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Evolution of the Geographic Range of a European Montane Leaf Beetle in Response to Climate Changes at the End of the Quaternary

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Abstract

Aim: During the past few 100,000 years, repeated cycles of glaciation and warming episodes have radically altered the range of organisms. Numerous phylogeographic studies have investigated the impact of past climate changes on the range of temperate organisms in Europe, but we know substantially less about organisms that are adapted to colder climates. Their distributions are often currently fragmented and limited to relatively high elevations in mountainous regions and to the north of Europe. Our inferences of their range during the last glaciation appear contradictory: some studies indicate a range expansion through colonisation of the lowlands, while others suggest a range contraction because they were restricted to small refugia. In this study, we wished to identify which of these two alternative hypotheses explains best the current distribution of genetic variation in a European montane leaf beetle whose current range spans several mountains.

Location: Four European mountain systems: Alps, Vosges, Massif Central, Pyrenees.

Time Period: End of the Quaternary, focusing mainly on the last glaciation and the Holocene.

Taxon: Gonioctena quinquepunctata [Fabricius, 1787] (Coleoptera; Chrysomelidae).

Results: Modelling of the potential current and last glacial maximum species distributions suggested that climatic conditions were more favourable in the lowlands during the last glaciation, opening the possibility that this leaf beetle has colonised them at the time. Characterising genomic variation across its current range using RAD- seq data and comparing alternative hypotheses about its evolution with coalescence models in a composite likelihood framework contradicted this hypothesis; however: these analyses inferred instead that the species was associated with a much smaller population size during the last glaciation, suggesting that its range was then restricted to the mountains' outskirts.

Main Conclusions: Comparing these results to those of other studies of European cold- adapted species, we argue that phylogeographic evidence points towards a similar glacial contraction of the range of many similar organisms.

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1. Introduction

Successive Quaternary cycles of glacial and interglacial periods spanning several hundred thousand years have driven major shifts in the range of organisms worldwide, shaping their current spatial pattern of genetic variation and the overall distribution of species diversity (Hewitt 2000, 2004; Rahbek et al. 2019; Schmitt 2007). In the last three decades, analysing DNA sequence variation has opened a window towards the evolution of the range of organisms during the late Pleistocene and Holocene (Avise 2000; Hewitt 2001), allowing biologists to explore more accurately the impact of climate change during that period. These phylogeographic studies have been particularly numerous for the northern hemisphere, especially for Europe, although most of them have focused on temperate climate organisms, that is, those that are currently found in the lowlands of temperate climate zones (e.g., the common beech, *Fagus sylvatica*, or the red deer *Cervus elaphus*). From these and from fossil evidence, it can be generally concluded that their distributions covered only a fraction of their current range during the last glaciation (i.e., they were subject to a range contraction), as they were restricted at the time to southern (mostly mediterrannean) refugia (Taberlet et al. 1998; Hewitt 2001; Tzedakis et al. 2002; Tzedakis et al. 2013; but see Bisconti et al. 2011 and Salvi et al. 2014 for counterexamples involving mediterrannean insular and coastal species) and sometimes to small central and northern cryptic refugia (Benke et al. 2009; Kotlik et al. 2006; Pedreschi et al. 2019; Provan and Bennett 2008; Schmitt and Varga 2012; Stewart and Lister 2001; Teacher et al. 2009). More recently, phylogeographic studies have focused on other types of European organisms: (1) cold- adapted species, defined here as those that are currently restricted to the North of Europe, from northern England, Germany and Poland to the Arctic Circle, and/or to the southern mountains of Europe (e.g., Dalén et al. 2005; Muster and Berendonk 2006; Quinzin et al. 2017; Theodoridis et al. 2017; Westergaard et al. 2011), and are thus associated with a strongly fragmented distribution; and (2) species inhabiting steppes and other dry grasslands (Kajtoch et al. 2016; Kirschner et al. 2020).

Cold- adapted organisms could have experienced a different evolution of their range compared to temperate organisms, because climatic conditions were presumably more favourable across most of Europe during glacial periods (Stewart et al. 2010). Therefore, their geographic range may have been larger during glacial periods (i.e., they experienced a range expansion) than during interglacial periods (Kropf et al. 2003; Stewart and Dalén 2008), although this may not have been universal (Schmitt 2007; Stewart et al. 2010). Even though the area associated with favourable climate may have been larger during glacial periods, geographic barriers to migration or their insufficient ability to disperse (Dalén et al. 2007) may have prevented some of these organisms from colonising all available territories at the time. The picture emerging from studies investigating the past distribution of cold-adapted organisms is less clear than for temperate climate organisms: some studies highlight strong genetic divergence among mountain systems and between southern mountains and northern Europe (Pauls et al. 2006; Schönswetter et al. 2003; Theissinger et al. 2013), suggesting that lowlands between mountains constituted a strong barrier against migration during the last glaciation (Eidesen et al. 2013); others have suggested on the contrary that these lowlands offered suitable climatic conditions during glacial periods and have therefore been colonised then by cold- adapted species (Birks 2008; Espíndola et al. 2012). If cold- adapted organisms, restricted

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during interglacial periods to mountains in the south and to portions of Northern Europe where the climate is suitable, colonised the lowlands during the last glaciation, it would have resulted in a glacial range expansion.

In agreement with this, when focusing on cold- adapted organisms currently living in mountains of temperate zones, three alternative historical hypotheses are considered to explain their distribution (Holderegger and Thiel-Egenter 2009): (1) they were distributed mainly in lowland regions during glacial periods, and have recolonised the mountain ranges at the end of the last glaciation (lowland refugia hypothesis); (2) they were mostly located in separate refugia in the mountain range's periphery during glacial periods, and have recolonised higher elevation areas of the same mountains at the end of the last glaciation (peripheral refugia hypothesis) and (3) they survived in nunataks, that is, ice- free mountain peaks that protruded from the ice sheet, during glacial periods (nunatak refugia hypothesis). Note that hypotheses 2 and 3 can in some cases be combined, as the current mountain range could have its source in both peripheral refugia and nunatak microrefugia (Lohse et al. 2011; Pan et al. 2020; Schneeweiss and Schönswetter 2011; Wachter et al. 2016).

Because both range expansion and range contraction during glacial periods have been hypothesised for cold-adapted organisms in Europe, whether they are currently present in the mountains of central and southern Europe or in the lowlands of the north (or both), it is important to continue gathering empirical data before we can draw general conclusions on the evolutionary history of such species. It is possible that both hypotheses are correct, each concerning different types of cold- adapted organisms. For example, we could consider separately organisms associated with boreo- montane or arctic- alpine distributions. Both types are adapted to cold climate, but arctic-alpine organisms should be adapted to lower temperatures than boreo- montane species. Also, some organisms have a tolerance to a wider range of temperatures than others, which may influence the evolution of their range. Another possibility is that the evidence that seemed to support one of the two hypotheses (range expansion or range contraction) has in fact been misinterpreted. In this regard, it is worth noting that many of the past phylogeographic studies in Europe were based on one or a few molecular loci and did not analyse genetic variation in a rigorous hypothesis- testing framework. This could be insufficient to infer reliable historical estimates. Indeed, coalescent theory shows that gene genealogies can vary widely among loci due to the stochastic nature of the coalescence process (Hudson 1990). It could even be misleading if the selected locus (or loci) is (are) not neutral or was (were) subjected to introgression in the past (Toews and Brelsford 2012). Recently, our ability to sequence DNA has made enormous progress, and inferring phylogeographic history based on a much larger fraction of the genome is likely to be more reliable and more informative (Carstens et al. 2012; Emerson et al. 2010; Garrick et al. 2015). New programmes allowing the analysis of such large amounts of genomic data with coalescence models for hypothesis testing have also become available (e.g., Excoffier et al. 2013, 2021; Gutenkunst et al. 2009). We thus wished to take advantage of these recent technical developments to further investigate the fate of cold-adapted organisms during the last glaciation by analysing genome- wide variation in a cold- adapted organism and comparing the alternative hypotheses mentioned above within a rigorous coalescence model framework.

More specifically, we focused on the phylogeographic history of a European montane leaf beetle, *Gonioctena quinquepunctata* [Fabricius], whose fragmented distribution spans multiple mountain ranges in central and southern Europe and extends to the northern lowlands of Europe, including the south of Fennoscandia (Boreo-montane distribution; Quinzin and Mardulyn 2014). Because it feeds on trees (to the best of our knowledge, only

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those from the genera *Sorbus* and *Prunus*; Quinzin and Mardulyn 2014; Schilthuizen et al. 2016), we felt confident we could reject a priori the nunatak refugia hypothesis, as it is extremely unlikely that the host trees were present above the ice sheet during glacial periods. Indeed, hindcasting the distribution of the host trees in Europe at the last glacial maximum suggested that they were absent from high elevation areas in the Alps (Quinzin et al. 2017). Instead, we asked whether this insect survived mainly in the mountain's periphery during the last glaciation and thus was already associated with a highly fragmented range then, or has colonised a large and continuous lowland area between mountains during that period, before recolonising the different mountain systems at the end of the glaciation.

It has been highlighted that the term cold- adapted species includes organisms associated with a tolerance to a large variety of temperature ranges (Theodoridis et al. 2017), and it may be necessary to contrast different types of cold- adapted organisms to understand the diversity of histories that are associated with different species. Because *G. quinquepunctata* is found only in the elevation range 600–1300 m in central and southern Europe and is thus associated with the forest belt of mountain systems, it can be considered as a montane species (Schmitt et al. 2010) there. As such, it constitutes an interesting intermediate model between temperate organisms that are currently found in the lowlands of the same region and alpine or arctic- alpine organisms that are associated with colder temperatures and are found at higher elevations in European mountains. Since *G. quinquepunctata* is found at lower elevation in mountains than alpine species, it may have been easier for it to reach the adjacent lowlands when the region transitioned to a colder climate. Thus, if this insect was unable to colonise the lowlands during the last glaciation, it is unlikely that arctic- alpine species, currently found at higher elevations, at least those with a similar capacity to disperse, would have been able to. For this reason, this study offers an interesting test of the likelihood of a lowland refuge hypothesis.

We therefore first used species distribution modelling (SDM) to estimate the potential current and last glacial maximum (LGM) distributions of *G. quinquepunctata* based on climatic and topographic variables, thereby testing whether climatic conditions in the lowlands of Europe during glacial periods were suitable for hosting this species. We then analysed genomic variation from numerous samples collected in the four main mountain ranges in which the species is most abundant, using a reduced representation sequencing approach (RAD- seq) to characterise genetic variation across the species range. Finally, we compared alternative historical hypotheses using coalescence modelling to (1) identify the most likely glacial refuge hypothesis (peripheral or lowland glacial refuge) and (2) estimate how long populations associated with different mountain ranges have been isolated. Once we have inferred how the species range has evolved in response to the climate changes of the end of the Quaternary, we compared our findings with patterns of genetic diversity uncovered for other cold- adapted European species. We then discuss whether a common interpretation is possible.

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2. Material and Methods

2.1 Species Distribution Models

We first used a total of 60 occurrences of the species across the four main mountain systems of its geographic range (Table S1), all from our own sampling record and including sampling sites used in this study. Nineteen bioclimatic and elevation (DEM) variables were downloaded from CHELSA V2.1 (Karger et al. 2020) at 30 arc-second resolution for present time (1981–2010). Several topographic predictors were generated with this DEM and the terrain function from the terra R package (Hijmans et al. 2023), using the eight surrounding cells (Table S2). While the 60 occurrences used for this analysis are highly reliable, they were exclusively located in the mountainous regions of the species distribution. Because the presence of the species is also recorded in the lowlands of northern Europe (although at a much lower frequency than its sister species *Gonioctena intermedia*), a second analysis was conducted on an expanded dataset that included additional occurrence points from the GBIF database. These occurrences are less reliable, especially for this species that is difficult to distinguish from its sister species (see below) but do include records from northern Europe. We therefore filtered all GBIF occurrences of this species, keeping only those that originated from trusted institutions (universities and museums of natural history) with records in Europe lowlands, as well as research grade iNaturalist data points (https:// doi. org/ 10. 15468/dl. dcse9u). The expanded dataset included a total of 192 occurrences, which reduced to 89 occurrences when keeping a single data point per pixel for the downstream analyses and included occurrences in the lowlands of northern Europe. Unfortunately, no GBIF occurrences were found for the Appennines (Italian peninsula) or for the Carpathian mountains, regions in which the presence of the species has been reported previously (Daccordi et al. 1991; Quinzin and Mardulyn 2014). Note that we have not extensively searched museum collections that could contain interesting georeferenced records. One limitation with such records is that older samples are often associated with geographic coordinates that are insufficiently precise for the type of SDM analyses conducted in this study. The accuracy of geographic coordinates is particularly important with mountain sites, in which a slight deviation in geographic distance often results in a major elevation shift, and thus in a strong climate difference.

To select predictors, we used the embedded variable selection developed by Adde et al. (2023) which consists of two steps:

(1) Collinearity filtering, which allows reducing collinearity issues (see Dormann et al. 2013) and (2) Model- specific embedding, which allows selecting the best combination of predictors. For the first step, we filtered our occurrence data to remove occurrences occurring in the same pixel via the dismo package (Hijmans et al. 2023). We also randomly sampled 10,000 background points across the geographic background using the function *Bp_Sampling* from the package "ESM" (Collart et al. 2024). We then conducted the collinearity filtering. We thus generated univariate generalised linear models (GLM) with a binomial distribution, allowing quadratic terms and setting the prevalence to 0.5 for each predictor. *p* values for each model were then ranked. The predictor with the smallest *p* value was kept and all predictors associated with a Pearson's correlation > 0.7 were removed (as recommended in Dormann et al. 2013). We continued this process until no more predictor remained. For the second step, we fitted multivariate GLM with elastic- net regularisation and guided regularised Random Forest,

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allowing quadratic terms. We then ranked predictors based on each model based on the absolute value of the regularised regression coefficients for GLM, and the Mean Decrease Gini index for RF. These ranks were then combined between the two models by ranking the sum of the two ranks for each predictor, beginning with the predictors that were selected by the two algorithms, and then adding the remaining ones. The number of predictors was set to 6 (one predictor per 10 species observations) to avoid overfitting issues.

After the predictor selection, we performed the species distribution models using the biomod2 package (Thuiller et al. 2023) via four modelling techniques: Generalised Linear Model (GLM), Random Forest (RF), Generalised Boosted Model (GBM) and Maximum Entropy (MAXNET). We used them with the default parameter set in biomod2 (v 4.2–4): for GLM, selection procedure via AIC, quadratic model, interaction level = 0, logit function; RF: 500 trees, a terminal node size of 5 and the square root of the number of variables as the number of variables randomly sampled as candidates at each split; for GBM: a Bernoulli distribution, 2500 trees, a tree complexity of 7, a minimum of five observations in the terminal nodes of the trees, a learning rate of 0.001, a bag fraction of 0.5 and 3 cross- validations; for MAXNET: a value of 1 to adjust regularisation and all possible features (linear, quadratic, hinge, product, threshold) were allowed. Prevalence in the models was set to 0.5, so that background and occurrence points were equally weighted. To evaluate the models, 100 replicates were run using 70% of the data to calibrate the models and 30% to evaluate them using the Area Under the Curve (AUC), maximum of the true skill statistic (MaxTSS) and the Boyce index (Guisan et al. 2017). We computed ensemble models (Araújo and New 2007), wherein all individual models with a MaxTSS ≤ 0 were removed, and wherein the other models contributed proportionally to their MaxTSS.

The models were then projected in the study area at present time and for LGM. For the latter, we downloaded the data from CHELSA following the CCSM4 scenario from the PMIP3 (Karger et al. 2023). The topographic predictors were generated for the LGM using the DEM provided by CHELSA Trace- 21 k for the period 22kbp (Karger et al. 2023). The procedure (variable selection, modelling, evaluation and projection) was also run with the expanded dataset that included GBIF data and the same 10,000 background points generated for the occurrence dataset derived from our own field work.

2.2 Sampling and DNA Extractions

We collected a total of 119 adult samples of *G. quinquepunctata* during May 2013 (Vosges) and May 2016 (Massif Central, Pyrenees and the Alps) in 30 locations across the four mountain ranges in which the species is mostly present: Vosges (*n* = 32), Alps (33), Massif Central (24) and Pyrenees (30) (Figure 1, Table S3). While it also occurs in the Carpathian Mountains and in some lowland regions of northern Europe such as southern Finland, it is much less abundant there, as these areas are dominated by its sister species *G. intermedia*. Note that samples were collected only in the western portion of the Alps, while the species is also present in the east of that mountain system. A previous analysis of genomic variation has shown that eastern populations of the species in the Alps are genetically different from those from the west, and the western region included in this study can therefore be considered a separate and independent population (Kastally et al. 2019). Similarly, we did not include samples from the Apennines in the genetic dataset. Beetles from the same locality were collected on different trees to avoid collecting related individuals. Individuals were preserved in 100% ethanol. We know that in the localities

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where the two sister species coexist, they occasionally hybridise. However, it was shown that this hybridisation has resulted only in a limited transfer of genetic material (Kastally et al. 2019): analysing genomic variation in both species in its hybrid zone indicated asymmetric introgression of the mitochondrial genome (Quinzin and Mardulyn 2014) and of only ~2% of the nuclear genome (Lukicheva and Mardulyn 2021) from *G. quinquepunctata* towards *G. intermedia*. Therefore, the genome of *G. quinquepunctata* does not include DNA from the sister species.

We extracted genomic DNA using the DNA Blood & Tissue kit from Qiagen, following the manufacturer's protocol. Sample DNA concentrations were estimated with a Qubit fluorometer, and DNA quality was verified via agarose gel electrophoresis.

The generation of RAD- seq data for the Vosges samples (Paired- End reads) was already described in Kastally et al. (2021). For all other samples, RAD- seq libraries of DNA fragments from 300 to 600 bp were prepared by Floragenex (http:// www. flora genex. com), generally following protocols described in Baird et al. (2008), Emerson et al. (2010) and Hohenlohe et al. (2010) and using the restriction enzyme sgrAI. Sequencing of the RAD- seq libraries was conducted by Floragenex on an Illumina sequencing platform (Single- End reads).

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2.3 RAD- Seq Raw Reads Processing

We separated the reads of each sample based on their barcodes using the tool process_radtags provided with STACKS (v2.62; Rochette et al. 2019). To harmonise our two RAD datasets (paired- end reads for the Vosges samples and single- end reads for all others), we used only the first read of each pair for the paired- end data (Vosges samples) and trimmed them to 91 bp. Reads were mapped to *Gonioctena quinquepunctata*'s reference genome (Lukicheva et al. 2021) using the BWA algorithm mem. We then used the program ref_map.pl pipeline

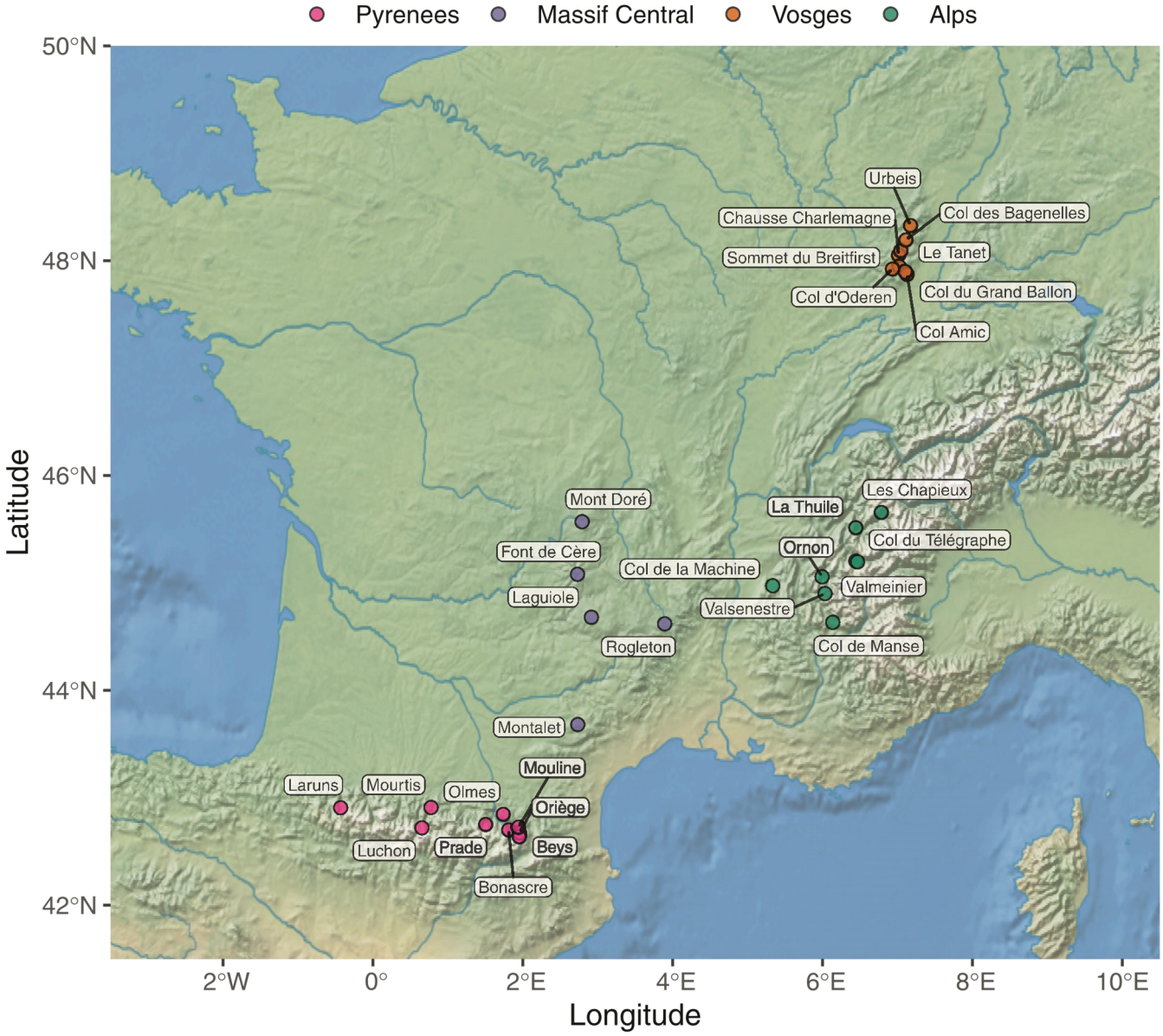

Figure 1 Sampling locations. Dots are coloured according to mountain range.

from STACKS for variant calling.

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We used the information in catalogue.call to exclude all variant and invariant sites with low sequencing depth (< 6) and retained only positions which had at least 50% of data in each of the four sampled regions (Alps, Massif Central, Vosges and Pyrenees). Furthermore, we excluded overlapping positions between independent radtags, which can lead to contradicting calls in STACKS. We excluded all RAD tags that had > 8 polymorphic sites and excluded variant sites with high heterozygosity (> 50%), as these likely contained incorrect calls stemming from duplicated loci that were erroneously grouped into a single locus. After this filtering step, we generated a bed file of the genomic coordinates sampled in our sequencing experiment. We further excluded the repetitive regions with BEDTOOLS v2.30.0 (Quinlan and Hall 2010) using the mask for the reference genome produced with RepeatMasker (Lukicheva et al. 2021).

Finally, we estimated the proportion of polymorphic sites and selected randomly one polymorphic site per RAD tag to reduce LD between sites. We also calculated the ratio of the total number of independent SNPs retained over the total number of polymorphic sites over all loci for estimating the portion of the sequenced genome, useful for some of the downstream analyses (demographic analyses with STAIRWAY PLOT 2 and model comparison with FASTSIMCOAL2).

2.4 Characterisation of Genomic Variation

Based on our characterisation of genetic structure, each mountain range was considered a different population (see Results). Nucleotide diversity for each population (Nei and Li 1979) and the count of all sequenced positions were computed from RAD tag sequences using custom scripts (https://github.com/ckastall/Gquinquepunctata_phylogeo_RADseq). Because STACKS only retains RAD tags that have at least one polymorphic site, we recovered the information on monomorphic RAD tag sequences in the intermediary file 'catalogue.calls' produced by the program. From this file, we performed the same filtering steps on the monomorphic sequences as we did on the variable RAD tags (i.e., excluding positions with depth < 6 and for which missing data within any given population were > 50%).

To characterise genetic structure within the studied area, we used ADMIXTURE v1.3 (Alexander et al. 2009) with default settings. We used the cross- validation procedure in this program to identify the number of clusters (K) that maximised the accuracy of the model. We also performed principal component analyses (PCA) using the R package ADEGENET v2.1.5 (Jombart and Ahmed 2011) with centering of the variables but without scaling. Pairwise F_{ST} between sampling locations and between regions and an overall F_{ST} (Weir and Cockerham 1984) were computed with ARLEQUIN v3.5.2 (Excoffier and Lischer 2010). The same program was used to run an analysis of molecular variance (AMOVA) as a weighted average over all loci and using a hierarchical framework with three levels: between mountain ranges, between sampling sites within mountain ranges, and between individuals within sampling sites. For this analysis, we excluded two locations (Rogleton and Oriège) because they included each a single individual.

Following Rousset (1997), we estimated the isolation by distance within mountain ranges by estimating the slope of the genetic distance (F_{st} / [1—F_{st}]) according to the $\log_{10}$ of the geographic distance (in km) between each pair of sampling locations.

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2.5 Demographic Inferences

We used STAIRWAY PLOT 2 (Liu and Fu 2020) to estimate the change of effective population size, N_e, over time from the site frequency spectrum (SFS) for each population separately. We estimated the folded SFS of each population and for the entire dataset using the hypergeometric distribution to project all SNPs to the same sample size *n* (50% of the initial sample size of the population, i.e., Alps = 33, MC = 24, Pyrenees = 30, Vosges = 32; Gutenkunst et al. 2009). We selected a typical insect mutation rate of 2.1×10^{-9} per base pair and per generation (Oppold and Pfenninger 2017) and counted a single generation per year (*G. quinquepunctata* is univoltine). We used the formula recommended in the manual to calculate the number of break points ('nrand'): '(n−2) / 4', '2(n−2)/4', '3(n−2)/4' and 'n–2' and the value 200 for the estimation (ninput). To determine the number of sites sequenced (parameter L), we summed the total number of base pairs retained in the entire set of RAD- tags (including both variable and invariable RAD- tags, see above) that we multiplied by the ratio of independent SNPs retained (one per RAD- tags) for building the folded SFS over the total number of SNPs.

2.6 Phylogeographic Inferences

A joint SFS was computed for each pair of populations using the down projection method described in the previous paragraph (Demographic inferences) to infer new sample sizes that maximised the total number of SNPs with no missing data.

To compare historical hypotheses, we used FASTSIMCOAL2 v2.7 (Excoffier et al. 2021) that implements a composite likelihood approach. Alternative hypotheses were designed based on SDM projections, our prior characterisation of genetic variation across the studied range and on our perception of how the beetles could have colonised its current range at the end of the last glaciation. In all tested evolutionary models, four current populations were defined, one per mountain range. While the Admixture analyses suggested that individuals from Massif Central and Pyrenees belonged to a single population (see Results), the two mountain ranges are geographically isolated from each other (i.e., they are separated by lowlands currently uninhabitable by the beetle). It is therefore more coherent to treat them as two separate populations, assuming that the lack of differentiation is caused by their recent separation.

In essence, we contrasted three main historical hypotheses (Figure 2): (1) a largely vicariant history, in which the current disjunct range resulted from the fragmentation of a former larger range sometimes in the past (Range fragmentation model); this is the most simple model we can think of that can explain the observed fragmented distribution of the species; we used it as a null model against which we compared the two following models that represent the hypotheses described at the end of the introduction; (2) colonisation of mountains at the end of the last glaciation, from a large lowland continuous population located around the mountain ranges during that glaciation (Lowland refuge model) and (3) current mountain populations originated from small refuge populations that existed during the last glaciation in the direct periphery of these mountain ranges (presumably in lower elevation localities; Peripheral refuges model). In this last case, multiple versions of the hypothesis were considered (Figure S1), in which colonisation of some mountain systems occurred from other mountain systems at various times in the species history: before, during, or just at the end of the last glaciation, from small peripheral

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refuges. In some versions of the model, the split of an ancestral population in two or more separate mountain ranges occurred via vicariance (peripheral refuges A–C, Figure S1), while in others, the split occurred via colonisation (i.e., involving a population bottleneck to account for the associated founder effect; peripheral refuges C–G, Figure S1). Note that in some models, the separation of the Massif Central and Pyrenees regions is more recent than in others, to account for our observation that they are currently genetically closer to each other. Each historical hypothesis was translated into a coalescence model defined in an input file for FASTSIMCOAL2 (Supporting Information). Prior intervals of parameter values associated with each model are reported in Table S4. In all models, we used a single parameter to define all current population sizes, with a population adjustment factor proportional to the relative size of the sampled portion of each mountain range (Alps: 1, Vosges: 0.10, Pyrenees: 0.55, Massif Central: 0.79). More specifically, we applied a grid on each mountain range and determined the number of grid cells (50 × 50 km) that spanned our sampled portion of that range and included an area between an altitude of 500 and 1500 m. While these relative values represent rough estimates, we believe they are sufficient for our purpose of comparing historical scenarios. In essence, we thus assumed that current population sizes were simply determined by the size of the sampled area in the corresponding mountain range. This means that if differences in genetic diversity among populations were not proportional to differences in their geographic spread, they would instead be attributed by the analysis to differences in their evolutionary histories. An alternative option would have been to infer each current population size as proportional to their genetic diversity. However, this would have explained the current differences in genetic diversities in terms of population size differences instead of in terms of population history differences, when there is a priori no reason to believe that one mountain range has currently a higher or lower population density than another (habitat and climatic conditions are similar). Each founder effect was modelled as an instant bottleneck, that is, a population size reduction lasting a single generation (parameter *instbot* in FASTSIMCOAL2).

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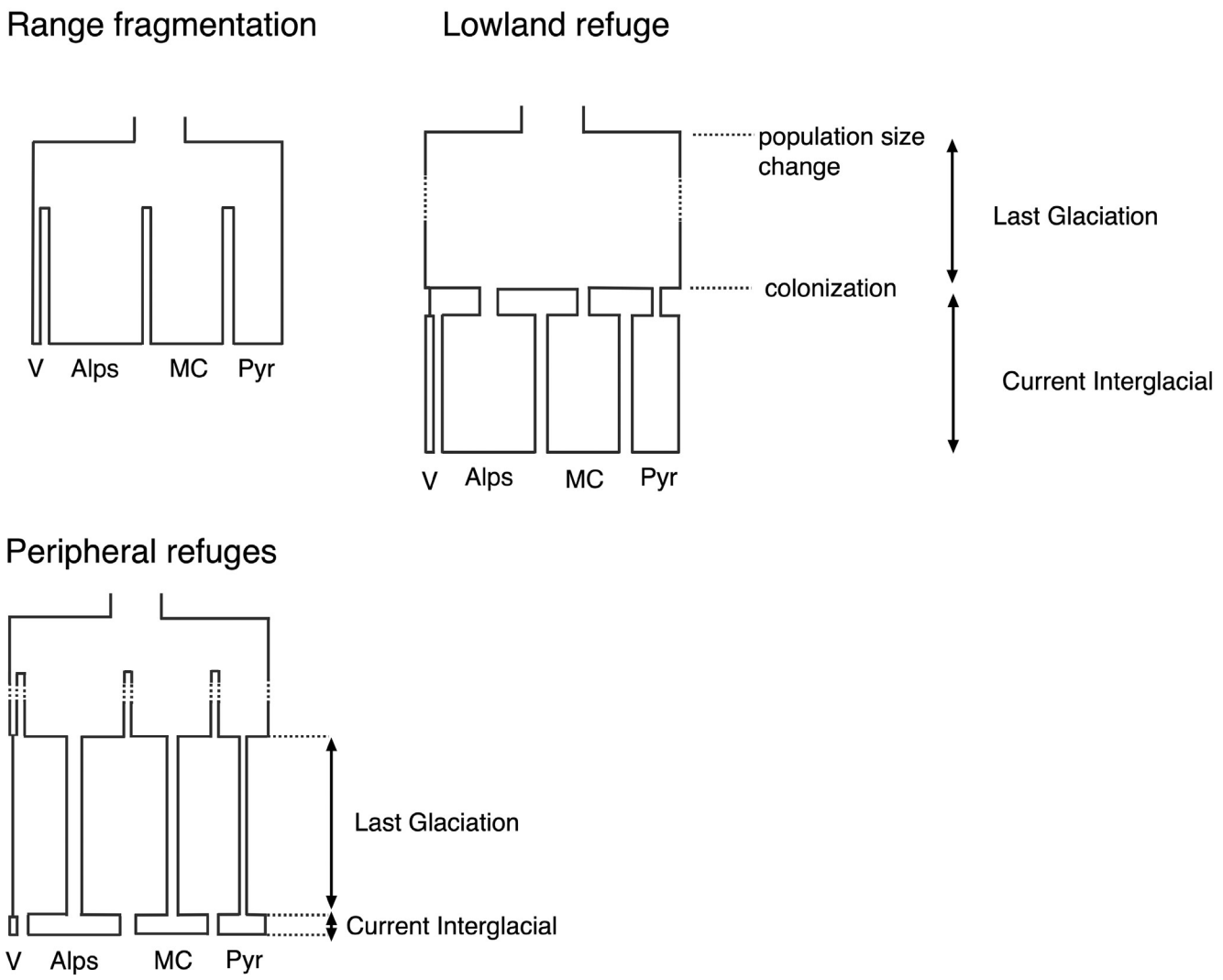

Figure 2 Coalescence models for three historical scenarios compared with FASTSIMCOAL2; V = Vosges, MC = Massif Central; Pyr = Pyrenees. Range fragmentation model: An ancestral continuous population is fragmented into four sub- populations (vicariance event), each associated with a different mountain range. The range of fragmentation time(s) for this model is wide and is not assumed to be associated with a specific climate event a priori. Lowland refuge model: It is assumed that the ancestral population was in the lowlands during the last glaciation; since the SDM suggested a more suitable climate in this area at the time, it is associated with a larger (or, at minimum, equal) size than today. Peripheral refuges model: Populations during the last glaciation are assumed to be geographically restricted to the mountain periphery and are thus associated with smaller sizes than today. In the two last models, colonisation of a mountain range is always associated with a bottleneck event (founder effect). Alternative versions of two models (Range fragmentation and Peripheral refuges) that were tested are described further in Figure S1.

For each model, 100 FASTSIMCOAL2 independent estimation runs were launched to ensure a proper exploration of the parameter space (i.e., to avoid being stuck on a local maximum likelihood peak), using the following command line: fsc27093 - t input.tpl - e input.est. - m - n 100,000 - L 40 - s 0 - M - c 8. The - n command indicates that 100,000 coalescent simulations were conducted to approximate the expected SFS for each set of parameter values, and the - L command specifies 40 optimisation (ECM) cycles per run. The best run among 100 runs for each model was selected by comparing their associated estimated likelihoods using a bash script found online.

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(https://raw.githubusercontent.com/speciationgenomics/scripts/master/fsc-selectbestrun.sh).

To compare hypotheses, we used the Akaike Information Criterion (AIC; Akaike 1974), that takes into account the number of parameters of each model, and the relative likelihood as estimated by the Akaike's weight of evidence

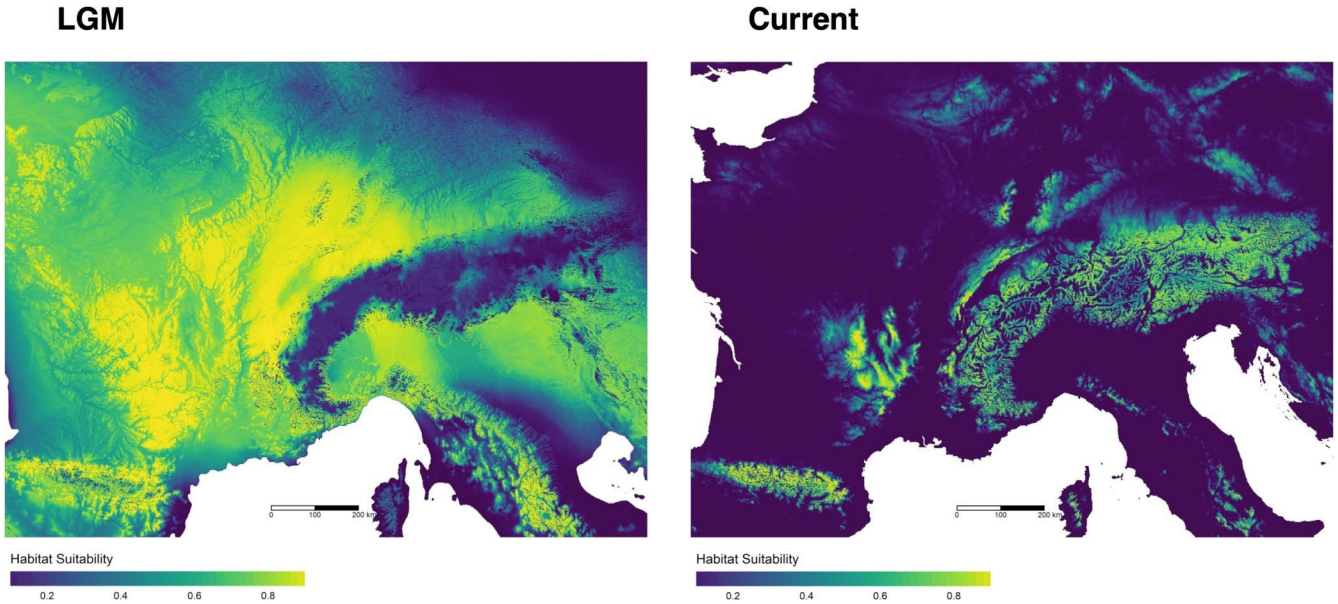

Figure 3 Habitat suitability for *G. quinquepunctata* as estimated via species distribution modelling, both for today and at the LGM.

in favour of the *i*- th model (Johnson and Omland 2004) as suggested by Excoffier et al. (2013). Following Meier et al. (2017), given the potential bias associated with nonzero LD between SNPs across RAD tags, we have inferred the likelihood distribution for each model by estimating the likelihood of 100 SFSs simulated using the same model and setting the parameters to their estimated values. A parametric bootstrap procedure was also used to estimate confidence intervals around the estimated parameter values for the best model, as suggested by Excoffier et al. (2013). Briefly, we simulated 100 SFSs using that model and setting the parameters to their estimated values. These 100 pseudo- observed datasets were analysed in the same way as the real data, but with 10 runs per simulated SFS (instead of 100 runs with the observed SFS). The confidence intervals were then computed as the range between the 2.5% and 97.5% quantiles of the distribution of each parameter.

3. Results

3.1 Species Distribution Modelling

The performance of all species distribution models based on the first dataset (60 reliable occurrences) was very good on average (± Standard deviation) with a Boyce Index of 0.82 ± 0.12, a max TSS of 0.88 ± 0.04 and an AUC of 0.97 ± 0.01 for the ensemble models (see Table S5 for individual performances of each separate algorithm used for generating the ensemble models). Habitat suitability, as estimated by projection of the SDMs, is shown for

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today and for the LGM in Figure 3. As expected, the current distribution is highly fragmented and the suitable habitat for the beetle is restricted to mountain ranges. On the contrary, at the LGM, mountain ranges, especially in the Alps, appear poorly suited for the species. Instead, a large area of lowlands around the mountain ranges seems to have been climatically suitable at the time. Overall, both the size of the suitable area and its level of suitability were projected to be stronger at the LGM. Climatic conditions seemed therefore more favourable for *G. quinquepunctata* at the time.

Species distribution models inferred from the expanded dataset resulted in a pattern of habitat suitability that was highly similar to the one produced with the first dataset (see Figure S2). Model performances were relatively similar with a Boyce Index of 0.88 ± 0.16, a max TSS of 0.79 ± 0.05, and an AUC of 0.94 ± 0.02 for the ensemble model. Including data from the lowlands of northern Europe, where the climate is largely similar to that of the mountains of central and southern Europe, did not significantly alter the estimates of the current and past distributions of the species climatic niche.

3.2 Mapping, Number of SNPs, Coverage

The STACKS analysis of raw data allowed us to identify 15,749 polymorphic sites across 5125 RAD tags for which at least 50% genotypes were available for each mountain range (compared to 6464 SNPs across 2072 RAD tags for which at least 75% genotypes were available per mountain range). These RAD tags covered a portion of the reference genome corresponding to 456,911 bp. For all but the estimation of nucleotide diversity, we retained only one SNP per RAD tag to limit the effect of linkage between SNPs, leaving us with a final set of 5125 SNPs. Given that we needed to know the portion of the genome on which these SNPs were discovered for the STAIRWAY PLOT and FASTSIMCOAL2 analyses, we adjusted the length of the genome used by multiplying it (456,911 bp) by the ratio of the number of SNPs retained over the total number of SNPs discovered (adjusted length: 456,911 × 5125/15,749 = 148,687 bp).

3.3 Characterisation of Genomic Variation

Nucleotide diversity was highest in the Alps (3.21×10^{-3}) and lower in the Massif Central and Pyrenees (1.12×10^{-3} and 1.21×10^{-3} respectively). This could be related to the more rugged terrain found in the Alps (at least compared to the Massif Central) or could mean that the Alpine population is older. On the other hand, it was surprisingly high in the Vosges (2.11×10^{-3}) given the much smaller size of this mountain range. Estimates of nucleotide diversity at the level of sampling sites (Figure S3, Table S6) are consistent with these patterns, with localities in the Alps and those in the Massif Central and the Pyrenees displaying, respectively, the highest and lowest values (Alps, Valmeinier: 4.0×10^{-3} and Massif Central, Mont Doré: 0.64×10^{-3}. Pyrenees, Laruns: 0.81×10^{-3}).

Calculated pairwise F_{ST}s (Table S7) were highest between the Alps and the Pyrenees (0.268) and lowest between the Vosges and Massif Central (0.033). The results of the AMOVA (Table S8) indicated that 17.1% of the molecular variance was explained by differences among mountain systems, 8.8% by differences among sampling sites within mountain ranges, 26% among individuals within sampling sites, and finally 48.1% within individuals. Overall, these

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results indicated that genetic structure was strongest among mountain ranges, although it was also present within them.

This was confirmed by the PCA graph (Figures 4 and S3), in which individuals from each mountain range were clearly separated from those of other mountain ranges, although there was a small overlap between clusters from the Massif Central and Pyrenees samples. The first principal component (PC1) explained 14% of the genomic variation, while the remaining 86% was spread out over several principal components. PC1 mainly differentiated the individuals sampled in the Alps from those sampled in the three

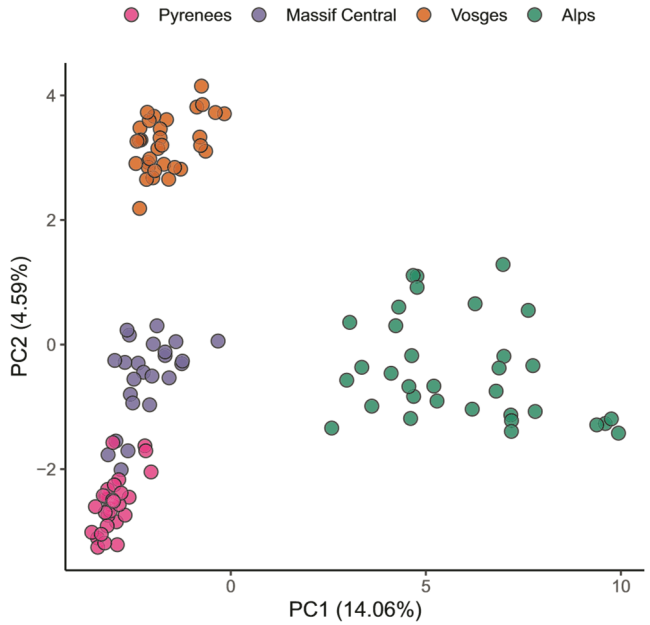

Figure 4 Results of the principal component analysis (centered data, not scaled) conducted with ADEGENET v2.1.5 (Jombart and Ahmed 2011), showing the two first principal components.

other mountain ranges and revealed substantial variation within the Alps (some Alpine individuals were genetically closer to some individuals from the other mountains than they were to the most distant Alpine individuals). PC2 differentiated individuals from the three other mountain systems. Admixture analysis revealed the best K value to be 3 (corresponding to that showing the minimum error in cross- validation analysis, see Figure S5), hinting at three genetic groups that corresponded to clear geographic entities: Alps, Vosges and a third group gathering individuals from Massif Central and the Pyrenees (Figure 5). The K value of 2 was associated with a cross- validation error only slightly larger than that of K = 3, and the corresponding bar graph differentiated individuals from the Alps from those of the three other mountain systems.

Our isolation by distance analysis (Figure S6, Table S9) showed that isolation by distance is strongest in the Alps (slope = 0.29) and lowest in the Massif Central (0.023), while within the Vosges and Pyrenees slopes were similar (0.096 and 0.093 respectively). The relative differences between mountains could be explained by differences in size, topography and historical contingencies (with more recent populations further away than older populations from the mutation- drift- migration equilibrium).

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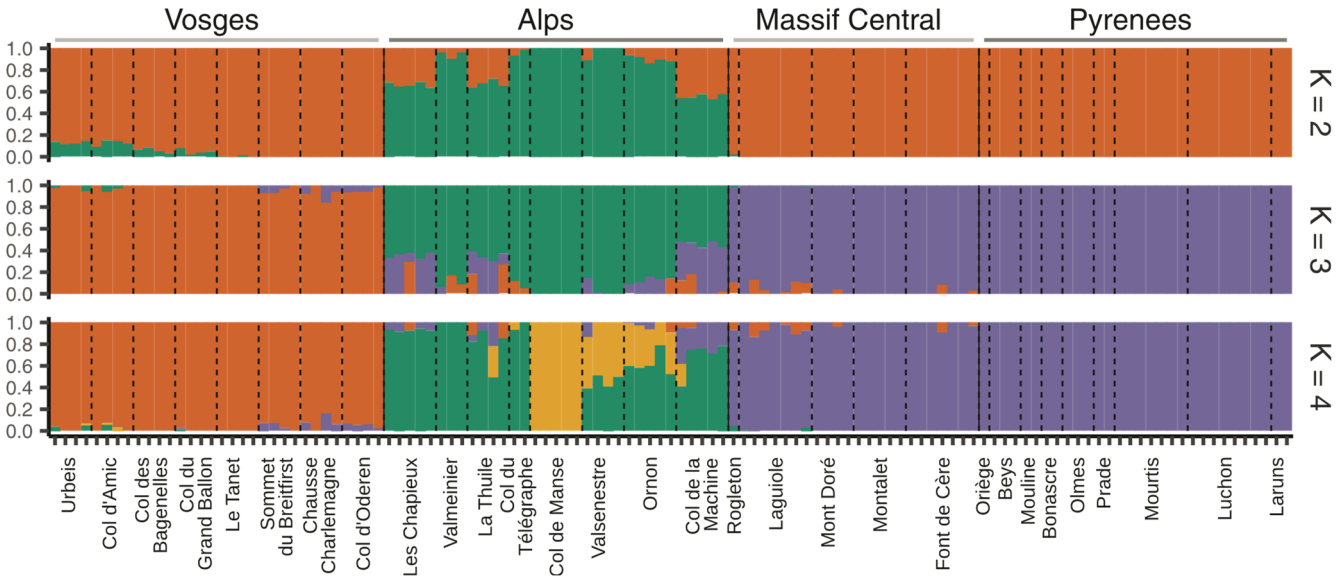

figure 5 Bar plots resulting from the ADMIXTURE analysis for K values from 2 to 4, with K = 3 identified as maximising model accuracy.

3.4 Demographic Inference Using Stairway Plot 2

STAIRWAY PLOT graphs (Figure 6) hinted at substantially lower population sizes in the past compared to current populations in all mountain ranges. A population expansion was inferred to have started between 100,000 and 50,000 years BP and lasted until between 30,000 (Alps) and 10,000 years BP (Vosges). The Pyrenees and Massif Central populations may have experienced a bottleneck (or founder) event around 50,000 years BP.

3.5 Phylogeographic Inferences

The analysis identified one version of the peripheral refuge model (model C in Figure S1), as associated with the highest composite likelihood and the lowest AIC value (Table S10). It implements an old fragmentation of an ancestral population into three separate mountain ranges (Alps, Vosges and Massif Central), and a strong size reduction in the three mountain ranges during the last glaciation, followed much later by a recent colonisation of the Pyrenees from the Massif Central. Comparing the AIC distributions obtained from the analysis of 100 expected SFS among models confirmed that this model was the best, as there was no overlap with the AIC distributions of other models (Figure S7). Figure 7 depicts this model again, this time displaying the estimated values for its different parameters (see Table S11 for all parameter estimates and their 95% confidence interval inferred from the parametric bootstrap analysis). The results depicted in Figure 7 agree with those from the stairway plots, that is, that population sizes were all substantially lower before the LGM compared to current population sizes. It suggests that the Alpine, Vosges and Massif Central populations were isolated for more than 100,000 years (95% confidence interval of 101,048—106,573), and that their divergence started just when the last two mountain ranges were colonised by individuals from the Alps at the beginning of the last glaciation. The Pyrenees and Massif Central populations were isolated for ~20,000 years (16,390– 20,799), when individuals from the Massif Central colonised the Pyrenees.

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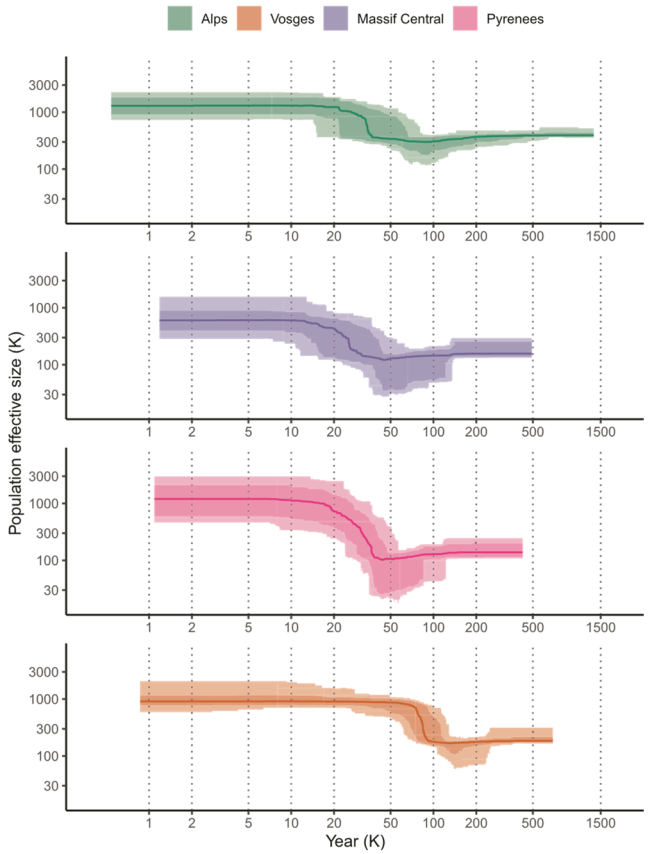

figure 6 Evolution of effective population size over time inferred by STAIRWAY PLOT separately for each mountain range. The main curve indicates the median estimate of Ne, while the outer and inner dashed lines represent the 95% and 75% confidence intervals respectively.

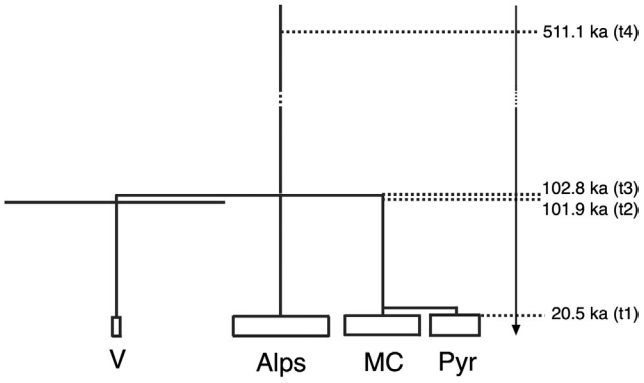

figure 7 Best scenario identified using FASTSIMCOAL2, including estimates for relative population sizes and divergence times (expressed in thousands of years). MC and V populations derived from an ancestral Alps population a little more than 100,000 years ago, Pyr population derived from MC population at the end of the last glaciation.

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It is worth noting that, at the beginning of the last glaciation, the Vosges population went through a brief but strong size increase (at time t2), and may have even been larger than today (although this size estimate is associated with a large 95% confidence interval; see Table S11).

4. Discussion

The current geographic range of the montane leaf beetle *G. quinquepunctata* across southern Europe displays a classic disjunct distribution for cold- adapted species, being present only in mountain systems there. Our characterisation of genetic variation across its range based on 5125 SNPs shows a relatively strong population structure that reflects this disjunct distribution, with strong differentiation observed among mountain systems. These results suggest the absence of current gene flow among mountain systems and that these have been isolated probably at least since the beginning of the Holocene.

As stated in the introduction, past global climatic changes are believed to have strongly impacted the distribution of species in Europe, and alternative historical scenarios have been proposed for cold- adapted organisms. To help us understand how climate change during the last glaciation has influenced the geographic distribution of *G. quinquepunctata*, we have hindcasted its potential distribution at the LGM and compared it to a SDM estimate of its current distribution. While the results indicated that climatic conditions at the LGM were unfavourable in a large portion of the Alps, it seems on the other hand that a wider area was available for the beetle, mostly thanks to the highly favourable climate in the adjacent lowlands around the Alps and other mountain systems. Comparing both the current and LGM maps thus suggests that the LGM was a climatically more suitable period for this beetle and that its distribution may have been wider at the time.

To formally test this hypothesis using genome variation data, we have designed coalescence models in which a large European population fragments into four isolated entities (mountain systems) sometimes during the Pleistocene (range fragmentation scenarios) or in which the four currently inhabited mountain systems are colonised at the end of the last glaciation from a large European population that existed at the time (lowland refuge scenario). These scenarios were compared to a series of others representing the peripheral refuges model, in which the geographic range of the species was much more restricted during the last glaciation and was essentially located in small refuges at the periphery of the mountain systems. Simulating genomic variation data using these coalescence models and comparing the simulated data to our RAD- seq dataset using a composite likelihood approach unequivocally resulted in rejecting models associated with a *G. quinquepunctata* population that was larger during the last glaciation than it is today. Instead, one of the peripheral refuges models was strongly favoured, in which the Vosges and Massif Central mountain systems were colonised from a source population in the Alps at the beginning of the last glaciation, and in which the Pyrenees were colonised from the Massif Central at the end of the last glaciation. The preference for a model in which the current population is larger than the one from the last glaciation, despite more favourable climatic conditions during the latter, is further supported by the stairway plot analyses, consistently estimating a larger population size for the Holocene populations than for the last glacial period, separately for each mountain system.

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Our genomic variation data therefore support a range expansion occurring at the end of the last glacial period, after the LGM, separately in all mountain systems, and probably from source populations located in peripheral refuges. This appears counterintuitive if we accept that environmental conditions in Europe were overall more favourable to this organism during the LGM than they are today, as hindcasted by SDMs. It does not necessarily contradict the SDM- based inferences, however, as SDMs reveal only the extent of the environmentally suitable range as opposed to the actual range of a species. Indeed, SDMs assume that species are not limited by their dispersal capacities (Zurell et al. 2020), thus that all suitable pixels are colonised by the species. Several reasons may explain why a species occupies a more restricted range than climatic conditions would suggest. First, other features than climatic or topographic variables that were not considered in the SDMs could influence the distribution of the species, such as soil structure or floral composition (which is obviously important in the case of a specialised phytophagous insect). Perhaps more importantly, the low capacity of an organism to disperse may prevent it from tracking the evolution of its environmentally suitable range (Dalén et al. 2007). Leaf beetles are indeed usually believed to be poor dispersers, as suggested by several genetic variation studies of other mountain species (Borer et al. 2010; Knoll et al. 1996; Rank 1992) that have revealed patterns of relatively strong genetic structure. Kastally et al. (2021) have recently studied genetic variation within the Vosges mountains for *G. quinquepunctata* with RAD- seq data and have uncovered relatively little population structure, showing that its migration inside this small mountain system is relatively unhindered by the rugged mountain terrain. Based on the results of this study, however, that spans four mountain ranges in Europe, it seems that the inferred relative ease to disperse within the Vosges cannot be generalised to dispersion between mountain systems. It is possible that some unknown barriers to migration, related to climate or topography, prevented *G. quinquepunctata* individuals located at the periphery of the mountains from moving further outside the mountain ranges to colonise the lowlands between mountains.

While this study includes genomic variation data from the four mountain systems where *G. quinquepunctata* is most abundant, our sampling design did not cover the entire geographic range of the species. Indeed, the species' range extends to northern Europe and the Carpathians, although the species is less abundant in these areas, where the sister species *G. intermedia* dominates. Moreover, our study did not include samples from the eastern Alps, nor from the Apennines, where the species is also known to occur (see Material and Methods). As a result, our study is likely to underestimate the genomic diversity characterising the species. Could this have potentially biased our phylogeographic inference? Because our genomic variation data showed strong differentiation among mountain ranges and estimated that they have been isolated for tens of thousands of years, and because a previous study highlighted an even stronger genomic differentiation between populations from the western and eastern Alps (Kastally et al. 2019), our demographic inferences of these populations during at least part of the last glaciation are unlikely to have been influenced by the evolution of the species in the other parts of its range. We therefore believe that our inference of a glacial range contraction in all four studied mountain ranges is likely to be unbiased by the lack of data from elsewhere. Of course, inferring what happened during the last glaciation in the unsampled regions of the distribution was not possible, but at least this lack of sampling should not bias our conclusions about the sampled mountain ranges.

The lack of occurrences from parts of the distribution for the SDM analysis may be more problematic, as this could create a niche truncation effect that would lead to underestimating the potential climatic niche of the species

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(Chevalier et al. 2021; Guisan et al. 2025). We have partially tested this hypothesis by inferring the SDM analyses for the current and LGM periods using two occurrence datasets, one including only the most reliable occurrences but that were limited to the four studied mountain ranges, and one with additional data points from public databases that extended the studied area to the lowlands of northern Europe. The resulting climatic niche distributions displayed surprisingly little difference however, and we suspect this is because populations from different parts of the species distribution are adapted to the same climatic conditions: more southern populations fulfil their climatic requirements by occupying higher elevation sites.

The lack of Apennines occurrence data points presents an additional challenge, as these are located at the southern edge of the species distribution. We do not know if the populations in this area adjust to the warmer climate of the south by restricting themselves to higher elevations in this mountain range than in the Alps. If not, the true potential climatic niche could actually be larger than estimated in our study. However, even if that was the case, it would actually increase our estimate of the potential distribution of the species at the LGM (since the species would be allowed to expand its potential distribution to warmer areas but would not decrease its presence in colder areas). Including Apennines samples in our SDM analyses would therefore have the potential to conclude to an even larger climatically suitable range for this species at the LGM, and would not change the main conclusions of our study.

Other phylogeographic studies of cold- adapted organisms in Europe have also highlighted strong genetic differentiation among mountain ranges. Although these were in some cases based on a limited number of markers (sometimes even on a single mtDNA locus) and did not always formally test historical hypotheses using coalescence models, the highlighted patterns suggest that the peripheral refugia hypothesis can accommodate the history of many of those species. This is for example the case for several freshwater cold- adapted organisms such as the caddisfly *Drusus discolor* (Pauls et al. 2006), the spring snail genus *Bythinella* (Benke et al. 2009), the plecopteran *Arcynopteryx dichroa* (Theissinger et al. 2013), the caddisfly *Thremma gallicum* (Macher et al. 2015) and the dipteran *Iponeura cinerascens* (Schröder et al. 2021), all currently distributed in several mountain systems. The butterfly *Erebia epiphron* has a disjunct arctic- alpine distribution and displays a similar pattern of genetic variation (Minter et al. 2020); Schmitt et al. (2006) have suggested that its humidity requirements have prevented it from colonising the lowlands during the last glaciation. Phylogeographic studies based on a few nuclear and mitochondrial loci of the closely related European montane leaf beetles *Gonioctena intermedia* (Quinzin et al. 2017) and G*onioctena pallida* (Mardulyn et al. 2009) also revealed strong genetic differentiation among mountain ranges and suggested populations from different mountain systems had been separated for more than 80,000 years. Similarly, a phylogeographic study of the glacier buttercup (*Ranunculus glacialis*) based on AFLP data (Schönswetter et al. 2003) suggested that the range of this arctic- alpine plant was restricted to mountains during the last glaciation, from which it recolonised the Arctic at the end of the last glaciation.

In all studies cited above, genetic variation seemed more compatible with a scenario of range contraction occurring during the last glaciation, despite these organisms being adapted to cold climatic conditions. More recently, Theodoridis et al. (2017) explicitly compared a hypothesis of glacial contraction with that of a glacial expansion for the montane plant *Primula farinosa* by analysing genome- wide variation (i.e., GBS) data in an ABC framework and also concluded in favour of a postglacial range expansion (and thus a glacial narrower range).

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While our study offers a similar conclusion, others, mostly of plants, have suggested instead that the range of cold-adapted species was larger during the last glaciation than it is today. A study on the evolution of the range of the dwarf willow (*Salix herbacea*), for example, observed relatively little population differentiation within Europe when analysing AFLP data and suggested, based on fossil and SDMs, that the central European lowlands served as a large continuous refugium for that species during the last glaciation (Alsos et al. 2009). In fact, Birks (2008) reviewed fossil evidence for arctic and alpine plants in Europe which suggest that those organisms moved southward from the northern ice sheet and to lower altitudes in the mountains during the last glaciation, thereby occupying the lowlands of central Europe. At the start of the Holocene, they retreated again to the north of Europe and to high altitudes, not so much because of warming temperatures that they can tolerate, but rather because of their inability to compete with trees and shrubs (Birks 2008). Espíndola et al. (2012) combined SDMs and genetic (AFLP) data to infer the past evolutionary history of the globe flower (*Trollius europaeus*) currently found in European mountains and in north Europe. They used their genetic data to delimit populations and conducted diffusion simulations that relied mostly on SDM projections to infer the evolution of the species range. Their results pointed to a range expansion at the onset of the LGM and to a range contraction following the LGM. It is worth noting that this last study used the genetic data only for the limited purpose of inferring current genetic structure and relied mostly on SDMs for inferring the evolution of the species range (i.e., they did not use coalescence models to infer the evolutionary history of the species).

In light of these results, it is worth wondering whether inferring a range expansion or a range contraction during the last glaciation is not related more to the type of evidence a study relies on: SDM- based inferences and fossil data (at least for plants) tend to support a large lowland European refugium during the last glaciation, while genetic data often support a range contraction during the same period. This is exemplified by our current study, in which SDM projections suggested a large suitable habitat in the lowlands of central Europe for the leaf beetle *G. quinquepunctata*, but the genomic variation data rejected the hypothesis of a large refugium occurring in that region during the last glaciation. While reconciling fossil data with genetic data may be challenging, reconciling the SDM and genetic evidence should not be problematic: identifying the environmentally suitable area for a species is not the same as identifying its actual range. In future works, it would be advisable to use SDM projections to delimit a priori the suitable area for a species at different time periods, but genetic variation data should then be further used to compare alternative scenarios about the evolution of the organism's range within the limit of the area set by the SDM projections. More generally, our results and those of other studies (e.g., Dellicour et al. 2014) suggest that we should be careful when trying to infer the evolutionary history of a species range exclusively from SDMs, as genetic data sometimes tell a different story.

In conclusion, our study of genetic variation for *G. quinquepunctata* thus indicated that this cold- adapted leaf beetle went through a strong range contraction during the last glaciation and was restricted at the time to one or several small refuges located at the periphery of the mountain systems it inhabited. Although climatic conditions appeared suitable in the large lowlands surrounding the mountains, the species was not able to take advantage of this territory, perhaps because of its limited capacity to disperse, as it did not colonise it. Patterns of genetic diversity uncovered for many other cold- adapted species associated with a similar geographic distribution (see above) are compatible with our conclusion of a limited peripheral glacial refuge, and we suggest we could generalise our inferences to many of these other cold- adapted organisms, at least to those characterised by low

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dispersal. This would mean that the last glaciation imposed a strong bottleneck to populations of many cold-adapted organisms in Europe, just like it did for most temperate species. Montane species were wiped out from the upper mountain areas that were covered by an ice sheet, and likely were forced to shift their range downward to survive. It is likely that many of them were unable to reach the lowlands that offered suitable climatic conditions. Squeezed in their peripheral refuge(s), their population size was then maintained at a low level for a 100,000 years, which in turn reduced considerably their overall genetic diversity. Others may have simply disappeared from these regions, a process contributing to the overall low species richness encountered in mountains from temperate zones (Rahbek et al. 2019). Before such a generalisation can be done, however, more studies on European cold- adapted organisms are needed. Indeed, only a few of the genetic variation studies mentioned above have been conducted with genome- wide data and in a hypothesis- testing framework using coalescence models, such as the current study or that of Theodoridis et al. (2017) on *Primula farinosa*, and it may be premature at this stage to infer general conclusions.

Author contributions

P.M. and C.K. designed the study and collected the samples. C.K. conducted the laboratory work (DNA extractions). M.S. and C.K. performed all analyses on the RAD- seq data. F.C. conducted the SDMs projections. All authors interpreted the data and contributed to writing the manuscript.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are openly available in DRYAD at https://datadryad.org, reference number doi.org/10.5061/dryad.ht76hdrs8.

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Supporting information

Additional supporting information can be found online in the Supporting Information section.