Annals of Botany **129**: 857–868, 2022
https://doi.org/10.1093/aob/mcac061, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

Tracking population genetic signatures of local extinction with herbarium specimens

Christoph Rosche[1,2,†], Annett Baasch[3,†,*], Karen Runge[3], Philipp Brade[3], Sabrina Träger[1,2], Christian Parisod[4,] and Isabell Hensen[1,2]

[1]Martin Luther University Halle-Wittenberg, Institute of Biology/Geobotany and Botanical Garden, Große Steinstraße 79/80, 06108 Halle (Saale), Germany, [2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Puschstraße 4, 04103 Leipzig, Germany, [3]Anhalt University of Applied Sciences, Department of Agriculture, Ecotrophology and Landscape Development, Strenzfelder Allee 28, 06406 Bernburg (Saale), Germany, and [4]University of Fribourg, Department of Biology, Chemin du Musée 10, 1700 Fribourg, Switzerland
** For correspondence. E-mail annett.baasch@hs-anhalt.de*
[†] Both authors contributed equally to this work and share first authorship.

Received: 13 February 2022 Returned for revision: 26 April 22 Editorial decision: 4 May 2022
Electronically published: 7 June 2022

• **Background and Aims** Habitat degradation and landscape fragmentation dramatically lower population sizes of rare plant species. Decreasing population sizes may, in turn, negatively affect genetic diversity and reproductive fitness, which can ultimately lead to local extinction of populations. Although such extinction vortex dynamics have been postulated in theory and modelling for decades, empirical evidence from local extinctions of plant populations is scarce. In particular, comparisons between current vs. historical genetic diversity and differentiation are lacking despite their potential to guide conservation management.
• **Methods** We studied the population genetic signatures of the local extinction of *Biscutella laevigata* subsp. *gracilis* populations in Central Germany. We used microsatellites to genotype individuals from 15 current populations, one *ex situ* population, and 81 herbarium samples from five extant and 22 extinct populations. In the current populations, we recorded population size and fitness proxies, collected seeds for a germination trial and conducted a vegetation survey. The latter served as a surrogate for habitat conditions to study how habitat dissimilarity affects functional connectivity among the current populations.
• **Key Results** Bayesian clustering revealed similar gene pool distribution in current and historical samples but also indicated that a distinct genetic cluster was significantly associated with extinction probability. Gene flow was affected by both the spatial distance and floristic composition of population sites, highlighting the potential of floristic composition as a powerful predictor of functional connectivity which may promote decision-making for reintroduction measures. For an extinct population, we found a negative relationship between sampling year and heterozygosity. Inbreeding negatively affected germination.
• **Conclusions** Our study illustrates the usefulness of historical DNA to study extinction vortices in threatened species. Our novel combination of classical population genetics together with data from herbarium specimens, an *ex situ* population and a germination trial underlines the need for genetic rescue measures to prevent extinction of *B. laevigata* in Central Germany.

Key words: *Biscutella laevigata* subsp. *gracilis*, conservation genetics, *ex situ* conservation, extinction vortex, functional connectivity, genetic diversity and differentiation, herbarium specimens, historical DNA, inbreeding depression, isolation by distance, isolation by environment, microsatellites.

INTRODUCTION

The Convention on Biological Diversity considers three levels of biodiversity as fundamental for protection: ecosystems, species and genes (Hoban *et al.*, 2020). Genetic diversity may be the first of these to vanish when anthropogenic activities threaten plant populations (Spielman *et al.*, 2004). Anthropogenic environmental changes such as habitat degradation and fragmentation decrease population sizes and limit gene flow among increasingly isolated populations (Young *et al.*, 1996; Heinicke *et al.*, 2016; González *et al.*, 2020). Together, these processes may lead to genetic erosion, including an increase of genetic drift and biparental inbreeding, and the accumulation of deleterious mutations (Young *et al.*, 1996; Hensen & Oberprieler, 2005; Hedrick & Garcia-Dorado, 2016).

While, for some species, low genetic diversity may foster selection (Rosche *et al.*, 2019), for most plant species genetic erosion is a major problem. This is because decreasing genetic diversity can limit the ability of populations to adapt to changing environmental conditions (Spielman *et al.*, 2004; Hoffmann *et al.*, 2017). In addition, loss of genetic diversity is frequently accompanied by reduced plant fitness due to inbreeding depression (reviewed by Leimu *et al.*, 2006). Since fitness inherently determines population growth, decreasing

© The Author(s) 2022. Published by Oxford University Press on behalf of the Annals of Botany Company.
All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

population sizes along with decreasing genetic diversity may accelerate a population's collapse. This process is coined in the term extinction vortex (Gilpin & Soulé, 1986). While the extinction vortex has been postulated in theory and modelling for decades, empirical evidence is still scarce due to missing monitoring data on population sizes and genetic diversity over many generations (Nabutanyi & Wittmann, 2021).

Many studies confirmed a positive relationship between population size and genetic diversity, but there were also contrasting results (reviewed by Leimu *et al.*, 2006). A recent meta-analysis found that the effects of habitat fragmentation and degradation on both genetic diversity and population size depend strongly on biogeography and plant functional traits such as life form, longevity and mating system (González *et al.*, 2020). A methodological issue in correlating genetic diversity with population size is that the mere counting of individuals may poorly estimate effective population sizes because numbers of individuals fluctuate throughout the seasons and across different years. Also, substantial parts of the genetic diversity of populations can be stored in seed banks (Plue *et al.*, 2017). As such, the assessment of population size and population genetics at a particular point in time does not necessarily mirror recent population dynamics (Münzbergová *et al.*, 2018).

Molecular analyses of historical DNA from herbarium specimens can help to overcome such methodological issues, as historical collections provide unique insights into recent population histories (James *et al.*, 2018; Lang *et al.*, 2019). Population genetic comparisons between historical and current populations allow us to study the adverse effects of recent habitat alterations on currently threatened populations (Meinicke *et al.*, 2018; Lang *et al.*, 2019; Albani Rocchetti *et al.*, 2021). In particular, such comparisons can document the consequences of genetic drift in populations that have become smaller and increasingly isolated, which may reveal if and how much genetic diversity has been lost through space and time (Cozzolino *et al.*, 2007). Herbarium specimens may also provide evidence of whether the extinction of distinct populations coincided with genetic erosion; so far, however, there are no empirical data available that have explicitly tested this. Yet, such data would be of great interest for conservation management of remnant populations and related policy decisions (Muniz *et al.*, 2019; Hoban *et al.*, 2021).

Reintroductions, for example using plant material from *ex situ* cultivation, may compensate for restricted gene flow and thus counteract genetic erosion (Hedrick & Garcia-Dorado, 2016; Bell *et al.*, 2019). However, reintroductions should follow natural patterns of gene flow to minimize risks of introducing maladapted genotypes and outbreeding depression (Holmes *et al.*, 2008; Barmentlo *et al.*, 2017). It is therefore crucial to understand the spatio-environmental determinants of gene flow using landscape genetic approaches (Manel & Holderegger, 2013). Gene flow can be affected by both isolation by distance (IBD; Hutchison & Templeton, 1999) and isolation by environment (IBE; Wang & Bradburd, 2014). The latter occurs where increasing differences in abiotic and/or biotic habitat conditions decrease the probability of migration between populations and thus constrain their functional connectivity. Because plant communities assemble predominantly according to the joint influences of abiotic and biotic filters (Diekmann, 2003), floristic dissimilarity may be a suitable predictor of functional connectivity. However, such analyses have rarely been done in landscape genetics, so far (but see Abraham *et al.*, 2015; Rosche *et al.*, 2018*a*), and have not yet been applied in decision-making for reintroduction actions.

Here we use a geographically isolated subspecies of buckler mustard [*Biscutella laevigata* subsp. *gracilis* Mach.-Laur. (Brassicaceae)] as a model to elaborate how comparisons of current vs. historical genetic patterns can help to guide restoration management. In our study area, Central Germany, *B. laevigata* is declining rapidly (Frank *et al.*, 2020). Given Germany's responsibility for the preservation of this endangered taxon (Welk, 2002), there is an urgent need for both *in situ* and *ex situ* conservation measures. We assessed the population genetic structures of current, *ex situ* and historical *B. laevigata* populations, recorded fitness proxies in the field and conducted a germination trial. To our knowledge, our study is the first that coherently combines such data. We specifically hypothesize:

(i) Decreasing population sizes have resulted in low genetic diversity and a high degree of biparental inbreeding in the current *B. laevigata* populations. We expect in particular that genetic diversity decreases through time.
(ii) Increasing habitat fragmentation has resulted in pronounced genetic differentiation among the current *B. laevigata* populations.
(iii) Gene flow among our study populations is determined by both non-adaptive drivers of genetic differentiation (i.e. IBD) and functional connectivity (i.e. IBE, measured as floristic similarity).
(iv) Increasing biparental inbreeding lowers reproductive fitness due to inbreeding depression.

Our multi-tied approaches are anticipated to reveal unique mechanistic insights into extinction vortex dynamics in a vanishing species. Our findings are anticipated to form the basis of practical measures for the species' conservation (e.g. genetic rescue measures and reintroductions).

MATERIALS AND METHODS

Study species

Biscutella laevigata subsp. *gracilis* is a perennial hemicryptophyte. It is diploid (see below for details on ploidy-level evaluation), strictly outcrossing (sporophytic self-incompatibility) and reproduces predominantly via seeds, although it is able to regenerate vegetatively from roots (Dannemann, 2000). Pollinators are generalist Diptera and Lepidoptera, while seeds are mainly dispersed through barochory in the immediate vicinity of mother plants (Parisod & Bonvin, 2008). *Biscutella laevigata* has a transient seed bank (Thompson *et al.*, 1997). The lack of a persistent seed bank may increase its vulnerability to random environmental fluctuations and global change (Kiss *et al.*, 2018). At low elevation, the ecological niche of *B. laevigata* is narrow and mainly limited by shade-intolerance and weak competitiveness (Dannemann, 2000). In our study area, *B. laevigata* occurs in open, nutrient-poor and xerothermic habitats where it is considered an indicator of historical old grasslands with high nature conservation value (Faulhaber & Partzsch, 2018). However, these grasslands are now jeopardized by increasing nitrogen deposition and the abandonment of sheep and goat grazing (Faulhaber & Partzsch, 2018).

While all our study populations clearly belong to one taxonomic entity (Dannemann, 2000), the taxonomy of the entire *B. laevigata*

complex is not completely resolved. In particular, there are several diploid lowland subspecies in Central Europe which are all considered as interglacial relicts, characterized by scattered occurrence with often strong geographical isolation. This isolation has led to strong morphological and genetic differentiation among these diploid taxa, resulting in different taxonomic concepts that have been controversially discussed (e.g. Manton, 1937; Dannemann, 2000; Tremetsberger *et al.*, 2002; Parisod & Besnard, 2007; Wierzbicka *et al.*, 2020). More specifically, it is not certain whether our Central German populations and some diploid populations from Poland and Czech Republic belong to the same taxonomic entity (Dannemann, 2000; Wierzbicka *et al.*, 2020). Regardless, the isolated *B. laevigata* populations from Central Germany are considered as a distinct conservation unit with a particularly high value for preservation (Welk, 2002). Because *B. laevigata* is rapidly declining in our study area, it is listed as 'endangered' in both the German Red List (Metzing *et al.*, 2018) and the regional Red List of Saxony-Anhalt (Frank *et al.*, 2020).

Sampling

We intensively studied floristic databases, herbarium specimens and literature on current and historical *B. laevigata* populations in Central Germany. We conducted field surveys and interviewed local floristic experts to check whether historical populations are still extant. Overall, we found that *B. laevigata* occurred at 53 grid cells (cell size: 5.6 × 5.7 km) along the Saale and Elbe valleys in the last 150 years. At the time of the study in 2018, the species was extinct in 50 of these grid cells (see Supplementary Data Fig. S1 for a raster map of extant and extinct occurrences). In the remaining three grid cells, there were 15 current populations of *B. laevigata* comprising 13 Saale valley populations (SV1–SV13) and two Elbe valley populations (EV1, EV2), all of which were sampled for this study (see Table S1 for details on the sampled populations).

During the flowering peak (beginning of May), population sizes were recorded by counting both flowering and non-flowering individuals. We compared the results between population size as the number of flowering individuals vs. population size as number of all individuals. Both population size proxies were highly correlated ($r = 0.997$, $P < 0.001$) and showed similar patterns in their relationships to genetic diversity and biparental inbreeding (compare Fig. 1 with Supplementary Data Fig. S2). We therefore use the number of flowering individuals as an approximation of population size to obtain estimates comparable to Dannemann (2000), who also recorded the number of flowering individuals 20 years ago.

To estimate fitness proxies in the field, we counted the number of flowering shoots from up to 25 randomly chosen flowering individuals per population. From these individuals, we also counted the numbers of flowers on one randomly chosen shoot. Both variables were multiplied to estimate the number of flowers per individual. One month later, we revisited the populations, collected seeds from the recorded individuals and counted their number per individual in the lab. At the time of seed set, we conducted a vegetation survey in each population. We located a 3 × 3-m plot in the area of the highest density of *B. laevigata* individuals, recorded all Spermatophyta species in the plot (mean = 29.1; s.d. = 7.4) and estimated their cover (%).

For our population genetic analyses, we collected leaves from up to 25 individuals per population that were evenly distributed across the population. In addition, we collected leaves from 24 individuals from one *ex situ* cultivation. Considering the immediate extinction risk of *B. laevigata* in the Elbe valley, we set up this *ex situ* cultivation in 2017 in the garden of the Middle Elbe Biosphere Reserve. The *ex situ* cultivation currently consists of 69 plants, representing the first generation of the source population EV1. The seeds were collected from ten mother plants in 2017.

In addition, historical *B. laevigata* samples were collected from leaf material from the following eight herbaria: HAL, HALN, JE, B, MNVD, MFNMD, GLM and DR (acronyms according to Thiers, 2016). We sampled only herbarium specimens that provided label description from which we could unambiguously identify the sampling locality. In total, we sampled leaves from 98 herbarium specimens (hereafter referred to as 'historical samples'). For these historical samples, we distinguished between samples from extinct vs. extant populations (see Supplementary Data Tables S2 and S3).

Microsatellite analyses

For both current and historical samples, DNA was extracted from dried leaves with the ATMAB method following the protocol given by Stein *et al.* (2014). Samples were genotyped at nine nuclear microsatellite loci that had been previously used for population genetic studies of pseudometallophyte *B. laevigata* populations from Poland (Babst-Kostecka *et al.*, 2014), following the procedures and PCR settings as described therein. Microsatellites are among the most commonly employed markers in conservation genetics because they are co-dominant, highly polymorphic and selectively neutral, and have high mutation rates (Hodel *et al.*, 2016). For our particular goal of reliably comparing heterozygosity through time, microsatellites have a crucial benefit over genome-wide or reduced-representation sequencing: microsatellites deliver locus-specific, multi-allelic data allowing manual scoring of fragment lengths. Compared to sequence data, these properties reduce data noise when working with samples that differ in DNA quantity and quality such as current vs. historical samples (see also Hodel *et al.*, 2016; Lang *et al.*, 2020). Note that we controlled for the quality of genotype scoring between historical and current samples when assessing the genotyping error, and through AMOVAs and STRUCTURE analyses (see below for details).

The PCR products were separated by size on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the internal size standard GSLIZ-600. Allele sizes were manually scored with Peak Scanner 2.0 (Applied Biosystems). We excluded samples of insufficient quality (e.g. minimum peak intensity threshold: 300) to avoid scoring incorrect alleles or recording false homozygotes in historical samples. We successfully amplified microsatellite profiles for all current samples, and for 81 out of the 98 historical samples. These 81 historical samples covered population genetic information from five extant and 22 extinct populations (see Supplementary Data Table S2). Forty samples originated from the Saale valley (27 from extant and 13 from extinct populations) and 41 samples originated from the Elbe valley (13 from extant and 28 from extinct populations). Their ages ranged from 23 to 162 years (mean: 97.1 ± 40.8) for the Elbe valley, and from 35 to 168 years (mean: 81.8 ± 39.1)

for the Saale valley (Fig. S3). Our final data set had a total of 0.7 % missing data (i.e. failed amplification of loci). To estimate genotyping error, we replicated 17 current and 17 historical samples from the DNA extraction to the allele scoring and recorded an error rate of 1.0 and 1.3 % for current and historical samples, respectively.

According to the microsatellite profiles, our samples appeared to be diploid. However, we double checked the ploidy status of one sample per population through flow cytometry with CyFlow Space (Sysmex Corporation, Kobe, Japan), using DAPI-labelled nuclei (CyStain UV Precise P; Sysmex Corporation). Samples from the diploid *B. laevigata* subsp. *varia* analysed in Geiser *et al.* (2016) were used as a reference and confirmed that all our investigated samples were diploid.

Genetic diversity and degree of biparental inbreeding in the current populations

To estimate genetic diversity within the current populations, we calculated allelic richness (A_R; i.e. the number of alleles rarefied to the minimum sample size of six individuals per population), expected heterozygosity (H_E) and observed heterozygosity (H_O) with SPAGeDi 1.4 (Hardy & Vekemans, 2002). In addition, inbreeding coefficients were calculated as $F_{IS} = 1 - (H_O/H_E)$.

Genetic diversity through time

Herbarium specimens are spatio-temporal explicit samples that do not allow assessment of estimates of genetic diversity such as A_R and H_E. To have a comparable estimate for genetic diversity between current and historical samples, we calculated mean observed heterozygosity across loci (H_O) for each historical sample.

Genetic differentiation through space and time

We performed an analysis of molecular variance (AMOVA) with Arlequin 3.5.1.2 (Excoffier & Lischer, 2010) to quantify the partitioning of genetic variation within and among current populations, and between the Saale and Elbe valleys. For this AMOVA, we used a data set with current samples only. In addition, we investigated spatio-temporal patterns of gene pool distribution using our full data set including all current populations and historical samples from populations for which we had at least seven historical samples ($EV1_{extant}$, $EV14_{extinct}$, $SV2_{extant}$ and $SV14_{extinct}$). We assigned all historical samples from a distinct location to one historical gene pool of a distinct population. We then ran another two AMOVAs that assessed the partitioning of genetic variation within and among populations, and at a third structural level: either between valleys or between sampling types (current vs. historical samples). Note that the historical populations consist of temporally heterogenic samples which consequently differ from the current populations in how they violate assumptions of AMOVAs (e.g. representative and random samples of distinct gene pools). We therefore suggest interpreting the presented estimates of our second and third AMOVAs with caution. However, the purpose of these AMOVAs was not to provide precise estimates of genetic differentiation but to draw conclusions on whether genotypes are more strongly determined by sampling type or by valley. This comparison may serve as a quality check on whether artificial differentiation overrides differentiation patterns (e.g. due to different amplification success of historical vs. current samples).

To examine the population genetic structure of the current populations, we used the Bayesian clustering program STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). We performed an admixture model with 20 replicate chains of 100 000 Markov chain Monte Carlo (MCMC) iterations and 100 000 burn-in iterations for each K (i.e. the number of tested genetic clusters). We used the ΔK approach to identify the most likely number of genetic clusters (Evanno *et al.*, 2005). Tested K-values ranged from 1 to 17 as the analyses involved 16 different populations (i.e. 15 current plus the *ex situ* cultivation). Individual mean posterior assignment probabilities to each cluster were inferred with CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007). Bar plots of assignment probabilities were created with DISTRUCT 1.1 (Rosenberg, 2004) and mapped on the distribution of our current populations using QGIS 3.16 (QGIS Development Team, 2021).

To study spatio-temporal patterns in genetic structure, we ran another STRUCTURE analysis with our full data set comprising all current and historical samples. Settings, analyses and visualization tools were the same as for the STRUCTURE analysis of the current data set.

Adaptive and non-adaptive drivers of gene flow in the current populations

We then investigated how gene flow among the current populations was affected by the relative influence of non-adaptive dispersal probabilities (which should be negatively correlated with spatial distance) and functional connectivity (which should be negatively correlated with environmental dissimilarity). For the latter, we assumed that populations that occur at communities with an increasingly similar floristic composition face increasingly similar environmental conditions (microclimate, soil conditions, management type, resident pollinator community, etc.). To explore differences in floristic composition among populations, we performed non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities with the *metaMDS* function implemented in the R-package vegan 2.5.-7 (Oksanen *et al.*, 2020). Details and results of the NMDS can be found in Supplementary Data Fig. S4.

We then performed two partial Mantel tests using the vegan package. First, we tested for correlation between the matrix of pairwise genetic distance $[F_{ST}/(1 - F_{ST})]$ and the matrix of pairwise $\log_e$-transformed spatial distances while correcting for Bray–Curtis dissimilarities of the floristic composition of the populations. Inversely, we then tested for correlation between genetic and floristic distance and corrected for spatial distance. The significance of correlations was determined based on 10 000 permutations. We also checked whether the floristic and spatial matrices were intercorrelated with another Mantel test.

The application of Mantel tests in landscape genetics has been criticized (Legendre *et al.*, 2015), and pairwise F_{ST}-values underlie eco-evolutionary assumptions that may be violated when populations have recently faced strong fluctuations in population size (James *et al.*, 2011). We therefore additionally applied a constrained ordination to confirm our findings with respect to the relative influence of non-adaptive dispersal probabilities and adaptive drivers of gene flow. In particular, we ran a Bray–Curtis distance-based redundancy analysis (dbRDA) with arcsinus square root-transformed allele frequencies. Predictor variables included the site scores of the first two axes of our vegetation NMDS (Veg_{NMDS1}, Veg_{NMDS2}) and the geographical position of our populations (i.e. latitude and longitude). We applied the *ordistep* function of the vegan package for forward model selection. This permutation approach opts to obtain the most parsimonious models by successively adding predictors which significantly improve model fit based on an inclusion threshold of $\alpha = 0.05$ (Borcard *et al.*, 2011).

Germination trial

To estimate fitness proxies under standardized conditions, we performed a germination trial. Sampled seeds were kept separated from mother plant (i.e. seed families). For each population, we used up to ten seed families (see Supplementary Data Table S1 for the numbers of involved seed families). Ten seeds from each seed family were spread in Petri dishes on filter paper resting on a plastic grid and moistened with tap water. Petri dishes were placed in germination chambers for 14 d (20 /10 °C with a 12-h photoperiod; settings based on Faulhaber & Partzsch, 2018). The germination trial lasted 14 d because a preliminary test with ten seeds from eight randomly chosen populations, which lasted 40 d, revealed that all observed germination occurred within the first 14 d. We assessed germination, removed emerged seedlings and randomized Petri dishes on a daily basis. To quantify germination, we recorded the total numbers of germinated and non-germinated seeds at the end of the germination trial.

Statistical analyses of population size, genetic diversity, degree of biparental inbreeding and fitness proxies

Statistical analyses were conducted with linear mixed-effects models using the R package lme4 1.1-26 (Bates *et al.*, 2015). Decisions on the transformation of variables depended on visual inspection of 'model-checking plots' in R for the models with transformed vs. untransformed variables. These plots allow us to check assumptions of the normality of residuals and variance homogeneity. Variables remained untransformed if not otherwise stated.

First, we tested for relationships between $\log_e$-transformed population size and the population estimates of genetic diversity and biparental inbreeding (four models: one for A_R, H_E, H_O and F_{IS} each). Valley (Saale vs. Elbe) was set as a random effect.

Then, we tested whether individual H_O differed between valleys and sampling types (current vs. historical samples). Population was set as a random effect. Here, we used valley as a fixed effect to explore whether H_O was more affected by sampling type or by valley, consistent with the AMOVA approach (i.e. as a quality check for comparable amplification success between sampling types). For three distinct populations, we had at least nine historical samples: the extant populations $SV2_{extant}$ and $EV1_{extant}$ and the extinct population $SV14_{extinct}$. For these populations, we investigated whether heterozygosity changed through time running three linear models that tested the effect of sampling year on H_O.

Visual assessment of the STRUCTURE analyses of the full data set for $K = 9$ revealed a cluster to be frequent in extinct but not in extant populations (see below considerations for the red cluster). We tested whether this relationship was significant using a generalized linear mixed-effects model with extinction (binominal: extinct vs. not extinct) as a response and population mean assignment probability to the red cluster as a predictor. Valley was set as a random effect.

As fitness proxies, we investigated the population means of seed set ($\log_e$-transformed), number of individual flowers ($\log_e$-transformed) and germination (binomial). We used (generalized) linear mixed-effects models to test whether our fitness proxies depend on $\log_e$-transformed population size and F_{IS}. Valley was set as a random effect.

RESULTS

Population sizes, current genetic diversity and degree of biparental inbreeding

Population sizes of the 15 current *B. laevigata* populations ranged from seven (SV3) to 893 (SV11) flowering individuals (mean: 210; Supplementary Data Table S1). Eight out of 15 populations comprised fewer than 100 individuals and 13 out of 15 populations were composed of fewer than 500 individuals. For eight populations from the Saale valley, we could compare our current population sizes with those 20 years ago from Dannemann (2000). Throughout the 20 years, we found decreasing population sizes for four populations (SV3, SV6, SV8 and SV9; see Table S4). Three of these populations already had population sizes below 100 individuals when recorded by Dannemann (2000). The other four populations had population sizes above 100 individuals 20 years ago, and increased in population size (SV2, SV4, SV7 and SV11).

Genetic diversity within the current populations was moderate: rarefied allelic richness (A_R) varied between 2.33 and 3.18 (mean = 2.76), expected heterozygosity (H_E) between 0.49 and 0.68 (mean = 0.58) and observed heterozygosity (H_O) between 0.40 and 0.67 (mean = 0.52). The inbreeding coefficients (F_{IS}) of the current populations were low to moderate and varied between −0.09 and 0.29 (mean = 0.11). There were significant positive effects of $\log_e$-transformed population size on A_R ($\chi^2_{(1)} = 11.24$, $P < 0.001$; Fig. 1A), on H_E ($\chi^2_{(1)} = 6.27$, $P = 0.012$; Fig. 1B), and on H_O ($\chi^2_{(1)} = 5.46$, $P = 0.019$; Fig. 1C), but no effect of $\log_e$-transformed population size on F_{IS} ($\chi^2_{(1)} = 0.02$, $P = 0.89$; Fig. 1D). Comparing our *ex situ* cultivation population with its source population EV1, we found almost identical values for H_E (0.47 vs. 0.49 for *ex situ* cultivation and EV1, respectively) and A_R (2.37 vs. 2.33) but a reduced H_O (0.44 vs. 0.53) and an increased F_{IS} (0.06 vs. −0.09).

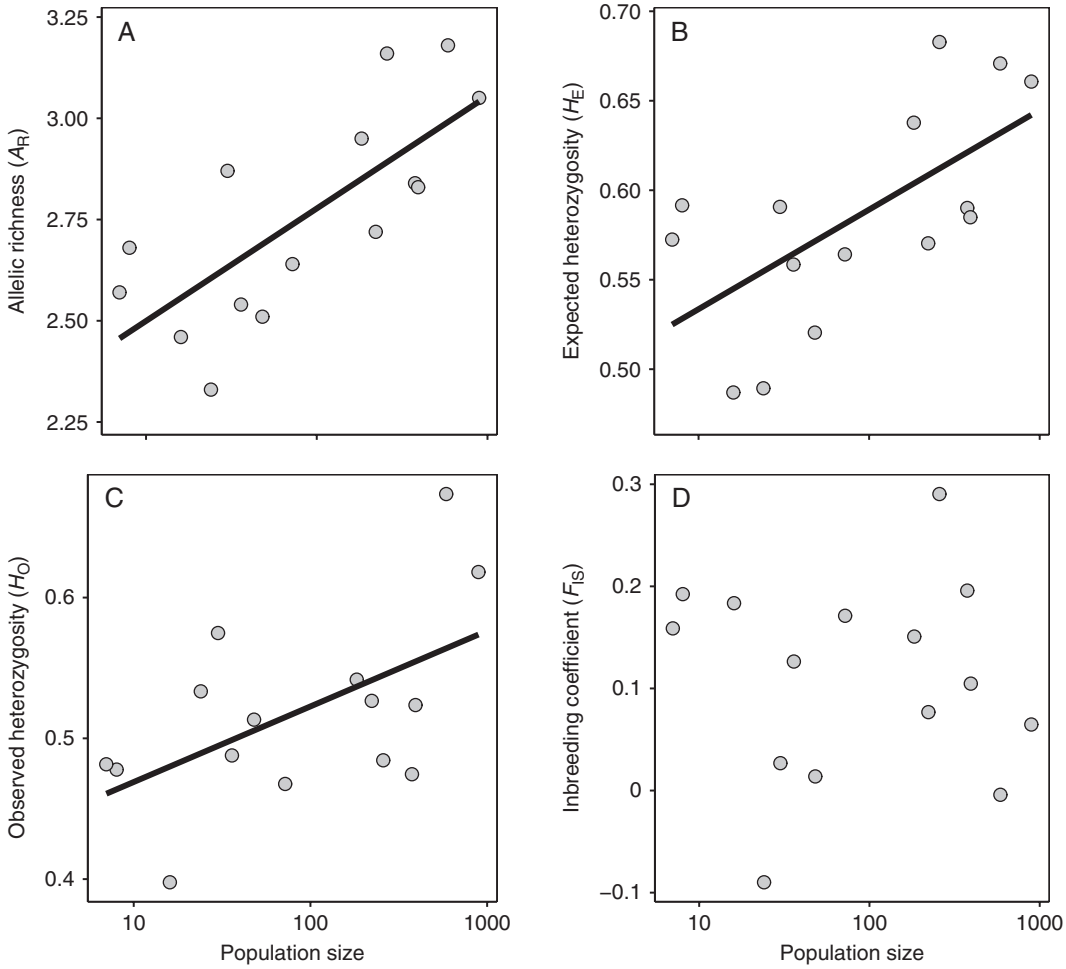

FIG. 1. Effect of $\log_e$-transformed population size on (A) allelic richness (A_R), (B) expected heterozygosity (H_E), (C) observed heterozygosity (H_O) and (D) inbreeding coefficient (F_{IS}) for 15 current *Biscutella laevigata* subsp. *gracilis* populations. Solid lines represent the model prediction for the significant relationships between genetic diversity estimates and population size. Shaded area around the line represents the 95 % confidence interval. Missing solid line for F_{IS} indicates the lack of a significant relationship.

Genetic diversity through time

Our historical samples showed a moderate H_O (mean = 0.48) that did not differ from that of our current samples ($\chi^2_{(1)} = 2.46$, $P = 0.12$). Instead, H_O differed between the valleys: H_O was higher in samples from the Saale valley than in samples from the Elbe valley ($\chi^2_{(1)} = 9.34$, $P = 0.002$). In other words, for H_O it was more important from which valleys the samples were drawn than whether they were from historical or current samples. This result, in accordance with our AMOVA findings (see below), indicates that amplification patterns were comparable between sampling types. We also pooled all current, all *ex situ* and all historical samples to compare overall gene pool estimates of these three sampling types ($N = 24$ for the A_R estimation). We found that genetic diversity estimates were comparable between current and historical samples ($A_R = 6.07$ vs. 6.73 and $H_E = 0.71$ vs. 0.72 for current vs. historical samples, respectively), yet both were substantially higher than estimates for the *ex situ* population ($A_R = 3.48$ and $H_E = 0.47$).

For the three distinct populations for which we had enough historical samples to test changes in H_O through time (ranges: $SV2_{extant}$ = 1854–1987, $EV1_{extant}$ = 1901–1999, $SV14_{extinct}$ = 1862–1965), we found that there was no effect of sampling year on H_O for the two extant populations $SV2_{extant}$ ($F_{1,23} = 0.19$, $P = 0.67$) and $EV1_{extant}$ ($F_{1,10} = 0.004$, $P = 0.95$). However, for the samples from population $SV14_{extinct}$ that became extinct recently (last record from 1965), we found that H_O significantly decreased through time ($F_{1,8} = 6.36$, $P = 0.036$; Fig. 2).

Genetic differentiation through space and time

The AMOVA of the current samples revealed that the molecular variance was kept mainly within populations (73 %) and to a lesser extent among populations within valleys (13.5 %) as well as between valleys (13.6 %; Table 1). The overall F_{ST}-value of 0.27 demonstrated substantial genetic differentiation. The AMOVA with current and historical samples, using region as a third grouping level, revealed almost identical results as compared with the AMOVA of the current populations (Table 1). In contrast, using sampling type as grouping for the AMOVA (i.e. current vs. historical samples) did not significantly partition variation. This lack of partitioning confirms that amplification of historical DNA was qualitatively comparable with the current DNA.

For the Bayesian inferences of genetic clusters, ΔK resulted in an optimal partitioning into $K = 2$ clusters for both the data set including only current populations and the full data set (Supplementary Data Fig. S5). For the current populations, the first cluster (blue) was distributed in the populations from the Saale valley (mean assignment probability of individuals from the Saale valley: $qK_1 = 0.97$), whereas the second cluster (yellow) was distributed in the Elbe valley populations ($qK_2 = 0.95$; Fig. S6). For the full data set, this valley-specific cluster assignment pattern was consistent across current samples (Fig. S7). Strikingly, the historical samples from extant populations also shared this valley-specific cluster assignment pattern ($qK_1 = 0.94$ and $qK_2 = 0.93$, for Saale and Elbe valley populations, respectively). In contrast, for historical samples from extinct populations, the valley-specific cluster assignment pattern was less apparent. Fifteen out of 23 extinct populations showed $qK < 0.8$ to the respective clusters, indicating that several extinct populations showed genetic clusters which do not match the current gene pools.

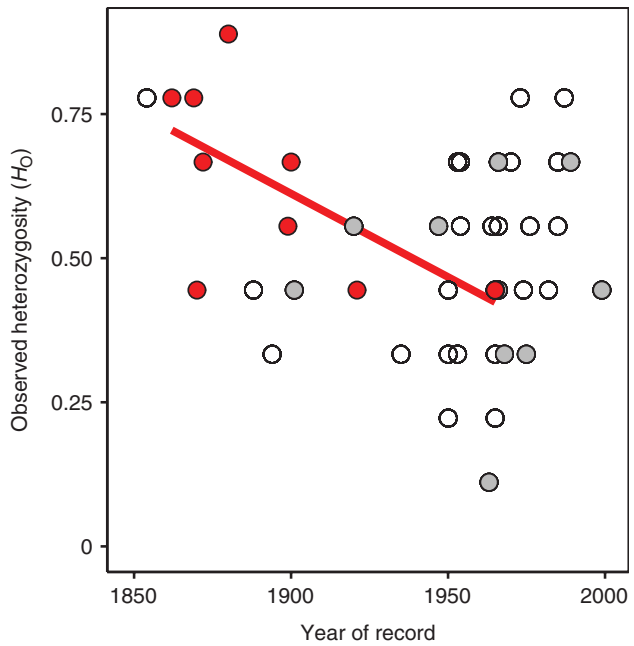

FIG. 2. Observed heterozygosity (H_O) through time for herbarium specimen samples from three distinct *Biscutella laevigata* subsp. *gracilis* populations. Two localities are extant: $SV2_{extant}$ (transparent dots) and $EV1_{extant}$ (grey dots), whereas the third locality $SV14_{extinct}$ (red dots) is already extinct (see Supplementary Data Table S2 for details on the historical samples). The solid line represents the model prediction for the significant relationship between year of collection and H_O in the already extinct population $SV14_{extinct}$. Shaded area around the line represents the 95 % confidence interval. Missing solid lines for $SV2_{extant}$ and $EV1_{extant}$ indicate the lack of a significant relationship for both populations.

For more in-depth information on the genetic structures of current vs. historical samples, we also checked resolutions with a higher number of clusters. This is important because Bayesian inferences of genetic clustering disproportionally often result in an optimal partitioning of $K = 2$ while higher resolutions could reveal more important information for conservation genetics (Janes *et al.*, 2017). We here plotted $K = 9$, which had the highest ΔK for K-values > 3. For current samples, this solution revealed that geographically close populations mainly shared similar genetic clusters (Fig. 3). Historical samples from extant populations shared the gene pools of the current samples from the same locality.

Regarding cluster assignments in extinct populations, we found that 16 out of 23 extinct populations were predominated by a red cluster (i.e. $qK_{red} > 50$ %) whereas no current population was predominated by this red cluster (Fig. 3). This shows that a previously abundant cluster appeared to have recently become extinct. In fact, there was a highly significant correlation between qK_{red} and the extinction of historical populations ($\chi^2_{(1)} = 12.19$, $P < 0.001$, Supplementary Data Fig. S8). Several of the extinct populations that were dominated by the red cluster are located near to towns [e.g. $EV3_{extinct}$ (Dresden), $EV4_{extinct}$ (Radebeul), $EV14_{extinct}$ (Aken), $SV14_{extinct}$ and $SV15_{extinct}$ (both Halle)]. In addition, SV2 which is located in the town of Halle shows the highest cluster assignment to the red cluster from the current populations. Note that we checked whether the red cluster comprised individuals of low H_O. Such a pattern might have occurred if the red cluster represented either an assemblage of genetically depleted populations or samples of low DNA quality (which would have decreased amplification success, and thus decreased H_O or increased the number of missing alleles). However, historical samples dominated by the red

TABLE 1. *Three analyses of molecular variance (AMOVAs) in the investigated* Biscutella laevigata *subsp.* gracilis *populations. Historical samples comprised samples from four localities (i.e. populations for which we had at least seven historical samples: $EV1_{extant}$, $SV2_{extant}$, $EV14_{extinct}$ and $SV14_{extinct}$; see Supplementary Data Table S2 for details on the historical samples).* P*-values are based on 1023 permutations (default setting in Arlequin). Abbreviations: d.f., degrees of freedom; %Total, percentage of total variance.*

Analysis type	Partitioning	d.f.	% Total	*F*-value	*P*-value
Current samples only					
Grouping: Saale vs. Elbe valley	Between valleys	1	13.58	$F_{CT} = 0.14$	<0.001
	Among populations	14	13.46	$F_{SC} = 0.16$	<0.001
	Within populations	676	72.97	$F_{ST} = 0.27$	<0.001
Current and historical samples					
Grouping: Saale vs. Elbe valley	Between valleys	1	11.99	$F_{CT} = 0.12$	<0.001
	Among populations	18	12.52	$F_{SC} = 0.14$	<0.001
	Within populations	782	75.49	$F_{ST} = 0.25$	<0.001
Current and historical samples					
Grouping: Current vs. historical samples	Between sampling type	1	−1.48	$F_{CT} = -0.01$	0.85
	Among populations	18	18.84	$F_{SC} = 0.19$	<0.001
	Within populations	782	82.64	$F_{ST} = 0.17$	<0.001

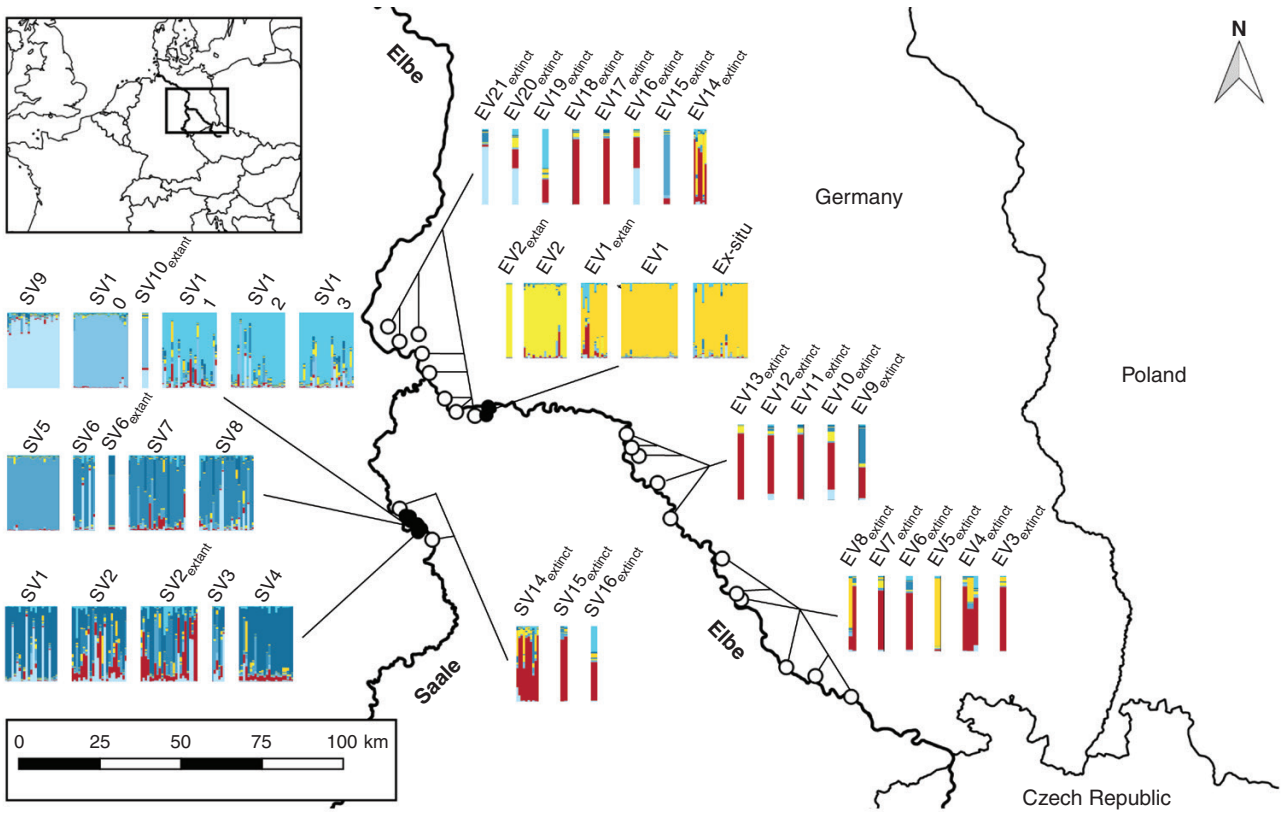

FIG. 3. Geographical distribution and genetic structure of the current and historical samples of *Biscutella laevigata* subsp. *gracilis* populations from the Saale valley and the Elbe valley. The stacked bar plots result from a Bayesian cluster analysis (STRUCTURE) and show the individual posterior assignment probabilities to nine clusters. Details on the populations are presented in Supplementary Data Tables S1 and S2 for current and historical samples, respectively. Historical samples are denoted with a subscript for extinct and extant populations. Inset map (upper left) shows Western and Central Europe with a rectangle indicating the location of the study region.

cluster did not differ in H_O from the other historical samples ($\chi^2_{(1)} = 0.33$, $P = 0.567$). Moreover, there was no linear relationship between H_O and qK_{red} ($\chi^2_{(1)} = 0.47$, $P = 0.493$). We also did not detect a relationship between the frequency of missing alleles and qK_{red} ($\chi^2_{(1)} = 0.25$, $P = 0.618$).

Adaptive and non-adaptive drivers of gene flow in the current populations

The first partial Mantel test suggested that genetic distances were significantly correlated with $\log_e$-transformed spatial distance ($r_M = 0.77$, $P < 0.001$, Supplementary Data Fig. S9), while correcting for functional connectivity using floristic distance as a proxy for environmental dissimilarity. The second partial Mantel test suggested that genetic distances were significantly correlated with floristic distance ($r_M = 0.34$, $P = 0.017$), while correcting for non-adaptive migration patterns using $\log_e$-transformed spatial distance as a proxy for dispersal probabilities. Floristic distance and spatial distance were not correlated ($r_M = -0.1$, $P = 0.73$).

The results of the partial Mantel tests were congruent with our dbRDA. We found that both floristic composition and geographical position of the populations were significantly associated with the population genetic structure (Supplementary Data Fig. S10). In particular, the model fit was significantly improved by adding latitude ($F = 2.97$, $P = 0.005$), longitude ($F = 3.57$, $P = 0.005$) and Veg_{NMDS2} ($F = 1.84$, $P = 0.04$), but not by adding Veg_{NMDS1} ($F = 1.56$, $P = 0.08$).

Effects of population size and biparental inbreeding on fitness proxies

The fitness proxies in the field were not affected by $\log_e$-transformed population size, as shown by both $\log_e$-transformed number of flowers ($\chi^2_{(1)} = 3.32$, $P = 0.069$) and $\log_e$-transformed seed set ($\chi^2_{(1)} = 0.04$, $P = 0.844$). Similarly, there was no effect of F_{IS} on $\log_e$-transformed number of flowers ($\chi^2_{(1)} = 0.99$, $P = 0.318$) and $\log_e$-transformed seed set ($\chi^2_{(1)} = 0.09$, $P = 0.764$). However, germination was significantly affected by $\log_e$-transformed population size ($\chi^2_{(1)} = 6.48$, $P = 0.011$) and by F_{IS} ($\chi^2_{(1)} = 21.57$, $P < 0.001$, Supplementary Data Fig. S11).

DISCUSSION

Conserving genetic diversity is crucial to ensure population viability, the adaptive potential of species and ultimately the stability of ecosystem processes in changing environments (Hoban *et al.*, 2020). However, the potential of population genetic information for conservation management remains underutilized (Liu *et al.*, 2020; Hoban *et al.*, 2021). Here we present a novel combination of classical population genetics together

with genetic data from herbarium specimens and an *ex situ* population, and data on plant fitness proxies. These combined data allow us to draw empirical conclusions about apparent extinction vortex dynamics and potentially about counteracting restoration measures.

Population sizes, genetic diversity and degree of biparental inbreeding

Many of the current *B. laevigata* populations were very small (<100 individuals). Comparisons with older records of population sizes (Dannemann, 2000) showed that populations that were already very small 20 years ago became even smaller, while four of five populations larger than 100 individuals increased in size over the past 20 years. Genetic diversity (mean $H_E = 0.58$) was moderate and comparable to similarly declining *B. laevigata* populations from Poland (mean $H_E = 0.57$; Babst-Kostecka *et al.*, 2014), yet was lower than the average for outcrossing plants in microsatellite studies ($H_E = 0.65$; reviewed in Nybom, 2004). For strictly outcrossing plants with a perennial life cycle, moderate levels of genetic diversity despite small population sizes may be expected when previously large and continuous populations have declined only in recent decades (Rosche *et al.*, 2018*b*).

In accordance with our first hypothesis and with many case studies (reviewed by González *et al.*, 2020), we found a negative relationship between population size and genetic diversity. However, inbreeding coefficients were not affected by population size, indicating selection against biparental inbreeding. Such selection against inbred offspring may occur where strict self-incompatibility prevents mating between close relatives, as shown for *Biscutella neustrica* (Leducq *et al.*, 2010), and also where inbreeding depression leads to fitness decreases in inbred compared to outbred offspring (Swindell & Bouzat, 2006).

Genetic diversity through time

Regarding temporal dynamics in genetic diversity, we found that genetic diversity has remained constant over the last 165 years in two extant populations, probably promoted by the perennial life cycle and selection against biparental inbreeding. Strikingly, for the already extinct population $SV14_{extinct}$ (last record from 1965), we detected a significant decline of H_O through time, indicating the existence of a certain threshold in population size accompanied by significant declines in genetic diversity and its negative feedback on population survival (i.e. minimum viable population size; Gilpin & Soulé, 1986). Modelling approaches using long-term monitoring data on both population sizes and allele frequency-based estimates of genetic diversity would be promising tools to predict the minimum viable population size.

Our study is, to our knowledge, the first that has tracked the population genetic signatures of the local extinction of a distinct population, and is among very few studies that have investigated temporal changes in genetic diversity using historical samples (reviewed by Albani Rocchetti *et al.*, 2021). From previous research, only one study also reported decreasing genetic diversity through time, investigating chloroplast haplotype diversity in *Anacamptis palustris* (Cozzolino *et al.*, 2007). This lack of empirical evidence is remarkable as recent conceptual frameworks particularly call for such data (e.g. Nualart *et al.*, 2017; Liu *et al.*, 2020; Albani Rocchetti *et al.*, 2021), underlining the need for future research on genetic diversity in current vs. historical samples. However, there are limitations in the use of herbaria related to collection biases (reviewed by Lang *et al.*, 2019). Sampling efforts may differ through time (i.e. temporal bias) and space (i.e. geographical bias). Such collection biases may limit the application of our approaches for studies focusing on larger geographical scales or on regions that have not been frequently and constantly sampled in the past.

Genetic differentiation through space and time

In accordance with our second hypothesis, we found strong genetic differentiation, despite small spatial distance among our study populations (range: 1–45 km). This confirms low genetic connectivity in increasingly fragmented landscapes (Young *et al.*, 1996; González *et al.*, 2020). Sheep grazing may have facilitated occasional gene flow among populations in the past, but is increasingly being abandoned in our study area (Faulhaber & Partzsch, 2018). Still, the AMOVA and STRUCTURE analyses demonstrated that the spatial–genetic structure was consistent through time and was predominately differentiated by the river valleys. However, we also found that a genetic cluster that was predominant in several extinct populations has been lost through time. This irreversible loss of a distinct gene pool underlines the need to conserve genetic diversity of plant populations when preserving biodiversity (Walisch *et al.*, 2015; Hoban *et al.*, 2020). While the red cluster appeared to be more frequent in populations that were located near towns, there were no clear patterns in habitat conditions between populations with vs. without pronounced assignment to the red cluster. Also, our neutral genetic data cannot explain why the red cluster was associated with extinction. This conundrum should be addressed in future research.

Adaptive and non-adaptive drivers of gene flow in the current populations

In line with our third hypothesis, the partial Mantel tests and the dbRDA revealed that both IBD and IBE dictate gene flow among our current study populations. IBD is a non-adaptive dispersal probability pattern which is commonly reported in plants but was not evident in *B. laevigata* populations from Poland (Babst-Kostecka *et al.*, 2014). Independently of spatial distance, we found that increasing difference in the habitat conditions of populations (i.e. increasing floristic dissimilarity) indirectly hampers gene flow among populations (Wang & Bradburd, 2014). Such IBE suggests that genotypes are locally adapted in our study area, which also means that genetic differentiation among genetic clusters should be accounted for regarding re-introductions. While IBE has often been reported for macroclimatic gradients across large spatial scales (e.g. Rosche *et al.*, 2019), our study is among very few that have used floristic dissimilarity in this context (Abraham *et al.*, 2015; Rosche *et al.*, 2018*a*). We emphasize its suitability for studying the

relative importance of adaptive vs. non-adaptive drivers of gene flow and recommend using this tool more frequently.

Effects of population size and biparental inbreeding on fitness proxies

In contrast to our fourth hypothesis, fitness proxies in the field were not correlated with population size or F_{IS}. For seed set, this result suggests that populations are not significantly affected by pollen limitation. However, we found a positive effect of population size and a negative effect of F_{IS} on germination. In other words, inbreeding depression lowers germination, which is in line with many case studies (reviewed by Angeloni *et al.*, 2011). Inbreeding depression may therefore explain selection against biparental inbreeding in our current *B. laevigata* populations. In this context, it is important to note that our *ex situ* cultivation showed comparable values of genetic diversity but had a strongly increased F_{IS} as compared with its source population EV1. Thus, selection against inbred progeny may be less pronounced in cultivation than under harsher natural conditions, possibly due to inbreeding–environment interactions (Enßlin *et al.*, 2011).

Many of our *B. laevigata* populations show very small population sizes with limited mating partners. In these very small populations, biparental inbreeding may be the only possible mating type and inbreeding depression may consequently lower their potential for rejuvenation. As such, extant populations below a minimum viable population size threshold may be prone to extinction vortex dynamics due to environmental stochasticity, increased biparental inbreeding and their interactions (Nabutanyi & Wittmann, 2021).

CONCLUSION

Our study is the first to show that genetic erosion coincides with the local extinction of a plant population and to document the extinction of a distinct genetic cluster. We thus highlight the potential of herbarium collections to track the genetic signature of local extinction. Recent advances may promote population genomic applications for herbarium specimens, which may allow us to determine whether loss of genetic diversity through time is more pronounced at adaptive or neutral loci (Jordon-Thaden *et al.*, 2020; Lang *et al.*, 2020). Such analyses may, for example, help to unravel mechanisms beyond the association between cluster assignment and population extinction.

The rapid and ongoing decline of *B. laevigata* populations in Central Germany mirrors their high vulnerability and underlines the urgent need for conservation measures (Welk, 2002; Frank *et al.*, 2020). Our data suggest that extinction vortex dynamics have contributed to the local extinction of *B. laevigata*. Genetic rescue measures could help to maintain genetic diversity and thereby augment survival probabilities and evolutionary resilience of the extant *B. laevigata* populations (Ralls *et al.*, 2018; Bell *et al.*, 2019). In addition, the re-introduction of extinct populations may counteract population loss and landscape fragmentation to facilitate gene flow across populations. Based on our data, re-introductions should be preferentially conducted between geographically close sites that share similar plant communities to avoid the introduction of maladapted genotypes (Barmentlo *et al.*, 2017). Also, *ex situ* cultivation should mimic natural conditions to avoid increasing inbreeding coefficients in cultivated offspring. Our upcoming re-introduction studies will consider these findings and may help to support the survival of *B. laevigata* in our study region.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Fig. S1: Raster map of extant and extinct occurrences. Fig. S2: Effect of population size on genetic diversity and inbreeding coefficient. Fig. S3: Histogram showing the age distribution of herbarium specimens. Fig. S4: NMDS of the floristic composition. Fig. S5: ΔK assessments. Fig. S6: STRUCTURE analysis for $K = 2$ for the current populations. Fig. S7: STRUCTURE analysis for $K = 2$ for the full data set. Fig. S8: Relationship between red cluster assignment and extinction of historical populations. Fig. S9: Correlation plots between geographical, floristic and genetic distances. Fig. S10: Plot of dbRDA. Fig. S11: Effect of population size and inbreeding coefficient on germination. Table S1: Population genetic samples from current populations. Table S2: Population genetic samples from historical populations. Table S3: Label information on the herbarium specimens. Table S4: Comparisons of current population sizes with those 20 years ago.

ACKNOWLEDGEMENTS

We thank K. Kittlaus and B. Müller for their invaluable efforts during the germination trial and the genetic lab work. We also thank all institutions and people who have provided us with herbarium specimens. Author contributions: A.B. and I.H. conceived the ideas for the study and designed the methodology. K.R. and P.B. collected the field data. C.R. analysed the data and interpreted the results with input from S.T. and C.P. C.R., I.H. and A.B. led the writing. All authors contributed critically to the drafts and gave final approval for publication. We declare no conflict of interest. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.t76hdr837

FUNDING

This paper is based on field data obtained under a research project carried out at the Anhalt University of Applied Sciences and financed by the Landesverwaltungsamt Sachsen-Anhalt (407.1.11.-60128/630116000020) within the framework of ELER regulation [commission regulation (EC) No 1303/2013].

LITERATURE CITED

Abraham EM, Ganopoulos I, Giagourta P, *et al.* 2015. Genetic diversity of *Lotus corniculatus* in relation to habitat type, species composition and species diversity. *Biochemical Systematics and Ecology* **63**: 59–67.

Albani Rocchetti G, Armstrong CG, Abeli T, *et al.* 2021. Reversing extinction trends: new uses of (old) herbarium specimens to accelerate conservation action on threatened species. *New Phytologist* **230**: 433–450.

Angeloni F, Ouborg NJ, Leimu R. 2011. Meta-analysis on the association of population size and life history with inbreeding depression in plants. *Biological Conservation* **144**: 35–43.

Babst-Kostecka AA, Parisod C, Godé C, Vollenweider P. 2014. Patterns of genetic divergence among populations of the pseudometallophyte *Biscutella laevigata* ssp. *gracilis* from southern Poland. *Plant and Soil* **383**: 245–256.

Barmentlo SE, Meirmans PG, Luijten SH, Triest L, Oostermeijer JGB. 2017. Outbreeding depression and breeding system evolution in small, remnant populations of *Primula vulgaris*: consequences for genetic rescue. *Conservation Genetics* **19**: 545–554.

Bates D, Martin M, Ben B, Steve W. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Bell DA, Robinson ZL, Funk WC, *et al.* 2019. The exciting potential and remaining uncertainties of genetic rescue. *Trends in Ecology & Evolution* **34**: 1070–1079.

Borcard D, Gillet F, Legendre P. 2011. *Numerical ecology with R*. Berlin: Springer.

Cozzolino S, Cafasso D, Pellegrino G, Musacchio A, Widmer A. 2007. Genetic variation in time and space: the use of herbarium specimens to reconstruct patterns of genetic variation in the endangered orchid *Anacamptis palustris*. *Conservation Genetics* **8**: 629–639.

Dannemann A. 2000. Der Einfluss von Fragmentierung und Populationsgröße auf die genetische Variation und Fitness von seltenen Pflanzenarten am Beispiel von *Biscutella laevigata* (Brassicaceae). *Dissertationes Botanicae* **330**: 1–151.

Diekmann M. 2003. Species indicator values as an important tool in applied plant ecology – a review. *Basic and Applied Ecology* **4**: 493–506.

Enßlin A, Sandner TM, Matthies D. 2011. Consequences of ex situ cultivation of plants: Genetic diversity, fitness and adaptation of the monocarpic *Cynoglossum officinale* L. in botanic gardens. *Biological Conservation* **144**: 272–278.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611–2620.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564–567.

Faulhaber M, Partzsch M. 2018. *Biscutella laevigata* L.-Ein postglaziales botanisches Relikt in Mitteldeutschland: Populationsstruktur und Interaktionen. *Hercynia* **51**: 58–79.

Frank D, Brade P, Elias D, *et al.* 2020. Rote Listen Sachsen-Anhalt. 7. Farne und Blütenpflanzen (Pteridophyta et Spermatophyta). *Berichte des Landesamtes für Umweltschutz Sachsen-Anhalt* **1**: 151–186.

Geiser C, Mandáková T, Arrigo N, Lysak MA, Parisod C. 2016. Repeated whole-genome duplication, karyotype reshuffling, and biased retention of stress-responding genes in Buckler mustard. *The Plant Cell* **28**: 17–27.

Gilpin ME, Soulé ME. 1986. Minimum viable populations: processes of species extinction. In: Soulé ME, ed. *Conservation biology*. Sunderland, MA: Sinauer Associates, 19–34.

González AV, Gómez-Silva V, Ramírez MJ, Fontúrbel FE. 2020. Meta-analysis of the differential effects of habitat fragmentation and degradation on plant genetic diversity. *Conservation Biology* **34**: 711–720.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618–620.

Hedrick PW, Garcia-Dorado A. 2016. Understanding inbreeding depression, purging, and genetic rescue. *Trends in Ecology & Evolution* **31**: 940–952.

Heinicke S, Hensen I, Rosche C, *et al.* 2016. Fragmentation and environmental constraints influence genetic diversity and germination of *Stipa pennata* in natural steppes. *Flora* **224**: 42–49.

Hensen I, Oberprieler C. 2005. Effects of population size on genetic diversity and seed production in the rare *Dictamnus albus* (Rutaceae) in central Germany. *Conservation Genetics* **6**: 63–73.

Hoban S, Bruford M, Jackson JD, *et al.* 2020. Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. *Biological Conservation* **248**: 108654.

Hoban S, Campbell CD, da Silva JM, *et al.* 2021. Genetic diversity is considered important but interpreted narrowly in country reports to the convention on biological diversity: current actions and indicators are insufficient. *Biological Conservation* **261**: 109233.

Hodel RG, Segovia-Salcedo MC, Landis JB, *et al.* 2016. The report of my death was an exaggeration: a review for researchers using microsatellites in the 21st century. *Applications in Plant Sciences* **4**: 1600025.

Hoffmann AA, Sgrò CM, Kristensen TN. 2017. Revisiting adaptive potential, population size, and conservation. *Trends in Ecology & Evolution* **32**: 506–517.

Holmes GD, James EA, Hoffmann AA. 2008. Limitations to reproductive output and genetic rescue in populations of the rare shrub *Grevillea repens* (Proteaceae). *Annals of Botany* **102**: 1031–1041.

Hutchison D, Templeton A. 1999. Correlation of pairwise genetic and geographic distance measure: inferring the relative influences of gene flow and drift on distribution of genetic variability. *Evolution* **53**: 1898–1914.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801–1806.

James PMA, Coltman DW, Murray BW, Hamelin RC, Sperling FAH. 2011. Spatial genetic structure of a symbiotic beetle-fungal system: Toward multi-taxa integrated landscape genetics. *PLoS ONE* **6**: e25359.

James SA, Soltis PS, Belbin L, *et al.* 2018. Herbarium data: Global biodiversity and societal botanical needs for novel research. *Applications in Plant Sciences* **6**: e1024.

Janes JK, Miller JM, Dupuis JR, *et al.* 2017. The $K = 2$ conundrum. *Molecular Ecology* **26**: 3594–3602.

Jordon-Thaden IE, Beck JB, Rushworth CA, *et al.* 2020. A basic dd RAD seq two-enzyme protocol performs well with herbarium and silica-dried tissues across four genera. *Applications in Plant Sciences* **8**: e11344.

Kiss R, Deák B, Török P, Tóthmérész B, Valkó, O. 2018. Grassland seed bank and community resilience in a changing climate. *Restoration Ecology* **26**: 141–150.

Lang PLM, Weiß CL, Kersten S, *et al.* 2020. Hybridization ddRAD-sequencing for population genomics of nonmodel plants using highly degraded historical specimen DNA. *Molecular Ecology Resources* **20**: 1228–1247.

Lang PL, Willems FM, Scheepens JF, Burbano HA, Bossdorf O. 2019. Using herbaria to study global environmental change. *New Phytologist* **221**: 110–122.

Leducq JB, Gosset CC, Poiret M, Hendoux F, Vekemans X, Billiard S. 2010. An experimental study of the *S*-Allee effect in the self-incompatible plant *Biscutella neustriaca*. *Conservation Genetics* **11**: 497–508.

Legendre P, Fortin MJ, Borcard D. 2015. Should the Mantel test be used in spatial analysis? *Methods in Ecology and Evolution* **6**: 1239–1247.

Leimu R, Mutikainen PIA, Koricheva J, Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* **94**: 942–952.

Liu CN, Li YY, Wang R, Chen XY. 2020. Genetic factors are less considered than demographic characters in delisting species. *Biological Conservation* **251**: 108791.

Manel S, Holderegger R. 2013. Ten years of landscape genetics. *Trends in Ecology & Evolution* **28**: 614–621.

Manton I. 1937. The problem of *Biscutella laevigata* L. II. The evidence from meiosis. *Annals of Botany* **1**: 439–462.

Meineke EK, Davis CC, Davies TJ. 2018. The unrealized potential of herbaria for global change biology. *Ecological Monographs* **88**: 505–525.

Metzing D, Hofbauer N, Ludwig G, Matzke-Hajek G. 2018. *Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands, Band 7: Pflanzen. Naturschutz und Biologische Vielfalt 70/7*. Bonn-Bad Godesberg: Bundesamt für Naturschutz.

Muniz AC, Lemos-Filho JP, Buzatti RSdO, Ribeiro PCC, Fernandes FM, Lovato MB. 2019. Genetic data improve the assessment of the conservation status based only on herbarium records of a Neotropical tree. *Scientific Reports* **9**: 1–11.

Münzbergová Z, Šurinová M, Husáková I, Brabec J. 2018. Strong fluctuations in aboveground population size do not limit genetic diversity in populations of an endangered biennial species. *Oecologia* **187**: 863–872.

Nabutanyi P, Wittmann MJ. 2021. Models for eco-evolutionary extinction vortices under balancing selection. *The American Naturalist* **197**: 336–350.

Nualart N, Ibáñez N, Soriano I, López-Pujol J. 2017. Assessing the relevance of herbarium collections as tools for conservation biology. *The Botanical Review* **83**: 303–325.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–1155.

Oksanen J, Blanchet FG, Friendly M, *et al.* 2020. *Vegan: Community Ecology Package*. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Parisod C, Besnard G. 2007. Glacial in situ survival in the Western Alps and polytopic autopolyploidy in *Biscutella laevigata* (Brassicaceae). *Molecular Ecology* **16**: 2755–2767.

Parisod C, Bonvin G. 2008. Fine-scale genetic structure and marginal processes in an expanding population of *Biscutella laevigata* ssp. *gracilis* L. (Brassicaceae). *Heredity* **101**: 536–542.

Plue J, Vandepitte K, Honnay O, Cousins SA. 2017. Does the seed bank contribute to the build-up of a genetic extinction debt in the grassland perennial *Campanula rotundifolia*? *Annals of Botany* **120**: 373–385.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Molecular Ecology Notes* **155**: 945–959.

QGIS Development Team. **2021.** *QGIS Geographic Information System. Open Source Geospatial Foundation Project*. http://qgis.osgeo.org

Ralls K, Ballou JD, Dudash MR, *et al.* 2018. Call for a paradigm shift in the genetic management of fragmented populations. *Conservation Letters* **11**: 1–6.

Rosche C, Heinicke S, Hensen I, *et al.* 2018*a*. Spatio-environmental determinants of the genetic structure of three steppe species in a highly fragmented landscape. *Basic and Applied Ecology* **28**: 48–59.

Rosche C, Hensen I, Schaar A, *et al.* 2019. Climate outweighs native vs. nonnative range-effects for genetics and common garden performance of a cosmopolitan weed. *Ecological Monographs* **89**: e01386.

Rosche C, Schrieber K, Lachmuth S, *et al.* 2018*b*. Sex ratio rather than population size affects genetic diversity in *Antennaria dioica*. *Plant Biology* **20**: 789–796.

Rosenberg NA. 2004. Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes* **4**: 137–138.

Spielman D, Brook BW, Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences* **101**: 15261–15264.

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. *Journal of Tropical Ecology* **30**: 199–208.

Swindell WR, Bouzat JL. 2006. Selection and inbreeding depression: effects of inbreeding rate and inbreeding environment. *Evolution* **60**: 1014–1022.

Thiers B. 2016. *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih

Thompson K, Bakker JP, Bekker RM. 1997. *The soil seed banks of North West Europe: methodology, density and longevity*. Cambridge: Cambridge University Press.

Tremetsberger K, König C, Samuel R, Pinsker W, Stuessy TF. 2002. Infraspecific genetic variation in *Biscutella laevigata* (Brassicaceae): new focus on Irene Manton' s hypothesis. *Plant Systematics and Evolution* **233**: 163–181.

Walisch TJ, Colling G, Bodenseh M, Matthies D. 2015. Divergent selection along climatic gradients in a rare central European endemic species, *Saxifraga sponhemica*. *Annals of Botany* **115**: 1177–1190.

Wang IJ, Bradburd GS. 2014. Isolation by environment. *Molecular Ecology* **23**: 5649–5662.

Welk E. 2002. Arealkundliche analyse und bewertung der Schutzrelevanz seltener und gefährdeter Gefäßpflanzen in Deutschland. *Schriftenreihe für Vegetationskunde* **37**: 1–337.

Wierzbicka M, Pielichowska M, Bemowska-Kałabun O, Rostański A, Wąsowicz P. 2020. A new taxon within *Biscutella laevigata* L. (Brassicaceae) endemic to calamine areas in southern Poland. *PhytoKeys* **160**: 123–129.

Young A, Boyle T, Brown T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution* **11**: 413–418.