Global Ecology and Conservation 66 (2026) e04090

Contents lists available at ScienceDirect

Global Ecology and Conservation

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

Original research article

Genetic monitoring of an endangered species: Associations between population characteristics and environmental factors in a dioecious perennial plant

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ARTICLE INFO

Keywords:
Antennaria dioica
Central Germany
Climate
Extinction debt
Herbarium specimens
Sex ratio

ABSTRACT

Understanding the genetic and ecological factors influencing population decline is essential for the conservation of threatened species. Our study species *Antennaria dioica* (L.) Gaertn. is a dioecious perennial with frequent clonal growth and has undergone significant population decline across much of Europe, making it a suitable model for studying genetic diversity and ecological interactions in declining species. This study combines over a decade of genetic monitoring with environmental data and herbarium specimens spanning up to 200 years. Field surveys in 2010 and 2022/2023 recorded population size, sex ratio, seedling percentage, vegetation height, bare soil cover, and soil depth. We analyzed genetic diversity and population structure using ddRAD sequencing on contemporary and historical samples and examined associations between population characteristics and environmental conditions. While overall genetic diversity in central Germany remained moderate, populations showed clear differentiation without strong large-scale spatial genetic structure. Populations sampled in 2010 and later classified as 'potentially extinct' or 'still extant' in 2022/2023 differed significantly in their genetic cluster composition, indicating long-term demographic and genetic shifts. Herbarium specimens revealed a male-biased sex ratio under drought conditions and a phenological shift likely driven by habitat loss rather than climate change. Our findings highlight the importance of population size and balanced sex ratios for the persistence of dioecious, partially clonal species and demonstrate the value of integrating genetic, historical, and ecological data for potential conservation.

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https://doi.org/10.1016/j.gecco.2026.e04090
Received 4 August 2025; Received in revised form 19 December 2025; Accepted 26 January 2026
Available online 28 January 2026

1. Introduction

The decline in biodiversity is a major ecological concern. Since the 1960s, over 70 % of plant species in central Europe have declined, with native species being primarily affected (Eichenberg et al., 2021; Jandt et al., 2022). Efficient biodiversity conservation must consider variation at the ecosystem, species, and genetic levels, with the latter being crucial for plant populations to adapt to environmental changes (Hoban et al., 2020). Genetic diversity is closely linked to population size and reproductive strategy (Leimu et al., 2006). Its erosion is often the result of biparental inbreeding and small population sizes, which in turn reduce the adaptive capacity of populations and increase the risk of local extinction (Mualim et al., 2024).

Population size is crucial for maintaining genetic diversity: larger populations experience weaker genetic drift, while smaller ones are more susceptible to random allelic loss (Schou et al., 2017). This erosion of diversity can reduce reproductive fitness and limit adaptive potential, thereby reinforcing the 'extinction vortex' (Gilpin and Soulé, 1986; Leimu et al., 2006). Although endangered species often show low genetic diversity (Frankham, 2005), exceptions exist (Honnay and Jacquemyn, 2007; Makuch et al., 2023). Genetic diversity loss often lags behind population decline (He et al., 2024), especially in long-lived or clonal species, contributing to an 'extinction debt'—a delayed extinction following past environmental change (Honnay and Bossuyt, 2005; Kuussaari et al., 2009). Yet, clonal reproduction can be crucial for a short- and mid-term population persistence under poor pollination conditions, in the absence of reproductive partners, or in degraded habitats (Lin et al., 2016; Carvalho et al., 2024). However, clonality can distort sex ratios in dioecious species, reduce genetic diversity, limit adaptive potential (Honnay and Bossuyt, 2005; Lin et al., 2016), and lead to pronounced genetic differentiation among populations (Carter and Sytsma, 2001). Thus, clonality can ensure persistence in the short-term but threaten genetic viability in the long-term (Honnay and Bossuyt, 2005).

While genetic diversity and population size are crucial for population persistence, life history and reproductive traits also influence species survival (Hamrick and Godt, 1996; García, 2008). In dioecious plants, which require both male and female individuals for reproduction, sex ratio is especially important (Song et al., 2020). An equal ratio is often optimal, although deviations may reflect adaptive responses to environmental factors like nutrients, light, or water (Liu et al., 2021; Buschmann, 2024). Males generally invest less into sexual reproduction and better tolerate resource limitations (Segalla et al., 2021). Additionally, male-biased ratios are common in long-lived species, while female bias is typical of clonal herbs (Field et al., 2013). The sex ratio likely balances two opposing pressures: more females boost seed output, while males are still essential for pollination and genetic exchange (Lloyd, 1974).

Environmental conditions can influence reproductive strategies in plants, thereby affecting population size and genetic diversity. Regional variation can shape the genetic structure across populations (Durka et al., 2025). Climate factors such as temperature and precipitation affect population size, genetic diversity, sex ratios, and phenology, all of which influence reproductive success (Li et al., 2023; Karbstein et al., 2023; Park et al., 2019; Liu et al., 2021; Xie et al., 2023). Additionally, soil conditions and surrounding vegetation are important drivers of population dynamics (Lehmair, 2020; Shuyskaya et al., 2020). Many of these natural factors are negatively impacted by anthropogenic influences like climate change and eutrophication. However, targeted management—such as protected areas or controlled grazing—can support population persistence by mitigating human pressure, though, if inappropriately established, causing unintended harm for endangered species or specific ecosystem (Souza and Prevedello, 2020; Rybashlykova et al., 2022).

Even though long-term population monitoring is crucial, it remains rare. Therefore, herbarium data are important tools for understanding species decline and shaping conservation measures (Viveiros-Moniz et al., 2025). Herbarium specimens are a valuable complement to data collected in field surveys, providing historical records of species occurrences and ecological changes (Rocchetti et al., 2021; Davis, 2023). They enable reconstructions of species distribution dynamics over time and space (Rosche et al., 2025; Vörös et al., 2025) and allow genetic analyses to uncover spatio-temporal patterns in diversity and structure (Rosche et al., 2022). Herbarium data also help to identify global environmental changes and phenological shifts, such as altered flowering times (Lang et al., 2019; Park et al., 2019; Willems et al., 2022). All these insights are critical for developing effective conservation strategies under global change (Greve et al., 2016; Rocchetti et al., 2021).

We used *Antennaria dioica* (L.) Gaertn. as a model species to investigate genetic consequences of population decline and to identify abiotic environmental drivers of these changes. The combination of dioecy, clonal growth, and perenniality makes *A. dioica* an ideal model for studying genetic responses to population decline. Its reliance on outcrossing increases genetic diversity but also sensitivity to demographic imbalances like biased sex ratios. Clonal and perennial reproduction allow genotypes to persist despite limited recruitment, enabling long-term genetic analyses. This endangered species has declined in central Germany over the past two centuries (Vörös et al., 2025), highlighting the need for its conservation while offering a rare opportunity to study a still widespread threatened species. The availability of genetic and field data from 2010 (Rosche et al., 2014) as well as herbarium specimens enables monitoring temporal changes in population genetic structure and environmental conditions related to the extinction risk of *A. dioica*. Our hypotheses are as follows:

Despite the recent rapid decline in populations, the genetic diversity of contemporary *A. dioica* populations remains moderately high due to the species' perennial growth, its dioecious reproduction system, and its historically continuous distribution, resulting in low genetic differentiation among populations and indicating a scenario of 'extinction debt'.

Genetic diversity in *A. dioica* is primarily associated with the sex ratio and population size, while environmental factors play a role in shaping population size.

Population characteristics (genetic diversity, population size, sex ratio, clonality, percentage of seedlings) and environmental factors have remained stable on contemporary time scales (2010 and 2022/2023), whereas corresponding comparisons with data

from historical *A. dioica* samples (herbarium specimens) show a decline in genetic diversity and a higher degree of isolation in the genetic structure.

2. Material and methods

2.1. Study species

Antennaria dioica is a diploid (2 n = 28; Bayer, 1984; genome size = 6304 Mbp/2 C; Smarda et al., 2019), hemicryptophyte inhabiting dry, nutrient-poor meadows, grasslands, and open woodlands (Blachnik, 2009; Müller et al., 2021). It occurs from the Pyrenees to the Urals (Bauer et al., 2013), but the populations are declining in many parts of the occurrence area, especially in lowland, mainly due to land-use changes, eutrophication, and shrub encroachment (Asdal, 2005; Walker, 2014; Frank and Schnitter, 2016). The species is classified as *Near Threatened* in several countries, e.g. in Finland (Hyvärinen et al., 2019), France (UICN France, FCBN, AFB & MNHN, 2018), and Hungary (Király, 2007), and as *Endangered*, e.g. in Germany (Haeupler and Muer, 2000) and the Czech Republic (Grulich and Chobot, 2017). In many other countries where the species is not considered nationally threatened, it is regionally threatened or even extinct in parts of its former range, e.g. in England (Walker, 2014), Austria (Pflugbeil, 2024), and Norway (Asdal, 2005). Small population sizes and sex-ratio imbalances result in poor survival prospects for most populations (Richter and Blachnik, 2013; Rosche, 2018). Sex is morphologically distinguishable by the presence of androecium or gynoecium, which may appear in either white or pink flowers (Supplementary Figure 1a and 1b; Vörös et al., 2025). Large populations often exhibit female-biased sex ratios (Rosche et al., 2018). Vegetative reproduction via runners results in the formation of patches consisting of only one to a few clonal ramets (Rosche et al., 2014).

2.2. Study area and field data

The study focused on *A. dioica* populations in Saxony-Anhalt, Germany, surveying all sites reported in the past 20 years ('Datenbank Farn- und Pflanzen Sachsen-Anhalt'). Additional sites previously sampled by Rosche et al. (2014) in Bavaria, Brandenburg, Saxony, Thuringia, and the Czech region Ústecký kraj were also included. Fieldwork in spring 2022 and 2023 covered 61 sites, including 28 revisited sites from 2010. However, *A. dioica* individuals were found at only 23 sites, with 11 matching populations sampled in 2010 (Supplementary Figure 2).

Each site was examined once. Patch number and vegetation cover (bare soil, cryptogams, vascular plants, and litter) were recorded; however, only bare soil was used in the analyses due to strong intercorrelations between vegetation cover parameters. Patches were defined as groups of rosettes with a separation of $\geq$ 20 cm (Supplementary Figure 1c; Rosche et al., 2018). Up to 20 randomly selected patches per site were assessed for sex composition and size (used to calculate cumulative patch size). Within each patch, a 50 $\times$ 50 cm square was surveyed for generative, vegetative, and seedling individuals; if smaller, the whole patch was recorded. Due to correlations among life stages, only the seedling percentage was used in analyses. Maximum vegetation height within patches and soil depth (mean of five measurements per site) were recorded, since the species is shallow-rooted and weak in competition (Rosche et al., 2014); no significant depth differences were found between the patch center and the edge.

Additionally, data from 2010 collected using the same methodology (Rosche et al., 2014) were used for temporal comparisons. Leaf samples were collected from up to 20 individuals (from a single rosette) per site. Whenever possible, individuals were sampled from different patches; if this was not feasible, samples were taken from spatially distinct locations within patches to maximize the number of genetic individuals collected. Samples were stored in silica gel for DNA analysis. We also included DNA extracted from individuals collected in 2010 from 28 populations (Supplementary Table 1).

Climate data (ppt = precipitation, def = climate water deficit, mint = minimum temperature, maxt = maximum temperature, soil = soil moisture, and PDSI = Palmer Drought Severity Index) were extracted from TerraClimate (Abatzoglou et al., 2018) via the R function *get_terraclim* (TerraclimateR v0.1.0; Selke, 2024) based on data from WorldClim (Fick and Hijmans, 2017). However, only soil, mint, and PDSI were retained for the analyses due to strong intercorrelations among the other variables. For each population, we calculated mean values over the 12 months preceding sampling. Additionally, habitat type (grassland, open woodland, or edge) was recorded for each population. Information on grazing type (none, sheep, cattle, other) was obtained directly from the respective site managers, while data on protection status (Habitat Directive site, nature reserve, landscape protection area) were obtained from the German Federal Agency for Nature Conservation (BfN, 2025) and local authorities in the Czech Republic.

2.3. Herbarium samples

To trace spatial-temporal changes in historical *A. dioica* populations, 327 herbarium specimens from nine herbaria were examined (B, MNVD, DR, GAT, GERA, GLM, HAL, JE, LZ; abbreviations per Thiers, 2024). Of the total specimens, 285 —collected between 1820 and 1994— could be georeferenced and dated. These specimens yielded data on collection site, collection date (as Day of Year, DOY), number and sex of collected individuals, totaling 2031 inflorescences. Specimens averaged six individuals per specimen (range: 1–58). Additionally, we obtained the climate data (soil, mint, and PDSI) for each specimen in exactly the same way as described above for the contemporary samples (see 2.2). As climate data were only available from 1958 onwards, not all herbarium specimens could be included, resulting in a reduced number of specimens used in the climatic analyses to 51. Ultimately, based on proximity and natural area classification (BfN, 2025), specimens were grouped into spatial populations. For groups with at least three individuals collected in at least three different years within a 30-year period, 2–5 leaves per specimen were sampled. In total, 71 herbarium leaf samples were

used for DNA extraction. However, only 43 could be included in the sequencing due to poor DNA quality.

2.4. Molecular work and bioinformatics

DNA from 2022/2023 samples was extracted using the NucleoSpin Plant II kit (Macherey-Nagel); 2010 samples had been extracted using the ATMAB method (Stein et al., 2014) and stored at –80 °C. Herbarium DNA was extracted using the CTAB method (Doyle and Doyle, 1990) with a sorbitol prewash (Inglis et al., 2018). No bias is expected due to the extraction method.

The double digest Restriction-site Associated DNA (ddRAD) protocol from Peterson et al. (2012) was applied as described in Durka et al. (2025) to identify SNP (single nucleotide polymorphism) markers. In total, 480 samples were processed: 43 historical, 139 from 2010, and 298 from 2022/2023, including 10 duplicates (5 from the historical samples and 5 from 2022/2023) to quantify the extant of clonal reproduction. In short, 100 ng of DNA were restricted with *Eco*RI and *Msp*I enzymes, adapters containing one of 96 7-bp barcodes were ligated to fragments, which were then pooled, size selected to 350–450 bp, PCR-amplified, thereby introducing a plate-specific 5 bp index, with intermediate and final cleanup steps. Multiple plates were finally equimolarly multiplexed for sequencing on an Illumina Novaseq 6000, resulting in an average of 4483,114 sequences per sample.

SNP identification was performed using dDocent (Puritz et al., 2014) as described in Durka et al. (2025). After SNP identification, filtering of samples and SNPs was performed following the guidelines of O'Leary et al. (2018). After removing indels, we kept only biallelic SNPs with a minimum allele count (mac) of 3, a minimum genotype read depth (minDP) of 3, a minimum mean sequence quality (minQ) of 30 and maximum missingness across individuals (max_missing) of 50 %. We filtered putatively fixed heterozygous SNPs that had a p-value $< 10^{-15}$ for heterozygosity excess and skipped individuals with $>$ 75 % missing values. In order to exclude paralogous SNPs we filtered SNPs according to allele balance (AB $>$ 0.2 & AB $<$ 0.8 | AB $<$ 0.01 | AB $>$ 0.99), strandedness (SAF / SAR $>$ 100 & SRF / SRR $>$ 100 | SAR / SAF $>$ 100 & SRR / SRF $>$ 100), mapping quality ratio of the two alleles (MQM / MQMR $>$ 0.9 & MQM / MQMR $<$ 1.05), and properly pairing of alleles (PAIRED $>$ 0.05 & PAIREDR $>$ 0.05 & PAIREDR / PAIRED $<$ 1.75 & PAIREDR / PAIRED $>$ 0.25 | PAIRED $<$ 0.05 & PAIREDR $<$ 0.05). We then filtered SNPs to maximum missingness of 34 % (max_missing 0.66), the minimum minor allele frequency of (maf) 0.05, minimum mean read depth (min-meanDP) of 20, and a maximum mean depth (max-meanDP) of 1000. In the end, we retained only one single SNP per contig. The final dataset included 6317 SNPs with 8.15 % missing data across 471 samples (34 herbarium samples from four spatial populations, 139 from 2010, 298 from 2022/2023), plus five duplicates.

2.5. Data analysis

Unless otherwise mentioned, all following analyses were conducted in R 4.5.0 (R Core Team, 2025).

Genotype data in vcf format were converted into genlight objects using the vcfR package (v1.15.0; Knaus and Grünwald, 2017). Nei's genetic distances (StAMPP v1.6.3; Pembleton et al., 2013) were calculated and compared at multiple hierarchical levels: duplicates, within patches, within populations, and among populations. Distances among duplicates did not exceed 0.0425, establishing this as the clonal threshold. Genetic differentiation was assessed using PERMANOVA (999 permutations; *adonis2*; vegan v2.6–4; Oksanen et al., 2022) based on Nei's distances. Genetic indices (H_o = observed heterozygosities, H_e = expected heterozygosity, uH_e = unbiased expected heterozygosity, F_{IS} = inbreeding coefficient) were calculated for each contemporary population (2010, 2022/2023) using *gl.report.heterozygosity* (dartR.base v2.9.7; Mijangos et al., 2022), excluding clonal individuals, reducing the sample size to 324 (containing 308 different genets; some of the genets are present in more than one population). The clonal diversity index (P_D) was calculated as the number of genotypes divided by the total sample size, following Ellstrand and Roose (1987).

To explore relationships among contemporary samples, Principal Component Analysis (PCA) was performed using *glPca* (adegenet v2.1.11; Jombart and Ahmed, 2011). For genetic group identification and population structure, model-based Bayesian clustering was done with 'ADMIXTURE' (Alexander et al., 2009) for all samples (contemporary and historical). Based on cross-entropy results (K = 2–22; Supplementary Figure 3), we selected most appropriate cluster numbers (K = 6). Isolation by distance (IBD) was tested by correlating standardized genetic differentiation ($F_{ST}/(1\text{-}F_{ST})$, dartR v2.9.9.5), against geographic distances ($\log_e$-transformed, *distm*; geosphere v1.5–18; Hijmans, 2022) for contemporary populations. Data from 2010 and 2022/2023 were analyzed separately to avoid double testing. IBD significance was assessed using a Mantel test with 999 random permutations (vegan v2.6–10).

For testing our second hypothesis and to explore direct and indirect relationships between population characteristics ($\log_e$-transformed population size (defined as cumulative patch size sensu Rosche et al., 2014), sex ratio (calculated as females/(females+males), where female only = 1, male only = 0), clonality (P_D), percentage of seedlings, and environmental factors (altitude, percentage cover of bare soil ($\log_e$), habitat type, grazing (yes/no), protection area (yes/no), and soil moisture), we performed structural equation modeling (SEM) using *psem* (piecewiseSEM v2.3.0.1; Lefcheck, 2016) with data from 2022/2023 to avoid double testing. A unimodal relationship was hypothesized for sex ratio, assuming that the presence of both sexes is ecologically relevant; thus, both linear and quadratic terms were included in the model. From the calculated genetic indices, H_e was chosen as the indicator for genetic diversity (response variable) because, unlike H_o, it reflects the genetic diversity across the entire population. uH_e was not suitable for later analysis of herbarium samples (the sample sizes will be standardized across historical and contemporary populations). Although H_e is not independent of sample sizes, we argue that in the populations with very low sample sizes, exhaustive sampling was performed, capturing the full extent of genetic diversity. Clonality analysis confirmed that the sample size exceeded the number of distinct genotypes, supporting the data representativeness. Variance inflation factors (VIF) were used to assess multicollinearity among predictor variables (*vif*; car v3.1–3; Fox and Weisberg, 2019). Apart from the expected correlation between sex ratio and its quadratic term, all VIF values were below the threshold of 5. Therefore, all variables were retained in the SEM (see Supplementary Table 2 for full

results of the multicollinearity test). The SEM included the following 5 initial component models: (1) percentage of seedlings ~ protected area + habitat + soil moisture + altitude + grazing + percentage of bare soil + sex ratio, (2) sex ratio ~ protected area + habitat + soil moisture + altitude + grazing + percentage of bare soil, (3) population size ~ protected area + habitat + soil moisture + altitude + grazing + percentage of bare soil + clonality + percentage of seedlings, (4) clonality ~ protected area + habitat + soil moisture + altitude + grazing + percentage of bare soil + sex ratio, and (5) genetic diversity ~ protected area + habitat + soil moisture + altitude + grazing + percentage of bare soil + population size + sex ratio + percentage of seedlings (see Supplementary Figure 4 for a visualized model structure). Variable reduction on each model was performed using backward stepwise simplification based on Akaike Information Criterion (AIC) values.

To explore climate effects on sex ratio, we modelled the proportion of male and female individuals per herbarium specimen as a binomially distributed variable using GLMs with climate predictors (mint, soil, PDSI). To approximate flowering phenology, we analyzed the effect of abiotic factors (year, altitude, climate) on the day of year (DOY) of collection, a common proxy for flowering time

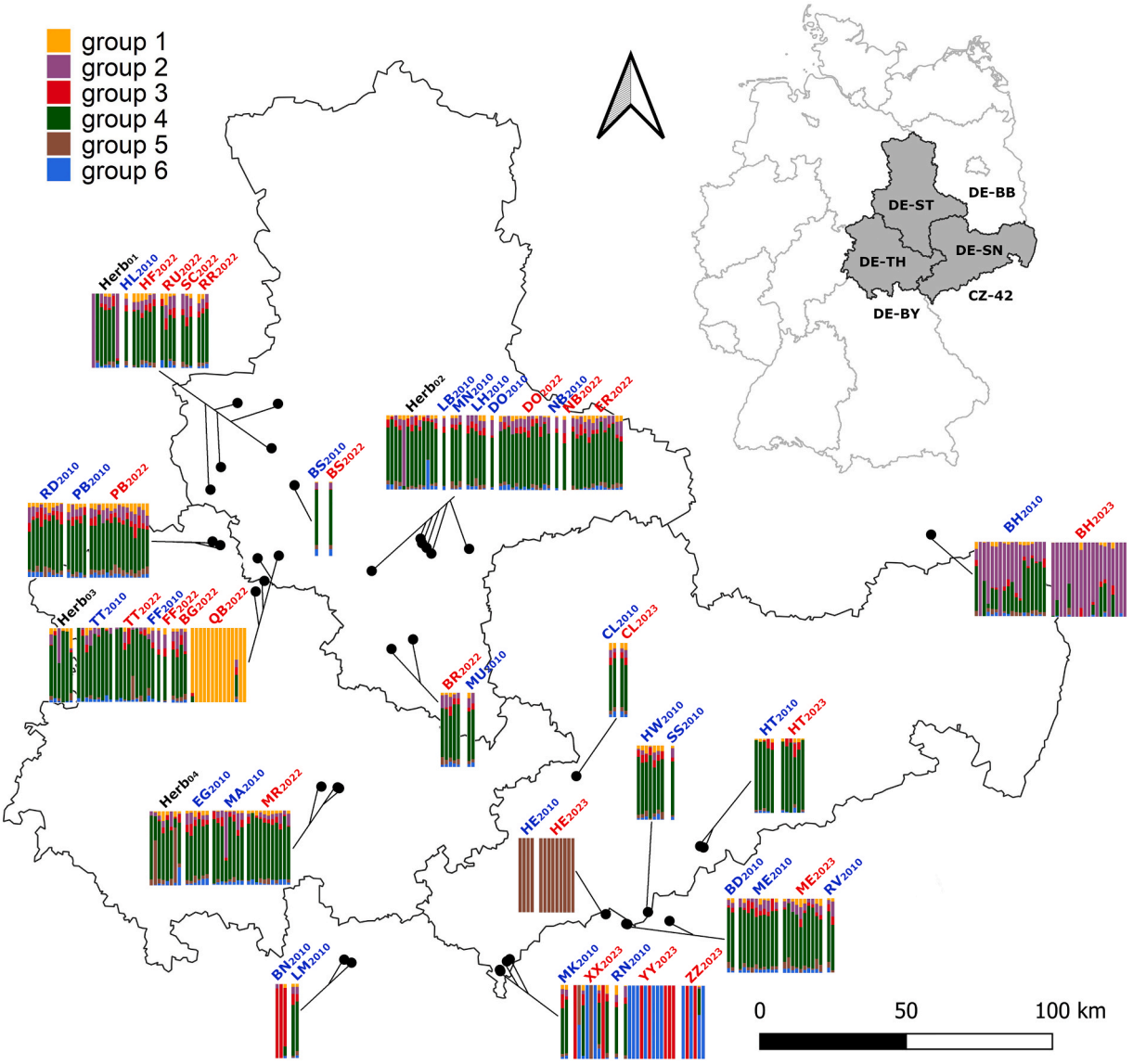

Fig. 1. Genetic structure and geographic distribution of historical and contemporary Antennaria dioica populations from central Germany and adjacent regions (DE-BY = Bavaria, Germany; DE-BB = Brandenburg, Germany; DE-SN = Saxony, Germany; DE-ST = Saxony-Anhalt, Germany; DE-TH = Thuringia, Germany; CZ-42 = Ústecký Kraj, Czech Republic). The bar plots from the ADMIXTURE analysis (K = 6) show the assignment probabilities to five genetic clusters, with each color representing one cluster (see legend). Populations are labelled with abbreviations and sampling year; names are color-coded: red (2022/2023), blue (2010), and black (herbarium samples). Herbarium samples were grouped into four spatial groups, and point locations therefore represent approximate rather than exact collection sites. Population abbreviations are listed in Supplementary Table 1.

in short-flowering perennial herbs (Lee et al., 2022).

To test our third hypothesis and to investigate temporal changes, we compared population characteristics (H_e, population size, sex ratio, percentage of seedlings) and environmental factors (soil depth, bare soil cover, vegetation height) using two approaches: (1) populations sampled both in 2010 and 2022/2023 were directly compared between time points, and (2) 2010 populations were categorized as 'still extant' or 'potentially extinct' by 2022/2023 and compared accordingly. We further examined whether H_e in 2022/2023 or its change over time (ΔH_e) was related to population size in 2010 or its change over time (Δ population size). Additionally, we assessed temporal shifts in genetic diversity by comparing 34 sequenced historical samples (grouped into four spatial groups) with contemporary samples from the same regions. Since H_e was sensitive to call rate we filtered the loci in the SNP data set to a call rate ≥ 0.9. To standardize sample sizes, we applied rarefaction to six individuals per group and calculated H_e over 20 iterations, which were used for comparisons between historical and current spatial groups using *t*-test. Lastly, we tested whether cluster assignment probabilities (from the ADMIXTURE analysis) differed between: (1) 2010 vs. 2022/2023, (2) 'still extant' vs. 'potentially extinct' populations, and (3) historical vs. contemporary samples.

3. Results

3.1. Genetic diversity, differentiation, and structure of contemporary A. dioica populations

Genetic diversity of our *A. dioica* populations was moderately high in both sampling periods, with mean $H_e \pm$ SD of 0.25 ± 0.05 in 2010 and 0.25 ± 0.04 in 2022/2023. Inbreeding coefficients were similar for the population collected in 2010 and 2022/2023 ($F_{IS} = -0.03$ in 2010; -0.02 in 2022/2023; see Supplementary Table 4 for full results e.g., other estimates and number of used samples). Within patches, Nei's genetic distances ranged from 0.01 to 0.18, indicating the presence of multiple clones per patch. Distances within and among populations were higher, averaging around 0.15 and reaching up to 0.25 (Supplementary Figure 5). In 2022/2023, three populations consisted entirely of a single clone, and in some cases, genetically identical individuals were found in both 2010 and 2022/2023 (see Supplementary Table 4). The analysis of genetic differentiation revealed that 31.6 % of the genetic variation occurred among populations, 43.1 % among patches within populations, and 25.3 % within patches ($F = 4.29$; $p = 0.001$). The Mantel test showed no significant relationship between genetic and geographic distance for populations collected in 2010 nor in 2022/2023 ($p > 0.06$; Supplementary Figure 6).

The PCA revealed no pronounced large-scale genetic structure (Supplementary Figure 7), with most populations clustering closely and a few exceptions (e.g., CL_{2010}/CL_{2023}, HE_{2010}/HE_{2023}, YY_{2023}, ZZ_{2023}). Successive PC axes revealed further outlier populations. Notably, populations sampled in both 2010 and 2022/2023 showed high similarity. The ADMIXTURE analysis (K = 6) supported these results: some populations (e.g. BH_{2010}/BH_{2023}, HE_{2010}/HE_{2023}, QB_{2022}, YY_{2023}, ZZ_{2023}) formed distinct clusters, but most populations belonged to one main cluster (Fig. 1). Temporal replicates were consistently assigned to the same clusters, similar to the PCA results.

3.2. Associations between population characteristics and environmental factors

The results of the structural equation modelling (SEM), which were based solely on data from 2022/2023, show that population size was positively correlated with H_e ($p = 0.034$; Fig. 2a). Sex ratio showed a significant unimodal relationship with both H_e ($p = 0.025$; peak at 0.56; Fig. 2b) and clonality (P_D; $p = 0.001$; peak at 0.54; Fig. 2c), indicating the highest genetic diversity and

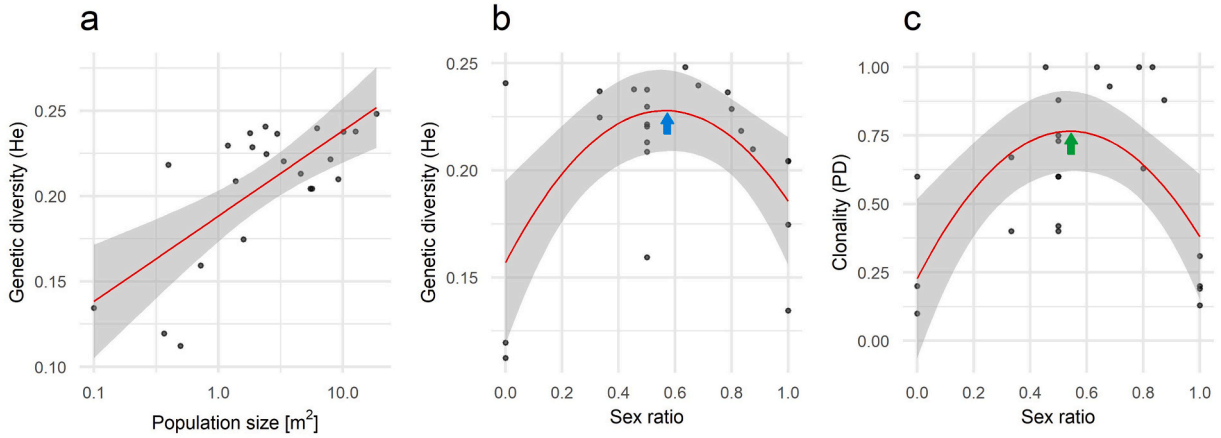

Fig. 2. Significant results from structural equation modeling: (a) Positive linear relationship between population size ($\log_e$-transformed cumulative patch size) and genetic diversity (H_e); (b) Unimodal relationship between sex ratio [females/(females+males), female-only = 1, male-only = 0] and H_e, with maximum H_e (blue arrow) at sex ratio 0.56; (c) Unimodal relationship between sex ratio and clonality (P_D), with maximum P_D (green arrow) at sex ratio 0.54, indicating lowest clonal growth. P_D was calculated as the number of genotypes divided by the total sample size, following Ellstrand and Roose (1987). Red lines show fitted regressions; gray areas represent confidence intervals.

lowest clonality at intermediate sex ratios. No indirect effects of environmental factors on genetic diversity were detected, as none of the environmental factors significantly influenced other population characteristics (see Supplementary Table 5 for full SEM results).

The assessment of the association between climate parameters (soil, mint, PDSI) and the sex ratio, based on herbarium data, reveals that lower soil moisture (Fig. 3a, $p < 0.001$) and higher minimum temperature (Fig. 3b, $p = 0.010$) are related to the occurrence of more male-dominant populations (see Supplementary Table 6 for all results).

The analysis of the collection day of herbarium specimens (DOY, day of the year) revealed a significant positive correlation with year (Fig. 4a; $p = 0.023$). Furthermore, DOY showed a negative correlation with the mean minimum temperature (Fig. 4b, $p = 0.046$), and a positive correlation with altitude (Fig. 4c; $p < 0.001$; see Supplementary Table 7 for full results).

3.3. Temporal changes in population characteristics and environmental factors

No significant differences were found between populations sampled in 2010 and 2022/2023 regarding population characteristics (H_e, population size, sex ratio, percentage of seedlings) and environmental factors (soil depth, percentage of bare soil, maximum vegetation height; Supplementary Table 8). Changes in genetic diversity were not associated with changes in population size (Supplementary Table 9). There were also no significant differences in population characteristics and environmental factors between populations sampled in 2010 that were 'potentially extinct' (not found in 2022/2023) and those that were 'still extant' in 2022/2023 (Supplementary Table 10). The comparison of genetic diversity (H_e) between historical (herbarium specimens) and contemporary data (collected in 2010 and 2022/2023) for four spatial groups showed no significant results ($t = 2.759$, $p = 0.070$).

Moreover, for populations sampled twice (2010 and 2022/2023), there were no significant differences in admixture cluster affiliations between the two time points. However, significant differences were found between 'potentially extinct' and 'still extant' populations from 2010: 'potentially extinct' populations showed higher proportions of Cluster 1 ($p = 0.030$) and Cluster 3 ($p = 0.001$), while 'still extant' populations showed higher proportions of Cluster 2 ($p = 0.002$). Historical samples did not differ significantly in admixture cluster assignment compared to contemporary samples (Supplementary Table 11).

4. Discussion

Antennaria dioica exhibits moderate genetic diversity in central Germany and does not have a clear large-scale genetic structure. However, some populations are genetically distinct. These patterns likely reflect the species' longevity, historically continuous distribution, and a possible extinction debt (Rosche et al., 2018). Genetic diversity is associated with population size and sex ratio, the latter also being linked to clonality. Environmental factors showed no significant association with current population characteristics. However, herbarium data suggest that climate may influence sex ratio and that lowland populations have declined, as indicated by the reduced number of recent collection records. 'Potentially extinct' and 'still extant' populations differ significantly in the composition of their genetic clusters.

4.1. Genetic diversity, differentiation, and structure of contemporary A. dioica populations

Rare species are often assumed to have low genetic diversity due to their small population sizes (Ellstrand and Elam, 1993; Leimu et al., 2006), but this is not always the case (Honnay and Jacquemyn, 2007). In our study, *A. dioica* showed moderate genetic diversity despite declining populations, low germination rates (Feldt, 2008), and the species' very limited seed colonization ability (Walker, 2014), confirming findings of Rosche et al. (2018), especially in previously large, continuous populations. Some of our study populations consisted of a single clone, which poses a high risk of extinction. However, identical genotypes were found across samples

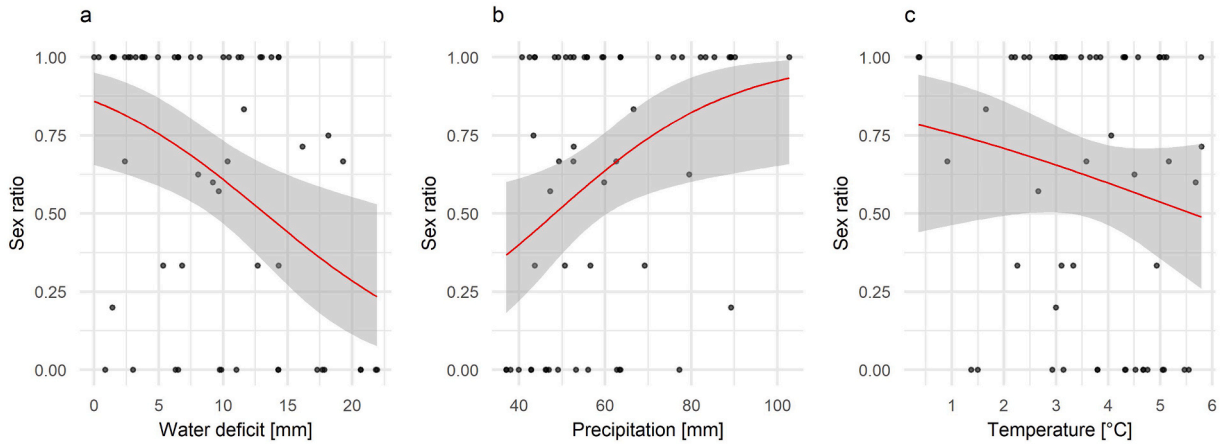

Fig. 3. Relationship between climate and sex ratio (where female only = 1, male only = 0) based on herbarium specimens: (a) Effect of soil moisture; (b) Effect of mean minimum temperature. The red line represents the trend, and the gray shading indicates the 90 % confidence interval.

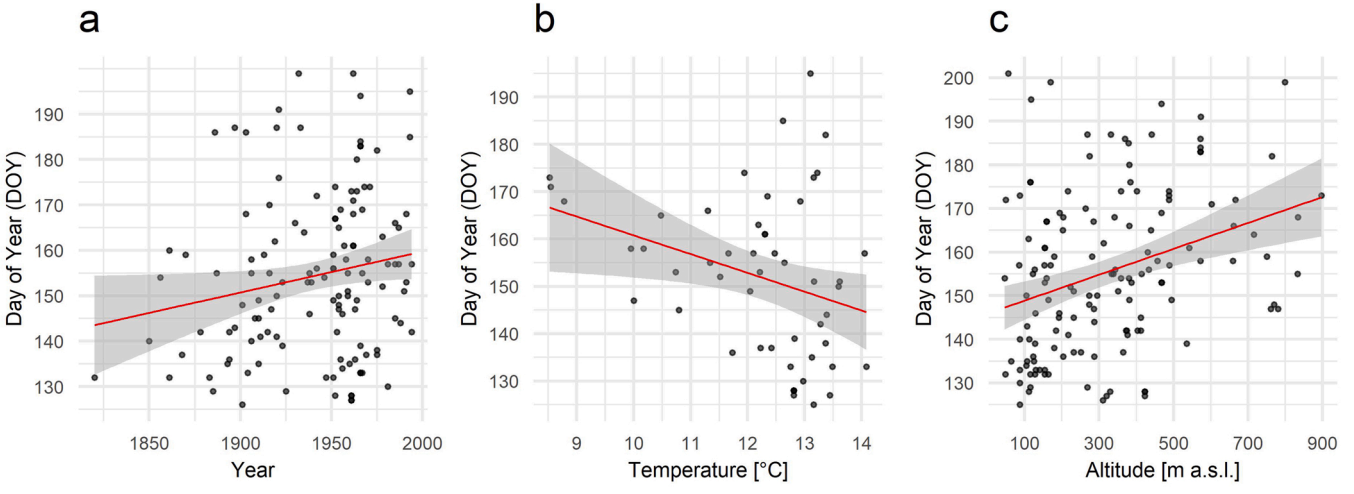

Fig. 4. Relationship between collection day (day of year; DOY; a common proxy for flowering time in short-flowering perennial herbs) and abiotic factors: (a) Effect of collection year; (b) Effect of mean minimum temperature; (c) Effect of altitude. The red line represents the trend, and the gray shading indicates the 90 % confidence interval.

from 2010 and 2022/2023, indicating long-term clone persistence. These results show that clonal growth has not yet reduced diversity, likely due to the species' longevity. Long-lived endangered species often retain higher genetic diversity despite declines in their populations (González-Robles et al., 2020). Genetic erosion may lag behind demographic declines (Gargiulo et al., 2024; Mualim et al., 2024), and clonal species often maintain greater diversity than expected (Xia et al., 2002). We found that the populations are not exclusively clonal, although our sampling method likely minimized clone numbers as we selected distant or separate sites. While PCA and ADMIXTURE analyses did not reveal a pronounced large-scale structure in central Germany, some local differentiation was apparent. The non-significant Mantel tests may reflect a dominant influence of genetic drift due to fragmentation and restricted gene flow (Frankham et al., 2017). Although populations may persist despite a shrinking distribution, they are at a high risk of extinction in the long term, which is referred to as 'extinction debt' (Hanski and Ovaskainen, 2002; Honnay and Bossuyt, 2005). Populations that are close to extinction and species with long generation times are particularly vulnerable to extinction debt (Kuussaari et al., 2009). Additionally, clonal growth can ensure survival when sexual reproduction fails (Honnay and Bossuyt, 2005).

4.2. Associations between population characteristics and environmental factors

Our study shows a correlation between population characteristics—such as population size, sex ratio, and clonality—and genetic diversity. Larger populations had higher genetic diversity, as observed in many other plants (reviewed by Leimu et al., 2006). An important finding is the unimodal relationship between sex ratio and genetic diversity, with the highest diversity at a balanced sex ratio. A similar pattern is observed between sex ratio and clonality. These results suggest that a balanced representation of both sexes supports higher genetic diversity and, consequently, long-term population survival. Although only a few studies established a direct link between sex ratio and genetic diversity, existing ones mostly report a balanced ratio (e.g., *Pistacia atlantica* Desf.– Nosrati et al., 2012; *Osmanthus fragrans* Lour.– Hu et al., 2014; *Populus nigra* L. – Çiftçi et al., 2020). However, in large, intact *A. dioica* populations, the sex ratio is often female-biased (0.67–0.71; Öster and Eriksson, 2007; Varga and Kytöviita, 2011; Rosche et al., 2014), and Rosche et al. (2018) did not find a unimodal relationship between population size and sex ratio but showed that the presence of more females can have a positive effect on genetic diversity. Our findings contrast with these results, likely because our analyses included a broader range of sex ratios, including more extreme values approaching 0 and 1.

Contrary to our expectations, environmental factors had no significant effect on population size or other population characteristics. Vörös et al. (2025) also found no influence of climate and soil parameters on *A. dioica* populations in central Germany, although they considered fewer variables (e.g., no protection status, habitat type, altitude, and grazing) and defined population size as the number of patches rather than the cumulative patch area. The lack of significance in our study may partly reflect the difficulty of accurately estimating population size in clonal species (Tepedino et al., 2012). More importantly, habitat loss due to extensive land use is likely a major driver of population declines of *A. dioica* (Frank and Schnitter, 2016), but data on land use intensity were not available for our analyses, leaving this as an open question for future research.

Analysis of herbarium specimens revealed an association between sex ratio and climate: drought conditions (low soil moisture and high temperature) can lead to a surplus of male individuals, which has been linked to male-biased sex ratios due to higher female sensitivity (Bierzychudek and Eckhart, 1988; Munné-Bosch, 2015; Hultine et al., 2018). This result coincides with experiments by Varga and Kytöviita (2008), who found that female plants of *A. dioica* were indeed more sensitive to drought. Thus, drier conditions could promote the dominance of males. However, opposite patterns also occur: males are more drought-sensitive in *Silene otites* (Soldaat et al., 2000), and *Fragaria moschata* shows more female offspring under drought (Buschmann, 2024). This contrasts with our findings and suggests species-specific responses. Nevertheless, as the climate in the study area becomes drier and extreme weather becomes more frequent (e.g., extreme drought in 2018), the sex ratio may undergo a worrying change, as the highest genetic diversity was found in populations with a balanced sex ratio.

The significant correlations between collection date (DOY) and year, temperature, and elevation suggest a shift in phenology. Although DOY does not directly reflect peak flowering, it is frequently used to estimate flowering phenology in short flowering perennial herbs (Lee et al., 2022). Our results show a negative relationship between temperature and DOY, which is consistent with earlier studies (e.g., Park et al., 2019). Park et al. (2019) and Büntgen et al. (2022) found that warming leads to earlier flowering. However, *A. dioica* shows a positive link between DOY and year which means that specimens have been collected later in recent decades—contrary to expectations based on climate warming. This contradiction can be explained by the spatio-temporal changes in the distribution of *A. dioica*: DOY increases with elevation and recent collections are more likely to come from cooler, high-elevation areas (e.g., Harz and Ore Mountains) while earlier specimens were more likely to come from the lowlands than today. We suspect that this reflects a long-term range shift, with lowland populations declining over the last 200 years (see Fig. 1a in Vörös et al., 2025), likely due to land use, eutrophication, and habitat fragmentation. Since *A. dioica* is naturally alpine, only populations in well-managed, nutrient-poor lowland areas are still able to survive. Thus, changes in DOY are likely to reflect range shifts rather than true phenological changes.

4.3. Temporal changes in population characteristics and environmental factors

The genetic structure of historical (herbarium) and contemporary samples (2010 and 2022/2023) shows no changes over time and similarly, population characteristics and environmental factors have remained stable over the past 12 years. This stability likely reflects *A. dioica's* formerly wide distribution in central Germany (Rosche et al., 2018) and its mainly clonal reproduction, allowing long-lived individuals to persist (Rosche et al., 2014). As a result, the genetic structure may have remained unchanged for centuries, with shifts occurring only after significant environmental changes. This is consistent with the concept of extinction debt, where the

negative effects of small population size or poor reproduction occur with a delay -although current conditions suggest short-term stability, long-term risks remain (Hanski and Ovaskainen, 2002; Honnay and Bossuyt, 2005). Since individual clones can survive for over a decade, short-term genetic changes are unlikely to be detected.

To date, only three studies have compared genetic diversity and structure over time using herbarium and contemporary samples (Gavrilenko et al., 2022; Nygaard et al., 2022; Rosche et al., 2022). Of these, only two studies examined declining plant species. Rosche et al. (2022) found reduced genetic diversity over time in extinct populations of *Biscutella laevigata* L. *subsp. gracilis*, while Nygaard et al. (2022) reported only minimal change in *Dracocephalum ruyschiana* L. As our findings, both studies revealed a similarity in genetic structure between historical and contemporary populations. However, the lack of further examples highlights the urgent need for long-term genetic monitoring in plants (Aravanopoulos, 2011; Pearman et al., 2024).

However, significant differences in the proportions of genetic clusters between "still extant" and "potentially extinct" populations denote the role of genetic structure in maintaining population persistence. This supports the findings of Rosche et al. (2022), who linked a specific genetic cluster to extinction probability in *Biscutella laevigata* subsp. *gracilis*. Certain genetic lineages may be more susceptible to environmental pressures due to differences in adaptive potential (Chung et al., 2023). This vulnerability can lead to shifts in genetic cluster composition, signaling early stages of genetic erosion before extinction becomes evident. Such patterns underscore the importance of conserving genetic diversity within plant populations to enhance their resilience and adaptive capacity in changing environments (Hoban et al., 2020)

5. Conclusion

This study shows that the loss of genetic diversity in threatened plant species may not be immediately apparent, while extinction risks can be reflected in shifts in genetic cluster composition. We also demonstrate that climate may influence population sex ratios, although environmental change does not appear to be the primary driver of the current decline, which is more likely related to habitat loss due to extensive land use. Additionally, our study underscores the importance of long-term species monitoring, including genetic data and herbarium specimens, to track past population structures and changes in sex ratios. Such an approach is essential for guiding conservation efforts.

CRediT authorship contribution statement

Jochen Müller : Writing – review & editing. **Sabrina Träger :** Writing – review & editing, Supervision, Methodology, Conceptualization. **Isabell Hensen:** Writing – review & editing, Conceptualization. **Marcus Lehnert :** Writing – review & editing, Funding acquisition, Conceptualization. **Daya Södje :** Investigation. **Karin Schrieber :** Writing – review & editing. **Weronika A. Vörös:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christoph Rosche:** Writing – review & editing, Methodology, Conceptualization. **Walter Durka:** Writing – review & editing, Methodology, Formal analysis. **Stefan G. Michalski:** Writing – review & editing, Formal analysis.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT in order to improve the readability and language of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Funding

The project was financed by the Federal State of Saxony-Anhalt through the MLU|BioDivFund (project R02020830).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We sincerely thank D. Frank (LAU), F. Richter (LfULG), T. Lemke (TLUBN), and staff from conservation and environmental authorities in Brandenburg, Erzgebirgskreis, Kyffhäuserkreis, Nordhausen, Saalfeld-Rudolstadt, Vogtlandkreis, and Zwickau for access to location data and sampling permissions. We are grateful to botanists W. Dietrich, H. Hertel, H.-U. Kison, J. Pusch, P. Rode, J. Stolle, and E. Welk for help in locating populations, and to F. Karimiyeganeh and W. Gelfgat for fieldwork assistance. We also thank the curators and staff of the herbaria in Görlitz (P. Gebauer), Gera (A. Gerth), Gatersleben (D. Harpke), Dessau (T. Karisch), Leipzig (P. Otto), Berlin (J. Paule), and Dresden (F. Müller, S. Wagner). Special thanks to H. Pereira and M. Méndez Camarena (iDiv) for support with historical DNA extraction. W.A.V., W.D., C.Ro., I.H., M.L., and S.T. acknowledge the support of iDiv funded by the German Research Foundation (DFG– FZT 118, 202548816). Many thanks also to the two anonymous reviewers and M. Galetti, the handling editor for their valuable and insightful comments.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2026.e04090.

Data availability

Data will be made available on request.

References

Abatzoglou, J.T., Dobrowski, S.Z., Parks, S.A., Hegewisch, K.C., 2018. Terraclimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958-2015. Sci. Data.

Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19 (9), 1655–1664. https://doi.org/10.1101/gr.094052.109.

Aravanopoulos, F.A., 2011. Genetic monitoring in natural perennial plant populations. Botany 89, 75–81. https://doi.org/10.1139/b10-087.

Asdal, Å., 2005. Changes in grassland management and its effect on plant diversity. Crop Wild Relat. 3, 15–17.

Bauer, P., Brabec, J., Kubát, K., Machová, I., Müller, F., Ondrácek, C., Richter, F., Tájek, P., Walczak, C., 2013. Seltene Pflanzen im Erzgebirge - Situation in Böhmen und Sachsen. CDSM.cz. Ústí nad Labem 9–20.

Bayer, R.J., 1984. Chromosome numbers and taxonomic notes for North American species of *Antennaria* (Asteraceae: Inuleae). Syst. Bot. 74–83. https://doi.org/10.2307/2418409.

BfN, 2025. Bundesamt für Naturschutz. Daten und Fakten. Retrieved from⟨https://www.bfn.de/daten-und-fakten⟩, on 08.10.2024.

Bierzychudek, P., Eckhart, V., 1988. Spatial segregation of the sexes of dioecious plants. Am. Nat. 132 (1), 34–43.

Blachnik, T., 2009. Arnika und Katzenpfötchen - Ein Artenhilfsprojekt im Bayerischen Vogtland. Regier. Von. Oberfrank. 3–10.

Büntgen, U., Piermattei, A., Krusic, P.J., Esper, J., Sparks, T., Crivellaro, A., 2022. Plants in the UK flower a month earlier under recent warming. Proc. R. Soc. B 289 (1968), 20212456. https://doi.org/10.1098/rspb.2021.2456.

Buschmann, S., 2024. The musk strawberry (Fragaria moschata Weston) in Central Europe – sex ratios, diversity and synanthropic distribution. PhD thesis, Senckenberg Museum für Naturkunde Görlitz and Technische Universität Dresden, Germany, 42-56.

Carter, M.C., Sytsma, M.D., 2001. Comparison of the Genetic Structure of North and South American Populations of a Clonal Aquatic Plant. Biol. Invasions 3 (2), 113–118. https://doi.org/10.1023/A:1014510730268.

Carvalho, C., da, S., Nascimento da Costa, L.E., Leal, B.S.S., Silva, K.R., Valentin-Silva, A., Costa, A.C.G., Tyski, L., Gomes dos Santos, F.M., Watanabe, M.T.C., 2024. Mating system, morphological, and genetic evidence endorse clonality as an essential reproductive mode in *Daphnopsis filipedunculata* (Thymelaeaceae), a dioecious and endemic species from the Amazon. Aob Plants 16 (5). https://doi.org/10.1093/aobpla/plae048.

Chung, M.Y., Merilä, J., Li, J., Mao, K., López-Pujol, J., Tsumura, Y., Chung, M.G., 2023. Neutral and adaptive genetic diversity in plants: An overview. Front. Ecol. Evol. 11, 1116814. https://doi.org/10.3389/fevo.2023.1116814.

Çiftçi, A., Yelmen, B., Değirmenci, F.Ö., Kaya, Z., 2020. Impact of biased sex ratio on the genetic diversity, structure, and differentiation of *Populus nigra* (European black poplar). Botany 98 (10), 603–613. https://doi.org/10.1139/cjb-2020-0046.

Davis, C.C., 2023. The herbarium of the future. Trends Ecol. Evol. 38 (5), 412–423. https://doi.org/10.1016/j.tree.2022.11.015.

Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. Focus 12, 13–15.

Durka, W., Michalski, S.G., Höfner, J., Bucharova, A., Kolář, F., Müller, C.M., RegioDiv Consortium, 2025. Assessment of genetic diversity among seed transfer zones for multiple grassland plant species across Germany. Basic Appl. Ecol. 84, 50–60. https://doi.org/10.1016/j.baae.2024.11.004.

Eichenberg, D., Bowler, D.E., Bonn, A., Bruelheide, H., Grescho, V., Harter, D., Jansen, F., 2021. Widespread decline in Central European plant diversity across six decades. Glob. Change Biol. 27 (5), 1097–1110. https://doi.org/10.1111/gcb.15447.

Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Syst. 24 (1), 217–242. https://doi.org/10.1146/annurev.es.24.110193.001245.

Ellstrand, N.C., Roose, M.L., 1987. Patterns of genotypic diversity in clonal plant species. Am. J. Bot. 74 (1), 123–131. https://doi.org/10.1002/j.1537-2197.1987.tb08586.x.

Feldt, C., 2008. Beiträge zur Populationsdynamik von *Antennaria dioica* (L.) GAERTN. im Bereich der mittleren Schwäbischen Alb. Disseration 137, 155.

Fick, S.E., Hijmans, R.J., 2017. Worldclim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol.

Field, D.L., Pickup, M., Barrett, S.C.H., 2013. Comparative analyses of sex-ratio variation in dioecious flowering plants. Evolution 67 (3), 661–672. https://doi.org/10.1111/EVO.12001.

Fox, J. & Weisberg, S. (2019). An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. ⟨https://www.john-fox.ca/Companion/⟩.

Frank, D. & Schnitter, P., 2016. Planzen und Tiere in Sachsen-Anhalt. Ein Kompendium der Biodiversität. – Natur+Text, Rangsdorf. P. 215.

Frankham, R., 2005. Genetics and extinction. Biol. Conserv. 126 (2), 131–140. https://doi.org/10.1016/j.biocon.2005.05.00.

Frankham, R., Ballou, J.D., Ralls, K., Eldridge, M.D.B., Dudash, M.R., Fenster, C.B., Sunnucks, P., 2017. Population fragmentation causes inadequate gene flow and increases extinction risk. Genet. Manag. Fragm. Anim. Plant Popul. 87–112. https://doi.org/10.1093/oso/9780198783411.003.0004.

García, M.B., 2008. Life history and population size variability in a relict plant. Different routes towards long-term persistence. Divers. Distrib. 14 (1), 106–113. https://doi.org/10.1111/j.1472-4642.2007.00429.x.

Gargiulo, R., Budde, K.B., & Heuertz, M., 2024. Mind the lag: Understanding delayed genetic erosion. 2. Version of Preprint. https://doi.org/10.1016/j.tree.2024.10.008.

Gavrilenko, T., Chukhina, I., Antonova, O., Krylova, E., Shipilina, L., Oskina, N., Kostina, L., 2022. Comparative analysis of the genetic diversity of Chilean cultivated potato based on a molecular study of authentic herbarium specimens and present-day gene bank accessions. Plants 12 (1), 174. https://doi.org/10.3390/plants12010174.

Gilpin, M.E., Soulé, M.E., 1986. Minimum viable populations: processes of species extinction. Conservation Biology. Sinauer, Sunderland, MA, pp. 19–34.

González-Robles, A., Manzaneda, A.J., Salido, T., Valera, F., García, C., Fernández-Ocaña, A.M., Rey, P.J., 2020. Spatial genetic structure of a keystone long-lived semiarid shrub: historical effects prevail but do not cancel the impact of recent severe habitat loss on genetic diversity. Conserv. Genet. 21, 853–867. https://doi.org/10.1007/s10592-020-01291-5.

Greve, M., Lykke, A.M., Fagg, C.W., Gereau, R.E., Lewis, G.P., Marchant, R., Marshall, A.R., Ndayishimiye, J., Bogaert, J., Svenning, J.C., 2016. Realising the potential of herbarium records for conservation biology. South Afr. J. Bot. 105, 317–323. https://doi.org/10.1016/j.sajb.2016.03.017.

Grulich, V., Chobot, K., 2017. Red list of threatened species of the Czech Republic: Vascular plants. Příroda 35, 1–178.

Haeupler, H., Muer, T., 2000. Bildatlas Der Farn-und Blütenpflanzen Deutschlands, 542. Eugen Ulmer, Stuttgart, p. 138.

Hamrick, J.L., Godt, M.W., 1996. Effects of life history traits on genetic diversity in plant species. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 351 (1345), 1291–1298. https://doi.org/10.1098/rstb.1996.0112.

Hanski, I., Ovaskainen, O., 2002. Extinction debt at extinction threshold. Conserv. Biol. 16 (3), 666–673. https://doi.org/10.1046/j.1523-1739.2002.00342.x.

He, Z.-Z., Stotz, G.C., Liu, X., Liu, J., Wang, Y.-G., Yang, J., Li, L.-F., Zhang, W., Nan, P., Song, Z., 2024. A global synthesis of the patterns of genetic diversity in endangered and invasive plants. Biol. Conserv. https://doi.org/10.1016/j.biocon.2024.110473.
Hijmans, R., 2022. Geosphere: Spherical Trigonometry_. R package (version 1.5-18). ⟨https://CRAN.R-project.org/package=geosphere⟩.
Hoban, S., Bruford, M., Jackson, J.D.U., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P.A., Laikre, L., 2020. Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biol. Conserv. 248, 108654. https://doi.org/10.1016/j.biocon.2020.108654.
Honnay, O., Bossuyt, B., 2005. Prolonged clonal growth: escape route or route to extinction? Oikos 108 (2), 427–432. https://doi.org/10.1111/j.0030-1299.2005.13569.x.
Honnay, O., Jacquemyn, H., 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. Conserv. Biol. 21 (3), 823–831. https://doi.org/10.1111/j.1523-1739.2006.00646.x.
Hu, S., Wu, S., Wang, Y., Zhao, H., Zhang, Y., 2014. Genetic diversity and genetic structure of different types of natural populations in *Osmanthus fragrans* Lour. and the relationships with sex ratio, population structure, and geographic isolation. Sci. World J. 2014 (1), 817080. https://doi.org/10.1155/2014/817080.
Hultine, K.R., Bush, S.E., Ward, J.K., Dawson, T.E., 2018. Does sexual dimorphism predispose dioecious riparian trees to sex ratio imbalances under climate change? Oecologia 187 (4), 921–931.
Hyvärinen, E., Juslén, A., Kemppainen, E., Uddström, A., Liukko, U.M., 2019. Suom. lajien uhanalaisuus Punainen Kirja 2019.
Inglis, P.W., Pappas, M.D.C.R., Resende, L.V., Grattapaglia, D., 2018. Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. PloS One 13 (10), e0206085. https://doi.org/10.1371/journal.pone.0206085.
Jandt, U., Bruelheide, H., Jansen, F., Bonn, A., Grescho, V., Klenke, R.A., Wulf, M., 2022. More losses than gains during one century of plant biodiversity change in Germany. Nature 611 (7936), 512–518. https://doi.org/10.1038/s41586-022-05320-w.
Jombart, T., Ahmed, I., 2011. Adegenet: new tools for the analysis of genome-wide SNP data (version 2.1.10). Bioinformatics. https://doi.org/10.1093/bioinformatics/btr521.
Karbstein, K., Römermann, C., Hellwig, F.H., Prinz, K., 2023. Population size affected by environmental variability impacts genetics, traits, and plant performance in Trifolium montanum L. Ecol. Evol. 13. https://doi.org/10.1002/ece3.10376.
Király G. (ed.) (2007): Vörös Lista. A magyarországi edényes flóra veszélyeztetett fajai. [Red list of the vascular flora of Hungary]. – Saját kiadás, Sopron, 73 pp.
Knaus, B.J., Grünwald, N.J., 2017. vcfr: a package to manipulate and visualize variant call format data in R. Mol. Ecol. Resour. 17 (1), 44–53. https://doi.org/10.1111/1755-0998.12549.
Kuussaari, M., Bommarco, R., Heikkinen, R.K., Helm, A., Krauss, J., Lindborg, R., Steffan-Dewenter, I., 2009. Extinction debt: a challenge for biodiversity conservation. Trends Ecol. Evol. 24 (10), 564–571 https://doi.org/0.1016/j.tree.2009.04.011.
Lang, P.L., Willems, F.M., Scheepens, J.F., Burbano, H.A., Bossdorf, O., 2019. Using herbaria to study global environmental change. N. Phytol. 221 (1), 110–122. https://doi.org/10.1111/nph.15401.
Lee, B.R., Miller, T.K., Rosche, C., Yang, Y., Heberling, J.M., Kuebbing, S.E., Primack, R.B., 2022. Wildflower phenological escape differs by continent and spring temperature. Nat. Commun. 13 (1), 7157. https://doi.org/10.1038/s41467-022-34936-9.
Lefcheck, J.S., 2016. PiecewiseSEM: Piecewise structural equation modeling in R for ecology, evolution, and systematics. Methods Ecol. Evol. 7 (5), 573–579. https://doi.org/10.1111/2041-210X.12512.
Lehmair, T., Pagel, E., Poschlod, P., Reisch, C., 2020. Surrounding landscape structures, rather than habitat age, drive genetic variation of typical calcareous grassland plant species. Landsc. Ecol. 35, 2881–2893. https://doi.org/10.1007/s10980-020-01120-7.
Leimu, R., Mutikainen, P.I.A., Koricheva, J., Fischer, M., 2006. How general are positive relationships between plant population size, fitness and genetic variation? J. Ecol. 94 (5), 942–952. https://doi.org/10.1111/j.1365-2745.2006.01150.x.
Li, Y., Zhao, X., Mao, X., Wei, X., Hou, H.-W., 2023. Temperature is a cryptic factor shaping the geographical pattern of genetic variation in *Ceratophyllum demersum* across a subtropical freshwater lake. Plant Divers. https://doi.org/10.1016/j.pld.2023.08.002.
Lin, C.-H., Miriti, M.N., Goodell, K., 2016. Demographic consequences of greater clonal than sexual reproduction in Dicentra canadensis. Ecol. Evol. 6 (12), 3871–3883. https://doi.org/10.1002/ECE3.2163.
Liu, M., Korpelainen, H., Li, C., 2021. Sexual differences and sex ratios of dioecious plants under stressful environments. J. Plant Ecol. 14 (5), 920–933. https://doi.org/10.1093/jpe/rtab038.
Lloyd, D.G., 1974. Female-predominant sex ratios in angiosperms. Heredity 32 (1), 35–44.
Makuch, W.A., Wanke, S., Ditsch, B., Richter, F.A., Herklotz, V., Ahlborn, J.A., Ritz, C.M., 2023. Population genetics and plant growth experiments as prerequisite for conservation measures of the rare European aquatic plant *Luronium natans* (Alismataceae). Front. Plant Sci. 13. https://doi.org/10.3389/fpls.2022.1069842.
Mijangos, J.L., Gruber, B., Berry, O., Pacioni, C., & Georges, A., 2022. dartR v2: An accessible genetic analysis platform for conservation, ecology and agriculture. Methods in Ecology and Evolution (version 2.9.7.). ⟨https://doi.org/10.1111/2041-210X.13918⟩.
Mualim, K.S., Spence, J.P., Weiss, C.L., Selmoni, O., Lin, M., Exposito-Alonso, M., 2024. Genetic diversity loss in the Anthropocene will continue long after habitat destruction ends, 2024-10 bioRxiv. https://doi.org/10.1101/2024.10.21.619096.
Müller, F., Ritz, C.M., Welk, E., 2021. Rothmaler-Exkursionsflora von Deutschland. In: Wesche, K. (Ed.), Gefäßpflanzen: grundband, 472, pp. 27–28, 860.
Munné-Bosch, S., 2015. Sex ratios in dioecious plants in the framework of global change. Environ. Exp. Bot. 109, 99–102.
Nosrati, H., Husainpourfeizi, M.A., Khorasani, M., Razban-Haghighi, A., Nikniazi, M., 2012. Sex ratio and genetic diversity in the dioecious *Pistacia atlantica* (Anacardiaceae). J. Agrobiol. 29 (1), 41. https://doi.org/10.2478/v10146-012-0006-2.
Nygaard, M., Kopatz, A., Speed, J.M., Martin, M.D., Prestø, T., Kleven, O., Bendiksby, M., 2022. Spatiotemporal monitoring of the rare northern dragonhead (*Dracocephalum ruyschiana*, Lamiaceae)—SNP genotyping and environmental niche modeling herbarium specimens. Ecol. Evol. 12 (8), e9187. https://doi.org/10.1002/ece3.9187.
Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., … Weedon, J., 2022. vegan: Community Ecology Package_. R package (version 2.6-4). ⟨https://CRAN.R-project.org/package=vegan⟩.
O'Leary, S.J., Puritz, J.B., Willis, S.C., Hollenbeck, C.M., Portnoy, D.S., 2018. These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. *3459-3459* Mol. Ecol. 28 (14). https://doi.org/10.1111/mec.14792.
Öster, M., Eriksson, O., 2007. Sex ratio mediated pollen limitation in the dioecious herb *Antennaria dioica*. Ecoscience 14 (3), 387–398. https://doi.org/10.2980/1195-6860(2007)14[387:SRMPLI]2.0.CO;2.
Park, D.S., Breckheimer, I., Williams, A.C., Law, E., Ellison, A.M., Davis, C.C., 2019. Herbarium specimens reveal substantial and unexpected variation in phenological sensitivity across the eastern United States. Philos. Trans. R. Soc. B 374 (1763), 20170394. https://doi.org/10.1098/rstb.2017.0394.
Pearman, P.B., Broennimann, O., Aavik, T., Albayrak, T., Alves, P.C., Aravanopoulos, F.A., Bruford, M., 2024. Monitoring of species' genetic diversity in Europe varies greatly and overlooks potential climate change impacts. Nat. Ecol. Evol. 8 (2), 267–281. https://doi.org/10.1038/s41559-023-02260-0.
Pembleton, L.W., Cogan, N.O.I., Forster, J.W., 2013. StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Version 1. 6. 3. Mol. Ecol. Resour. 13 (5), 946–952.
Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PloS One 7 (5), e37135. https://doi.org/10.1371/journal.pone.0037135.g001.
Pflugbeil, G. (2024) Österreichs Gefäßpflanze des Jahres 2025, Gewöhnliches Katzenpfötchen / Antennaria dioica https://www.flora-austria.at/Docs/Pflanzen%20des%20Jahres/2025/ Folder%202025-5%20AL%20-%20Antennaria%20dioica.pdf.
Puritz, J.B., Hollenbeck, C.M., Gold, J.R., 2014. dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ 2, e431. https://doi.org/10.7717/peerj.431.
R Core Team, 2025. R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. Version 4.5.0; ⟨https://www.R-project.org/⟩.

Richter, F., & Blachnik, T., 2013. Antennaria dioica – Vorschlag für ein länderübergreifendes Erhaltungsprojekt im Vogtland und Frankenwald. Unveröffentlichtes Gutachten im Auftrag des Bayerischen Landesamtes für Umwelt (LfU), 41 S., Augsburg.

Rocchetti, G.A., Armstrong, C.G., Abeli, T., Orsenigo, S., Jasper, C., Joly, S., Bruneau, A., Zytaruk, M., Vamosi, J.C., 2021. Reversing extinction trends: new uses of (old) herbarium specimens to accelerate conservation action on threatened species. N. Phytol. 230 (2), 433–450. https://doi.org/10.1111/NPH.17133.

Rosche, C., Baasch, A., Runge, K., Brade, P., Träger, S., Parisod, C., Hensen, I., 2022. Tracking population genetic signatures of local extinction with herbarium specimens. Ann. Bot. 129 (7), 857–868. https://doi.org/10.1093/aob/mcac061.

Rosche, C., Broennimann, O., Novikov, A., Mrázová, V., Boiko, G.V., Danihelka, J., Mráz, P., 2025. Herbarium specimens reveal a cryptic invasion of polyploid *Centaurea stoebe* in Europe. N. Phytol. 245 (1), 392–405. https://doi.org/10.1111/nph.20212.

Rosche, C., Schrieber, K., Hensen, I., Seidler, G., Hirsch, H., Blachnik, T., Richter, F., 2014. Sexuelle Reproduktion und klonales Wachstum in kleinen Populationen von *Antennaria dioica* (L.) Gaertner. *Hercynia-Ökologie und Umwelt in Mitteleuropa*, 47 (1), 59–86.

Rosche, C., Schrieber, K., Lachmuth, S., Durka, W., Hirsch, H., Wagner, V., Hensen, I., 2018. Sex ratio rather than population size affects genetic diversity in *Antennaria dioica*. Plant Biol. 20 (4), 789–796. https://doi.org/10.1111/plb.12716.

Rybashlykova, L.P., 2022. Effect of conservation and grazing on the structure of cenopopulations in the community of *Festuca valesiaca Gaudin*. Izv. â Timirâzevskoj Sel. ˈskohozâjstvennoj Akad. (1), 52–60. https://doi.org/10.26897/0021-342x-2022-1-52-60.

Schou, M.F., Loeschcke, V., Bechsgaard, J., Schlötterer, C., Kristensen, T.N., 2017. Unexpected high genetic diversity in small populations suggests maintenance by associative overdominance. Mol. Ecol. 26 (23), 6510–6523. https://doi.org/10.1111/mec.14262.

Segalla, R., Pinheiro, F., Barônio, G.J., Morellato, L.P.C., 2021. Male-biased effective sex ratio across populations of the threatened *Zamia boliviana* (Zamiaceae). Plant Ecol. 222 (5), 587–602. https://doi.org/10.1007/S11258-021-01127-3.

Selke, J., 2024. TerraclimateR: Download TerraClimate Data in Tidy Format. Version 0 (1), 0.

Shuyskaya, E.V., Nukhimovskaya, Yu, D., Lebedeva, M., Churilina, A.E., Kolesnikov, A.V., 2020. Effect of Soil Conditions on the Level of Genetic Diversity in the *Xerohalophyte kochiaprostrata* (L.) Schrad. (Chenopodiaceae). Russ. J. Ecol. 51 (2), 118–126. https://doi.org/10.1134/S1067413620020101.

Smarda, P., Knapek, O., Brezinova, A., Horova, L., Grulich, V., Danihelka, J., Vesely, P., Smerda, J., Rotreklova, O., Bures, P., 2019. Genome sizes and genomic guanine plus cytosine (GC) contents of the Czech vascular flora with new estimates for 1700 species. Preslia 91 (2), 117–142. https://doi.org/10.23855/preslia.2019.117.

Soldaat, L.L., Lorenz, H., Trefflich, A., 2000. The effect of drought stress on the sex ratio variation of Silene otites. Folia Geobot. 35 (2), 203–210.

Song, Y., Xu, L., Duan, J., Zhang, W., Shentu, X., Li, T., Dong, M., 2020. Sex ratio and spatial pattern of Taxus fuana, a wild plant with extremely small populations in Tibet. Biodivers. Sci. 28 (3), 269. https://doi.org/10.17520/BIODS.2019102.

Souza, A., Prevedello, J.A., 2020. The importance of protected areas for overexploited plants: Evidence from a biodiversity hotspot. Biol. Conserv. 243, 108482. https://doi.org/10.1016/J.BIOCON.2020.108482.

Stein, K., Rosche, C., Hirsch, H., Kindermann, A., Köhler, J., Hensen, I., 2014. The influence of forest fragmentation on clonal diversity and genetic structure in *Heliconia angusta*, an endemic understorey herb of the Brazilian Atlantic rain forest. J. Trop. Ecol. 30 (3), 199–208. https://doi.org/10.1017/S0266467414000030.

Tepedino, V.J., 2012. Overestimating population sizes of rare clonal plants. Conserv. Biol. 26 (5), 945–947. https://doi.org/10.1111/j.1523-1739.2012.01886.x.

Thiers, B., 2024. Index Herbariorum: A global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium.

UICN France, FCBN, AFB & MNHN (2018). La Liste rouge des espèces menacées en France – Chapitre Flore vasculaire de France métropolitaine. Paris, France.

Varga, S., Kytöviita, M.M., 2011. Sex ratio and spatial distribution of male and female *Antennaria dioica* (Asteraceae) plants. Acta Oecologica 37 (5), 433–440. https://doi.org/10.1016/j.actao.2011.05.011.

Viveiros-Moniz, M., García-Muñoz, A., Matias, L., Abdelaziz, M., Muñoz-Pajares, A.J., 2025. Monitoring biodiversity in the global change era: The importance of herbaria and genetic diversity. Perspect. Plant Ecol. Evol. Syst., 125862 https://doi.org/10.1016/j.ppees.2025.125862.

Vörös, W.A., Hensen, I., Träger, S., Rosche, C., Müller, J., Römermann, C., Lehnert, M., 2025. From herbarium specimens to contemporary surveys: Tracing the extinction dynamics and performance of endangered plant species in central Germany. Flora 323, 152664. https://doi.org/10.1016/j.flora.2024.152664.

Walker, K.J. (2014). Antennaria dioica (L.) Gaertn. Mountain Everlasting. Species Account. Botanical Society of Britain and Ireland.

Willems, F.M., Scheepens, J.F., Bossdorf, O., 2022. Forest wildflowers bloom earlier as Europe warms: lessons from herbaria and spatial modelling. N. Phytol. 235 (1), 52–65. https://doi.org/10.1111/nph.18124.

Xia, L.-Q., Li, J.-Q., Li, W., 2002. Genetic diversity of clonal plant. Chin. Bull. Bot. 19 (04), 425.

Xie, Y., Thammavong, H.T., Berry, L.G., Huang, C.H., Park, D.S., 2023. Sex-dependent phenological responses to climate vary across species' ranges. Proc. Natl. Acad. Sci. USA 120 (48), e2306723120. https://doi.org/10.1073/pnas.2306723120.