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RESEARCH ARTICLE

Variable time lags in genetic response of three temperate forest herbs to 70 years of agricultural landscape change

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Abstract

Context Agriculture-driven land-use changes over the past decades have not only reduced the amount of habitat for species but also influenced the genetic exchange among the remaining fragmented populations. Many recent studies have found a delayed response in population genetic diversity and differentiation of species in fragmented habitats to past landscape disturbances, a so-called time lag. However, the specific role of species' individual reproductive traits and the population genetic measures used remain poorly understood.

Objectives We examined the impact of past and current agricultural landscape composition in temperate Europe on the population genetic structure of three long-lived, slow-colonizing forest herb species – Anemone nemorosa, Oxalis acetosella and Polygonatum multiflorum, which vary in their reproductive traits.

Methods We considered four time points in history (mid-1900s, 1985, 2000 and 2017) to identify the potentially different length of time that is needed by each species to respond to landscape change. We also explored the impact of using different genetic measures in quantifying the time lags.

Results Our findings show that despite substantial landscape alterations about 70 years ago, the mid-1900s landscape composition was not reflected in the current genetic diversity and differentiation of the three species. This indicates a possible unexpected quick genetic adjustment of these species. Nevertheless, by combining the signals of multiple genetic measures, we found that O. acetosella, which

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reaches sexual maturity earlier than the other two species and is self-compatible, showed signs of faster genetic adjustment to these landscape changes. In contrast, A. nemorosa and P. multiflorum, which take longer to reach sexual maturity, might exhibit longer time lags that were beyond this study's time frame.

Conclusions This study underscores the importance of considering the species' reproductive traits and especially the role of temporal scales of different genetic measures when investigating the impact of landscape history on current population genetic structures.

Keywords Time lag · Sexual maturity · Reproductive traits · Population genetic measures · Land-use change

Introduction

Anthropogenic alterations to landscapes, particularly common in those dominated by agriculture, pose significant threats to wild animals and plants in many ways (Fischer and Lindenmayer 2007), among others by affecting population genetic structure (Wang et al. 2017). Landscape disturbance, including land-cover change, habitat fragmentation and land-use intensification, can lead to a reduction of population sizes, restricted dispersal and genetic exchange among populations—key factors for maintaining a stable population genetic structure and thus for the long-term survival of populations (Keyghobadi 2007).

Disturbances both within habitats and in the surrounding landscape affect population genetic structure due to the altered conditions within the habitat itself and the affected gene flow occurring across the landscape. Studies have shown that historical habitat quality (Honnay et al. 1999; Vere et al. 2009), habitat connectivity, habitat size or habitat loss events have left imprints in the current genetic diversity (Spear and Storfer 2008; Plue et al. 2017; Reisch et al. 2017). Meanwhile, changes in the landscape matrix in which the habitat is embedded are often neglected or considered as hostile, despite their constant changing and their importance for movement of individuals, dispersal of gametes and recruitment of juveniles or seedlings (Murphy and Lovett-Doust 2004).

Due to disturbances, the landscape may evolve more rapidly than the corresponding shifts in population genetic structure, which often lags behind environmental changes (Spear et al. 2016). If neglected, this time lag may cause misinterpretation of the role of the current landscape in forming the population genetic structure and may mislead conservation management (Manel and Holderegger 2013; Keller et al. 2015). A time lag is measured by the time passed between a disturbance event and the corresponding response in the population`s genetic structure (Epps and Keyghobadi 2015). The cycle of disturbances and responses is, however, rarely completed. More often, the next disturbance has already happened before the reaction to the previous disturbance becomes detectable (Caplins et al. 2014; van Rees et al. 2018). Furthermore, except for drastic catastrophes like volcanic eruptions, the current landscape often bears strong resemblance to the previous one since landscape changing is an ongoing process (Palang et al. 2000). Additionally, landscape development is not homogeneous across time and space, with some elements changing more rapidly or extensively than others (Manley et al. 2009). Therefore, it can be challenging to identify the impact of the entire landscape change from a specific point in time. By establishing a contrast between only two points in time, one representing the current landscape and another one a certain point in the past (Honnay et al. 2006; Helm et al. 2009; Aavik et al. 2017; Reisch et al. 2017, but see Münzbergová et al. 2013; Baessler et al. 2010; Zellmer and Knowles 2009), we risk overlooking potentially important landscape changes between or prior to the selected time points and over-simplifying the temporal dynamics of different landscape elements. Instead, considering each landscape element with its individual temporal scale, while also accounting for the overall development of the surrounding landscape over a certain period, may be more appropriate for quantifying time lags.

The detectable time lag in response to landscape changes can vary significantly among species due to their reproductive traits, such as generation time and dispersal ability (Epps and Keyghobadi 2015). For instance, species with overlapping generations tend to react more slowly to fragmentation compared to those with no overlapping generations (Lloyd 2013). Organisms that are highly mobile and have a short life span can quickly show the impact of recent landscape change (Epps et al. 2013; Blair

et al. 2015). Conversely, species with longer life spans or those that are sedentary, such as many perennial plants, typically exhibit longer time lags (Epps et al. 2013; Aavik et al. 2019). An important aspect of generation time is the age at which an organism reaches sexual maturity. Combined with lifespan, this factor influences how quickly the demography of a population can change and thus how fast the population genetic structure can react to landscape change (Lee et al. 2011). However, this important aspect remains underexplored in current studies.

Studies addressing dispersal ability in mobile organisms often focus on landscape elements that significantly influence their movement patterns and consequently shape their gene flow (Cushman et al. 2006; Epps et al. 2007). In contrast, dispersal ability in plants is not determined by active movement but by passive pollen and seed dispersal through various vectors (Auffret et al. 2017). Different landscape elements can influence the vectors variably, and even the same element can have opposite effects depending on the vector species involved (Baessler et al. 2010; Naaf et al. 2022). Plant species that rely on highly mobile pollen or seed vectors benefit from their efficiency in transporting pollen or seeds over large distances, which helps maintain the population genetic diversity across fragmented habitats (Castilla et al. 2017). However, these highly mobile pollen or seed vectors could be particularly vulnerable to habitat loss and fragmentation due to their high degree of specialization or species-specific behaviours (Jauker et al. 2009; Torres-Vanegas et al. 2019). This vulnerability can induce faster responses to landscape changes and consequently, faster shifts in the population genetic structure of the associated plant species compared to species with less mobile vectors (Landguth et al. 2010). The complexity increases as different vector species may interact with the landscape in diverse ways, and thus affect the gene flow among plant populations differently (Jauker et al. 2009). Additionally, identifying landscape features that influence individual vector movement is insufficient to quantify the overall distribution of dispersal events in plant populations, which result from cumulative movements of multiple dispersing individuals (Dyer 2016).

Measuring time lags becomes even more complicated when we consider that different population genetic measures may take varying amounts of time to reach a new equilibrium after a disturbance (Epps and Keyghobadi 2015). Allelic richness has been shown by simulation to react quicker to disturbance than heterozygosity (Lloyd et al. 2013). F_{ST}, originally an estimation of inbreeding coefficient, and its related measures such as G_{ST}, reflect rather the gene flow that occurred in the historical landscape (Aparicio et al. 2012; Epps et al. 2013), while individual-based genetic distance measures, such as D_{PS}, were used to detect recent landscape changes (Landguth et al. 2010; Murphy et al. 2010), although its rate of approaching an equilibrium is still largely unstudied. Additionally, within-population heterozygosity reaches an equilibrium slower than heterozygosity-related differentiation measures such as G''_{ST} (Pannell and Charlesworth 2000). It is thus important to consider the very different time lags that can be expected and combine different measures to cover the potential range of time lags (Epps and Keyghobadi 2015).

Comparative landscape genetics of multiple species in a shared landscape is a relatively unexplored area (Waits et al. 2016). This is particularly true for the influence of species' reproductive traits and different population genetic measures on the length of time lags. We conducted a multi-species study across three agricultural landscape windows, each of which contained all three species and went through constant anthropogenic disturbances since the mid-1900s. We chose three common forest herb species *Anemone nemorosa* L., *Oxalis acetosella* L. and *Polygonatum multiflorum* (L.) All., which share similar characteristics as slow colonizers (Honnay et al. 2005), but differ in their time to reach sexual maturity and in their associated pollinators. We used various measures of genetic diversity (A_r, H_o) and differentiation (G''_{ST}, D_{PS}) to quantify the legacy of the past landscape composition from multiple points in time in the population genetic structure of these species.

Specifically, we tested the following hypothesis:

(1) The past landscape composition explains the current genetic diversity and differentiation of forest herb populations better than does the present-day landscape composition.
(2) The time lags differ among the three species, depending on their traits. Specifically, we expect that (a) *P. multiflorum* and *A. nemorosa*, which need a long time to reach sexual maturity, exhibit a longer time lag compared to *O. acetosella*,

which can reproduce after the first year, and (b) *P. multiflorum*, which is pollinated by highly mobile pollinators, exhibits a shorter time lag than *A. nemorosa*, which is associated with less mobile pollinators.

(3) The time lags differ between alternative population genetic measures given their different reaction times. In particular we expect that (a) allelic richness exhibits shorter time lags than does heterozygosity, and (b) D_{PS} exhibits shorter time lags than does G''_{ST}.

Methods

Study species

The three studied species (*Anemome nemorosa*, *Oxalis acetosella*, *Polygonatum multiflorum*) are common perennial temperate forest herbs that share a similar life history of being slow-colonizing forest specialists (Verheyen et al. 2003; Schmidt et al. 2014). They all flower in spring (Klotz et al. 2002) and can propagate vegetatively besides seedling recruitment (Holderegger et al. 1998; Berg 2002; Kosiński 2012). However, the species differ in their number of chromosomes, with *P. multiflorum* and *O. acetosella* being diploid while *A. nemorosa* being tetraploid (Baumberger 1971). They also differ in other reproductive traits.

Anemone nemorosa and *Polygonatum multiflorum* both take mostly 10 years or more to reach their sexual maturity (Shirreffs 1985; Kosiński 2015) and depend on pollinators for sexual reproduction (Müller et al. 2000; Kosiński 2012). *A. nemorosa* is visited by different groups of insect pollinators (Shirreffs 1985; Erbar and Leins 2013), with solitary bees and hoverflies being the most important ones (Naaf et al. 2021). These insect groups typically have limited foraging distances and are unlikely to cross the agricultural matrix between forest patches frequently (Feigs et al. 2022). In contrast, *P. multiflorum* is mainly pollinated by long-tongued bumblebees (Kosiński 2012; Feigs et al. 2022), which can cover several hundred meters and traverse the agricultural matrix between forest patches regularly (Darvill et al. 2004; Knight et al. 2009; Redhead et al. 2016).

The third species, *Oxalis acetosella*, can already reproduce sexually after the first year (Berge et al. 1998). It is considered to produce most of its seeds from cleistogamous flowers (Packham 1978; Berg and Redbo-Torstensson 2000). However, our previous research indicated that *O. acetosella* is mostly out-crossing (Naaf et al. 2021) with potential flower visitors including flies, thrips, beetles, bees and bumblebees (Packham 1978; Willemstein 1987).

Population genetics and attributes

We conducted the data collecting in spring of 2018 within three landscape windows of 5 km × 5 km, namely western Germany (GeW), eastern Germany (GeE) and southern Sweden (SwS) (Fig. 1A). All landscape windows represent typical agriculture landscapes, in which forest fragments are embedded in an agricultural matrix (see the change of the landscape along the time in Figure S1).

In each landscape window, we aimed to sample six populations per species, each of which should be at least 100 years old. We defined a population as a spatially distinct group of shoots > 100 m apart from other shoots and estimated the age of populations by assessing the persistence of their habitat, forests in this case, using historical aerial-photographs. Habitat persistence may not directly indicate population age but serves as a suitable proxy due to the three studied species being slow-colonizing forest specialists (Verheyen et al. 2003). In each population, we aimed to take leaf samples from 20 healthy flowering individuals which were at least 10 m away from each other to avoid repeated sampling of the same clone. A total of 1075 leaf samples were included in this study (Table S2). We extracted total genomic DNA from the leaf samples and genotyped them based on sets of nuclear microsatellite markers (Table S2). These markers were developed for congeneric species (*A. nemorosa* and *P. multiflorum*) and newly developed for *O. acetosella* by AllGenetics & Biology SL (Spain) on demand. The applied marker sets comprised six, ten and six markers with a total number of 96, 47 and 136 alleles for *A. nemorosa*, *O. acetosella* and *P. multiflorum*, respectively. Samples for which genotyping failed at more than one locus were excluded. Twenty three percent of populations had fewer than 20 samples (Table S2), either due to a small population size or genotyping failure. We repeated the genotyping procedure for 10% of the samples to estimate the multi-locus genotyping

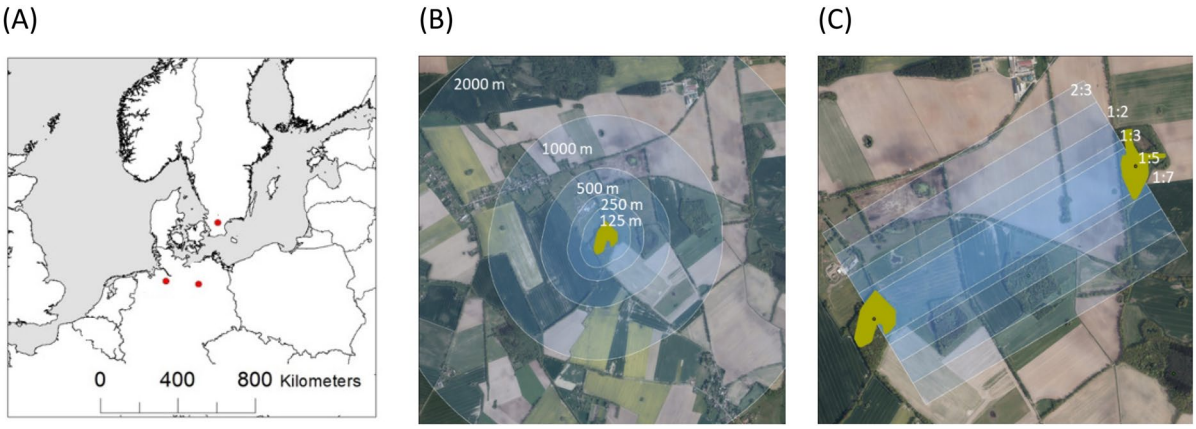

Fig. 1 **A** Location of the three landscape windows (GeE, GeW, SwS) in Europe. **B** Buffer zones around focal populations (node level) with five different buffer distances: 125 m, 250 m, 500 m, 1000 m, 2000 m. **C** Land strips between two populations (link level) with five different width-to-length ratios: 1 to 2, 1 to 3, 1 to 5, 1 to 7 and 2 to 3

error rate (3.7%, 2.7% and 4.0% for *A. nemorosa*, *O. acetosella* and *P. multiflorum*, respectively). Finally, we excluded all repeated multi-locus genotypes (MLG) in a population as assumed clones from our analysis. Repeated MLG were randomly distributed across all regions. The complete allele tables are provided in Supplementary Information (Table S2).

In each sampled population, we also estimated census population size (PopSize) (Table S2), since population size is an important basic determinant of population genetic diversity (Young et al. 1996). For *A. nemorosa* and *O. acetosella*, we estimated this attribute by extrapolating flower density from a known area to the complete population area. The complete population area was either the corresponding forest patch area, or demarcated in the field by marking the outmost flowering shoots of a population with a GPS device. For calculating flower density, we counted flowering shoots along a two-meter-wide transect until reaching 40 flowers, then measured the transect length and calculated the density as 40/(2 m × length). The flower density of the population was then averaged across five randomly placed transects within the population. For *P. multiflorum*, we calculated the census population size by counting all flowering shoots in the population area since *P. multiflorum* individuals tend to grow in small patches rather than in a carpet-like fashion across the population area, which is typical for *A. nemorosa* and *O. acetosella*. Similarly, we included geographical distance (GeoDist) between populations as a covariable in determining the effect of landscape metrics on genetic differentiation, since geographical distance often influences genetic differentiation (Slatkin 1985) (Table S2).

For all three species, we calculated two measures of genetic diversity within populations, i.e. allelic richness (A_r) and observed heterozygosity (H_o). Since allelic richness is only comparable among similar sample sizes, we calculated rarefied allelic richness based on the mean number of MLG per population across three landscape windows, i.e. 19, 18 and 19 samples for *A. nemorosa*, *O. acetosella* and *P. multiflorum*, respectively. We used the mean instead of the minimum sample size as a trade-off to avoid losing too much information, given the fact that in some populations, the number of samples with distinct MLG were very small (Table S2). We sampled every detectable genet in very small populations so that these populations were 100% represented despite the small sample size, thus the allelic richness is not biased through extrapolation.

Further, we used two measures to quantify pairwise genetic differentiation among populations i.e. G''_{ST} and D_{PS}. G''_{ST} is based on heterozygosity, like traditional F_{ST} and G_{ST}. It is recommended to be used with microsatellite markers and for small sample sizes (Meirmans and Hedrick 2011). D_{PS} is calculated using the complement of the proportion of shared alleles (Bowcock et al. 1994) and is therefore easy to interpret.

For details on genetic analyses and the calculation of population genetic variables see Naaf et al. (2021).

Past landscape composition

In order to detect potential time lags, we selected four points in time for which aerial photographs were available (Table 1, Figure S1): the middle of the twentieth century, around 1985, around 2000 and 2016/2017 (hereafter referred to as "mid-1900s", "1985", "2000" and "2017"). If a significant response of the population genetic structure was detected, this would yield potential time lags of about 70 years, 35 years, 20 years and 0 years, respectively.

We georeferenced and digitized the aerial photographs from these four points in time to quantify the past and recent landscape composition in each of the three landscape windows. The exact years of the available aerial photographs vary from landscape window to landscape window and are listed in Table 1. The temporal category addressing the time points instead of the exact year will be used hereafter for the sake of clarity.

For each time point and landscape window, we defined two spatial units: (a) buffer zones around each plant population (node level, Fig. 1B); (b) rectangular land strips connecting the centres of each pair of plant populations (link level, Fig. 1C). The node level was used to analyse the effects of landscape metrics on genetic diversity, while the link level was employed to their impact on genetic differentiation. We then quantified landscape composition using three types of metrics. Area-based metrics measured the percentage cover of different land-use types within buffer or strip area, while length-based metrics assessed the relative length of linear landscape elements, expressed as the total length of a given element divided by the area of the buffer or strip. Additionally, structure-based metrics incorporated all land-use types (Table 2). We applied several different buffer distances to reflect ranges of sizes and forage distances of potential pollen dispersal vectors: 125 m, 250 m, 500 m, 1000 m, and 2000 m. Similarly, we used five different width-to-length ratios of the rectangular land strips: 1to2, 1to3, 1to5, 1to7, 2to3 (Fig. 1B and C).

Table 1 Available aerial photographs of each landscape window categorized into four points in time: mid-1900s, 1985, 2000 and 2017 in order to allow the alignment and comparison of the landscape windows

Landscape Window	Temporal category			
	mid-1900s	1985	2000	2017
GeE	1953	1985	2002	2017
GeW	1963	1987	2000	2016
SwS	1947	1986	2004	2017

Data analysis

In order to determine the time lag of each species and to quantify the contributions of the past and contemporary landscape composition to explaining the current population genetic patterns of the forest herbs, we applied linear mixed-effects models (LMMs) with landscape window as a random intercept term.

For each species, we modelled population genetic diversity (node level) and differentiation (link level) as a function of a set of landscape metrics. At the node level, we included population size (PopSize), and at the link level, the edge-to-edge geographical distance (GeoDist) as a basic population genetic determinant, which remained in the model throughout the analysis.

Table 2 Landscape metrics that were included in the analysis and their descriptions

Metric names	Description
Area-based	
	Percent cover of…
INTENSIVE	Intensively used agricultural land (incl. intensive grassland and arable land)
FOREST	Forest, including coniferous and deciduous forests
ORCHARD	Traditional orchards
SEMVEG	Semi-natural vegetation (incl. ruderal vegetation, heath, swamps etc.)
SEMGRASS	Semi-natural grassland
SETTLE	Settlement area
Length-based	
	Relative length of…
LWATER	Water courses (incl. draining ditches)
LWOOD	Woody elements (incl. hedgerows and tree lines)
LROAD	Roads
Structure-based	
EDGEDEN	Density of edges of all land-use parcels
SHANNON	Shannon index of area land-use types

Additionally, at the link level, we accounted for the correlation among population pairs that included a shared population by defining a correlation structure within the lme function using the function corMLPE (Pope 2022). Prior to modelling, all variables were Box-Cox transformed to increase the symmetry of their distribution and then centred and scaled to yield standardized regression coefficients.

We designated the most recent point in time, 2017, as the reference time point and allowed landscape metrics from this time to enter the global model first. Subsequently, landscape metrics from earlier points in time were allowed to enter the global model step by step (Fig. 2). We then identified the time lag based on the oldest time point in the model that significantly lowered the AIC_c (details provided below). In all steps, before entering the global model, landscape metrics were selected using the following procedure:

Each landscape metric was assigned with one buffer distance (node level) or one width-to-length ratio (link level) that yielded the lowest AIC_c by fitting LMMs individually. We compared the same landscape metric over different points in time and selected the one with the lowest AIC_c to enter the global model. Given the large number of landscape metrics, we only considered those metrics in the global model that showed a significant effect in the single-metric models at a significance level of $\alpha = 0.15$, based on a likelihood ratio test against the reduced model with only the respective basic determinant. We then fitted the global models for all subsets of the predictors with two restrictions (1) any correlations among predictors ≥ 0.7 were not tolerated, and these terms were not allowed to enter the global model simultaneously; (2) we allowed a maximum of two and four landscape metrics in models at the node and link level, respectively, given the limited sample size.

The single-best model with the lowest AIC_c at each step was selected as the final model. In the step involving only landscape metrics from 2017, we designated this model as Model Ref. In subsequent steps, the other three final models were named as follows: Model I (incl. 2017 and 2000), Model II (incl. 2017, 2000 and 1985) and Model III (incl. 2017, 2000, 1985 and mid-1900s) respectively (Fig. 2). We then compared models involving past landscape metrics against Model Ref, in the sequence of Model I, Model II, and Model III as our hypothesis was that including historical landscape metrics would better explain genetic patterns than using only the most current landscape metrics. The first model to achieve a decrease in AIC_c of 2 or more ($\Delta AIC_c \geq 2$) was considered optimal, and the earliest time point included in this model denoted the corresponding time lag. In a second step, we compared the subsequent models against this optimal model to assess whether including landscape metrics based on earlier points in time could further improve model's explanatory power, using $\Delta AIC_c = 2$ as a threshold. Should this be the case, the time lag would be adjusted accordingly.

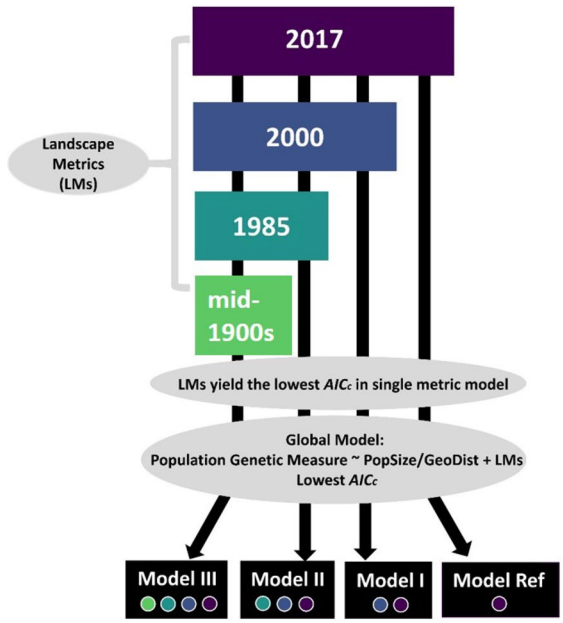

Fig. 2 Flowchart illustrating the process of selecting four final models (Model I, II, III and Model Ref). These models incorporate landscape metrics (LM) from different time points and population size (PopSize) to explain genetic diversity or geographical distance (GeoDist) to explain genetic differentiation. The four colours represent the four time points (mid-1900s, 1985, 2000 and 2017) at which the landscape metrics were included

Since all alternative models following the single-best model with $\Delta AICc \leq 2$ were considered equivalent in explaining variance, we refitted all candidate models of the step defining the time lag, and submitted all models with $\Delta AICc \leq 2$ to conditional model averaging (Grueber et al. 2011; Harrison et al. 2018). The averaged coefficient of each term, i.e. landscape metric, then reflected the effect size of this term when

it was included and the sum of Akaike weights over all component models in which this term appeared represented the general likelihood of this term's relevance.

All analyses were conducted in R (R version 4.1.3).

Results

Landscape changes since the mid-1900s

Intensively used agricultural land (INTENSIVE), i.e. arable land and intensive grassland were the dominant land use types at all points in time (Figure S3, Table S6) but slightly decreased across the four points in time. Semi-natural grassland (SEMGRASS) decreased in eastern Germany but increased in Sweden until 1985, while forest cover increased in all three landscape windows (Figure S3). Semi-natural vegetation (SEMVEG) tended to increase, while traditional orchard (ORCHARD) decreased, with both consistently comprising only small portions of the overall landscape. The total length of linear landscape elements generally increased over time in all three landscape windows, although there were some time points where a decrease was observed. The main increment came from woody elements (LWOOD) (Figure S4, Table S6). Generally, the most significant relative change in terms of the percentage of each land-use type occurred between the mid-1900s and 1985 (Table S7). Also, during this time, edge density of land-use parcels (EDGEDEN) of GeE and GeW decreased most strongly (Figure S5), while land-use diversity (SHANNO) remained largely stable with minor fluctuations in Germany (GeE, GeW).

Landscape metrics calculated within different buffers around the studied populations and within land strips between the studied populations showed similar trends (Figure S8) and reflected the compositional changes of the entire landscape windows (Figure S3, S4 and S5).

Time lags in current population genetic diversity and genetic differentiation

For all three species, models that were used to explain observed heterozygosity (H_o) had a lower AIC_c when incorporating past landscape metrics in addition to landscape metrics from 2017 (Table 3). Specifically, adding landscape metrics from both 2000 (Model I) and subsequently from 1985 (Model II) improved the model for *P. multiflorum* ($\Delta AIC_c = 7.0$), which corresponds to a time lag of 35 years. For *O. acetosella*, Model I was by far the best in explaining H_o ($\Delta AIC_c = 14.3$) and the time lag was identified to be 20 years. For *A. nemorosa*, the improvement in model quality was not significant when past landscape metrics were included ($\Delta AIC_c = 0.9$). However, *A. nemorosa* was the only species for which the inclusion of past landscape metrics significantly lowered the AIC_c of the models that were used to explain allelic richness ($\Delta AIC_c = 2.6$) (Table 3). The corresponding time lag was 35 years.

Models including past landscape metrics explained G''_{ST} and D_{PS} better for *O. acetosella*, but not for the

Table 3 The difference in AIC_c (ΔAIC_c) of the final models that included only present landscape metrics (Model Ref), and those including both present and past landscape metrics (Model I: incl. 2017 and 2000; Model II incl. 2017, 2000 and 1985; Model III: incl. 2017, 2000, 1985 and mid-1900s)

		III (mid-1900s + 1985 + 2000 + 2017)	II (1985 + 2000 + 2017)	I (2000 + 2017)
A_r				
	A. nemorosa	2.6	**2.6**	1.5
	O. acetosella	− 1.0	− 1.4	− 2.4
	P. multiflorum	1.6	1.6	0.5
H_o				
	A. nemorosa	0.9	0.6	0.4
	O. acetosella	14.3	14.3	**14.3**
	P. multiflorum	7.0	**7.0**	**4.7**

Models were used to explain allelic richness (A_r) and observed heterozygosity (H_o) of *A. nemorosa*, *O. acetosella* and *P. multiflorum*. A reduction of AIC_c compared to the previous step by at least 2 were marked bold

other two species (Table 4). The identified time lag was 20 years ($\Delta AIC_c = 18.5$ and $= 7.2$, respectively). Past landscape metrics from 1985 and the mid-1900s did not significantly improve the models for *P. multiflorum* and *A. nemorosa* (Table 4).

Detailed modelling results are provided in Tables S9–S11.

Effects of the past landscape on genetic diversity and differentiation

Among the various landscape metrics that contributed significantly to explain population genetic diversity and differentiation, semi-natural grassland (SEMGRASS), traditional orchards (ORCHARD), Shannon diversity of land-use types (SHANNO), woody elements (LWOOD), and semi-natural vegetation (SEMVEG) had significant past effects (Figs. 3 and 4).

Discussion

Past landscape characteristics, mainly from 2000 and 1985, have left their traces in the current population genetic diversity of all three forest herb species, and in the genetic differentiation among populations of *O. acetosella*. The landscape metrics from the mid-1900s were not needed to explain the herbs' population genetic structure, despite the fact that most strong relative changes of the landscape composition occurred between the mid-1900s and 1985 (Table S7). This finding was surprising given the long life span of all three species. Conversely, we did not detect any signals of time lag using genetic differentiation measures in *A. nemorosa* and *P. multiflorum*. This raises the question of which temporal scale of time lags we should actually expect, and leaves the second and third hypothesis (species-specific lags based on traits, and genetic measure differences, with shorter lags for allelic richness and D_{PS}), partly untested.

Lacking evidence for legacies of the mid-twentieth century

It was surprising that in our study, whenever we detected a time lag, landscape metrics from the mid-1900s did not significantly contribute to explaining any of the population genetic measures of the species examined. This absence of a signal from the mid-1900s may suggest that early landscape changes before the mid-1900s, particularly those related to the landscape matrix, are already manifested in the population genetic structures of the three studied species. Although it is often expected that species with a long life span and a low dispersal ability exhibit long time lags, sometimes exceeding decades (Münzbergová et al. 2013; Reinula et al. 2021, 2024), the actual response time reflected in population genetic measures is often affected by population attributes, e.g. population size. A small effective population size, which is not uncommon and often overestimated in partially clonal species (Trepdino 2012; Gargiulo et al. 2023), can accelerate the process of reaching a new equilibrium (Lloyd et al. 2013; Epps and Keyghobadi 2015). Furthermore, we

Table 4 The difference in AIC_c (ΔAIC_c) of the final models that included only present landscape metrics (Model Ref), and those including both present and past landscape metrics (Model I: incl. 2017 and 2000; Model II incl. 2017, 2000 and 1985; Model III: incl. 2017, 2000, 1985 and mid-1900s)

		III (mid-1900s + 1985 + 2000 + 2017)	II (1985 + 2000 + 2017)	I (2000 + 2017)
G''_{ST}				
	A. nemorosa	1.0	1.0	0
	O. acetosella	14.13	18.5	**18.5**
	P. multiflorum	1.2	1.2	0.3
D_{PS}				
	A. nemorosa	1.3	1.3	0.1
	O. acetosella	7.4	7.4	**7.2**
	P. multiflorum	0.5	0.5	0.5

Models were used to explain G''_{ST} and D_{PS} of *A. nemorosa*, *O. acetosella* and *P. multiflorum*. A reduction of AIC_c compared to the previous step by at least 2 were marked bold

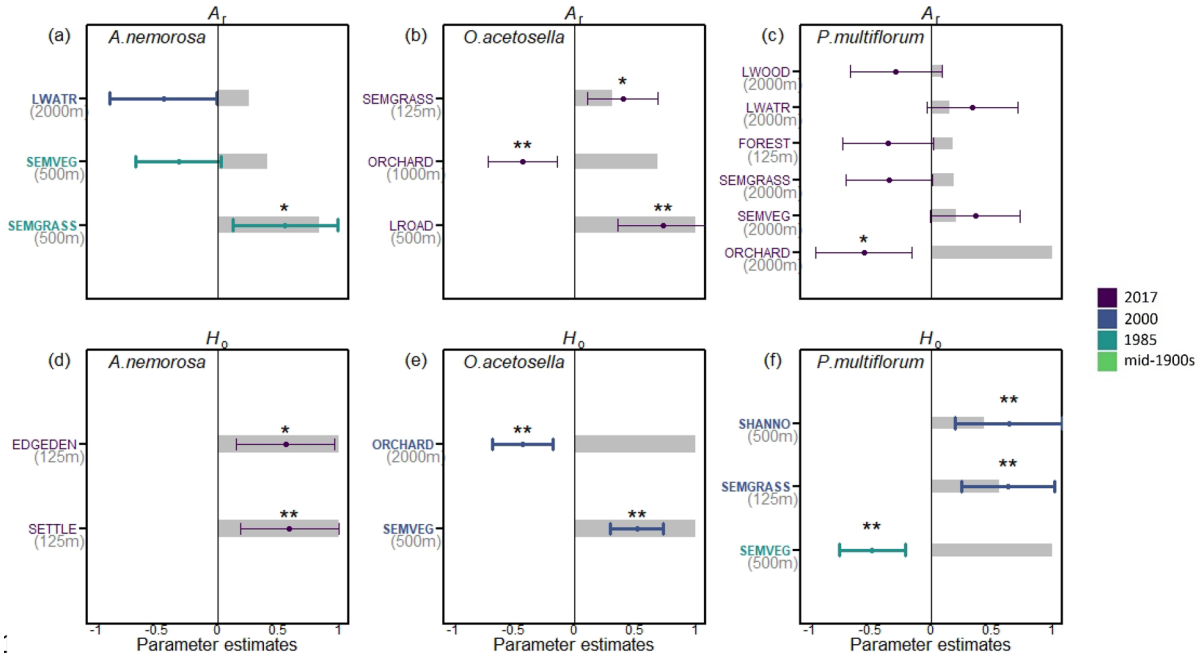

Fig. 3 Effects of current and/or past landscape metrics on genetic diversity (A_r: allelic richness, H_o. observed heterozygosity) of *A. nemorosa, O. acetosella* and *P. multiflorum*. Illustrated are the conditionally averaged coefficients of all models with $\Delta AIC_c \leq 2$ at the step that defined the time lag. If no time lag was identified, the conditionally averaged coefficients represent the present landscape only. Shown are landscape metrics with their sum of Akaike weights (grey bars), regression coefficient (points), 95% confidence interval (error bars), and statistical significance according to conditionally averaged models (**: $p < 0.01$; *: $p < 0.05$). The effects of past landscape metrics were marked in bold

might underestimate the role of seedling recruitment within the populations of the studied species (Berg 2002; Verheyen and Hermy 2004). A high seedling recruitment may alter population demography, resulting in a dominance of younger individuals. Consequently, the population genetic structure may reflect more recent landscapes.

This result also raised the question of whether the intensity or magnitude of the landscape change is relevant to the detectable duration of time lags, considering the strong relative changes of landscape composition that occurred between the mid-1900s and 1985 (Table S7). It was assumed that a strong change in landscape composition would also lead to a significant alteration in functional connectivity (Auffret et al. 2015). Drastic landscape changes relevant for gene flow were often found being reflected in genetic diversity even after a relatively short exposure period (Vandergast et al. 2007; Zellmer and Knowles 2009). However, significant changes in structural connectivity do not necessarily lead to substantial changes in functional connectivity (Aavik et al. 2014), which is essential for gene flow in heterogeneous landscapes. This may be due to the robustness and resilience of the pollination and seed dispersal community to environmental perturbations (Bascompte et al. 2006; Buono et al. 2023). Thus, despite the considerable changes in landscape composition, we could not detect distinctive effects of the landscape from the mid-1900s on current population genetic structure.

Different time lags depending on species' reproductive traits

Keyghobadi et al. (2005a) detected a heterozygosity time lag of 40 years in a pine species that reaches sexual maturity at a similar time as *A. nemorosa* and *P. multiflorum.* We used the same measure and detected a time lag of 35 years in *P. multiflorum,* while *O. acetosella* showed a time lag of 20 years (Table 3, Fig. 3). This finding partly supported our

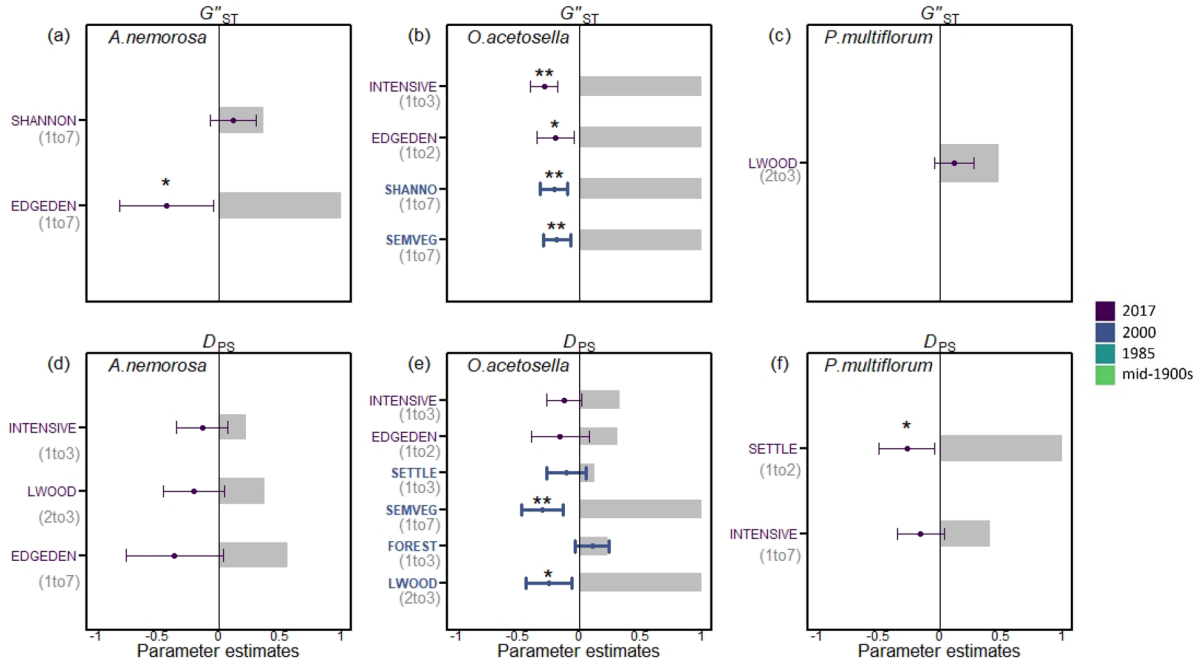

Fig. 4 Effects of current and/or past landscape metrics on genetic differentiation (G''_{ST}, D_{PS}) of *A. nemorosa, O. acetosella* and *P. multiflorum*. Illustrated are the conditionally averaged coefficients of all models with $\Delta AIC_c \leq 2$ at the step that defined the time lag. If no time lag was identified, the conditionally averaged coefficients represent the present landscape only. Shown are landscape metrics with their sum of Akaike weights (grey bars), regression coefficient (points), 95% confidence interval (error bars), and statistical significance according to conditionally averaged models (**: $p < 0.01$; *: $p < 0.05$). The effects of past landscape metrics were marked in bold

hypothesis that species with a shorter generation time exhibit shorter time lags. However, the absence of a significant signal in *A. nemorosa* introduced uncertainty regarding the question in how far other traits might counterbalance the effects of generation time. Additionally, we found no clear evidence that *A. nemorosa*, which is pollinated by less mobile pollinators, exhibits longer time lags than *P. multiflorum*, which is pollinated by highly mobile pollinators.

Nevertheless, we detected a tendency for the AIC_c to become lower when including landscape metrics from the mid-1900s to explain H_o of *A. nemorosa*, although without achieving the threshold of 2. Combined with simulation results suggesting that H_o requires a longer time to respond (Lloyd et al. 2013), we speculate that the time lag of *A. nemorosa* may correspond to landscape structures further back in time not covered within the range of our study. This speculation is even more plausible, considering that polyploidy can buffer the genetic response of plants to habitat fragmentation (Plue et al. 2018), and that *A. nemorosa* is tetraploid (Shirreffs 1985). Specifically, in our study, this suggests that *A. nemorosa* might have a time lag exceeding 70 years using H_o, which is longer than that of *P. multiflorum*. This possibility is further supported by the result on allelic richness, which showed a time lag of 35 years only for *A. nemorosa*, but not for *O. acetosella* and *P. multiflorum*.

In contrast to the results on genetic diversity, the results on genetic differentiation, where we found no signal for any time lags for *A. nemorosa* and *P. multiflorum* (Table 4, Fig. 4), provide ambiguous information, making it difficult to compare time lags across species with different reproductive traits. The result could indicate: (a) there is no time lag in genetic differentiation in *A. nemorosa* and *P. multiflorum*; or (b) the time lags of both species exceed 70 years. Our results thus raised the question of which time lags we should expect in genetic differentiation measures compared to those in genetic diversity measures.

Different time lags using different genetic measures

Using H_o as a genetic diversity measure, we found a time lag of 20 years in *O. acetosella* and a time lag of 35 years in *P. multiflorum*, but not when measuring genetic diversity with A_r. Heterozygosity can stay stable for over 200 generations after fragmentation events (Lloyd et al. 2013) and is often used to detect historical effects (Münzbergová et al. 2013), but this only holds true when the population size is sufficiently large. In small populations, heterozygosity declines rapidly (Lloyd et al. 2013), which might explain the time lag of a few decades detected in our study. Conversely, allelic richness is more responsive to recent landscape changes (Epps et al. 2005) and reacts faster than heterozygosity (Caplins et al. 2014; Aavik et al. 2017). This could explain the missing time lag signal in *P. multiflorum* and *O. acetosella* in our study, suggesting that their allelic richness, influenced by previous landscape changes, has already reached a new equilibrium.

Using both G''_{ST} and D_{PS}, we detected a time lag of 20 years in *O. acetosella*. Another study using G'_{ST} and D_{PS} detected a similar time lag of 20–40 years with the coastal tailed frog (*Ascaphus truei*), which is also restricted in dispersion (Spear and Storfer 2008). Despite the theoretical sensitivity of D_{PS} to recent landscape changes due to its reliance on allelic diversity (Landguth et al. 2010), this was not reflected in our results (Table 4, Fig. 4).

What remained puzzling is the absence of signals in *P. multiflorum* and *A. nemorosa* using either measure. Although Keyghobadi et al. (2005b) demonstrated that, compared to heterozygosity, genetic differentiation measures can detect relatively recent landscape changes, this is generally true after reconnection events, where previously isolated populations become connected through habitat restoration or increased dispersal opportunities (Landguth et al. 2010). In contrast, genetic differentiation tends to react more slowly following isolation events, where new barriers to gene flow emerge, for instance, due to habitat fragmentation (Wang 2004; Landguth et al. 2010; Alcala et al. 2013). It might thus be reasonable to speculate that the signals of *A. nemorosa* and *P. multiflorum* using differentiation measure lie further back in time.

Impact of landscape elements in a short and long term

Surprisingly, despite intensive agricultural land use, with arable fields and intensively managed grasslands comprising up to 75% of the whole landscape, it was the semi-natural landscape elements, such as semi-natural grassland, other vegetation, linear woody elements, and traditional orchards that contributed most to explaining genetic diversity and differentiation of the forest herb populations. Moreover, most of these semi-natural landscape elements showed long-lasting effects in that their past composition was still reflected in the current population genetic structures (Figs. 3 and 4).

We believe two characteristics are important for a landscape metric to have a detectable legacy effect on the population genetic structure. First, whether or not a certain landscape metric has a historical effect is not determined by its absolute proportion, but by the relative change in its proportion over time (Metzger et al. 2009). For a landscape metric to exhibit a detectable historical effect, it must have undergone some changes. Otherwise, distinguishing between past and present conditions is impossible. For instance, semi-natural grassland (SEMGRASS) and semi-natural vegetation (SEMVEG) made up only a small portion of the landscape compared to arable fields (INTENSIVE), however their percentage change was substantial (Table S6 and S7). Second, the landscape element must be functionally important for the focal species. While semi-natural elements may not necessarily serve as habitat or increase the habitat connectivity for forest specialist species (Liira and Paal 2013), they provide a wide range of potential nesting and foraging resources for pollen and seed dispersers (Eeraerts et al. 2021). Even small patches of semi-natural habitat or scarce flower resources can be utilized by many species (Jauker et al. 2009), influencing their behaviour and even community composition. This, in turn, can potentially have a long-lasting effect on the genetic diversity and differentiation of various wild forest herb populations (Cruzan and Hendrickson 2020; Feigs et al. 2022; Naaf et al. 2022).

Conclusion

Our study provides important insights in respect of our hypotheses, but at the same time raised questions for further research. First, we found limited evidence of time lags beyond 35 years. This may indicate that the population structure of long-lived forest herb species can react relatively fast to landscape changes, which contradicted our expectations that these species exhibit time lags of many decades. However, there were some indications that the time lags for *A. nemorosa* and *P. multiflorum* could potentially exceed the temporal scope of our study, which leaves our interpretation uncertain and highlighted the importance of carefully considering the chosen time scale when addressing time lags. Resolving this issue requires further investigation with an extended temporal scale beyond that of the current study.

Secondly, we observed variability in detected time lags and historical landscape effects among the three species and the genetic measures used. Our findings suggest that *P. multiflorum* may exhibit longer time lags than *O. acetosella*, likely due to its later sexual maturity. However, this inference is limited by our inability to detect signals for *A. nemorosa,* which shares the attribute of having late sexual maturity. Comparing the time lag between *P. multiflorum* and *A. nemorosa* given their differences in associated pollinators proved even more challenging. Our results partially supported the third hypothesis that heterozygosity has a longer time lag than allelic richness. However, further investigation is needed to understand the difference in temporal scales between the two differentiation measures G''_{ST} and D_{PS}. Given the inconsistency in identifying time lags in dependence of species' reproductive traits using four genetic measures, with some measures showing no signals of time lag at all, we conclude that it is important to consider multiple measures in detecting time lags and to account for the time scales of these measures.

Our results also indicated that whether a landscape element has a long-lasting effect is not explained by its dominance in the landscape but rather its proportional change over time and its functional relevance. Semi-natural landscape elements have a more enduring effect on shaping the genetic diversity and differentiation of forest herb populations than have intensively used agricultural landscape elements.

In conclusion, our results emphasized that agricultural landscapes have a historical dimension that constantly shapes the genetic patterns of present-day wild populations and influences their long-term persistence, even for the forest-dwelling species. Recognizing these long-lasting effects is essential for effective conservation planning.

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Author contributions TN, SIJH conceived and designed the study. All authors except JL and SOAC were involved in site selection, field work and sampling. SH, JTF and TN performed the molecular lab work and did the allele scoring. SH analysed the data and wrote the first draft of the manuscript with contributions of TN, JTF and SIJH. All authors contributed to the revisions and gave final approval for publication.

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Data availability The datasets generated during and/or analysed during the current study are available in the supplementary information. The R code for analysing the data are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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