroduced populations of lowest effective population size had the highest proportions of the genome affected by ROH (Pearson’s product-moment correlation, df = 19, r = −0.70, p = 0.0004, Fig. 3d and Supplementary Fig. 3). The $R_{xy}$ statistics showed a strong deficit of highly deleterious mutations in the Alpi Marittime population suggesting that the most deleterious mutations were efficiently purged (Fig. 3e and see below).

Fig. 3 Genomic consequences of the Alpine ibex recolonization. a The recolonization history and population pedigree of Alpine ibex. Locations include also zoos and the population Pilatus (pi), which was not sampled for this study. am: Alpi Maritime, gp: Gran Paradiso; ih: Zoo Interlaken Harder; al: Albris; bo: Bire Oschinen; br: Brienzer Rothorn; ob: Oberbauenstock; pl: Pleureur; rh: Rheinwald; wh: Weisshorn; pi: Pilatus; pp: Wildpark Peter and Paul. The gray circle represents a population that was founded from more than one population. Figure elements were modified from Biebach and Keller (2009) with permission. b Principal component analysis of all Alpine ibex individuals included in the study. c Nucleotide diversity per population. d Proportion of the genome within runs of homozygosity (ROH) longer than 2.5 Mb. e $R_{xy}$ analysis contrasting the strongly bottlenecked Alpi Maritime population with all other Alpine ibex populations across the spectrum of impact categories. $R_{xy}$ < 1 indicates a relative frequency deficit of the corresponding category in the Alpi Maritime population. $R_{xy}$ distributions are based on jack-knifing across chromosomes. Circles with a black outline indicate the first three reintroduced populations in Switzerland that were used for all subsequent population reintroductions of Alpine ibex. Colors associate founder and descendant populations. Box plot elements are defined as in Fig. 1. Source data are provided as a Source Data file.
Bottlenecks affect the landscape of deleterious mutations by randomly increasing or decreasing allele frequencies at individual loci. We find that individuals from populations that underwent stronger bottlenecks carry significantly more homozygotes for nearly neutral and mildly deleterious mutations (i.e. modifier, low and moderate impact mutations; Fig. 4a). In contrast, individuals showed no meaningful difference in the number of homozygotes for highly deleterious (i.e. high impact) mutations across populations. The stability in the number of homozygotes for highly deleterious mutations through successive bottlenecks despite a step-wise increase in the number of homozygotes for weaker impact mutations, supports that purging occurred over the course of the Alpine ibex reintroductions. We repeated the analyses using an alternative scoring of mutations based on the phylogenetic conservation of the region in which the mutations were found (i.e. Genomic Evolutionary Rate Profiling; GERP). The number of homozygotes for mutations in highly conserved regions showed a slight upwards trend still indicating purifying selection but not necessarily purging for this category of mutations (Supplementary Fig. 15). Interestingly, GERP scores (as well as other conservation-based scores) seem to perform well at distinguishing neutral mutations from mutations under selection, but less so to discriminate weakly from highly deleterious mutations. Hence, this suggests that the mutational impact (e.g. premature stop codons) rather than degree of conservation predicts whether purging is likely to occur. This suggests also that mean fitness should be more directly assessed using e.g. simulation datasets (see below). Because the above findings are contingent on a model where deleterious mutations are recessive, we also analyzed the total number of derived alleles per individual. We find a consistent but less pronounced increase in total number of derived alleles per individual for nearly neutral and mildly deleterious mutations (Fig. 4b). In contrast, the total number of derived alleles for highly deleterious mutations did not correlate with the strength of bottleneck and was lowest in the most severely bottlenecked Alpi Marittime population (Fig. 4b), suggesting that the most deleterious mutations were purged in this population. The Rxy statistics showed a corresponding strong deficit in the Alpi Marittime population (Fig. 3e). We repeated the Rxy analyses using four additional mutation scoring methods (i.e. SIFT, REVEL, CADD, and VEST3) and found a consistent deficit of the highest impact category in the Alpi Marittime (Supplementary Fig. 12). Overall, we find evidence for more purging in the most bottlenecked Alpine ibex population.

We analyzed the impact of highly deleterious mutations by predicting the protein truncation using homology-based inferences. Focusing on mutations segregating in Alpine ibex, we found that nearly all mutations disrupted conserved protein family (PFAM) domains encoded by the affected genes (Fig. 5). This shows that highly deleterious mutations not only are altering the length of open reading frames but that evolutionarily conserved protein domains are affected by the mutations.

**Individual-based simulations under a realistic demographic scenario.** We further analyzed the impact of bottlenecks on different mutation classes using an individual-based forward simulation model parametrized with the demographic record (Fig. 6a). The model realistically reproduced the reintroduction history and populations were parametrized with the actual founder size (Fig. 6a, Supplementary Fig. 16, Supplementary Table 1). We used Rxy to analyze the evolution of deleterious mutation frequencies through the reintroduction bottlenecks. The simulations showed a deficit of highly deleterious mutations, but an increase of mildly deleterious mutations after the reintroduction bottlenecks (Fig. 6b). This is consistent with our empirical evidence for purging during the species bottleneck (Fig. 2e). We computed genetic load defined as the mean individual fitness in
females and found an increase in Alpine ibex following the species bottleneck (Supplementary Fig. 17). Hence, the accumulation of mildly deleterious mutations was reducing overall fitness despite purging. The simulations also supported purging at the level of individual populations as found in the extremely bottlenecked Alpi Marittime population (Fig. 3e). The number of derived mildly deleterious homozygotes increased with the strength of drift experienced by individual populations (Fig. 6c, Supplementary Figs. 18–21). In contrast, the median number of homozygote counts for high impact mutations were lower for Alpi Marittime than Gran Paradiso but not statistically significant (Fig. 6c, Mann–Whitney U, $p = 0.39$). The highly deleterious allele counts were significantly lower for Alpi Marittime compared to Gran Paradiso (Supplementary Fig. 19, Mann–Whitney U, $p = 0.001$). We also analyzed $R_{xy}$ for the simulation data and found that high impact mutations were indeed relatively less frequent in Alpi Marittime compared to all other Alpine ibex populations (Fig. 6d) and compared to Gran Paradiso (Supplementary Fig. 22). Overall, the realistically parametrized model recapitulated all major empirical findings of mutation accumulation and purging across bottlenecks.

**Discussion**

Ibex species with recently reduced population sizes accumulated deleterious mutations compared to closely related species. This accumulation was particularly pronounced in the Iberian ibex that experienced a severe bottleneck and Alpine ibex that went nearly extinct. We show that even though Alpine ibex carry an overall higher mutation burden than related species, the strong bottlenecks imposed by the reintroduction events purged highly deleterious alleles. The highly deleterious allele counts were significantly lower for Alpi Marittime compared to Gran Paradiso (Supplementary Fig. 19, Mann–Whitney U, $p = 0.001$). We also analyzed $R_{xy}$ for the simulation data and found that high impact mutations were indeed relatively less frequent in Alpi Marittime compared to all other Alpine ibex populations (Fig. 6d) and compared to Gran Paradiso (Supplementary Fig. 22). Overall, the realistically parametrized model recapitulated all major empirical findings of mutation accumulation and purging across bottlenecks.

**Fig. 5 Homology-based inference of the impact of highly deleterious mutations.** The localization of protein family (PFAM) domains are highlighted in dark. Red dots indicate the relative position of a highly deleterious mutation segregating in Alpine ibex. The frequencies of highly deleterious mutations are summarized for Iberian ibex and three groups of Alpine ibex. Allele frequencies are shown as boxplots per group representing the outcome of downsampling to the smallest group (Alpi Marittime, $n = 3$) based on 100 replicates. The frequencies of highly deleterious mutations tend to be higher in Alpine ibex because mutation ascertainment was performed in this species. Box plot elements are defined as in Fig. 1. Source data are provided as a Source Data file.
deleterious mutations. Importantly, purging was only effective against highly deleterious mutations. Mildly deleterious mutations actually accumulated through the reintroductions. Hence, the overall number of deleterious mutations increased with bottleneck strength. This is consistent with the finding that population-level inbreeding, which is a strong indicator of past bottlenecks, is correlated with lower population growth rates in Alpine ibex.

Empirical evidence for purging in the wild is scarce. Here, we show that a few dozen generations were sufficient to reduce the burden of highly deleterious mutations. Purging may occur widely in populations undergoing severe bottlenecks contingent on populations surviving the consequences of inbreeding depression. Failure to purge under extreme bottlenecks can have severely deleterious consequences for wild populations such as shown for the Isle Royal wolves. As predicted from theory, Alpine ibex reintroduction bottlenecks allowed mildly deleterious mutations to accumulate in contrast to highly deleterious mutations. Our empirical results are also in line with predictions that populations with an effective size below 100 individuals can accumulate a substantial burden of mildly deleterious mutations.

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**Fig. 6 Individual-based simulations of the reintroduction history of Alpine ibex.**

- **a** The demographic model was parametrized using census data and historical records. Bold numbers show effective population sizes. Numbers not in bold indicate the number of individuals released to found each population. If a population was established from two source populations, the individual numbers are separated by commas. * Upwards adjusted harmonic means of the census size (historical records were ih = 16, Zoo Interlaken Harder, and pp = 20, Wildpark Peter and Paul). The adjustment was necessary to prevent extinction of zoo populations. ** Census numbers were estimated based on historical records of the population but no long-term census data were available.
- **b** Relative frequency comparison ($R_{xy}$) of Alpine ibex just before and after the species bottleneck and recolonization. **c** Individual homozygote counts per impact category. Boxplots summarize 100 population means across simulation replicates. Colors associate founder and descendant populations (see also Fig. 3a).
- **d** $R_{xy}$ analysis contrasting the strongly bottlenecked Alpi Marittime population with all other Alpine ibex populations across the spectrum of impact categories. Box plot elements are defined as in Fig. 1. Source data are provided as a Source Data file.
Iberian ibex supports the notion that even population sizes of ~1000 still accumulate mildly deleterious mutations. High loads of deleterious mutations have been shown to increase the extinction risk of a species. Thus, conservation efforts aimed at keeping effective population sizes above a minimum of 1000 individuals are critical for the long-term survival of managed species.

Methods

Genomic data acquisition. DNA samples from 29 Alpine ibex, 4 Iberian ibex, 2 Nubian ibex, 2 SIER ibex and 1 Markhor individuals were sequenced on an Illumina HiSeq2500 or HiSeq4000 to a depth of 15–38 (median of 17). Supplementary Table 2 shows individual sampling locations. Libraries were produced using the TruSeq DNA Nano kit. Illumina sequencing data of 6 Bezoar and 16 domestic goat (coverage 6x–14x, median 12x) were generated by the NextGen Consortium (https://nextgen.epfl.ch). The corresponding raw data were downloaded from the EBI Short Read Archive: ftp://ftp.sra.ebi.ac.uk/vol1/fastq/.

Read alignment and variant calling. Trimmomatic v.0.36 was used for quality and adapter trimming before reads were mapped to the domestic goat reference genome (version CHIRI12) using Bowtie2 v.2.3.5.5. MarqDuplicates from Picard (http://broadinstitute.github.io/picard/v1.130) was used to mark duplicates. Genotype calling was performed using HaplotypeCaller and GenotypeGVCF (GATK, v3.6.2.5). VariantFiltering of GATK was used to remove single nucleotide polymorphisms (SNP) if QD < 2.0, FS > 40.0,SOR > 5.0, MQ < 20.0, −3.0 > ReadDepth > −3.0: ReadDepth > 30.0 and AFI < 80% (of all Alpine ibex individuals). Indels up to 10 bp were also retained and filtered using the same filters and filter parameters, except for not including the filter MQRankSum, because this measure is more likely to be biased for indels of several bp. Filtering parameters were chosen on genome-wide quality statistics distributions (see Supplementary Figs. 23–25). We further followed the following categorizations adopted for human SFS analyses were performed for SnpEff and GERP categories and two other options: (i) −2 (reverse stranded) −f (count reads at the exon level), −O (assign reads to all their overlapping features), −C (excluding read pairs mapping to different chromosomes or the same chromosome but on a different strand). The R package edgeR was used to calculate FPKM (Counts Per Kilobase of Exon Per Million Mapped) per each gene and organ. For variant sites that were included in more than one exon, the highest FPKM value was used. We found that 16,013 out of 17,998 genes showed transcriptional activity of at least one exon (FPKM > 0.3). Overall 166,973 out of 178,504 exons showed evidence for transcription. In a total of 1928 genes, one or more exons showed no evidence for transcription. Retained SNPs were found among 118,756 and 17,685 genes. Overall 611,711 out of 677,578 SNPs were located in genes with evidence for transcription.

Deleterious mutations are assumed to be overwhelmed by derived mutations. We used all ibex species except Alpine and Iberian ibex as an outgroup to define the derived state. For each bi-allelic site, which was observed in alternative state in Alpine ibex or Iberian ibex, the alternative state was defined as derived if its frequency was zero in all other species (a total of 44,730 autosomal SNPs). For loci with more than two alleles, the derived state was defined as unknown. For comparisons among all species, we only used the following criteria to select SNPs (370,853 bi-allelic SNPs retained; transcriptional activity (FPKM > 0.3) and that were considered as a maximum of 3 bp to 7 bp as extreme effects. We also required a minimal distance to the next SNP of 3 bp to avoid confounding effects of potential multi-nucleotide polymorphisms (MPM).

Population genetic analyses. SFSs were calculated using the R package phyloseq and dp yn. SFS analyses were performed for SnpEff and GERP categories and two additional conservation scores: phyloDF60 and phastCons67. We chose a cutoff of 0.1 to distinguish conserved from less conserved sites. In the case of phyloP, sites with slightly lower but the qualitative trends hold among species and population (Supplementary Figs. 41 and 42).
a score above 1 were defined as conserved. For phastCons, sites with a score equal to 1 (the maximum observed value) were considered as conserved.

For individual counts of derived alleles or homozygotes, we used all biallelic sites polymorphic either in Alpine or Tibetan ibex (or both) for which the derived state was known with a maximal missing rate per locus of 10%. We retained all sites matching these criteria for any downstream analyses even if a particular site was not polymorphic in any given population. The effective rate of missing data per locus was less than 0.03–0.07% (Supplementary Fig. 43) and no correlation was found between missing rate per population and counts. We found no qualitative differences if we included only loci with a 100% genotyping rate (Supplementary Fig. 44).

We calculated the relative number of derived alleles Rxy28 for the different categories of mutations. Rxy compares the number of derived alleles found at sites within a specific category. Following23,24, we used a random set of 65529 intergenic SNPs for standardization, which makes Rxy robust against sampling effects and population substructure. We performed the Rxy analysis for the four SnpElF categories as well as four additional mutation scoring methods: SIFT,76,77 REVEL,78, CADDS95 and VEST37. All scores mapped to hg38 chromosomal positions were retrieved using the web interface of the Variant Effect Predictor (VEP) by ensemble72. The scores were mapped to chromosomal positions in the domestic goat reference genome using LiftOver (http://hgdownload.cse.ucsc.edu, v.287) with the chain file accessed from http://hgdownload.cse.ucsc.edu/goldenPath/caprH1/.

As we applied these scores outside of humans, we did not have pathological evidence underpinning score cut-offs for deleteriousness. We conservatively used score cut-offs proposed as best-practices by the tool developers (REvel: 0.75, CADDS: 20, SIFT: 0.05) or used a very conservative percentile (99%, 0.91 VEST3). The number of segregating deleterious mutations and heterozygosity at deleterious categories of mutations.

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