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ORIGINAL ARTICLE

Genetic diversity in a long-lived mammal is explained by the past's demographic shadow and current connectivity

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Abstract

Within-species genetic diversity is crucial for the persistence and integrity of populations and ecosystems. Conservation actions require an understanding of factors influencing genetic diversity, especially in the context of global change. Both population size and connectivity are factors greatly influencing genetic diversity; the relative importance of these factors can, however, change through time. Hence, quantifying the degree to which population size or genetic connectivity are shaping genetic diversity, and at which ecological time scale (past or present), is challenging, yet essential for the development of efficient conservation strategies. In this study, we estimated the genetic diversity of 42 colonies of *Rhinolophus hipposideros*, a long-lived mammal vulnerable to global change, sampling locations spanning its continental northern range. Here, we present an integrative approach that disentangles and quantifies the contribution of different connectivity measures in addition to contemporary colony size and historic bottlenecks in shaping genetic diversity. In our study, the best model explained 64% of the variation in genetic diversity. It included historic bottlenecks, contemporary colony size, connectivity and a negative interaction between the latter two. Contemporary connectivity explained most genetic diversity when considering a 65 km radius around the focal colonies, emphasizing the large geographic scale at which the positive impact of connectivity on genetic diversity is most profound and hence, the minimum scale at which conservation should be planned. Our results highlight that the relative importance of the two main factors shaping genetic diversity varies through time, emphasizing the relevance of disentangling them to ensure appropriate conservation strategies.

KEYWORDS

bottleneck, connectivity, conservation, genetic diversity, isolation-by-distance

Lehnen & Jan equally contributed to this work as first authors. Petit & Puechmaille equally contributed to this work as senior authors.

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1 | INTRODUCTION

The significance of genetic diversity as a prerequisite for long-term population viability and evolution has long been acknowledged in conservation biology (DeWoody et al., 2021; Frankel & Soulé, 1981). Reduced heterozygosity, a concomitant effect of inbreeding (Falconer & Mackay, 1996), can have negative fitness effects due to, for example, the accumulation of deleterious alleles (O'Grady et al., 2006; Saccheri et al., 1998; Vandewoestijne et al., 2008) or loss of phenotypic variation (Neaves et al., 2015). Ultimately, low genetic diversity can thus result in extinction (Frankham, 2005), particularly under stressful conditions (Armbruster & Reed, 2005), but even in benign environments (Markert et al., 2010). Genetic diversity furthermore represents the raw material upon which selection is acting, allowing species to respond to changing environments and thereby alleviating extinction risk (Allendorf et al., 2012; Polechová, 2018). Its preservation has never been more urgent given ongoing rapid global change, as it is essential for resilience against extinction (González-Suárez & Revilla, 2013), resistance against invasive species (Scheepens et al., 2017), successfully coping with degraded or changing environments (Markert et al., 2010; Perrier et al., 2017), the successful colonization of novel habitats (Forsman, 2014; Polechová, 2018; Szűcs et al., 2017) and ecosystem recovery (Reusch et al., 2005). As a key factor of biosphere integrity, which is among the most imperilled of the nine planetary boundaries, genetic diversity is also inextricably linked to earth-system stability and the sustenance of humankind (Steffen et al., 2015). Consequently, we face a pressing need to identify the major causes of reduced levels of genetic diversity in wild populations in order to develop effective conservation strategies that mitigate its worldwide loss (Benson et al., 2016; Lewin et al., 2018; Polechová, 2018; Steffen et al., 2015).

Population size and genetic connectivity between populations influence effective population size and thus, genetic drift (Allendorf et al., 2012; Frankham, 2015; Polechová, 2018). Therefore, both represent key targets for any conservation planning focused on addressing genetic diversity issues. In theory, small and disconnected populations can be expected to harbour the lowest genetic diversity, while large, stable and well-connected populations should accommodate the highest genetic diversity (Allendorf et al., 2012; Lynch et al., 1995). In practice, populations can be anything within this spectrum of combinations of population size and connectivity, which makes it difficult to pinpoint the main driver of genetic diversity. The identification of the dominating feature determining genetic diversity, however, is crucial for efficient strategies for the protection of genetic diversity, because a decrease in population size and a reduction or loss of gene flow require distinct conservation measures (Frankham, 2015; Westemeier et al., 1998).

Quantifying the relative contribution of population size and connectivity to genetic diversity is not trivial because both may change over time. Disregarding this temporal variation can introduce a considerable bias in the estimated importance of each factor due to time lags in the response of genetic diversity levels. Studies investigating drivers of genetic diversity increasingly incorporate population history by accounting for past changes in population size, commonly referred to as bottlenecks (e.g. Pelletier et al., 2017; Shirley & Austin, 2017). However, genetic bottleneck signals¹ can originate not only from demographic crashes or expansions, but also from total disconnection, that is, complete disruption of gene flow (Broquet et al., 2010). The unmet demand for a method to pinpoint the process behind a bottleneck signal thus has been precluding the quantification of the relative importance of historic population sizes and connectivity for current genetic diversity.

We address this issue by developing and testing a method to distinguish between demographic and disconnection-induced bottlenecks based on their respective effects on isolation-by-distance, IBD (Broquet et al., 2006; Rousset, 1997). Following the principles of population genetics theory, gene flow should still occur between populations after a demographic crash, albeit with a lower number of migrants exchanged between populations. This implies that an IBD pattern would still exist, but with a reduced genetic similarity between populations, resulting in a higher IBD slope. Total disconnection on the other hand implies a complete disruption of gene flow. With time, genetic drift would then progressively erase the effect that geographic distance once had on genetic similarity, and decrease the IBD slope. Consequently, the ratio between IBD slopes for populations that underwent a bottleneck and those that did not ought to be higher than one in case of bottlenecks caused by demographic crashes, while the ratio is expected to be less than one in the case of disconnection bottlenecks. We test this hypothesis by combining data from simulations and an empirical data set based on *Rhinolophus hipposideros* colonies located along the species' continental leading edge in Europe.

This long-lived Palaearctic bat species of conservation concern (Biedermann et al., 2012) has been predicted to respond to global warming via range shift (Rebelo et al., 2010). For many species, the genetic diversity of leading edge populations plays a crucial role for range shift success (Chaine & Clobert, 2012; Szűcs et al., 2017). This also applies to *Rhinolophus hipposideros*, because range expansion

¹In this article, we use the term "bottleneck" to refer to a reduction in a population's genetic diversity caused by a past event (see e.g., Broquet et al., 2010; Faurby & Pertoldi, 2012; Girod et al., 2011; Williamson-Natesan, 2005). The events we consider here to potentially cause a bottleneck are either a reduction in population size (which may or may not be followed by demographic regrowth) or a sudden disconnection (see "Bottleneck impacts on IBD slopes" section).

can be expected to occur mainly from leading edge populations (Dool et al., 2013) given the species' limited dispersal capability (Dool et al., 2016). Despite its ability to fly, *Rhinolophus hipposideros* is highly sensitive to forest fragmentation (Dool et al., 2016; Reiter et al., 2013; Tournant, 2013), and severe declines have been reported for many of its populations in North Western Europe in the 20th century (Bontadina et al., 2000). Consequently, a pressing need exists to identify the dominating current and historic drivers of genetic diversity of *Rhinolophus hipposideros* leading edge populations, that is, to quantify the relative importance of population size as well as connectivity in present and former times for the genetic diversity of those populations.

Identifying the impacts of variation in ecological parameters between populations requires a sampling scheme of sufficient extent (Gurevitch et al., 2016). To determine the drivers of genetic diversity of *Rhinolophus hipposideros* leading edge populations, we thus genetically sampled 42 sites across an area of nearly 1500 km along the species' continental European leading edge (Figure 1), and assessed the role of connectivity and bottlenecks in shaping the observed genetic patterns.

2 | MATERIALS AND METHODS

The data sets, tools and methods described in the sections below are graphically summarized in Figure 2.

2.1 | Sampling and quantification of genetic diversity

To quantify the genetic diversity of *Rhinolophus hipposideros* leading edge populations, we noninvasively collected fresh faeces samples from 42 nursery colonies along the species' North-Western continental distributional leading edge (Figures 1 and 2, S1) for genetic analysis, which corresponds to 3.8% of all known colonies in this area (1116 colonies, cf. section "Estimation of connectivity measures"). Great Britain and Ireland were excluded from the sampling to avoid potential bias introduction due to island effects. The target area of this study has been recolonized by *R. hipposideros* from the same glacial refugia after the Pleistocene (Dool et al., 2013), but shows differences regarding the more recent demographic history of the sampled colonies: For Belgium and Germany, severe population declines and extinction events have occurred in the second half of the 20th century (Bontadina et al., 2000), but many of these populations seem to have started recovering since the late 1990s (Bontadina et al., 2006; Tress et al., 2012). Colonies sampled in these countries therefore include recovering colonies that are currently growing (Jan et al., 2019), and a few which are known or suspected to have been temporarily extinct and re-established within the last three to four decades. Only part of the French colonies are reported to have been affected by the 20th century declines (Bontadina et al., 2000), with a relatively stable distribution since 1950 and no reported sign of recent range expansion.

Nursery colonies are aggregations of mainly female bats gathering in late spring to early summer, before parturition (Biedermann et al., 2012). In *Rhinolophus hipposideros*, nursery colonies (from now on referred to simply as "colonies") can also harbour some males (Dool et al., 2016; Gaisler, 1966; Lehen et al., 2018; Zarzoso-Lacoste et al., 2018). *Rhinolophus hipposideros* females are highly philopatric and in most cases use their natal roost as a nursery roost throughout their lives (Biedermann et al., 2012; Jan et al., 2019). We hence infer population genetic diversity by sampling colonies, which represent a discrete unit of organisms of the same species interacting with each other and occupying a particular space at a particular time (Burland & Worthington Wilmer, 2001; Waples & Gaggiotti, 2006).

Faeces collection and the DNA extraction protocol were as described by Zarzoso-Lacoste et al. (2018). To estimate the genetic diversity of the sampled colonies, DNA was extracted from 40 faecal samples (= single droppings) per colony in most cases. For five colonies, synergism with other projects allowed us to process more samples, and for two colonies, colony size estimates based on visual counts suggested that fewer samples would suffice to sample all individuals of the colonies (see Table S1 for exact sample sizes). Sampling took place before parturition to sample only adults. Colony size was estimated based on visual counts or, in two cases where count data were not available, based on the Capture Mark Recapture estimator developed by Puechmaile and Petit (2007), using within-sampling-session recapture rates to estimate colony size. This estimator has been demonstrated to yield colony size estimates that are consistent with visual counts in *Rhinolophus hipposideros* (Zarzoso-Lacoste et al., 2018). The genotyping procedure using eight neutral microsatellite loci, bioinformatic pipeline processing, and genotyping quality assessment were carried out as described by Zarzoso-Lacoste et al. (2018, 2020). To allow a consequent number of samples to be analysed, the protocol was specifically designed and optimised in a single multiplex reaction with eight microsatellites. The number of coamplified loci was limited by the necessity to have nonoverlapping fragments length for each fluorophore and, ideally, short fragments as they better amplify for noninvasively collected DNA (Broquet et al., 2007). In total, we genotyped 1,800 samples (Table S1) to capture a sufficient proportion of individuals (see Puechmaile & Petit, 2007) in each of the 42 sampled colonies.

Departure from Hardy-Weinberg proportions was assessed at the population level via F_{IS} and tested with the corresponding permutation test using the software GENETIX. Genetic diversity (expected heterozygosity) was calculated in R version 3.4.2 (R Core Team, 2017) via the *adegenet* package version 1.3-1 (Jombart, 2008; Jombart & Ahmed, 2011, see Figure 2). Distinct genotypes detected in the respective colonies have been deposited in Dryad (<https://doi.org/10.5061/dryad.27q46m2>; Lehen et al., 2020).

2.2 | Detection of genetic bottlenecks

We tested for bottleneck signals in each of the 42 colonies in our empirical data set with the program MSVAR v.1.3. (Beaumont, 1999;

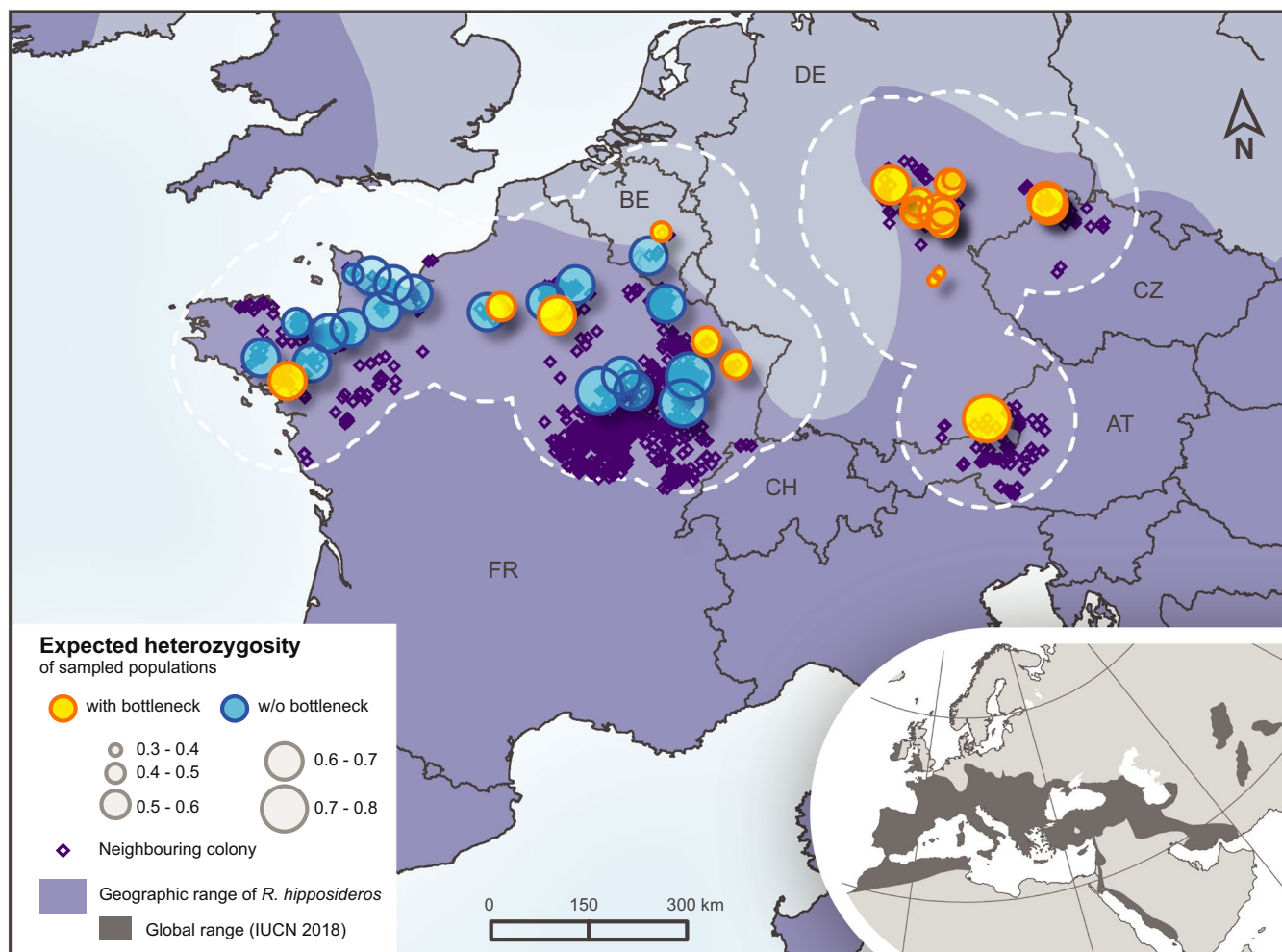


FIGURE 1 Location of investigated *Rhinolophus hipposideros* colonies. Coloured dots represent genetically sampled colonies with bottleneck signal (yellow) or without (blue) bottleneck signal, with circle size proportional to expected heterozygosity. Purple diamonds correspond to colonies located in a 140 km radius (dashed white lines) around sampled ones. Background colours indicate the species distribution range (International Union for Conservation of Nature [IUCN]; Taylor, 2016). AT, Austria; BE, Belgium; CH, Switzerland; CZ, Czech Republic; DE, Germany; FR, France

Storz & Beaumont, 2002, see Figure 2), which draws on a Bayesian method to infer mutational and demographic parameters via simulations. The most likely mutation rate (μ), time since the bottleneck (t_f), and the ratio of the original population size to the observed population size (r) conditional on the observed population size and allele frequencies are estimated. MSVAR has been shown to reliably detect demographic changes such as population declines, provided that they are not too recent or too weak, and to be relatively robust to moderate departures from a strict stepwise mutation model (Girod et al., 2011). However, wide credibility intervals are usually obtained for estimates of the time since bottleneck occurrence and its strength. Therefore, we exclusively focused on the detection of a signal for reductions in effective colony size as a binary variable (presence or absence of a detected bottleneck). Three independent chains with different priors were run for each colony. Priors differed among the three chains (File S1), considering reductions in effective colony size of distinct severity and/or distinct time periods since the reduction, but were identical for each colony (with the exception of

the random seed). The first 50,000 values of each chain were discarded as burnin before the computation of Bayes factors (BF) and the Gelman & Rubin's convergence diagnostic (Brooks & Gelman, 1998). The BF was computed by combining the three Markov chains before dividing the number of states in which the population declined by the number of states in which the population increased (Girod et al., 2011; Storz & Beaumont, 2002). Since without prior knowledge about mutation rates and metapopulation structure, MSVAR is prone to detect spurious bottleneck signals (Chikhi et al., 2010), we chose a more conservative threshold than usual and only considered bottlenecks for colonies exhibiting BF's higher than 10, which corresponds to a "strong support" (Jeffreys, 2000). Genetic diversity of bottlenecked colonies was lower on average (see Results), and we checked if this difference was significant with a one-sided permutation test. We permuted the bottleneck detection status (presence or absence of a detected bottleneck) between colonies (10,000 permutations), considering the null hypothesis of equal genetic diversity for bottlenecked colonies when compared to nonbottlenecked ones.

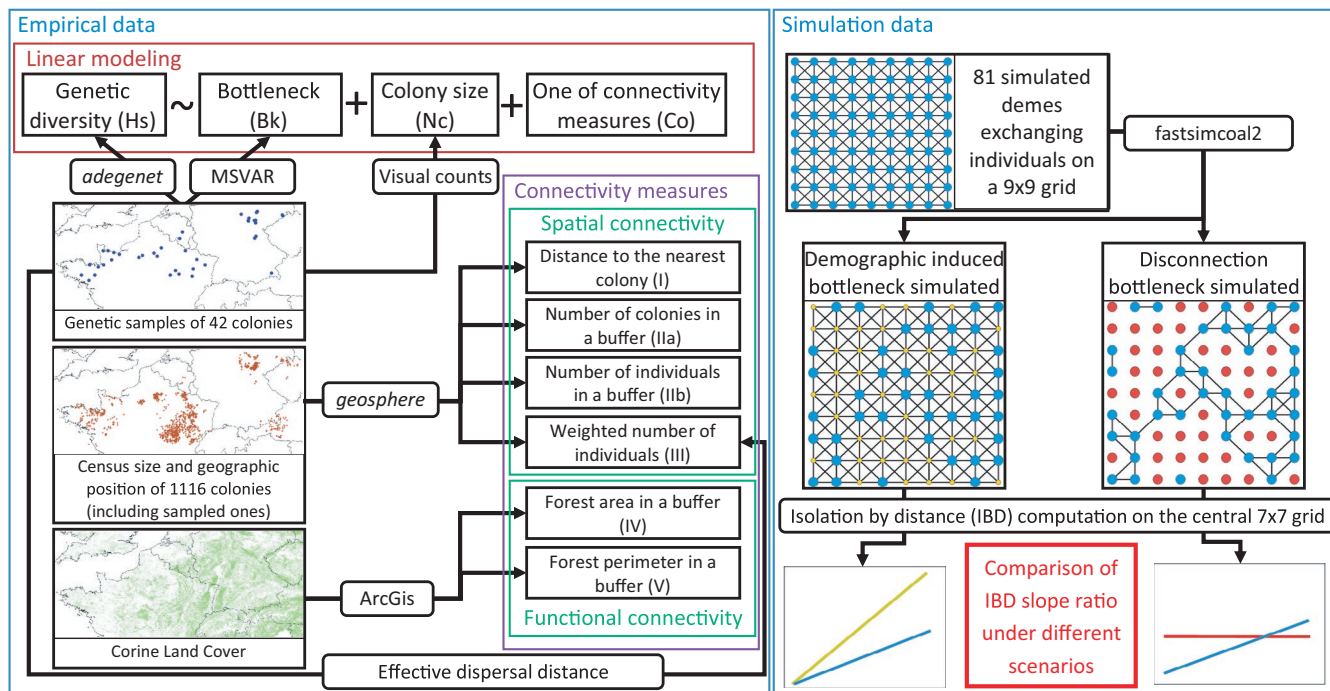


FIGURE 2 Simplified summary of our methods, presenting the purpose of each data set with associated key tools and computation

To account for discourse regarding the power and suitability of different methods of bottleneck detection, we also screened for bottlenecks using a moment-based method using the M-ratio as proposed by Garza and Williamson (2001) in addition to MSVAR. Overall, the results obtained using the MSVAR and the M-ratio methods were consistent (Table S1). Given the better performance (lower Akaike Information Criterion, AIC) of MSVAR-based compared to M-ratio-based models in our study system (Figures 3, S5, S9–S10) and several caveats regarding the M-ratio reported in the literature (Supporting Information File S2), we focus on results obtained via MSVAR in the main manuscript (results and discussion).

2.3 | Estimation of connectivity measures

Our study focussed on how genetic connectivity (*sensu* Lowe & Allendorf, 2010) is influenced by landscape connectivity (*sensu* Taylor et al., 1993). Genetic connectivity relates to the influence of gene flow, which is mediated by individuals immigrating into empty habitat patches (colonization) or into extent populations (dispersal), on evolutionary processes. How landscape features influence the probability that individuals successfully found a new colony or disperse is the focus of landscape connectivity studies, and a priority in the conservation genetics research agenda (Baguette et al., 2013). Ecological studies draw on a plethora of indirect connectivity measures or proxies due to the numerous challenges and difficulties associated with directly determining landscape connectivity (Moilanen & Nieminen, 2002). A meta-analysis investigating the power of three classes of landscape connectivity measures to predict colonization events detected pronounced differences in their

performance, which were further confirmed with two empirical data sets (Moilanen & Nieminen, 2002).

While the connectivity concept of interest differs when investigating genetic diversity (genetic connectivity) and patch colonization (demographic connectivity), ultimately, both depend on the movement of individuals across the landscape from one patch to another. The influence of landscape variables is the common factor between landscape demography (Gurevitch et al., 2016) and landscape genetics (Manel et al., 2003). We thus drew on the different connectivity measures suggested by Moilanen and Nieminen (2002) to identify the most informative one for our study system, testing and comparing the performance of the different connectivity measures in explaining the genetic diversity of our focal colonies.

We used the three main classes of connectivity metrics described by Moilanen and Nieminen (2002): (1) nearest neighbour measures, which only take into account the population which is closest to the focal one; (2) buffer measures, which consider surrounding populations within a certain buffer but not their respective distances from the focal population; and (3) incidence function model measures, where the impact of surrounding populations is weighted depending on their distance from the focal one.

For the nearest neighbour category, we considered (I) the distance of the nearest neighbouring colony to the focal one (Moilanen & Nieminen, 2002, equation 1a). The buffer metrics we used were (IIa) the number of colonies within a certain buffer area around the focal colony and (IIb) the cumulative census size of all colonies within a certain buffer area around the focal one. The study by Moilanen and Nieminen (2002) approximated source population size by patch area in combination with scaled emigration/immigration rates (equation 2a). When using approximated emigration/immigration rates

because the actual rates are not known, Moilanen and Nieminen (2002) found the performance of the buffer measure to be highly sensitive to the choice of the emigration/immigration rate values (Moilanen & Nieminen, 2002). We therefore used census size as a more robust proxy for colony size instead. From the incidence function model category, we used (III) the cumulated census size of all colonies surrounding the focal one, weighted by the negative exponential dispersal kernel (Moilanen & Nieminen, 2002, equation 3a).

To construct the negative exponential dispersal kernel (Nathan et al., 2012) based on the effective dispersal distance, we investigated the isolation-by-distance (IBD) pattern of our data set. For this purpose, we first quantified the linear relationship between genetic distance $F_{ST}/(1-F_{ST})$ and the logarithm of Euclidean geographic distance, that is, isolation-by-distance, and calculated the 95% confidence interval with the bootstrap method implemented in the Genepop software (Raymond & Rousset, 1995). Because the relationship between the slope of IBD and the dispersal distance assumes that populations are at migration-drift equilibrium (Rousset, 1997), we only considered nonbottlenecked colonies to infer the dispersal kernel. Population density was calculated for each region monitored by a certain NGO (see below) by dividing the total census size in this region by the geographic area covered in the census assessment (removing sea/ocean surfaces). Because visual counts of adults in nurseries mainly include females (Zarzoso-Lacoste et al., 2018) and sex ratios are usually balanced in this species (Gaisler, 1966), we assumed the total number of individuals in a region to be twice the number of individuals counted in colonies. Effective population size is, on average, 10 times smaller than adult census size in wild populations (Frankham, 1995), so we divided population density by 10 to estimate the effective density, D .

We used the resulting IBD (Figure S2) and density estimates to calculate the effective dispersal distance, σ , based on the equation provided by Rousset (1997):

$$\sigma^2 = \frac{1}{b4D\pi}$$

where b is the slope of the linear isolation-by-distance-relationship and D is the effective population density. The resulting dispersal kernel is presented in Figure S3. We used the R packages *adeigenet* version 1.3-1 and *hierfstat* version 0.04-22 (Goudet, 2005; Goudet & Jombart, 2015) to determine pairwise F_{ST} and IBD regression line slopes and to perform the Mantel test.

The information on distance to the nearest neighbouring colony (I), number of colonies (IIa), and number of individuals (IIb and III) was taken from a database built by assembling locations and adult census sizes of colonies provided by local nature conservation NGOs in France, Belgium, Germany, Austria and the Czech Republic. This database allowed us to estimate the distribution and abundance of *Rhinolophus hipposideros* in a maximum radius of 140 km around every sampled colony. Only colonies hosting a minimum of five adults and with evidence for reproduction were considered, resulting in a data set of 1,116 colonies recorded in up to 140 km distance from the genetically sampled ones, far beyond the maximum movement

recorded for our study species in this region (81 km, Fairon, 1967). The buffer area within which the number of colonies (IIa) or their census size (IIb) were considered increased in incremental steps of 1000 km². We also computed two small buffers of 100 and 500 km² around sampled colonies, resulting in a total of 62 different radii. Spatial connectivity measures were computed with the help of the R package *geosphere* version 1.5-7 (Hijmans, 2017).

All of the hitherto described measures are based solely on the availability of potential immigrants, considering homogenous gene flow across the landscape. However, landscape composition and configuration can considerably impact the movement of individuals between patches/colonies. Following the recommendation by Moilanen and Nieminen (2002) to account for habitat matrix quality and spatial configuration, we therefore additionally tested two additional connectivity measures that explicitly take landscape features into account (Tischendorf & Fahrig, 2003; Watson et al., 2017). We focused on mixed and deciduous forest, because *Rhinolophus hipposideros* forages nearly exclusively in broadleaved woodland (Reiter, 2004). We therefore hypothesized that this landscape type could also affect the connectivity between colonies. We chose the following metrics: (IV) the total area of mixed and deciduous forest patches around the focal colony as a composition metric, and (V) the total perimeter of forest patches around the focal colony as a configuration metric. Both metrics were calculated for each of the 42 sampled colonies for the same 62 buffers previously used for connectivity measures IIa and IIb. For the calculation of forest area and perimeter, we used ArcGis 10.6 (ESRI) to build a simplified land cover data set from the Corine Land Cover (CLC, 2012) vector database (European Environment Agency). The different classes of CLC corresponding to mixed and deciduous forest were merged into a new single Forest class. To handle the high topologic complexity of the new dissolved polygons, the data set was then simplified by reducing the number of vertices while preserving polygon shape (see File S3).

Moilanen and Nieminen (2002) found that the performance of all tested connectivity measures considerably increased when including local patch size. In contrast to their approach, we did not integrate local patch size into the respective connectivity measures, but included local census size as an extra explanatory variable in the linear model framework (cf. next section).

2.4 | Demographic declines, connectivity, and genetic diversity

To quantify the relative importance of different factors influencing contemporary genetic diversity, we used a linear model framework considering different combinations of explanatory variables (Figure 2). The fit of the genetic diversity data to a normal distribution was visually assessed via the package *fitdistrplus* version 1.0-11 (Delignette-Muller & Dutang, 2015, see Figure S4) and the normality of the best model's residuals was tested with a Shapiro-Wilk test. The explanatory variables tested included census size (N_c) of the focal colony to consider local demography, the category bottlenecked (B_k)

to assess the impact of population history, and current connectivity (Co). Every combination of those variables was tested in linear models. Each connectivity measure was tested in a separate set of models, so that models including Co always considered only one of the six different connectivity measures described above. For buffer measures of connectivity, we tested each of the 62 radii separately in the linear model framework.

Model fit was compared based on AIC. The proportion of variance explained by the respective models/model variables was assessed by calculating the adjusted R^2 values of the linear models. All statistical analyses were performed in R version 3.4.2, and the effect of each predictor included in the best model was displayed using the R package *effects* version 4.0-0 (Fox, 2003).

Only models considering expected heterozygosity (H_s) as a response variable are included in the main document, because results were similar when estimating genetic diversity via allelic counts corrected for sample size (allelic richness) instead (Figure S5).

2.5 | Bottleneck impacts on IBD slopes

To test our hypotheses regarding how IBD slopes are affected by bottlenecks caused by reductions in colony size or a cessation of gene flow, respectively, we simulated demes (corresponding to colonies in our empirical data set) evolving in a strict stepping stone model of gene flow using the coalescent method implemented in *fastsimcoal2* version 2.5.2.21 (Excoffier et al., 2013). As a compromise between the much more complex spatial distribution and higher number of demes existing in our empirical data set (Figure 1) and computational feasibility, we simulated 81 demes in a 9×9 grid (Figure 2).

Mimicking the two scenarios leading to bottleneck signatures described by Broquet et al. (2010), we simulated (1) demes that underwent a reduction in population size and (2) well-connected demes that suddenly became isolated without a change in deme size. We simulated two different deme sizes (100 and 500 diploid individuals) and gene flow rates (1 and 10 immigrants per generation), resulting in four basic simulation scenarios (Table S2). In each simulation, we randomly bottlenecked (either by reducing population size or by setting the number of immigrants to zero) 40 of the 81 demes (Figure 2), which approximately corresponds to the proportion of colonies for which we found a bottleneck signature in our empirical data set.

When simulating disconnection-induced bottlenecks, only total disconnection was considered, because incomplete reductions of gene flow do not lead to bottleneck signatures (Broquet et al., 2010). We assumed that disconnection was definitive: disconnected demes remained isolated for the whole simulation after disconnection had occurred.

Demographic crashes were simulated to mimic the effects of reductions in deme size. We reduced deme sizes using two different crash magnitudes by applying two reduction size factors (deme sizes divided 10 and 25 times). Demes may or may not recover their initial size after a demographic crash. We thus distinguished between scenarios in which demes could regrow to their original size immediately

after the demographic crash, and scenarios in which growth after the demographic crashes was not allowed. These situations are the most extreme cases, between which most realistic situations resulting in bottleneck signatures are likely to occur. We chose an intrinsic growth rate of 1.1 based on the available literature for bats (Froidevaux et al., 2017; O'Shea et al., 2011).

Finally, we explored different times at which the bottlenecks occurred (20, 100, and 1000 generations back in time) for both types of bottlenecks. The resulting 60 parameter sets (see Table S2) were replicated 100 times each, resulting in 6000 simulated data sets. To avoid edge effects, we only sampled the 49 (7×7) demes located at the central part of the 9×9 simulation grid. We sampled 35 individuals from each deme and classified demes as bottlenecked or nonbottlenecked according to their demographic history. To test our hypothesis that the two kinds of bottlenecks have diverging effects on the IBD slope, we recorded, for each simulation replicate, the ratio of the slope of the IBD regression between bottlenecked demes over the slope of the IBD regression between nonbottlenecked demes (Figure 2).

To determine the major process behind the bottleneck signal detected in our empirical data set, we calculated the IBD slope ratio between bottlenecked and nonbottlenecked colonies in the same manner, and compared the resulting value to those obtained for the different simulated scenarios. A geometric bootstrap confidence interval for the IBD slope ratio of the empirical data set was obtained by constructing conservative confidence regions, following the method described by von Luxburg and Franz (2009) for general distributions.

As the distribution of geographic distances between pairs of bottlenecked colonies differed from the distribution of geographic distances between pairs of nonbottlenecked colonies in our empirical data set, we performed additional analyses to verify that the IBD patterns observed for the genetically sampled colonies were not caused simply by such differences in geographic distance. A more conservative approach using only that subset of colonies whose geographic distance to other colonies was within the range of geographic distances observed for nonbottlenecked colonies yielded an even higher IBD slope ratio, supporting the hypothesis that IBD slope differences between bottlenecked and nonbottlenecked colonies are linked to the bottleneck occurrence itself (Figure S6).

3 | RESULTS

The genetic diversity of the sampled *Rhinolophus hipposideros* colonies varied from 0.36 to 0.73 (Figure 1, Table S1), and was within the range reported for other terrestrial vertebrate species (Figure S7). The average genetic diversity (0.57 ± 0.018) of bottlenecked colonies (22 out of 42; Table S1) was significantly lower than that of nonbottlenecked colonies (0.65 ± 0.014 ; $p < .001$, Figure 4a). Correspondingly, models including the binary variable bottlenecked (*Bk*) consistently outperformed according models without this variable in explaining genetic diversity (Figure 3). In addition to *Bk*, including the explanatory variables connectivity (*Co*) and census size (*Nc*) greatly

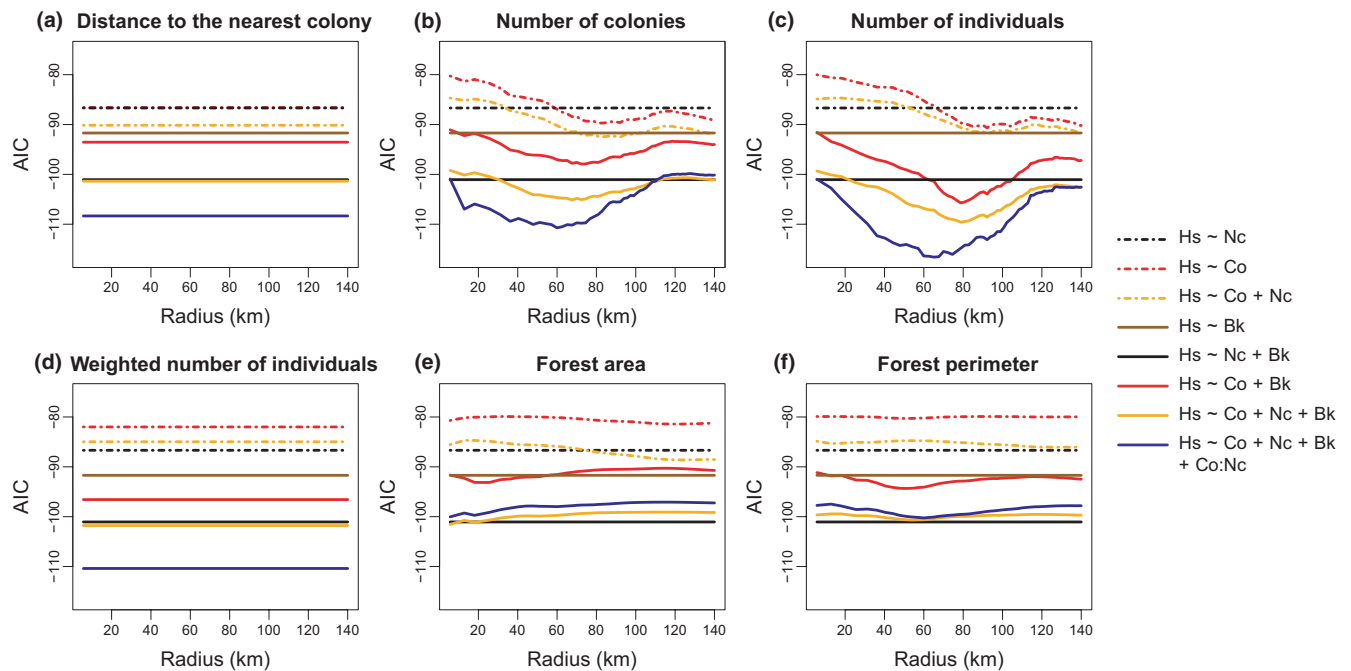


FIGURE 3 AIC of models explaining the genetic diversity (expected heterozygosity, H_e) of *Rhinolophus hipposideros* populations. The explanatory variables considered include census size of the focal colony (N_c), the category bottlenecked (B_k), and the following six connectivity measures (Co): (a) the distance to the nearest colony (I), (b) the number of colonies in the buffer (IIa), (c) the number of individuals within the buffer around the focal colony (IIb), (d) the number of individuals within the buffer around the focal colony weighted by the dispersal kernel (III), (e) total forest area in the buffer (IV), (f) total forest perimeter in the buffer (V). The model indicated in blue also includes the interaction between Co and N_c . Models including buffer measures of connectivity were run for 62 different radii. Details of the different connectivity measures are described in the section “Estimation of connectivity measures”.

improved model performance for each connectivity measure category (Figure 3). For the two buffer-based connectivity measures (IIa and IIb), the contribution of Co was especially pronounced for radii in the range of 60–80 km (Figure 3b and c). No interaction between explanatory variables resulted in an increase in model performance except the negative interaction between N_c and Co (Figure 3).

The overall best performance was observed for a model considering B_k , N_c , Co calculated as the number of individuals within a 65 km radius (connectivity measure IIb), and the interaction between N_c and Co (Figure 3c). Residuals of this model were normally distributed (Shapiro-Wilk test; $p = 0.50$). Both N_c and Co had a positive effect on genetic diversity, while B_k had a negative effect (Figure 4). Together, the explanatory variables of this model explained 64% of the variation in genetic diversity. The positive effect of Co was particularly pronounced for small colonies (i.e., $N_c \leq 60$, Figure 4b). Effect sizes for this model indicated that for the average N_c observed in our data set ($\bar{N}_c = 45$, $n = 1116$ colonies), increasing connectivity from 1000 to 5000 individuals within the buffer corresponded to an increase of almost 0.1 in expected heterozygosity (Figure 4b).

The simulated data sets mimicking demographic- and disconnection-induced bottlenecks confirmed the prediction that demographic bottlenecks increase the slope of IBD, resulting in average slope ratios above one (Figures 5 and S8, which show simulation results for disconnection bottlenecks together with demographic bottlenecks of either 10- or 25-fold reduction in effective sizes for demographic crashes, respectively; note that the y-axis

is transformed for visualization purpose, see legend of the figure). In contrast, disconnection bottlenecks, as predicted, led to slope ratios below one, with a clearer signal when the time since disconnection was large and for higher effective population sizes (Figures 5 and S8). Lower effective population sizes led to a high variance in slope ratios because, as expected, the variance in IBD slopes became very high for disconnected populations (data not shown). Allowing the regrowth of crashed populations, however, erased the bottleneck signal in many cases, also resulting in slope ratios of approximately one for demographic bottlenecks or even in slope ratios that were similar to values observed following disconnection-induced bottlenecks (Figures 5 and S8). The IBD slope ratio between bottlenecked and nonbottlenecked colonies in our empirical data set was 3.4, with a confidence interval that did not encompass one, the value expected under the null hypothesis (95% confidence interval: 1.4–435.1).

4 | DISCUSSION

4.1 | Relevance of different connectivity measures for genetic diversity

The pronounced positive impact of local census size (N_c) and particularly landscape connectivity (Co) on genetic diversity confirms that the number of individuals, both locally and within a certain radius around focal colonies, has a profound positive effect on

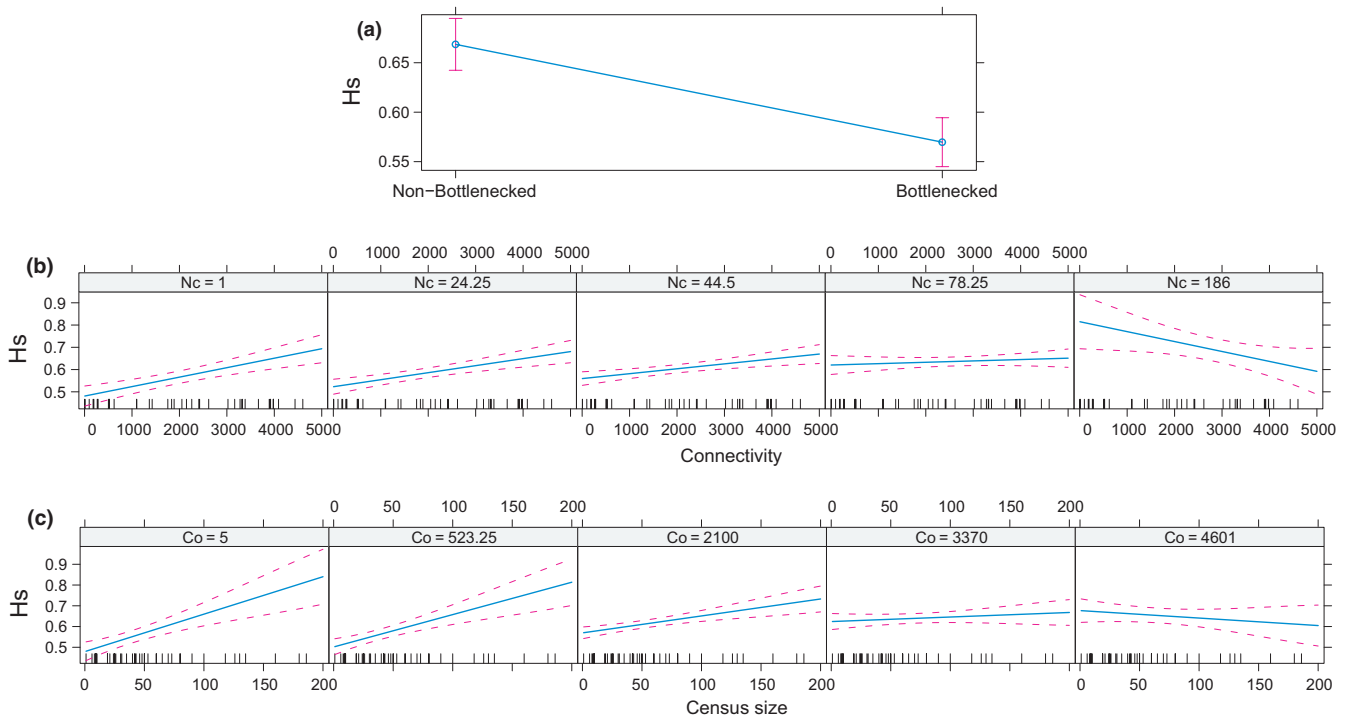


FIGURE 4 Effect sizes of factors affecting genetic diversity in *Rhinolophus hipposideros*. (a) the category bottlenecked (Bk), (b) connectivity (Co), and (c) census colony size (Nc). Effect sizes are shown for the best model, which considers all tree variables and the interaction Co:Nc for a radius of 65 km (Figure 3c). The effect of the interaction is illustrated by presenting the effect size of concerned variables (connectivity, census size) with varying values of the interacting variable in different panels (minimum, first quartile, median, third quartile, maximum, from left to right).

genetic diversity. The observed improvement in model performance when including the negative interaction between local census size and connectivity is concordant with the expectation that the larger a population is, the less it depends on connectivity to maintain high levels of genetic diversity, and that vice versa, well-connected populations can be smaller without forfeiting genetic diversity. Given that the influence of connectivity is strongest for colonies with a census size of 60 or less, and that 83% of the 1116 colonies that we considered here to calculate Co fall into this census size range, connectivity can be regarded as the main driver of genetic diversity for the majority of the *Rhinolophus hipposideros* colonies registered in this study.

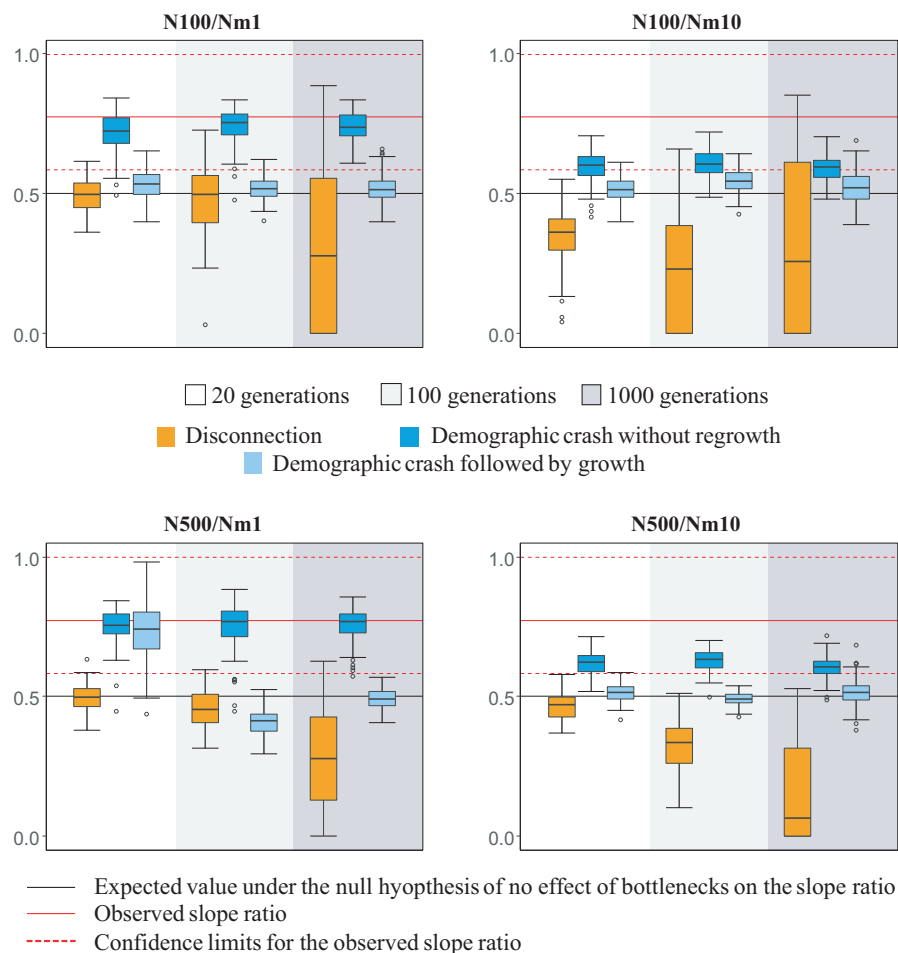
In our study system, the optimal connectivity measure (IIb) differs from those suggested by Moilanen and Nieminen (2002). In their study on two butterfly species, the best performance among the tested connectivity measures was observed for the incidence function model category. The according connectivity measure (based on the negative exponential dispersal kernel, III) however performs comparatively poorly for our empirical data set. In our study, the number of individuals within a radius of more than 30 km (IIb) explains variation in genetic diversity better than the corresponding number of individuals weighted by their distance from the focal colony (III), suggesting that long distance dispersers have a pronounced influence on local gene diversity. This is consistent with the observed isolation-by-distance pattern, in which colonies are more genetically distinct if they are geographically further apart (Figure

S2). Influx of genes from distant colonies is accordingly more likely to introduce novel alleles, resulting in a more pronounced amelioration of genetic diversity.

Moilanen and Nieminen (2002) furthermore stressed the importance of more complex measures for modelling connectivity, considering, for example, habitat matrix quality and spatial configuration. We implemented this suggestion by calculating two connectivity measures that take into account the surface area (IV) and perimeter (V) of mixed and deciduous forest around the focal colony, which represent the main foraging habitat types of *Rhinolophus hipposideros* (Bontadina, 2002; Reiter, 2004). Models considering either of these connectivity measures however are clearly inferior in explaining genetic diversity to those considering the number of individuals within a 65 km radius around the focal colony (IIb), suggesting that the proportion of woodland in the habitat matrix is not a suitable predictor of genetic diversity in our study system.

This finding confirms the results of a previous study on *Rhinolophus hipposideros* in Ireland, where the suitability of the habitat matrix between colonies did not explain genetic differentiation better than geographic distance alone (Dool et al., 2016). A shared drawback of the study by Dool et al. (2016) and ours however is the limited resolution of the land cover data used to assess the impact of woodland, which prevents testing the influence of linear elements such as tree lines or hedgerows. Such landscape elements, while small, could still play an important role for foraging and the functional connectivity of habitat patches for *Rhinolophus hipposideros*.

FIGURE 5 Effect of disconnection-induced and demographic bottlenecks on isolation-by-distance (IBD) slopes. Simulated bottlenecks considered original population size (N), gene flow (Nm), time since the bottleneck (in generations), and the possibility for populations to regrow after a bottleneck or not. Boxplots show the ratio of the slope of IBD computed between bottlenecked colonies compared to the slope of IBD between nonbottlenecked colonies. Negative slope ratios, which may occur stochastically when there is no isolation-by-distance between bottlenecked populations were given null values. Slope ratios were transformed using the $x/(x + 1)$ function to make all values easily viewable on the $[0;1]$ interval and symmetric around the expected value under no effect of bottlenecks (0.5, shown as a black line on the figure). In this figure, demographic bottlenecks correspond to a reduction factor in population size of 10 (see Figure S8 for a reduction factor of 25). The value observed in our empirical data set and its confidence interval limits are shown in red.



Due to the current lack of sufficiently fine-grained land use data for the extensive area covered by our sampling scheme, a potential influence of small-scale landscape elements eludes detection in our study, but warrants future investigation.

Our study shows that the absence of a strong link between the area of available forest habitat in the matrix between habitat patches and gene flow between colonies is shared between *Myotis bechsteinii* and *R. hipposideros*, two forest-dwelling bat species (Kerth & Petit, 2005). Furthermore, our findings are in concordance with the hypothesis that crossing unsuitable habitat during mating or dispersal is a common behaviour in mobile animals (Keeley et al., 2017). Altogether, the apparent discrepancy between the optimal connectivity measure in our study and those suggested by Moilanen and Nieminen (2002) highlights that the optimal type of connectivity measure may differ between study species and systems, and depend on whether the focus is on genetic or demographic connectivity. Nevertheless, the difference in performance of the different connectivity measures can be informative and provide insights into the mechanisms at play, as in our study.

4.2 | Importance of the radius considered for connectivity

Overall, the number of individuals within a buffer zone around the focal colony (IIB) explains the largest part of genetic diversity among

the connectivity measures tested here, but model performance considerably varies with the radius considered. Alongside small local census sizes, the positive effect of connectivity becomes most apparent for radii of 60–80 km in our study system. These results suggest that connectivity at scales of less than 60 km is insufficient to uphold genetic diversity; an unexpected and important finding considering the rather small movement distances reported for *Rhinolophus hipposideros* based on capture-mark-recapture (CMR) studies: While the maximum movement distance recorded is 153 km (Heymer, 1964), dispersal over such long distance seems to be very exceptional. A long-term (25 years) CMR study performed in a part of our sampling region has shown that 90% of dispersing individuals ($n = 98$) were recaptured less than 30 km from their original colony, with the maximum observed distance being 81 km (Fairon, 1967). Furthermore, the diminishing importance of gene flow for sustaining genetic diversity for radii larger than 80 km indicates that *Rhinolophus hipposideros* colonies become disconnected when distances to other colonies exceed this connectivity threshold.

4.3 | Past bottlenecks and their impact on present genetic diversity

Detecting changes in population size solely based on genetic data is a challenging task (e.g., Chikhi et al., 2010; Faurby & Pertoldi, 2012)

also faced in our study when classifying colonies as bottlenecked or nonbottlenecked. First, as ubiquitously encountered with population genetic methods, MSVAR relies on assumptions that are only partially fulfilled in empirical data sets (Chikhi et al., 2010). However, our results should be robust to mutation model misspecifications as the same loci were analysed in all populations and we concentrated on relative differences, hampering the effects of deviations from the stepwise mutation model (Faurby & Pertoldi, 2012). Second, when investigating the presence/absence of a bottleneck signal, we investigated each colony independently because no analytical method specifically accounting for population structure currently exists (Grusea et al., 2019; Leblois et al., 2014). We chose to limit the risk of false positive bottleneck detection by increasing the Bayes Factor that defined which colonies were classified as having decreased in size, but a more sophisticated classification approach could evolve as new analytical tools are developed. Models using MSVAR output offered a better fit to our data (lower AIC) than those provided using the alternative M-ratio method (File S2), but results obtained with the two methods were qualitatively consistent, further consolidating the robustness of our results. Third, the number of microsatellite markers used was somewhat low, which may have hampered both methods' power to detect bottlenecks. Although no study has specifically tested the impact of the number of loci used to recover signals of bottleneck with MSVAR, this program has been shown to perform well when a set of 10 loci was used, a number similar to the number classically used in empirical studies (e.g., Girod et al., 2011). Those simulations, added to the fact that we detected bottleneck in nearly half of the colonies, makes us confident in the ability of our eight loci set to detect past bottlenecks.

Apart from present connectivity and local census size, a colony's genetic diversity in our study system largely depends on whether or not it underwent a genetic bottleneck in the past, with significantly lower genetic diversity in bottlenecked colonies. This concerns more than half of the sampled colonies, resulting in a considerable improvement of model performance when including the variable bottlenecked (*Bk*). The geographic proximity of some of the sampled populations makes it likely that some of them have a common history regarding past bottlenecks: populations which are closer together are more likely to have been exposed to the same environmental circumstances, with more beneficial conditions in some regions and bottleneck-inducing ones in other regions (e.g., pesticide application, cf. section "Implications for conservation"). Due to this potential spatial auto-correlation between some sampled populations, the increase in model performance (AIC and variance explained) by considering bottlenecks may be partially overestimated. Nevertheless, our results clearly show that the historic bottlenecks identified in our study are also an important factor shaping present genetic diversity in European *Rhinolophus hipposideros* leading edge populations.

4.4 | Bottleneck origin classification: disconnection versus local demographic crash

As expected, different effects on IBD slopes can generally be observed for the simulated demographic- and disconnection-induced bottlenecks,

with an overall increase in IBD slopes resulting from demographic bottlenecks and an overall IBD slope decrease for disconnection-induced ones. However, the effect of IBD slopes can elude detection if the demographic crash occurred very long ago and/or if population sizes recovered afterwards (Figures 5 and S8). While IBD slope ratios of approximately one or less thus prevent unambiguous bottleneck classification, slope ratios higher than one provide strong support for the bottleneck signal originating from a demographic bottleneck.

The IBD slope ratio of our empirical data set significantly exceeds one, indicating that the overall bottleneck signal detected in our sampled colonies most likely originated from a demographic collapse, rather than the disruption of gene flow (Figure 5). This finding applies to all populations analysed together. However, while the signal of demographic bottlenecks clearly dominates in our data set, we cannot exclude that some of the bottlenecks (albeit a small proportion) may be due to disconnection. While it is challenging to control for various sources of bias when investigating wild populations, the dominance of demographic bottlenecks inferred for the empirical data set is concordant with historical records of severe declines reported for many North Western European *Rhinolophus hipposideros* colonies in the 1950–1970s (Bontadina et al., 2000; Ohlendorf, 1997).

4.5 | Bottleneck classification: possible applications, current limitations, future potential

Identifying drivers of genetic diversity loss can be difficult when neither connectivity loss nor decreases in population size can be ruled out (e.g., Pacioni et al., 2015). The approach for bottleneck classification presented here can greatly contribute to a better understanding of factors causing genetic diversity loss for study systems meeting the requirements imposed by its current limitations. However, the approach we developed to distinguish demographic from disconnection-induced bottlenecks based on their respective effects on IBD slopes currently will not be informative for data sets where the observed IBD slope ratio is one or less, because such patterns can arise from both disconnection and demographic crashes followed by population growth. In those cases, additional independent data are required to tease these two scenarios apart. Furthermore, the grid-structure and parameters used in our simulations (deme size, reduction factors, full or no regrowth) are simplified approximations that deliver qualitative results only. Future, more comprehensive simulation studies accounting for the spatial distribution of populations (here, colonies) and the resulting patterns of migrant exchanges may contribute to the development of a yet more robust approach allowing unambiguous bottleneck classification.

4.6 | Implications for conservation

Reductions in either population size or connectivity can negatively impact genetic diversity and drive populations into an extinction vortex

(Benson et al., 2016; Blomqvist et al., 2010). Consequently, both represent legitimate conservation targets, but resource limitations may require to prioritise one of these two aspects for conservation purposes. The approach presented here can help to identify the dominating driver of genetic diversity still prevalent in present times, which is most likely to have a tangible positive effect on genetic diversity.

The pronounced negative impact of demographic bottlenecks for genetic diversity detected in our study confirms the importance of regular monitoring, as it provides an early-warning system to rapidly detect demographic declines, and can therefore act as a trigger to identify causes and appropriate countermeasures (Fritze & Puechmaille, 2018). Currently, monitoring data suggest a positive growth trend for the majority of bottlenecked colonies in our study system (Tress et al., 2012; Van der Meij et al., 2015), suggesting that the initial cause of the past demographic collapse has already been rectified. This is in concordance with better protection of roosts (Marnell & Presetnik, 2010) and the banning of DDT in Europe (Council Directive 79/117/EEC, 1978), whose use has been suggested to be one of the major causes of the species' population declines (Bontadina et al., 2000).

By comparing a broad variety of connectivity measures, and considering different spatial scales, we could show that present-day connectivity is a major driver of genetic diversity in our study system. Our findings confirmed the importance of considering different spatial scales in conservation planning for the protection of genetic diversity (Ishiyama et al., 2015), also for *Rhinolophus hipposideros*. Elucidating the geographic scale at which the positive impact of connectivity on genetic diversity is most profound (60–80 km, Figure 3) revealed that the protection of one or few colonies at a local scale is insufficient for long-term preservation of genetic diversity. At the same time, colonies in key locations that spatially connect clusters of colonies (e.g., Vex1 and Pic17 in Figure S1) warrant prioritization in conservation efforts, as gaps of more than 60–80 km between colonies result in their disconnection.

Taken together, our results thus highlight that today, large-scale connectivity dominates over local colony size in maintaining genetic diversity of European leading edge populations of *Rhinolophus hipposideros* (Figure 3c), whereas in the past, the influence of demography most likely prevailed (Figure 5). Given that within-species variations in genetic diversity are ubiquitous, yet poorly understood, applying similar approaches as presented here could help to understand major drivers of genetic diversity. This knowledge can provide a foundation for the development of sustainable conservation strategies to mitigate the ongoing massive loss of genetic diversity in many species worldwide.

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AUTHOR CONTRIBUTIONS

E.J.P. and S.J.P. designed the project. E.J.P., G.K., and S.J.P. acquired funding and supervised the project. Project administration fell to E.J.P. and S.J.P., E.J.P. and G.K. provided facilities. L.L., P.N., M.B., and W.S. collected samples. L.L. and P.-L.J. carried out the formal analyses with help from A.-L.B. and D.F. (genetic and statistical analyses, respectively). E.J.P., L.L., P.-L.J., and S.J.P. interpreted the results. E.J.P. and P.L.J. developed simulations and models, respectively. E.J.P., D.F., P.-L.J., and S.J.P. visualized the results. L.L. and P.-L.J. wrote the first draft, which was subsequently edited by L.L., P.-L.J., G.K., E.J.P., and S.J.P., L.L., P.-L.J., G.K., E.J.P., and S.J.P. revised the manuscript. All authors approved the final version of the manuscript.

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CONFLICTS OF INTERESTS

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The distinct genotypes detected in the respective colonies and the empirical data used for measuring connectivity are available from Dryad (<https://doi.org/10.5061/dryad.27q46m2>).

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SUPPORTING INFORMATION

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