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Genetic erosion and projected habitat loss in the protected Alpine moth *Actias isabellae galliaegloria* (Lepidoptera: Saturniidae)

Erosion génétique et perte d'habitat chez le papillon alpin protégé *Actias isabellae galliaegloria* (Lepidoptera: Saturniidae)

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Abstract

1. Montane and specialist insects are particularly vulnerable to habitat loss and climate change. The Spanish Moon Moth (*Actias isabellae*), a rare and protected species, hosts an isolated Alpine population (subspecies *galliaegloria*), whose conservation status remains unclear.
2. We combined Restriction-site Associated DNA sequencing (RADseq) and Ecological Niche Modelling (ENM) to assess its genetic diversity, population structure and environmental vulnerability.
3. Genomic analyses revealed extremely low genetic diversity and inbreeding in the Alpine subspecies, consistent with a historical bottleneck, reducing adaptive potential.
4. Species distribution models predict a major contraction of suitable habitat by 2050 due to rising temperatures and declines in its primary host, Scots pine (*Pinus sylvestris*).

Carlos Lopez-Vaamonde and Jérémy Gauthier contributed equally to this work as senior authors.

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5. These findings highlight the compounded risks posed by genetic impoverishment and habitat loss, emphasizing the urgent need for targeted conservation measures.
6. Our study demonstrates the value of integrating genomic and ecological approaches to evaluate extinction risk and guide management strategies for montane specialist insects under rapid environmental change.

KEYWORDS
conservation genomics, effective population size, genetic diversity, genetic structure, habitat suitability, species distribution models

INTRODUCTION

Global biodiversity is declining at an unprecedented rate, with insects appearing to be particularly affected (Díaz et al., 2019; Edwards et al., 2025; Warren et al., 2021). Increasing evidence highlights climate change, habitat loss and agricultural activities as the major drivers of insect decline worldwide (Dirzo et al., 2014; Hallmann et al., 2017; Wagner et al., 2021). Habitat loss occurs both directly, through agriculture intensification, urbanization and land-use changes, and indirectly, as climate change alters environmental conditions, species interactions and community composition (Wagner et al., 2021). These pressures lead to population declines, local extinctions, and fragmentation of remaining populations, increasing their vulnerability to stochastic events and accelerating biodiversity loss (Seibold et al., 2019).

Climate change exacerbates these threats by shifting suitable habitats poleward and upward in elevation, disproportionately affecting cold-adapted and habitat-specialist species. The rapid pace of contemporary climate change often exceeds species' adaptive capacity, resulting in demographic declines and range contractions (Breed et al., 2013; Shirey et al., 2024). Montane insects are particularly vulnerable, as their available habitat is inherently limited and may disappear entirely under future warming scenarios (Romo et al., 2023; Ursul et al., 2025).

Assessing the conservation status of insects remains challenging due to a lack of robust baseline data on population sizes, trends and connectivity (IUCN, 2012). In this context, genetic information has become a key component of conservation biology (Garner et al., 2020). Small and isolated populations are especially susceptible to inbreeding and genetic drift, which erode genetic diversity and can trigger an 'extinction vortex' (Blomqvist et al., 2010; Frankham, 2005; Palomares et al., 2012). Genetic analyses provide insights into population structure, levels of genetic diversity, inbreeding and effective population size, all of which are essential for informing conservation strategies and assessing extinction risk (González-Castellano et al., 2023; Hohenlohe et al., 2021; Marí-Mena et al., 2019; Sucháčková Bartoňová et al., 2023).

Ecological Niche Modelling (ENM), or Species Distribution Modelling (SDM), is a powerful approach to predict species responses to climate change. By linking occurrence data to environmental variables, ENMs identify suitable habitats and project their spatial shifts under past, present or future climate scenarios (e.g., Sistri et al., 2022). For geographically restricted and ecologically specialized species, ENMs are particularly informative, revealing potential range contractions, altitudinal shifts, or poleward movements. For example, research on lowland butterflies has shown upward range shifts (Habel et al., 2023), a pattern that has also been particularly observed in mountain butterflies as species track cooler climates (Rödder et al., 2021; Wilson et al., 2005). Long-term monitoring reveals that mountain species with narrow climatic niches are especially vulnerable to warming, as suitable habitats become fragmented or disappear altogether. ENMs, therefore, provide essential tools for forecasting distributional range shifts and prioritising conservation efforts (Guisan & Thuiller, 2005; Settele et al., 2008). However, ENMs may underestimate extinction risk if species show local adaptation or if non-climatic constraints, such as host-plant availability, limit realized distributions (Harte et al., 2004).

The Spanish Moon Moth, *Actias isabellae* (Graells, 1849) (Lepidoptera, Saturniidae), is a flagship example of a montane specialist insect considered vulnerable at the European level (Phelan et al., 2026). The species typically occurs at elevations between 700 and 1700 m, which correspond to its optimal ecological niche and provide climatic conditions suitable for its development. Marí-Mena et al. (2016) detected pronounced phylogeographic structure in *A. isabellae*. Nuclear markers resolved six distinct genetic clusters corresponding to major mountain systems: French Alps, western and eastern Pyrenees, Central System, Iberian System and Baetic Mountains. In contrast, mitochondrial variation showed a broader two-lineage pattern, grouping the Alps, Pyrenees and Iberian System versus the Central System and Baetic Mountains. The traditionally recognized subspecies: *A. isabellae galliaegloria* (French Alps), *A. isabellae paradisea* (eastern Pyrenees), *A. isabellae roncalensis* (western Pyrenees) and *A. isabellae ceballosi* (Baetic range), occupy regions that match the nuclear clusters. Its striking morphology and restricted distribution have made *A. isabellae* an iconic European moth, but also a target for collectors (Rougeot, 1974). Intense collecting pressure during the 1970s led the mayors of five municipalities in the Queyras region to issue municipal decrees banning the collection of *A. isabellae galliaegloria*. These decrees were issued in 1975, 1 year before the adoption of the first French nature protection law. This subspecies is currently listed under Article 3 of the Decree of 23 April 2007 establishing the lists of insects protected throughout the French national territory and the conditions for their protection.

In French Alps, the subspecies *A. isabellae galliaegloria* occurs mainly in the departments of Alpes-de-Haute-Provence and

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Hautes-Alpes, with a higher concentration in the Queyras and the Guil Valley (Breton et al., 2018; Maurel et al., 2013). The origin of the Alpine subspecies has long been debated. Some authors considered a native origin unlikely and suggested a human-mediated introduction from Spain during the early 20th century (Agenjo, 1943; Bollow, 1932; Chrétien, 1925; Fernández-Vidal, 1992; Templado et al., 1975), whereas others argued for a native origin (Cleu, 1925, 1939; Maso-Planas & Ylla-Ullastre, 1989; Nässig, 1991; Warnecke, 1943). More recent genetic analyses based on mitochondrial and microsatellite markers indicate that the Alpine subspecies is genetically unique and related to the eastern Iberian and Pyrenean populations (Marí-Mena et al., 2016). When it comes to foraging preferences, larvae of *A. isabellae galliaegloria* feed mainly on Scots pine (*Pinus sylvestris*), making the moth highly dependent on the distribution and health of its host plant. *A. isabellae galliaegloria* can be locally abundant in the Alps (Maurel et al., 2013) and may have benefited from extensive afforestation programmes during the 19th century using *P. sylvestris* and *Pinus nigra*, which may have facilitated colonization of new areas (Leraut, 2020; Maurel et al., 2013). But Scots pine is sensitive to drought and heat stress and has experienced stand-level dieback in southern and central Europe in recent decades (Bose et al., 2024). This raises concerns about the long-term viability of *A. isabellae galliaegloria* habitats under ongoing climate change, especially given that previous genetic studies revealed that the Alpine subspecies exhibits extremely low genetic diversity, compared to most Iberian populations (Marí-Mena et al., 2016). Despite this genetic impoverishment, these observations have led some authors to argue that the species should no longer be considered endangered in the Alpine region and that its protected status should be reconsidered (Leraut, 2020). Confirming the genetic status of the Alpine subspecies and understanding its demographic history and environmental constraints are, therefore, critical for assessing extinction risk and guiding conservation decisions.

In this study, we address two main objectives. First, we genetically characterise the French Alpine population of *A. isabellae galliaegloria* using RADseq (Restriction-site Associated DNA sequencing; Baird et al., 2008). This genomic approach provides much higher resolution than previous studies based on mitochondrial markers and microsatellites (Marí-Mena et al., 2016), allowing us to investigate population structure, genetic diversity and demographic history and to assess the extent of genetic erosion. Second, we investigate the ecological constraints shaping the current and future distribution of the Alpine subspecies using species distribution models that incorporate both climatic variables and the distribution of its host plant, *P. sylvestris*. Using ENM, we project habitat suitability under current conditions and future climate scenarios (2050) to evaluate potential range shifts and risks of habitat contraction.

By coupling genomic data and ENM, we aim to answer the following questions: (i) What is the current genetic status of the isolated Alpine subspecies of *A. isabellae*? (ii) How will climate change and host plant availability affect its future distribution and persistence in the Alps? Together, our results provide a robust framework for assessing the vulnerability of *A. isabellae galliaegloria* and for guiding conservation strategies for montane specialist insects facing rapid environmental change.

MATERIALS AND METHODS

Sampling

In total 62 samples of *A. isabellae* collected during previous studies (Marí-Mena et al., 2016) were included in this study (Table S1). These comprised 53 individuals of the Alpine subspecies *A. isabellae galliaegloria* from seven localities in the French Alps (Figure 1b), seven individuals of *A. isabellae paradisea* from two localities in the eastern Pyrenees and two individuals of *A. isabellae roncalensis* from one locality in the western Pyrenees (Table S1). To capture the genetic diversity of the French Alpine subspecies as comprehensively as possible, sampling was focused on its main distribution areas in the administrative departments of Alpes-de-Haute-Provence and Hautes-Alpes, especially in the Guil Valley and Queyras massif (Figure 1b) under the appropriate collecting permits: Arrêtés no. 2008.38.4 and no. 2012-156-0008 issued by the Préfecture des Hautes-Alpes, Arrêté no. 2012.978 issued by the Préfecture des Alpes-de-Haute-Provence, permit ref. LC/mp 24/2009/1638 issued by Gobierno de Aragón, and permit ref. 0152S issued by the Generalitat de Catalunya to CLV and MV. Sampling was non-lethal and consisted of removing a single leg or a piece of the hindwing tail following established protocols (Vila et al., 2009).

DNA extraction, RADseq library and sequencing

DNA was extracted using the Qlamp® mini kit from Qiagen, following the manufacturer's instructions and protocol. Legs were cut in small pieces with a scalpel and incubated with the buffer and proteinase K in a ThermoMixer for at least 17 h at 56°C and 400 rpm. The DNA was eluted in 50 µL of Nuclease-free water, with an incubation time of 30 min at room temperature before centrifugation. DNA concentrations were measured using the Qubit® 2.0 Fluorometer (ThermoFisher), using the dsDNA HS kit. DNA quality was also checked using the NanoDrop™ One spectrophotometer based on the 260/280 and 260/230 absorbance ratios. For the preparation of the RADseq library, genomic DNA was initially digested with the SbfI restriction enzyme, followed by size selection to retain fragments ranging from 200 to 400 bp using a PippinPrep HT 1.5% (SageScience). These steps were undertaken to selectively capture homologous loci for each individual. The sequencing library was subsequently constructed by incorporating indexes and barcodes for individual identification, along with sequencing Illumina adapters. The sequencing was performed using 100 bp single reads from the Illumina Novaseq6000 technology. The RADseq library preparation and sequencing were performed by the MGX-Montpellier GenomiX (Montpellier, France).

Population genetics analyses

The raw read quality was assessed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), demultiplexed and

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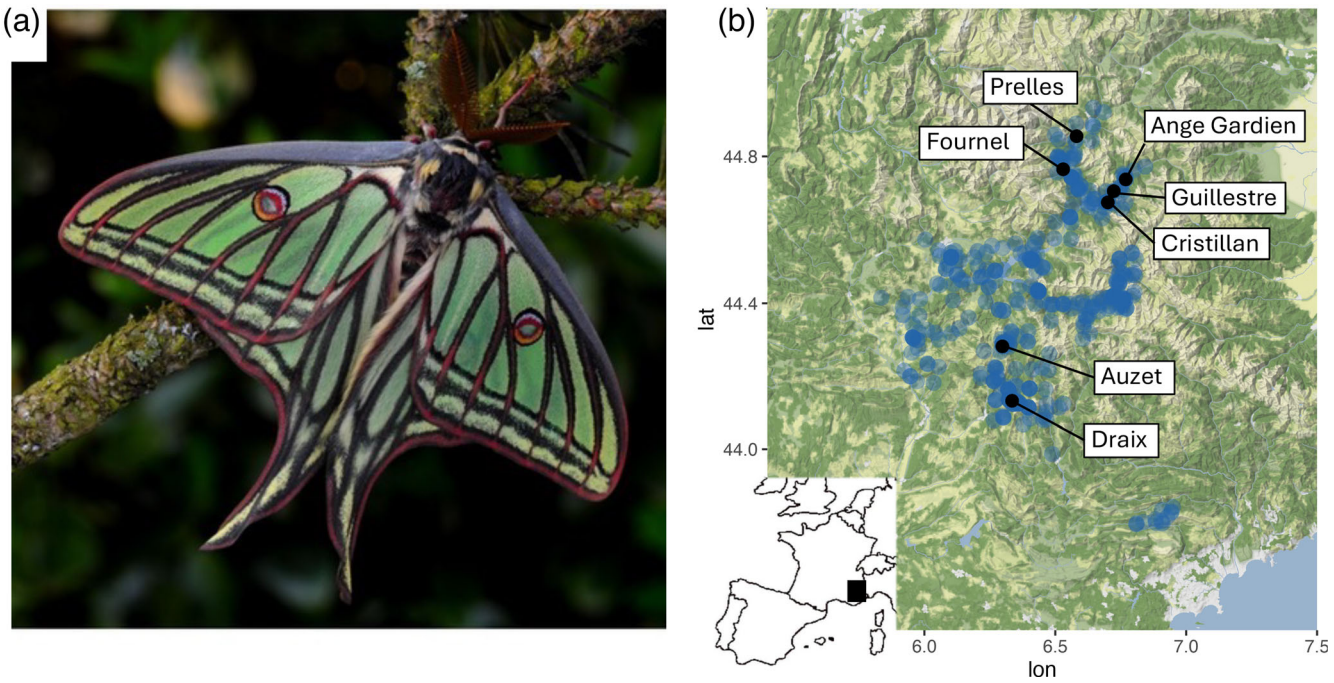


FIGURE 1 (a) Habitus of a male of *Actias isabellae galliaegloria* (credits: Thibaud Decaens). (b) Map of the distribution in the Alps, the blue outline reflects the known distribution of *Actias isabellae galliaegloria* using the GBIF occurrences (including the Gréolières locality in South of France) and black the localities included in the genetic analyses ($n = 7$ in Prelles; $n = 9$ in Fournel; $n = 9$ in Ange Gardien; $n = 6$ in Guillestre; $n = 7$ in Cristillan, $n = 6$ in Auzet and $n = 9$ in Draix).

cleaned using process_radtags from *Stacks* (Rochette et al., 2019). Reads were mapped on the reference genome of *A. isabellae* genome (Vila et al., 2025) using *bwa mem* (Li, 2013). Mapping files were filtered according to the mapping quality, that is, mapq <20, using samtools (Li et al., 2009), and indels were realigned using the *Picard suite* (Picard toolkit, 2019). Variants were called using freebayes 1.3.8 (Garrison & Marth, 2012). Bi-allelic SNPs shared by at least 60% of the samples and with a minimum quality of 50 were extracted using vcftools (Danecek et al., 2011).

Independent SNPs were extracted to perform genetic structure inference, keeping SNPs separated by at least 400 bp, thus corresponding to different RAD loci. To investigate genetic population structure, two different approaches were used. Population admixture was assessed using *STRUCTURE 2.3.3* (Pritchard et al., 2000) and testing *K*-value from 1 to 10. For each value, three runs were performed with a burn-in of 100,000 followed by 500,000 iterations. The Evanno method (Evanno et al., 2005) implemented in *Structure Harvester* (Earl & vonHoldt, 2012) was used to investigate the likelihood of the tested K-values. As a complementary approach, Principal Component Analysis (PCA) implemented for genetic data in the *adegenet* R package (Jombart & Ahmed, 2011) was used. Population differentiation was assessed using pairwise F_{ST} (Weir & Cockerham, 1984) with the *pairwise.WCfst* function from the *hierfstat* R package (Goudet, 2005).

Genetic diversity indices, that is, observed heterozygosity (H_o), expected heterozygosity (H_e) and the inbreeding coefficient (F_{IS}) were estimated using the *basic.stats* function from the *hierfstat* R package (Goudet, 2005). The contemporary and historical effective population size (N_e) were inferred using *GONE2* (Santiago et al., 2020) on the SNPs shared by all the samples. To optimize the number of samples that is too low at the locality scale, we combined all the samples from the Alps and performed a subsampling approach by resampling 30 individuals, 10 iterations, to test the robustness of the estimates. Genetic structure was accounted for using a model that considers a random set of individuals from a metapopulation composed of equally sized subpopulations, closely reflecting the studied system. A constant recombination rate of 3 cM/Mb across the genome was applied (Yamamoto et al., 2008).

Occurrence data

At the French national scale, occurrence data for *A. isabellae galliaegloria* were primarily obtained through a monitoring programme documenting its distribution in the French Alps (Breton et al., 2018; Maurel et al., 2013). This programme, initiated in 2012 and coordinated by INRAE Orléans in collaboration with the naturalist association Proserpine, was conducted during the emergence period in May and June using sticky traps baited with a synthetic sex pheromone (Millar et al., 2010). This monitoring method is highly sensitive, non-lethal, inexpensive and allows traps to remain in the field for several weeks. Males attracted to the pheromone leave scales and hairs on the adhesive surface, making their detection straightforward (Maurel et al., 2013). The occurrence dataset for the French Alps is therefore largely based on male records because sex pheromones attract

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exclusively males. Female records are much scarcer, as females are poor fliers and are only occasionally detected by light trapping flying during a brief period at dusk. However, males and females occupy the same habitat, and male records are therefore considered a reliable proxy for population occurrence. This pheromone-based survey covered a large part of the French Alps, including many areas where the species was suspected to occur but had never been confirmed. Importantly, the monitoring programme generated a substantial number of both presences and confirmed absences, resulting in a detailed distribution map (Breton et al., 2018; Maurel et al., 2013). These data were combined with records from previous studies in the French Alps and Pyrenees (Breton et al., 2018; Lopez-Vaamonde & Goussard, 2011; Marí-Mena et al., 2016, 2019; Maurel et al., 2013; Millar et al., 2010; Morichon et al., 2014; Vila et al., 2010) that allowed an accurate characterization of the species' realized niche.

Additional occurrence records were obtained from publicly available databases, including Artemisiae from Oreina (https://oreina.org/artemisiae/index.php) and the Global Biodiversity Information Facility (GBIF; https://doi.org/10.15468/dl.u54nhc; Flemons et al., 2007). To ensure data quality, points with imprecise coordinates (<1000 m), duplicates, erroneous localities and erratic male records (isolated records of males observed well beyond the known distribution area and at the end of the flight season) were removed. The French dataset contained 655 occurrences, resulting in 370 unique records after filtering. To reduce spatial autocorrelation and improve analytical robustness, the dataset was thinned to 262 occurrences using the *thin* function from the *spThin* package (Aiello-Lammens et al., 2015), selecting one occurrence per raster cell (~1 km^2) using randomization and iteration approaches. For the modelling approach (see below), 250 presence records were retained from the initial set of 262, after excluding the putative introduced population as explained in the result section, that is, Gréolières locality in southern France (Figure 1). For the PCA, an additional dataset composed of 186 Spanish occurrences was obtained from GBIF and filtered using the same criteria applied to the French dataset, resulting in a global dataset of 446 occurrences.

Environmental variables

To predict the potential current and future distribution of *A. isabellae galliaegloria* using ENM, we downloaded the 19 bioclimatic variables for the current period (1981–2010) and future projections for 2050 from the CHELSA database (https://chelsa-climate.org/; Brun et al., 2022) at a spatial resolution of 30 arc-seconds (~1 km^2). From the 19 bioclimatic variables, BIO8, BIO9, BIO18 and BIO19 were excluded due to spatial artefacts (Campbell et al., 2015), resulting in 15 remaining variables. Subsequently, a subset of these variables was transformed following CHELSA guidelines (scale and offset) to ensure that raster values matched their corresponding units of measurement. First, we performed PCA including these 15 variables using the *ade4* R package (Dray & Dufour, 2007) to compare the climatic niches of the Spanish and Alpine populations. To carry out the PCA, bioclimatic values were extracted at each occurrence point using the *RChelsa* package (Karger et al., 2017) and normalized.

To further perform the analyses at the study scale, we cropped the downloaded layers at the following spatial extent: longitude 4.960 to 7.186 and latitude 43 to 47.575. A dendrogram was then constructed using the *hclust* function from the R *stats* package. To reduce multicollinearity, variables with a pairwise Pearson correlation coefficient <0.7 were selected for ENM construction under current and future conditions. Three bioclimatic variables were retained: BIO1 (Annual Mean Temperature), BIO3 (Isothermality) and BIO15 (Precipitation Seasonality). Future climate projections were based on the IPCC Shared Socioeconomic Pathways (ssp1-2.6, ssp3-7.0, ssp5-8.5), representing different socioeconomic trajectories for the year 2050. Projections were obtained using the MPI-ESM1-2-HR model with CMIP6 simulations (Müller et al., 2018).

Current and future distributions of *P. sylvestris*, the primary host plant of *A. isabellae galliaegloria*, were also included. The current distribution was obtained from land-use data in BD Forêt® V2, available via the Institut national de l'information géographique et forestière geoservice (IGN, https://geoservices.ign.fr/bdforet). For future projections, we used a 2050 distribution under the rcp8.5 scenario provided by ClimEssences (ONF & CNPF, 2021). To ensure consistency with CHELSA layers, the ClimEssences projection was resampled to a spatial resolution of 30 arc-seconds.

Modelling approach

Species distribution models at the Alps scale were developed using the three selected bioclimatic variables and the *P. sylvestris* distribution, and projections were generated using the R package *biomod2* (Guéguen et al., 2025). To account for algorithmic variability in species distribution projections (Thuiller et al., 2019), we used an ensemble modelling framework (Araújo & New, 2007) incorporating eight statistical algorithms: Maximum Entropy (MaxEnt; Phillips et al., 2006), Random Forest (RF; Breiman, 2001), Random Forest downsampled (RFd), Flexible Discriminant Analysis (FDA; Hastie et al., 1994), Generalized Linear Model (GLM; Nelder & Wedderburn, 1972), Generalized Boosting Model (GBM; Friedman, 2001), Generalized Additive Model (GAM; Hastie & Tibshirani, 1986) and Extreme Gradient Boosting (XGBoost; Chen & Guestrin, 2016).

As these algorithms require either presence–absence data or presence-only data supplemented with pseudo-absences, and true absence data were unavailable, we first generated pseudo-absence points using a random sampling strategy with 10 replicates of 750 points each. Indeed, we generated three times more pseudo-absences than presence data based on advice from the *biomod2* authors. Model validation was performed using a random cross-validation strategy with five repetitions, allocating 80% of the data for training and 20% for testing. Equal weighting (0.5) was applied to presences and pseudo-absences, and variable importance was assessed through 10 permutations.

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A total of 400 single models were generated under current environmental conditions for each of two sets of variables: one using only climatic variables (first model) and the other combining climatic variables with *P. sylvestris* distribution (second model). Models were evaluated using the Boyce index (Hirzel et al., 2006), which is specifically suited for assessing predictive performance of presence-only models. This index is a continuous metric, with theoretical values ranging from −1 (model predicts the opposite of the observed occurrences) to 1 (perfect agreement between model predictions and observed occurrences) and with 0 corresponding to a performance no better than random. An ensemble modelling approach was then applied to combine individual models, retaining only those with a Boyce index above 0.7. Variable importance was estimated for the ensemble model using a permutation procedure repeated over 10 runs. Final ensemble models were constructed using three methods: weighted mean (EMwmean), coefficient of variation (EMcv) and committee averaging (EMca). Models with Boyce values >0.7 were retained for the final ensemble forecasts. Independent evaluation based on the Boyce index showed better performance of the EMwmean compared to the EMca. Consequently, only EWmean is presented in the remainder of the results and was used to project suitability and prediction presence. We defined areas of high habitat suitability by applying a conservative threshold of 0.6 to the EMwmean ensemble projections, retaining only pixels predicted as highly suitable across models. Habitat suitability was projected for present-day and future (2050) climatic scenarios. We measured spatial overlap between the predicted suitability (first vs. second model and current vs. future scenarios) using the *dismo* R package (Hijmans et al., 2017) by calculating Schoener's *D* and Warren's *I* overlap metrics (Warren et al., 2008). Those metrics range from 0 to 1, with values close to 1 indicating a larger overlap. In total, two current projections, with and without *P. sylvestris* distribution, and four future projections, under three climatic scenarios and including *P. sylvestris* for the corresponding one, ssp5-8.5, were generated.

RESULTS

Genetic characterization of *A. isabellae galliaegloria*

The RADseq protocol and subsequent sequencing resulted in a total of 46 million reads, that is, an average of 0.75 million reads per sample (SD = 0.18 million reads) (Table S1). The mapping of the reads on the reference genome revealed a high percentage of mapping, an average of 96.4%. The variant calling resulted in a total of 1.2 million raw genetic variations. The filtering retained 5007 shared SNPs suitable for performing population genomic estimates and characterising the state of the populations. Only one of the initial 62 samples was discarded due to an excessive proportion of missing data, (up to 20%).

Genetic structure analyses first revealed a strong differentiation between the Alpine subspecies, *A. isabellae galliaegloria*, and the two Pyrenean subspecies *A. isabellae paradisea* and *A. isabellae roncalensis*. This separation is evident in the admixture analysis, with the most likely number of clusters $K = 2$ (Figure 2b, Table S2), and in the PCA along PC1, which explains 31.07% of the genetic variance and clearly separates samples from the two mountain ranges (Figure 2c). High levels of genetic differentiation are also reflected in F_{ST} values, which exceed 0.5 for each pair of genetic clusters from the Alps versus the Pyrenees (Figure 3).

Within the Alpine range, a substructure among localities was detected. This substructure appears in both the admixture results at $K = 5$ (Figure 2b) and in the PCA, which identifies three genetic clusters (Figure 2c,d). In the PCA, PC2 (8.27% of variance) separates cluster 3 (Figure 2d), which includes the southern Alpine localities of Auzet and Draix (Figure 1c), while PC3 (6.40% of variance) separates the two northern Alpine clusters along an east–west gradient (Figure 1d). F_{ST} values among these three Alpine clusters are lower, ranging from 0.182 to 0.296, than between the Alpine and Spanish populations (Figure S2).

Although the western Pyrenean subspecies *A. isabellae roncalensis* was represented by a single locality (Gillué) and only two sequenced individuals, the $K = 5$ analyses (Figure 2b) revealed a clear genetic separation from the eastern Pyrenean subspecies *A. isabellae paradisea* (seven specimens from two localities, Baiasca and Embouni) (Figure S1 and Table S1). The pairwise F_{ST} value between these two Pyrenean lineages was 0.227, although this estimate should be interpreted with caution given the limited sampling available for *A. i. roncalensis*. Nevertheless, this pattern is consistent with previous microsatellite-based analyses showing genetic differentiation between the western and eastern Pyrenean subspecies (Marí-Mena et al., 2016). The level of differentiation observed here is comparable to that among the three Alpine genetic clusters. However, admixture proportions among the Pyrenean populations were more mixed, possibly reflecting ongoing gene flow or incomplete lineage sorting resulting from recent divergence.

Genetic diversity was assessed using observed heterozygosity (H_o) and mean gene diversity within populations (H_s) at both the locality and genetic cluster levels. H_o and H_s estimates were consistent among localities within the same Alpine cluster, and cluster-level estimates obtained by pooling localities yielded similar values (Table 1), indicating that relatively small sample sizes did not bias the results.

Among Alpine clusters, heterozygosity varied from $H_o = 0.039$ in the least diverse cluster (Auzet) to $H_o = 0.067$ in the most diverse cluster (Guillestre). Compared to the Pyrenean localities, Alpine populations exhibited substantially lower genetic diversity, up to fivefold lower in Auzet versus Embonui (Table 1). A similar pattern was observed for the inbreeding coefficient (F_{IS}), which was higher in Alpine localities, whereas Pyrenean populations showed values near zero, consistent with an absence of inbreeding. When samples were pooled at the cluster level, F_{IS} increased, potentially reflecting a Wahlund effect, where population subdivision leads to an apparent deficit of heterozygotes relative to Hardy–Weinberg expectations (Hartl & Clark, 1997), although this effect was not formally tested (Table 1).

The historical trajectory of effective population size (N_e) between 1655 and 1900 should be interpreted with caution, as estimates for this period are associated with greater uncertainty and wider confidence intervals (Figure 3). Nevertheless, the inferred pattern suggests

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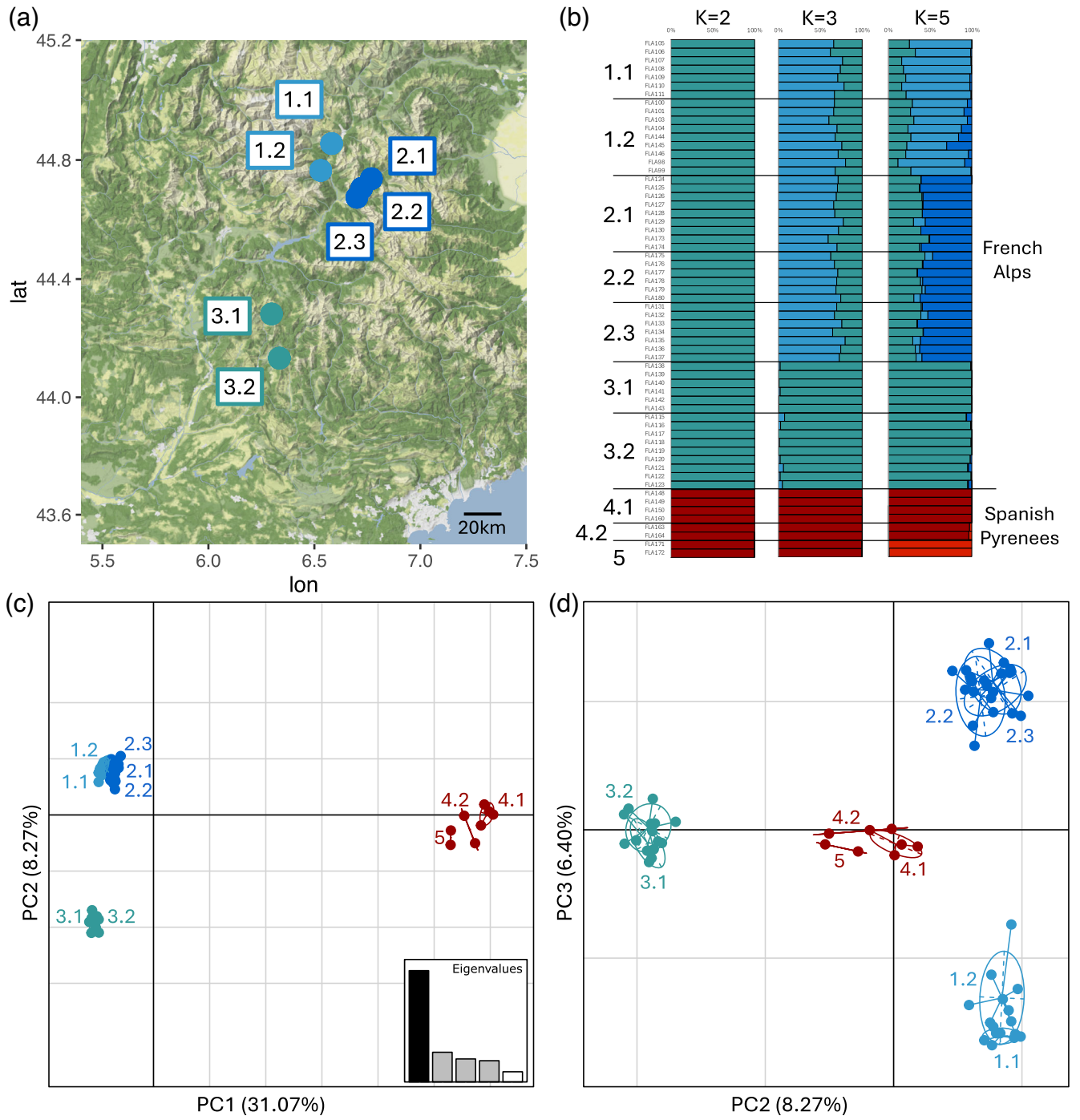


FIGURE 2 Genetic structure of French populations of *Actias isabellae*. (a) Geographic distribution of the three genetic clusters, 1.1 Prelles; 1.2 Fournel; 2.1 Ange Gardien; 2.2 Guillestre; 2.3 Cristillan, 3.1 Auzet, 3.2 Draix. (b) Barplot of the individual admixture levels for $K = 2$, $K = 3$ and $K = 5$. (c,d) Principal component analysis representing the sample distribution along the first PCs, respectively, on PC1/PC2 and PC2/PC3. The Alpine genetic cluster is depicted in blue tones, and the western and Eastern Pyrenean clusters in red.

a gradual demographic decline that is broadly consistent with the extensive historical loss and fragmentation of Alpine forests during this period. This was followed by an increase in N_e, reaching a maximum around 1930, a pattern that coincides temporally with large-scale afforestation programmes in the Alpine region. However, given the temporal resolution and uncertainty associated with LD-based N_e estimates, these historical events should be considered as potential contributing factors rather than demonstrated causes. The subsequent decline in N_e until the mid-1990s is also consistent with several possible pressures, including increased accessibility of populations and potential collecting pressure, although the relative contribution of these factors remains difficult to assess. Finally, the apparent increase in N_e after this period coincides with the legal protection of the subspecies in 1976 at a local scale, but this temporal association should

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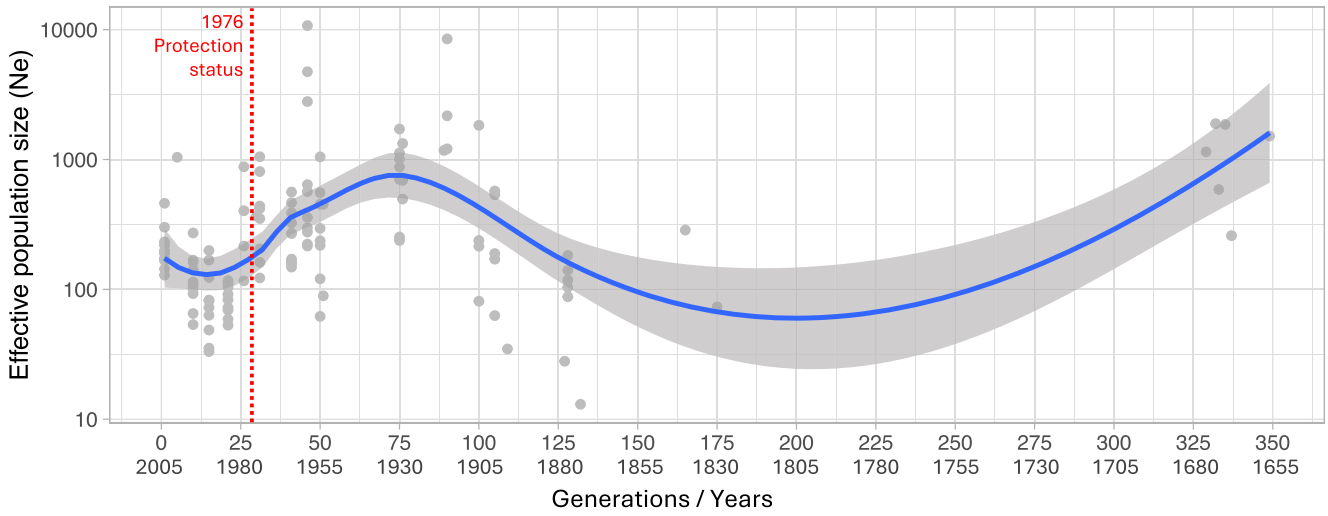


FIGURE 3 Effective population size (N_e) of the *Actias isabellae galliaegloria* population over time since 350 years ago. Effective population size is plotted from the present to the past. The blue line represents the median effective population size. The shaded area represents the 95% confidence interval (CI 95%).

TABLE 1 Descriptive statistics for *Actias isabellae* populations (genetic cluster, locality, number of samples, H_o observed heterozygosity, H_s mean gene diversity within populations, F_{IS} inbreeding coefficient).

Population		Locality	*N*	H_o (SE)	H_s (SE)	F_{IS} (SE)
1	1.1	Prelles	7	0.046 (0.002)	0.055 (0.002)	0.104 (0.018)
	1.2	Fournel	9	0.053 (0.002)	0.062 (0.002)	0.127 (0.017)
		Merged	16	0.050 (0.002)	0.061 (0.002)	0.189 (0.015)
2	2.1	Ange Gardien	9	0.064 (0.002)	0.074 (0.002)	0.132 (0.015)
	2.2	Guillestre	6	0.067 (0.002)	0.076 (0.002)	0.088 (0.016)
	2.3	Cristillan	7	0.063 (0.002)	0.073 (0.002)	0.104 (0.015)
		Merged	22	0.065 (0.002)	0.075 (0.002)	0.192 (0.013)
3	3.1	Auzet	6	0.039 (0.002)	0.047 (0.002)	0.130 (0.022)
	3.2	Draix	9	0.040 (0.002)	0.050 (0.002)	0.178 (0.019)
		Merged	15	0.039 (0.002)	0.049 (0.002)	0.234 (0.018)
4	4.1	Baiasca	4	0.168 (0.003)	0.190 (0.003)	0.049 (0.010)
	4.2	Embonui	2	0.192 (0.004)	0.213 (0.004)	−0.082 (0.014)
		Merged	6	0.174 (0.003)	0.202 (0.003)	0.086 (0.008)
	5	Gillue	2	0.200 (0.004)	0.228 (0.004)	−0.035 (0.013)

be interpreted cautiously given the uncertainty surrounding recent demographic estimates.

Niche characterization

Thanks to the rich occurrence dataset, from both the Alpine populations and the Spanish range where the species is widely distributed (Figure 4a), we were able to encompass the full environmental breadth of the species. As an exploratory step, we first performed a PCA on all environmental variables extracted for the 446 unique occurrences (Figure 4b). The first two principal components accounted for 92% of the variance in the dataset; clear separation between Alpine and Spanish occurrences is visible along PC1, with only limited overlap. These results suggest that the Spanish and Alpine populations occupy contrasted ecological niches.

It should be noted that an additional locality in southern France (Maritime Alps), Gréolières-les-Neiges, where males of *A. isabellae* have been recorded since June 2018 (unpublished data from C. Lopez-Vaamonde), was initially included in the Alpine dataset. However, this locality occupied an unexpected position in the PCA, clustering with the Spanish populations rather than with the other Alpine occurrences (Figure S3). Furthermore, the occurrence of this population more than 150 km south of the previously known Alpine distribution raises questions about its origin. This recently discovered population could represent either a natural southward range expansion of the species (Maurel et al., 2013) or the

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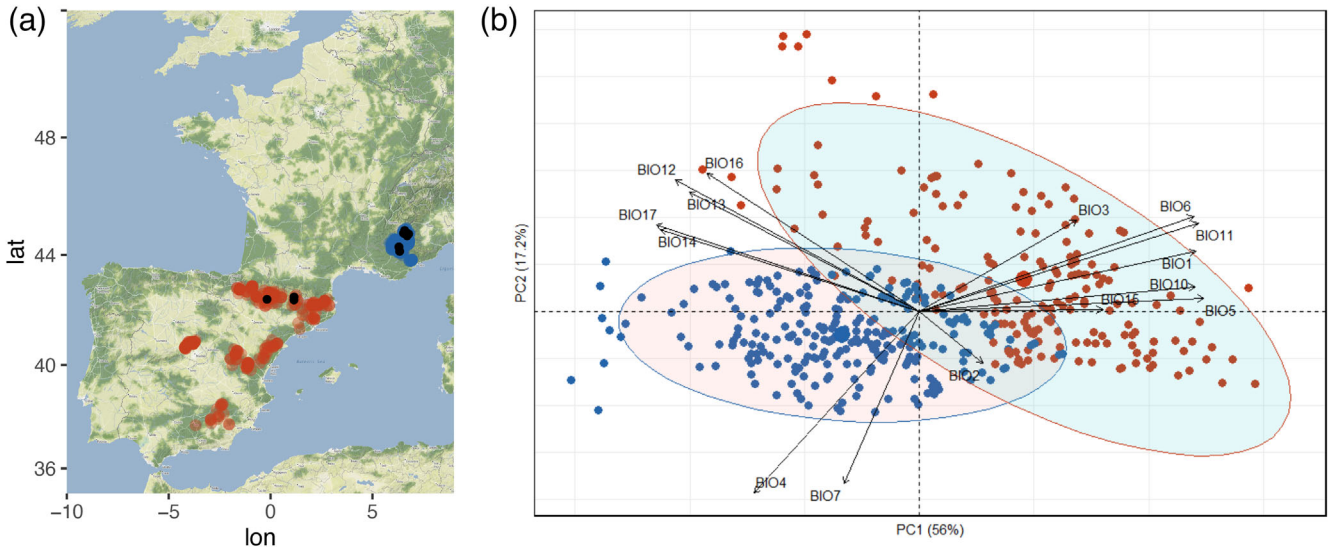


FIGURE 4 (a) Map of the occurrence distribution of *Actias isabellae* in Spain (in red) and Alps (in blue). Black dots indicate the locality sampled for the genetic analyses. (b) Climatic PCA with the 15 bioclimatic variables from Spanish (red) and French (blue) occurrences.

result of an accidental or deliberate introduction by amateur breeders, as has previously been documented for the Swiss population (Pro Natura–Ligue suisse pour la protection de la nature, 2005). A dedicated project coordinated by the *Parc naturel régional des Préalpes d'Azur* is currently investigating the status and origin of this population using ecological and genetic approaches. Given this uncertainty, and to avoid introducing potential bias into the estimation of the species distribution models, we adopted a precautionary approach and excluded the Gréolières-les-Neiges records from the final dataset pending the outcome of these investigations.

To precisely characterize the potential distribution of the Alpine population and its future evolution, an ENM approach was implemented. For both modelling approaches (climate-only and climate + *P. sylvestris*), all single models except the Generalized Additive Model (GAM) produced high predictive accuracies, as indicated by Boyce Index values ranging from 0.77 to 0.97 with an average of 0.91 and were incorporated into the ensemble modelling framework (Figure S4, Table S3). The GAM algorithm, with a Boyce index of 0.43 for the climate-only modelling approach, fell below the selection threshold of 0.7 and was therefore excluded from the ensemble. The strong Boyce values indicate robust model training and a high ability to discriminate suitable from unsuitable habitats under the current climatic conditions. Ensemble models (EMwmean, EMca and EMcv) integrated all high-performing single models, yielding spatially coherent predictions of suitable habitat for *A. isabellae* under current conditions. The convergence across ensemble metrics indicates low model uncertainty and high agreement among algorithms (Figure S4).

Drivers of *A. isabellae galliaegloria* distribution

Across algorithms, the relative contributions of the environmental predictors were consistent, suggesting stable model behaviour despite methodological differences among algorithms. For both modelling approaches, climatic predictors related to precipitation patterns were the dominant determinants of *A. isabellae* distribution. In particular, precipitation seasonality (BIO15) emerged as the most influential factor (accounting for >80% of total model contribution for climate-only), indicating that variation in rainfall across seasons strongly constrains habitat suitability for the species in the Alps. In contrast, temperature-related variables (BIO1 and BIO3) contributed less to model performance. Indeed, BIO1 exhibited a moderate contribution (20%), whereas BIO3 contributed only marginally (<1%), implying that the current range is more tightly associated with moisture regimes than with thermal conditions. When *P. sylvestris* distribution—which covers 13.36% of the 14,471 km^2 study area—was incorporated into the modelling framework, the explanatory power of precipitation variables remained dominant (>50%), while the Scots pine variable showed a secondary contribution (10%), suggesting the species' dependence on its host plant. This confirms that both climatic suitability and host plant availability jointly determine the realized niche of *A. isabellae*.

Under current climatic conditions, ensemble models consistently identified suitable habitats for *A. isabellae* throughout the region surrounding its current distribution area, namely the westernmost part of the Alps, and supporting the robustness of the ensemble predictions (Figure 1). The most favourable area is the meridional Pre-Alps, west of the Mercantour massif, which has more moderate altitudes (Figure 5). Further to the north-east, a dendritic pattern can be observed in the highest massifs, such as the Ecrins and Queyras, following the valleys and revealing that the favourable habitat does not cover the highest altitudes. There is also an extension of favourable habitat towards the north-west, reaching almost as far as the Vaucluse massif, even though the species has never been observed there. Low suitability values were observed both further north and further south. In the north, the predicted range shows a clear limit around 45° N, even in areas that might appear favourable, such as the

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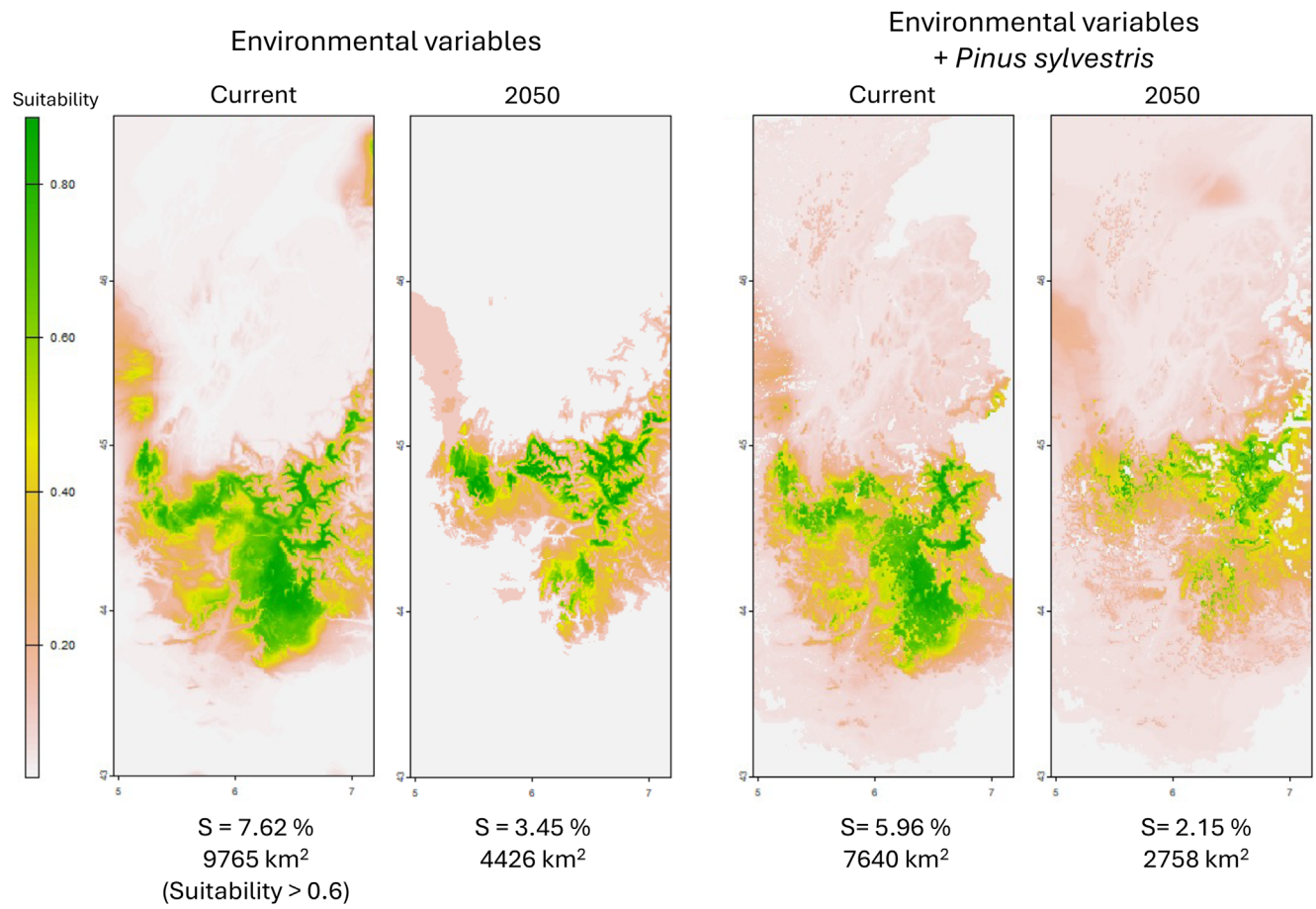


FIGURE 5 Species distribution models of *Actias isabellae* French population under current and future (2050) conditions, based on bioclimatic variables (BIO1, BIO3 and BIO15) and Scots pine distribution. Suitability values range from low (grey) to high (green). Percentages indicate the proportion of the study area with suitability >0.6 and the corresponding surface for each scenario.

Vanoise massif. In the south, including the Mercantour region, lower suitability likely reflects the stronger Mediterranean climatic influence. Across the entire study area (Figure 5), which covers a total of 128,160 km^2, the habitat area considered favourable, with a suitability >0.6, covers 9765 km^2, or 7.62%. When incorporating the distribution of *P. sylvestris*, the extent of suitable habitat was spatially congruent with the climate-only projections (Figure 5). In the same study area, excluding the edges in Italy and Switzerland due to the lack of data for the Scots pine distribution, the total area considered covers 99,777 km^2, of which 7640 km^2 are considered favourable, and representing 7.66% of the study area. This indicates that host-plant availability acts as an additional constraint refining the climatic niche envelope of *A. isabellae*. The overlap between both modelling approaches (climate-only vs. climate + *P. sylvestris*) was high (Schoener's $D = 0.81$; Warren's $I = 0.93$), suggesting that bioclimatic and biotic drivers operate synergistically to determine the realized distribution of the species. These results should be considered with caution, as the distribution of *P. sylvestris* used in the modelling framework was itself derived from a species distribution model based on occurrence data, but also on the same set of abiotic predictors.

The mean overlap between current and future distribution models was also high for the climate-only (Schoener's $D = 0.76$; Warren's $I = 0.95$) and for the climate + *P. sylvestris* (Schoener's $D = 0.66$; Warren's $I = 0.86$) approach, suggesting strong similarity in suitability patterns between current and future projections.

Prediction of suitable areas under future climatic conditions

The comparison of predicted suitable habitats for the current and the future (2050) reveals a contraction in suitable habitat area for the three ssp. scenarios tested, with variations in the intensity of this reduction (Figure 5). This difference in intensity is rather substantial, ranging from an 11% loss of suitable habitat (1085 km^2) with 8680 km^2 of remaining favourable habitat for the most optimistic scenario (ssp1-2.6) to a loss of nearly 55% (5339 km^2) for the most pessimistic scenario (ssp5-8.5), resulting in a suitable habitat area of 4426 km^2. The intermediate scenario (ssp3-7.0) also shows an intermediate loss of 36% of favourable habitat area (3524 km^2). In the three scenarios, the pattern of loss is consistent, with losses mainly

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occurring in the southern part of the distribution area (Figure 5). However, there is no counterbalancing effect towards the north with a putative favourable habitat extension above the 45° N limit. This artificial limit is still marked in the three inferences (Figure 5). Similarly, it does not appear to be any altitudinal extension of the distribution area, or if there is, it does not increase the total area of suitable habitat. The integration of the projected 2050 distribution of *P. sylvestris* into the modelling framework (using the RCP8.5 projections from ClimEssences) resulted in a further reduction of suitable habitat for *A. isabellae galliaegloria*, with the predicted suitable area declining by 64% to 2758 km^2. This result reflects the combined effects of future climatic suitability for the moth and the projected contraction of its principal host plant. Consequently, in addition to the bioclimatic constraints specific to *A. isabellae galliaegloria*, its strong dependence on *P. sylvestris* is expected to further constrain its future distribution and increase its vulnerability to climate change.

DISCUSSION

Current situation

The analysis of population genetic structure and the pronounced genetic differentiation between localities in the French Alps and the Pyrenees support the native origin of the Alpine lineage and refute the hypothesis of a human-mediated introduction of Iberian individuals in the early 20th century (Agenjo, 1943; Chrétien, 1925). The observed genetic structure is consistent with previous microsatellite-based results (Marí-Mena et al., 2016), which clearly separated the two Pyrenean subspecies. However, the higher resolution of the RADseq dataset (5007 SNPs vs. nine microsatellite loci) reveals finer-scale structure, identifying three distinct genetic clusters within the Alps. These clusters show a clear geographic structuring and indicate reduced gene flow across the mountain ranges. The fine-scale genetic substructure shown here for the Alpine subspecies must be integrated into conservation strategies, as it directly influences the spatial distribution and long-term maintenance of genetic diversity.

Similar topographic constraints on dispersal have been reported in *Lycaena* species (Trense et al., 2022), where the more sedentary *L. hippothoe* exhibited, in the Alps, strong population structure and localized genetic diversity, consistent with low dispersal ability and valley isolation. Landscape-scale genetic structuring shaped by topography and land-use barriers has also been documented in *Phengaris* butterflies (Gauthier et al., 2025). Although *A. isabellae* has a large wingspan and potentially higher dispersal capacity, female sedentary behaviour likely limits effective dispersal. Males actively search for pheromone-emitting females at the end of the flight period, when calling females are scarce, and occasionally disperse away from core areas (Maurel et al., 2013), with vagrant males observed in adjacent regions such as l'Oisans (Baillet, 2008). Despite male mobility, female behaviour largely defines species distribution, as shown in *Phengaris* (Plazio et al., 2020), where females preferred valley corridors and avoided crossing hills, unlike more mobile males.

The comparison of genetic diversity between Alpine and Pyrenean populations reveals substantially lower diversity in the Alps. These estimates are directly comparable, as they were obtained using the same methodology and a similar number of nuclear loci, providing robust insight into population status. They are consistent with previous microsatellite-based findings (Marí-Mena et al., 2016), with observed heterozygosity (H_o) two to three times higher in Pyrenean populations. They are also aligned with the low diversity observed at the mitochondrial level, with a single haplotype, which more specifically reflects the evolutionary trajectory of females. This reduced diversity in the Alps could be the result of a founder effect during the colonization of the Alps, but this process seems to have taken place a long time ago, and diversity should have emerged in a healthy population (Dapporto et al., 2024). The most likely mechanism linked to this low diversity would be recent effective population size variations, and more specifically declines and bottlenecks. The isolation of the Alpine population seems to have then led to inbreeding and amplified the effects of genetic drift, pushing progressively this population into a vortex of extinction (Bosse & van Loon, 2022).

Although cross-study comparisons of diversity metrics are often complicated by differing methodologies and species-specific life histories, the heterozygosity values observed in Alpine *A. isabellae* rank among the lowest reported for European butterflies (Després et al., 2019; Gauthier et al., 2025; Kebaïli et al., 2023; Trense et al., 2021), including species considered threatened (Dupuis et al., 2020) or critically endangered (Nakahama et al., 2024). Low genetic diversity constrains adaptive potential by limiting the allelic pool available for natural selection, thereby weakening responses to pathogens, climatic changes or habitat shifts. It also increases susceptibility to inbreeding depression, as reflected in elevated inbreeding coefficients. Isolation combined with reduced diversity raises the likelihood of matings between related individuals, increasing the risk of homozygosity for deleterious alleles, which typically manifests as reduced fitness through lower survival, fertility and developmental stability (Dussex et al., 2023). In conservation contexts, such patterns are widely recognized as warning signals of limited evolutionary resilience and heightened extinction risk (Nakahama et al., 2024).

Effective population size (N_e) is a key indicator of genetic health and accurately reflects genetic dynamics (Waples, 2025). In some Spanish *A. isabellae* populations, despite apparently healthy census sizes (N_c), genetic monitoring has revealed strikingly low N_e values. For instance, in one Pyrenean locality, N_e was estimated at only 27–49, despite ca. 1500 reported individuals (Marí-Mena et al., 2019). This N_e/N_c ratio implies that the population genetic effective size is much smaller than its census size, consistent with unequal reproductive success and/or a reduced effective number of breeders. This increases the rates of genetic drift and inbreeding and can accelerate the loss of neutral genetic diversity.

Historical demographic inference suggests a pattern of increasing N_e between 1800 and 1930, which is temporally consistent with extensive afforestation programmes in the Alps using both *P. sylvestris* and *P. nigra laricio* (Leraut, 2020). However, the uncertainty associated with historical estimates increases with increasing time depth, limiting

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our ability to distinguish among alternative demographic scenarios and to establish direct causal relationships with specific historical events. Alpine reforestation effectively began with the French law of 28 July 1860, which established both voluntary (subsidized) and compulsory (including possible expropriation) reforestation zones, followed by rapid implementation from 1861 onward with large designated areas, nurseries and seed facilities (France, Law of 28 July 1860; Fourchy, 1963). In Alpes-de-Haute-Provence alone, 500–1000 ha were reforested annually between 1895 and 1914 (Douguedroit, 1980). The more recent trajectory, from the beginning of the 20th century, appears to reflect the combined influence of several factors, including changes in forest management, habitat fragmentation and recent climatic changes. Smaller and isolated patches increase the risk of local extinctions and impede recolonization, reinforcing the evolutionary isolation of populations.

The collection pressures may have also played a role in the recent decline. Indeed, as an emblematic species, *A. isabellae* has been widely collected by amateur entomologists throughout its range and in particular the Alps (Rougeot, 1974), prompting local declines and the implementation of protective measures. By the mid-20th century, conservation concerns led to full legal protection in several countries, including France in 1976, where it became the first insect to receive such status initially locally and then at the national scale.

The benefits of this protection may already be observable, since the 1990s, N_e has increased slightly. However, short-term demographic gains may mask ongoing genetic erosion due to the lag between demographic recovery and genetic response. Genetic diversity does not collapse immediately after a population decline; rather, losses accumulate gradually over successive generations. This phenomenon, known as genetic extinction debt or drift debt, refers to genetic variation that is effectively doomed to disappear. Simulations and empirical studies (Liu et al., 2025) show that small populations continue to lose diversity long after a bottleneck, even if census sizes remain stable, due to the cumulative effects of drift and inbreeding. This process likely affects Alpine populations of *A. isabellae galliaegloria*, as evidenced by their low diversity and elevated inbreeding. Once lost, recovery of genetic variation is slow. Maintaining adaptive potential thus depends not only on current diversity but also on preserving sufficiently large and connected populations to ensure gene flow and minimise inbreeding. Long-term conservation will require the maintenance of favourable conditions to support these processes.

Putative evolution of the Alpine subspecies

The long-term persistence of Alpine populations of *A. isabellae galliaegloria* depends critically on maintaining sufficient genetic diversity, effective population sizes and habitat connectivity. However, these prerequisites appear increasingly challenged under projected future environmental conditions as revealed by this work. Indeed, our ENMs indicate a contraction of suitable habitat, largely driven by climate change and the patchy distribution of its primary host plant, *P. sylvestris*. Among the environmental variables tested, annual mean temperature and precipitation seasonality explained most of the variation in the current distribution of the Alpine subspecies. These factors collectively shape the species' ecological niche, confining it to mid- to high-elevation montane habitats where microclimatic conditions and host plant availability coincide.

Genetic structuring in mountainous species typically reflects historical isolation in glacial refugia, limited dispersal and topographic barriers (Trense et al., 2021). The genetic structure of the Alpine populations of *A. isabellae* further illustrates the influence of landscape configuration on evolutionary trajectories. Three distinct genetic clusters were identified, corresponding broadly to the northern, central and southern sectors of the French Alps. This substructure reflects the role of valleys and mountain ridges as barriers to gene flow, restricting dispersal and reinforcing genetic differentiation. In such fragmented landscapes, reduced gene exchange allows stochastic fluctuations in allele frequencies to accumulate among populations, making genetic drift a main driver of population differentiation. Such isolation, combined with small effective population sizes, may exacerbate the loss of allelic diversity over time, limiting adaptive potential and increasing vulnerability to stochastic events. Landscape connectivity is therefore a critical determinant of both genetic and demographic stability in these montane populations (González-Castellano et al., 2023).

ENMs also suggest that future climatic conditions will further constrain the distribution of *A. isabellae*, with a pronounced poleward and upward shift in suitable habitats. In addition to this latitudinal migration, a significant contraction of the overall range is predicted, particularly in lower-elevation valleys where warming and drought reduce host plant availability. Comparisons with other European montane insects indicate that upward or poleward shifts are common responses to warming, though local microclimatic refugia can buffer populations against broader trends (Biella et al., 2024). While ENMs provide a useful forecast, fine-scale topographic variation may create pockets of suitable habitat that enable local persistence despite overall range contraction (Stark & Fridley, 2022).

The distribution of *A. isabellae galliaegloria* is tightly constrained by the availability and continuity of *P. sylvestris*, its obligate larval host in France. The past demographic trajectory appears to follow the forest dynamics of *P. sylvestris* as mentioned above. But an additional factor potentially influencing the future trajectory of Alpine *A. isabellae* is the capacity for host-plant flexibility. While larvae predominantly feed on *P. sylvestris*, the species can also utilise other pine species as observed in Spanish populations (Gázquez et al., 2008; Maso-Planas & Ylla-Ullastre, 1989; Nässig, 1991; Sánchez-Fernández & de Arce-Crespo, 2017). In the context of climate warming, a host shift towards higher-altitude *Pinus uncinata* or more drought-tolerant *P. pinaster* could provide opportunities for range persistence or even expansion into new habitats (Ylla, 2026). Such flexibility may partially buffer populations against local declines in *P. sylvestris* due to drought, heat stress or forestry practices. Thus, while past population trends may have been influenced by the distribution and availability of the host plant, niche modelling suggests that the future distribution of *A. isabellae galliaegloria* will be primarily constrained by the climatic conditions defining its specific and narrower ecological niche.

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Taken together, these results suggest that the evolutionary trajectory of Alpine *A. isabellae galliaegloria* populations has been shaped by a combination of historical demographic events, landscape configuration and host plant availability. While the species exhibits some capacity for dispersal, especially in males, landscape barriers and habitat fragmentation may limit connectivity among populations and constrain their ability to respond to future environmental changes (González-Castellano et al., 2023). Conservation efforts should therefore focus not only on preserving existing populations but also on maintaining or restoring habitat connectivity, ensuring the persistence of *P. sylvestris* patches and identifying microclimatic refugia that may buffer populations against climate-driven range contractions. This integrated approach will be essential to reduce the combined threats posed by genetic erosion and host-plant decline, forest degradation, increasing wildfire frequency and extreme events such as heat waves, thereby supporting the long-term persistence of this emblematic montane insect. Beyond neutral demographic processes, local adaptation may also influence the persistence of alpine *A. isabellae* populations under ongoing environmental change. Future analyses based on whole-genome sequencing, combined with broader environmental and spatial sampling, would allow a more comprehensive assessment of genotype–environment associations and local adaptation. They could also provide more robust estimates of genomic offset (Fitzpatrick & Keller, 2015), thereby identifying populations whose current genetic composition may be poorly matched to future climatic conditions and helping to evaluate their adaptive vulnerability.

AUTHOR CONTRIBUTIONS

Flora Lambert-Auger: Formal analysis; writing – original draft; writing – review and editing; methodology; data curation; investigation. **Marina Querejeta:** Formal analysis; writing – review and editing; methodology; investigation. **Stéphane Boyer:** Methodology; writing – review and editing; investigation. **Marta Vila:** Resources; writing – review and editing; methodology; investigation. **Carlos Lopez-Vaamonde:** Conceptualization; funding acquisition; supervision; writing – original draft; methodology; resources; writing – review and editing; project administration; investigation. **Jérémy Gauthier:** Conceptualization; supervision; formal analysis; writing – original draft; project administration; writing – review and editing; investigation; methodology; data curation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw genomic data can be found under NCBI BioProjects PRJNA1512204 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1512204) (Lambert-Auger et al., 2026c). VCF files and custom scripts have been made available in Zenodo at https://doi.org/10.5281/zenodo.21934302 (Lambert-Auger et al., 2026a). The GBIF occurrence datasets are available at https://doi.org/10.15468/dl.u54nhc and https://doi.org/10.15468/dl.rk6bcs (Lambert-Auger et al., 2026b).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Descriptive statistics comprising sampling information (localities, coordinates), molecular statistics (concentration, purity), and sequencing.

Table S2. STRUCTURE likelihood.

Table S3. BOYCE statistics for each model used in the niche modelling approach.

Figure S1. Principal component analysis representing the sample distribution along the PC3 and PC4. The Alpine genetic cluster is depicted in blue tones, and the western and eastern Pyrenean clusters in red.

Figure S2. FST between localities and populations.

Figure S3. A PCA including all samples (from Spain, Alps and Gréolières locality) and using all 19 environmental variables B PCA including all 'natural' samples (without Gréolières locality) and using all 19 environmental variables C PCA including all 'natural' samples (without Gréolières locality) and using the three selected environmental variables (BIO1, BIO3, and BIO15).

Figure S4. Ensemble model construction and performance assessment of single models.

Figure S5. Species distribution models of *A. isabellae* under future (2050) conditions, based on bioclimatic variables (BIO1, BIO3 and BIO15) and three climatic scenarios, ssp126, ssp370, and ssp585. Suitability values range from low (grey) to high (green). Percentages indicate the proportion of the study area with suitability >0.6 and the corresponding surface for each scenario.

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