

Landscape determinants of genetic differentiation, inbreeding and genetic drift in the hazel dormouse (*Muscardinus avellanarius*)

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Abstract The dispersal process is crucial in determining the fate of populations over time, but habitat fragmentation limits or prevents it. Landscape genetic is an effective tool to assess the degree to which dispersal still occurs in fragmented landscapes. The purpose of this study was to investigate the landscape determinants of genetic differentiation in the hazel dormouse (*Muscardinus avellanarius*), a forest-dependent species of conservation concern. By comparing subpopulations in a continuous (SLR) and a fragmented (VTH) population, we (i) searched for the presence of Isolation-by-Resistance (IBR); (ii) estimated migration rates; (iii) evaluated the degree of inbreeding and genetic drift, and searched for their landscape determinants. We found an IBR effect in VTH, which heavily hindered the dispersal process. The overall number of migrants among VTH

subpopulations was very low (1 per generation, compared to 15 in SLR), although a between-patch displacement of about 4 km along a well-structured hedgerow probably occurred. The inbreeding ($F > 0.2$ in most subpopulations) and the genetic drift (four out five subpopulations showed private alleles on several loci, with relatively high frequencies) are of particular concern in VTH. However, they were found to be limited in large patches or in patches connected by hedgerows with a high number of neighbouring patches. As a conservation strategy in the VTH landscape, characterized by small patches, we suggest that the dispersal process among subpopulations is enhanced to sustain a functional metapopulation. For this purpose, an effective ecological network should be created by enhancing the continuity and the internal features of hedgerows.

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Introduction

Habitat destruction is recognized as one of the major causes of biodiversity loss worldwide (Fischer and Lindenmayer 2007). The mechanisms underlying this phenomenon are relatively complex to identify because key drivers of habitat changes act cumulatively or interactively, affecting the dynamics of populations from the local to the landscape scale (Giplin and Soulé 1986; Fahrig 2003). Habitat destruction, and the consequent process of habitat fragmentation, reduces the size of populations inhabiting residual patches and divides them into a network of spatially discrete subpopulations linked by dispersal (Hanski and Simberloff 1997). The amount of dispersal between

subpopulations depends on the degree to which landscape composition and configuration effectively facilitate individuals' passages among patches, i.e. the functional connectivity of the landscape (With et al. 1997; Taylor et al. 1993; Tischendorf and Fahrig 2000; Hilty et al. 2006). The disruption of dispersal movements, due to particularly hostile matrices embedding the residual habitat patches, leads to the ecological isolation of subpopulations, which lose both their demographic and their genetic connectivity (Lowe and Allendorf 2010). When the migrants' flow between small subpopulations is interrupted, genetic (inbreeding and genetic drift) and demographic (stochasticity affecting local recruitment) processes play a major role in increasing the risk of local extinction. Herein, genetic and demographic factors interact with each other, and with environmental stochasticity, through a positive feedback mechanism pushing populations into the so-called extinction vortex (Giplin and Soulé 1986).

The maintenance of genetic and demographic connectivity in fragmented landscapes (Lowe and Allendorf 2010) is thus essential for the long-term persistence of populations, and their assessment is a critical step to identify the actions required to counteract isolation among subpopulations (Lowe and Allendorf 2010).

The traditional methods used to evaluate animal dispersal in the field, such as radio telemetry or capture-mark-recapture, may not be the most cost-effective methods for studying functional connectivity at the landscape level (Tracey 2006). These methods are particularly time-consuming and provide information about the movement of individuals only. Conversely, molecular-markers, i.e. polymorphic proteins or DNA sequences, are often used to evaluate the functional connectivity of landscapes, since they can account for breeding events, and allow inferring migration rates and gene flow between subpopulations (Frankham 2006). In addition, molecular techniques require a less intensive sampling effort, as they usually rely on biological samples collected at a single time point (Neville et al. 2006). Among molecular markers, microsatellites (or Short Tandem Repeats, STRs), are ordinarily used to measure the genetic distances among or within populations, which are a critical piece of information when studying subpopulations in fragmented landscapes (e.g. Selkoe and Toonen 2006; Pisa et al. 2015). Indeed, by calculating the genetic distance between populations and by evaluating if it is more correlated with the geographical distance (as postulated by the Isolation-by-Distance theory, IBD) or with the environmental resistance opposed by the matrix to the gene flow (as postulated by the Isolation-by-Resistance theory, IBR; McRae 2006), the actual effect of fragmentation on populations can be quantified.

Forests are one of the most fragmented habitat types worldwide due to human activities and consequently

several forest-dwelling species are strongly threatened by this phenomenon (e.g. Wilcove et al. 1986; Saunders et al. 1991; Gustafsson et al. 2010). In particular, arboreal mammals, such as tree squirrels and dormice, are among the species most negatively affected by forest loss and fragmentation due to their low dispersal capability in the absence of connections between patches (Mortelliti et al. 2010). Various single-species studies carried out in Europe found similar responses in this guild of mammals (e.g. Rodriguez and Andr en 1999; Mortelliti 2013). The hazel dormouse (*Muscardinus avellanarius*), an arboreal rodent living in broadleaved woodlands and hedgerows (Juškaitis 2008; Ehlers 2012), is highly threatened by habitat loss and fragmentation (Bright et al. 1994; Mortelliti et al. 2011). Due to its poor conservation status and negative long-term trend in almost all Europe, the species is protected under the 92/43/ECC Directive (Habitat Directive) and is included in Annex IV, concerning the species that require a strict protection regime across their entire natural range within the EU.

The main goal of our research was to investigate, by means of a landscape genetic approach (Holderegger and Wagner 2006; Manel and Holderegger 2013), the effects produced by isolation on a hazel dormouse population inhabiting a fragmented landscape, and to compare it with a continuous population.

As previously found by Bani et al. (2017) in a study carried out on the same two populations, the observed heterozygosity showed a marked deficit in the fragmented population, both at all loci and at single locus level, but not in the continuous one. In addition, while the continuous population was characterised by an IBD effect, the genetic distance was not explained by the Euclidean distance in the fragmented population. This suggests that the population inhabiting the fragmented landscape was strongly affected by the ecological isolation of its subpopulations. Indeed, the genetic population structure found by Bani et al. (2017) highlighted the presence of six genetic clusters in the fragmented landscape, corresponding to an equal number of investigated subpopulations (i.e. sampling locations, SLs), while the five subpopulations of the continuous landscape showed a clear intermeshing pattern.

Starting from these evidence, in this paper we focused on discovering the causes of the observed genetic structuring and differentiation of the fragmented population. The general aim of the study was to investigate whether and how much the subpopulations in the fragmented landscape are isolated due to habitat fragmentation, compared to the continuous population. Moreover, we tried to identify the landscape determinants of the degree of inbreeding and genetic drift characterizing the subpopulations of the sampling populations. Indeed, inbreeding and genetic drift are often regarded as the major genetic parameters when

assessing the conservation status of small populations, and identifying their determinants could have important management implications.

Herein, we first tested the effects produced by landscape resistance on genetic distance (i.e. IBR) between each pair of SLs in the two landscapes separately. Moreover, in order to quantify the effect of the landscape matrix on dispersal, we evaluated the migration rate among SLs per generation within each sampling population.

Secondly, we quantified the effect of both patch size and isolation on the degree of inbreeding and genetic drift. As regards the area-effect, we expected an inverse relationship between the degree of inbreeding and genetic drift and the habitat patch size, according to the postulates of the Small Population Paradigm (Caughley 1994). On the other hand, as regards the isolation-effect, according to the Genetic Rescue Effect (*sensu* Brown and Kodric-Brown 1977), we expected that both inbreeding and genetic drift could be limited by immigration from adjacent habitat fragments, and thus by a higher number of neighbouring patches connected by hedgerows.

Methods

Study area and sampling design

The study was conducted in the Viterbo Province of the Latium Region (Central Italy, Fig. 1). Genetic data were collected from a continuous and a fragmented hazel dormouse sampling populations, approximately 25 km from each other. The first population inhabits the “Selva del Lamone” Regional Reserve, a unique and large broadleaved forest block (about 2700 ha) subjected to periodic partial coppicing, located in a hilly landscape (200–400 m a.s.l.). The second sampling population lives in fragmented broadleaved woodlands embedded in a hilly agricultural area of the Viterbo Hills (300–500 m a.s.l.), with interspersed urban areas. Here, the largest sampled patch measures about 250 ha, the two medium-sized patches are 20 and 30 ha large, while the smallest fragments vary from about 4–7 ha. A network of linear structures, such as hedgerows and tree lines, connects woodland patches (Fig. 1).

Tissue sampling was conducted in five sampling locations (SLs) within the SLR sampling population, and in six SLs corresponding to an equal number of woodland patches within the VTH sampling population.

We trapped dormice using wooden nest-boxes (average size 18 × 18 × 21 cm) placed in trees at a height of 1.5–2 m, with the entrance hole (3 cm of diameter) facing the trunk. We placed 343 nest-boxes according to a standard grid of 4 ha (6 × 6 nest-boxes, 40 m apart) in each SL, with the exception of those patches too small or too irregular in

shape to accommodate the grid. In those cases, the whole patch was sampled by maintaining the same density of nest-boxes as in standard grids, in order to obtain a constant sampling effort (nest-boxes/area). The distance between nest-boxes was comparable to that used in other studies on this species, and it has proven adequate to enhance the chance of individual detection, as several nest-boxes may be included in an individual’s home-range (Juškaitis 2007; Chanin and Gubert 2011). Overall, the maximum distance of nest-boxes from the SL grid centroid reached 172 m. The mean distance between the centroid of the SLs was 2053 m (ranging from 883 to 5474 m) for the SLR and 4451 m (ranging from 1081 to 7668 m) for the VTH sampling population. Nest-boxes were checked monthly from May 2010 to December 2012, but the survey was interrupted between January and March to avoid disturbance during hibernation (Juškaitis 2008; Chanin and Gubert 2011). For further details on the sampling design, see Mortelliti et al. (2014) and Bani et al. (2017).

Tissue collection and DNA analyses of microsatellite markers

We captured 139 individuals in the SLR sampling population and 104 individuals in the VTH sampling population. In order to minimize disturbance, females with litter were never handled. DNA was obtained from the tissue collected by cutting a part of the ear tip from adult or sub-adult individuals only, for a total of 213 individuals (68 males and 58 females in SLR and 40 males and 47 females in VTH). Hazel dormice were captured and handled under permit number PNM 0024822, granted to A.M. by the Ministry of Environment, Rome, Italy. Tissues were stored in 95% ethanol right after their collection in the field and, subsequently, at –20 °C in a laboratory. We genotyped all samples by Polymerase Chain Reaction (PCR) for 8 species-specific microsatellite markers (Alice Mouton, modified from Naim et al. 2009). We excluded microsatellite loci Mav6 from the analyses because we were not able to obtain clearly interpretable PCR products. For other details on laboratory analyses, see Bani et al. (2017).

IBR analyses

Geographic distances were measured considering the Euclidean distance between the grid centroid of each pair of SLs (Table S1a). Since Bani et al. (2017) discovered that the genetic distances between the SLs of the SLR population, but not those of the VTH population, was significantly explained by the Euclidean distance, in this study we searched for a possible relationship between genetic distance and ecological distance for each pair of SLs within each sampling population. The Euclidean distances were

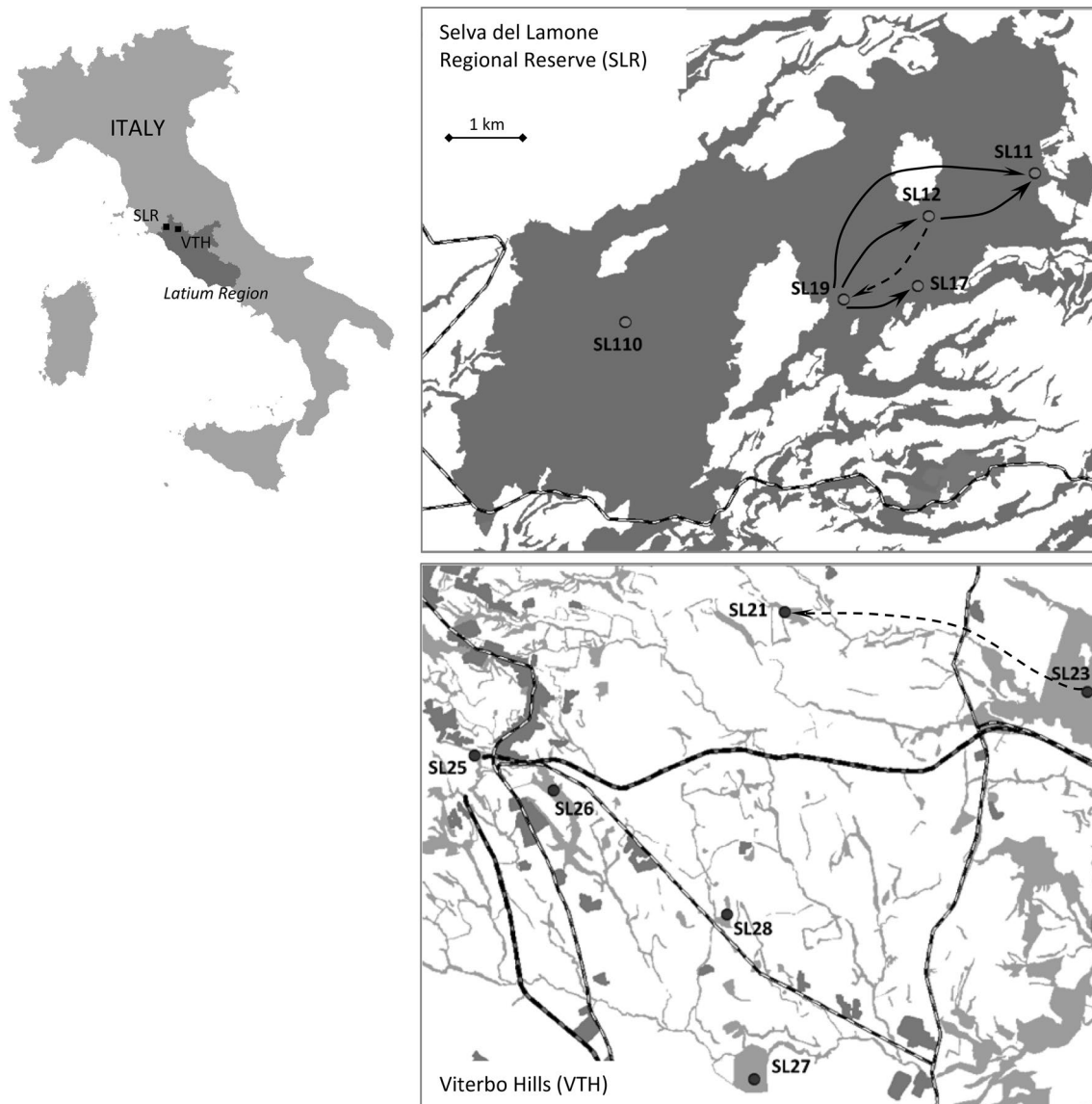


Fig. 1 On the *left side*, the Selva del Lamone Regional Reserve (SLR), and the Viterbo Hills (VTH) study areas (in *black*) within the Latium Region (*dark grey*) in Central Italy. On the *right side*, SLR (*above*) and VTH (*below*) sampling populations. *Light grey* forest areas; *black dots* centroid of Sampling Locations (SLs); *dark grey*

urban areas. *Thick black–grey dashes lines* railways; *thin black–with dashes lines* main roads. *Arrows* indicate the inferred significant ($p < 0.05$; *continuous arrows*) and marginally significant ($p < 0.1$; *dashed arrows*) pairwise migration rates between SLs (see *Table 2* for rate values)

calculated between the grid centroids of each pair of SLs (Table S1a). To calculate the pairwise ecological distances, we used a resistance model approach (McRae and Beier 2007; McRae et al. 2008), where landscape permeability was considered to be differently affected by the amount of suitable habitat and by the presence of barriers (e.g. Bani et al. 2015). For the analysis, we used the CORINE Land Cover 2006 map (<http://www.eea.europa.eu>) as land cover cartography. Moreover, for a more accurate estimation of the ecological distance among SLs, we digitalized all the hedgerows present in the two landscapes based on Google

Earth images (33 T 739,000 m E 4,710,000 m N. Google Earth, June 26, 2015. Accessed on November 18, 2016). Considering the maximum spatial detail available from the CORINE Land Cover map and from the digitalized hedgerows, we were able to work on a grid map with a 20 m resolution on the ground. All forest patches (almost entirely represented by broadleaved forests in the study area) and digitalized hedgerows were considered as suitable habitat for the hazel dormouse, while all the other land cover types were regarded as unsuitable (i.e. not permeable). As the scale at which habitat amount affects the hazel dormouse is

unknown, we performed a set of expert-based permeability maps calculating the fractional cover of suitable habitat in a buffer of 50, 100, 150 and 200 m radius. We considered 50 m as the minimum buffer radius as it corresponds to the radius of a circular individual's home range (Juškaitis 2007, 2008), and 200 m as the maximum buffer radius as it corresponds to the maximum dispersal ability of the species in unsuitable habitats. We obtained the latter value by averaging different documented values (100 m; Bright 1998; 250 m; Büchner 2008; 100–300 m; Mortelliti et al. 2013). We hypothesized two relationships describing the effect of habitat amount on permeability for dormice. First, we assumed that permeability increased positively and linearly with the habitat fractional cover within the buffer, and ranging from 0 (absence of suitable habitat within the buffer) to 1 (buffer completely filled by suitable habitat). We also assumed that a logarithmic relationship existed between habitat fractional cover and permeability. We tested these two relationships as they have different ecological meanings. A disproportional increase of landscape permeability with habitat amount (logarithmic relationship) is typical of species which use stepping stones to disperse, while a proportional relationship (linear relationship) indicates that the dispersal ability of the species studied is either so low that the use of stepping stones is prevented, or so high that stepping stones are useless (Saura and Rubio 2010).

For this purpose, we calculated the permeability as $\log(100 \times \text{habitat fractional cover} + 1)$, and we rescaled the obtained values between 0 and 1, in order to obtain the same range of variation as for the linear relationship. Then, we converted the two sets (linear and logarithmic) of the four scales permeability maps into resistance maps, assigning to each 20-m grid cell the value of one minus the permeability value. Thus, a pixel with no suitable habitat within the considered buffer was given a resistance value of 1, while a pixel centred in a buffer completely filled up by suitable habitat was given a resistance value of zero. Despite the two sets of maps (linear and logarithmic) had the same range of variation, for intermediate values of fractional cover they displayed noticeably different values of permeability, and thus of resistance. Moreover, we considered water surfaces, urban areas and the infrastructural network, i.e. main and secondary roads, and railways laying on the ground, as barriers to dispersal. The infrastructural network was obtained from the Open Street Map platform (<https://www.openstreetmap.org>). In order to ponder the possible barrier-effect, the resistance value of all the 20-m pixels crossed by at least one barrier were arbitrarily set to 10. Such high value was assigned to avoid that dispersal routes would cross barriers unless no other possibility of movement existed (Nogues and Cabarga-Varona 2014). We thus obtained two sets (linear and logarithmic) of eight resistance maps (four without barrier-effect, four

with barrier-effect). The 16 resistance maps were singularly used in the connectivity analysis to calculate the ecological distance between each pair of SLs. This analysis was performed using the Circuitscape software (Shah and McRae 2008; McRae and Shah 2009), which relies on the circuit theory that incorporates the concept of minimum movement distance and the availability of alternative pathways connecting nodes. Circuit resistance can be interpreted as an ecological resistance to dispersal, and so can the degree of hindrance to individuals' movement exerted by the amount and configuration of suitable habitat and barriers within the landscape (McRae et al. 2008; see Bani et al. 2015 for a landscape genetic approach). In our case, pairwise ecological resistance is a measure of the hindrance to gene flow and its value decreases as the amount of suitable habitat between two SLs increases, or if multiple connections between them are present. Using the Circuitscape pairwise mode, we estimated the SLs pairwise ecological distances (McRae and Shah 2009; it must be noted that the software cannot account for "zero" as resistance value, so we approximated it to the value of 10^{-3} , which is the minimum resistance value occurring in the resistance maps).

To investigate whether landscape resistance could explain the amount and scale of dispersal among SLs (i.e. if an IBR effect subsisted) within each sampling population separately, we ran one-tailed simple Mantel tests (Mantel and Valand 1970) between the genetic distance matrix and each of the 16 log-transformed ecological distance matrices. Moreover, in order to remove the effect of the Euclidean distance that positively correlates with the ecological distance, we ran one-tailed partial Mantel tests (Smouse et al. 1986) between the genetic distance matrix and each of the log-transformed ecological distance matrices, controlling for the log-transformed Euclidean distance. We used log-transformed Ecological and Euclidean distances, because in real landscapes dispersal is thought to occur in two dimensions (McRae 2006), as confirmed by real case studies (Ruiz-González et al. 2014; Bani et al. 2015). All the analyses were performed using the *ecodist* package (Goslee and Urban 2007) in R environment (R version 3.2.0; R Core Team 2015).

Private alleles analysis and estimation of migration rates per generation

For each sampling population, we performed a separate, private allele analysis at the SL level, in order to estimate the theoretical number of migrants moving between them in each generation (Barton and Slatkin 1986; Slatkin 1987; Yamamichi and Innan 2012). Indeed, as demonstrated by Slatkin (1981, 1985) the average frequency of rare alleles is inversely related to the effective number of migrants (Nm), where N is the local population size and m is the proportion

of migrants. To this aim, we counted the number of private alleles and calculated their frequency using Genepop 4.4 (Rousset 2015).

Moreover, for each sampling population, a further independent analysis of migration rates between SLs was performed using a MCMC-based assignment test, an approach that admits the deviation from the Hardy–Weinberg equilibrium (which is the case of VTH population; see Bani et al. 2017). This analysis was carried out using BayesAss 3.0 (Wilson and Rannala 2003). The convergence of the MCMC analysis was reached using 10,000,000 iterations, with a burn-in of 1,000,000 iterations. Moreover, we set the interval between samples to 1000 and, following the Rannala (2007) advices, we adjusted the mixing parameters in order to get optimal acceptance rates.

Although very promising, we did not use the new approach proposed by Sundqvist et al. (2016) in evaluating the migration among local subpopulations. Indeed, this method is still at an experimental stage and requires a large sample size and a high number of genotyped loci (Sundqvist et al. 2016). Such requirements were not fulfilled by our data.

Analysis of inbreeding and genetic drift landscape determinants

We estimated inbreeding as the probability of each individual inheriting two identical alleles from a common ancestor of its parents (Wright 1922). For this purpose, we evaluated the inbreeding coefficient (F) of each individual within each SL, using the *adegenet* package (Jombart and Ahmed 2011) in R. To account for the genetic drift, we assessed the number of effective alleles across all loci at the SL level using GenAIEx v. 6.501 (Peakall and Smouse 2006, 2012). We also evaluated the relatedness of individuals within and between SLs for each sampling population separately, using the Coancestry software (Wang 2001).

Subsequently, in order to identify the major drivers of inbreeding and genetic drift, we tested the effect of patch size and the degree of patch isolation. Specifically, we related the degree of inbreeding and the number of effective alleles with the \log_{10} of patch size and with the number of neighbouring fragments within 1000 m from each sampled patch connected by continuous hedgerows. Considering the magnitude of species dispersal, we chose 1000 m as the possible distance within which gene flow might occur in one or few generations.

We evaluated the effect of patch size and patch isolation on inbreeding by performing a linear regression with pseudo-replication considering all the sampled hazel dormouse individuals as statistical units and by adopting a linear mixed model to account for the nested sampling design (SLs nested within sampling populations). In order

to account for a possible difference in the mean value of inbreeding between the two sampling populations, we also included the sampling population in the model as a categorical factor. As one of the most important assumptions of linear mixed effect regression models is that predictors should not be strongly correlated among them (Quinn and Keough 2002; Zuur et al. 2009), we checked the correlation among the environmental variables, which was found to be not too strong and always lower than 0.549 (Dormann et al. 2013). Continuous independent variables (patch size and patch isolation) were scaled to improve model performance and to allow regression coefficient comparison (Zuur et al. 2009). Subsequently, starting from the full model, we performed a dredge procedure (Burnham and Anderson 2002) to select a set of top models, composed of all models with a difference of AICc (corrected Akaike Information Criterion; Anderson et al. 2000) lower than two compared to the model with the lowest AICc (Burnham and Anderson 2002; Grueber et al. 2011), and we performed a conditional model averaging (Burnham and Anderson 2002) on the set of top models. All the analyses were carried out using the *lme4* (Bates et al. 2015) and the *MuMIn* (Barton 2016) packages in R.

To evaluate the effect of patch size and patch isolation on genetic drift, we performed a generalized linear mixed regression model considering all the SLs as statistical units, patch size, patch isolation and sampling population as a fixed effect and sampling population as a random effect. Even in this case, we checked the correlation among the environmental variables, which was found to be always lower than 0.643 (Dormann et al. 2013), and we scaled continuous variables. Finally, we performed a model selection and model averaging procedure using the same framework adopted for the analysis of the inbreeding landscape determinants described above.

As expected, the relatedness (Table S3) within SLs resulted highly correlated to inbreeding (estimated as the average of individual F values at the SL level; Pearson's $r=0.939$), thus we did not perform a model aimed to evaluate its landscape determinants.

Results

IBR

Among the four buffers used to evaluate the ecological resistance in terms of suitable habitat fractional cover, the 150-m buffer was the most effective in explaining the effect played by the ecological distance on the genetic distance between each pair of SLs within each sampling population (see Table S1b, for the pairwise ecological distance between the SLs). Indeed, this value corresponds to the minimum

radius for which the relationship between genetic and ecological distances was found to be significant. Moreover, the linear function linking the amount of habitat within the buffer to the ecological resistance appeared more powerful compared to the logarithmic function. Finally, the ecological resistance accounting for the barrier-effect was found to be more efficient in explaining the genetic distances compared with resistance maps that did not consider barriers. Thus, we used as a resistance model the one developed using the 150-m buffer, the linear function, and a barrier-effect value of 10 (see Table S2 for all the Mantel test outputs). We found a marginally significant Mantel r between F_{st} and the ecological distance ($r=0.714$; $p=0.066$) for the SLR, and a strong relationship between the Euclidean and ecological distance ($r=0.959$; $p=0.018$). In the SLR landscape, the partial Mantel test relating genetic distance with ecological distance, controlled for the Euclidean distance, was not significant ($r=0.151$; $p=0.349$). Conversely, in the VTH fragmented landscape, we found a significant relationship between F_{st} and ecological distance ($r=0.450$; $p=0.041$), and even the partial Mantel test between F_{st} and ecological distance, controlled for the Euclidean distance, was found to be significant ($r=0.449$; $p=0.040$; the relationship between Euclidean and ecological distance was not significant).

Private alleles and migration rates per generation

The private allele analysis, performed for each sampling population separately, showed that only one out of five SLs in the SLR population was characterized by two private alleles, relatively rare ($FPa < 0.05$; Table 1), at one locus. Conversely, four out of six SLs showed private alleles in the VTH population, ranging from two (SL25 and SL26) to five (SL27) private alleles. In this case, the frequency of private alleles was nearly always found to be relatively high (Table 1). The theoretical number of migrants moving among SLs in each generation (Nm), estimated using the private allele method, amounted to 15.53 in the SLR landscape and to 0.65 in the VTH landscape.

Significant migration rates were found between several SLs within the SLR population (except one, marginally significant), while only one marginal significant and unidirectional migration was discovered from SL23 to SL21 in the VTH population (see Fig. 1). The inferred migration rates (with their standard deviations) are presented in Table 2. They correspond to the fraction of migrants from a sampling location (SL of origin) with respect to the number of individuals of sampling location where they immigrate (SL of destination). Based on the estimated fraction of migrants, we could extrapolate an overall number of about 15 migrants for the SLR sampling population and about 1 for the VTH sampling population for each generation,

Table 1 Results of the private alleles’ analysis performed on the hazel dormouse at the level of sampling location (SL) for each sampling population separately (Selva del Lamone Regional Reserve, SLR, and Viterbo Hills, VTH)

Sampling population	Sampling location (SL)	Locus	Number of private alleles	Frequency of private alleles (FPa)	
SLR	SL11	Mav3	2	0.021	0.011
		Mav1	2	0.069	0.138
	SL23	Mav5	1	0.115	
		Mav7	1	0.017	
		Mav3	1	0.167	
	SL25	Mav5	1	0.250	
		Mav2	1	0.036	
VTH	SL26	Mav7	1	0.206	
		Mav1	2	0.100	0.100
	SL27	Mav3	1	0.100	
		Mav4	1	0.125	
		Mav7	1	0.100	

Only SLs with private alleles are shown

which proves that the results of the two analyses on migration are highly consistent.

Inbreeding and genetic drift landscape determinants

Overall, the individual inbreeding coefficient (F) ranged between 0.118 and 0.683 within the SLR continuous landscape, and from 0.121 to 0.532 in the VTH fragmented landscape. Moreover, the average of individual F values was always lower than 0.13 in the SLs pertaining to the SLR sampling population, and always higher than 0.20 in the SLs pertaining to the VTH sampling population, except in one case (SL23), where the value was near 0.15 (Fig. 2). The mean relatedness was assessed by means of the triadic likelihood estimator, since it allowed obtaining lower variance values (similar variance values were also obtained using the dyadic likelihood estimator). Within SLs in the continuous SLR landscape the mean relatedness varied from 0.125 to 0.188, while resulted higher in the VTH fragmented population, whose values varied from 0.223 to 0.333 (see Table S3).

The dredge procedure applied to the inbreeding model selected four top models ($\Delta AICc < 2$), and all the independent variables were included in the top models (Table 3). Specifically, the number of neighbouring connected fragments appeared in three models, the \log_{10} of patch size in two models, and the sampling population in one model only (Table 3). As expected, in the conditional-averaged model both the \log_{10} of the patch size and the number of neighbouring connected fragments significantly and negatively influenced the individual F value (Table 4). Moreover, in

Table 2 Inferred (posterior mean) migration rates and standard deviations (sd, in brackets) estimated by BayesAss between each pair of sampling locations (SLs) for (a) Selva del Lamone Regional Reserve (SLR) population and (b) Viterbo Hills (VTH) population

(a)	Sampling location (SL) of destination					
	SL11	SL12	SL17	SL19	SL110	
Sampling location (SL) of origin						
SL11	<i>0.7537**</i> (0.039)	0.031 (0.029)	0.029 (0.022)	0.105* (0.052)	0.076 (0.051)	
SL12	0.105* (0.046)	<i>0.743**</i> (0.057)	0.067 (0.057)	0.089 ^a (0.053)	0.075 (0.059)	
SL17	0.046 (0.035)	0.028 (0.033)	<i>0.7005**</i> (0.027)	0.041 (0.037)	0.055 (0.047)	
SL19	0.074 (0.051)	0.174* (0.069)	0.170** (0.064)	<i>0.7101**</i> (0.038)	0.094 (0.064)	
SL110	0.022 (0.028)	0.025 (0.028)	0.034 (0.028)	0.055 (0.044)	<i>0.700**</i> (0.035)	
(b)	SL21	SL23	SL25	SL26	SL27	SL28
Sampling location (SL) of origin						
SL21	<i>0.722**</i> (0.048)	0.016 (0.016)	0.019 (0.018)	0.015 (0.015)	0.037 (0.033)	0.023 (0.022)
SL23	0.096 ^a (0.055)	<i>0.878**</i> (0.075)	0.032 (0.027)	0.017 (0.016)	0.051 (0.040)	0.051 (0.038)
SL25	0.042 (0.039)	0.012 (0.011)	<i>0.858**</i> (0.046)	0.019 (0.018)	0.085 (0.054)	0.029 (0.026)
SL26	0.086 (0.054)	0.011 (0.010)	0.042 (0.031)	<i>0.916**</i> (0.032)	0.032 (0.029)	0.050 (0.055)
SL27	0.027 (0.026)	0.011 (0.011)	0.018 (0.019)	0.015 (0.014)	<i>0.724**</i> (0.041)	0.021 (0.020)
SL28	0.029 (0.028)	0.072 (0.073)	0.032 (0.028)	0.017 (0.016)	0.071 (0.049)	<i>0.826**</i> (0.063)

The mean value represents the fraction of migrants from each SL of origin with respect to the number of individuals in the SL of destination. Along the diagonal (italic), the fraction of resident individuals for each sampling location

^aLower limit of the 90% credible intervals ($1.65 \times \text{sd}$) larger than zero

*Lower limit of the 95% credible intervals ($1.96 \times \text{sd}$) larger than zero

**Lower limit of the 99% credible intervals ($2.58 \times \text{sd}$) larger than zero

this model, the individual F was found to be on average significantly higher in the VTH compared with the SLR sampling population (Table 4).

The genetic drift was evaluated through the all loci number of effective alleles (N_e) per SLs, the values of which are reported in Table 5 (see Table S4 for single loci number of effective alleles and fixation indices). N_e varies from 3.45 to 4.07 in the SLs of the SLR population, and from 2.19 to 3.32 in the SLs of the VTH population.

The dredge procedure applied to the genetic drift model selected three top models containing all the three independent variables (Table 6). While the number of neighbouring connected fragments appeared in two models, the \log_{10} of patch size and the sampling population appeared in one model only (Table 6). As expected, in the

conditional-averaged model both the \log_{10} of the patch size and the number of neighbouring connected fragments significantly and positively affected the all loci N_e (Table 7). Moreover, in this model, the all loci N_e was found to be significantly lower in the VTH compared with the SLR sampling population (Table 7).

Discussion

In this study, we adopted a landscape genetic approach to investigate whether and how forest fragmentation affected a hazel dormouse population inhabiting a highly fragmented landscape (VTH population) in Central Italy, and we compared it with a population inhabiting a continuous forest

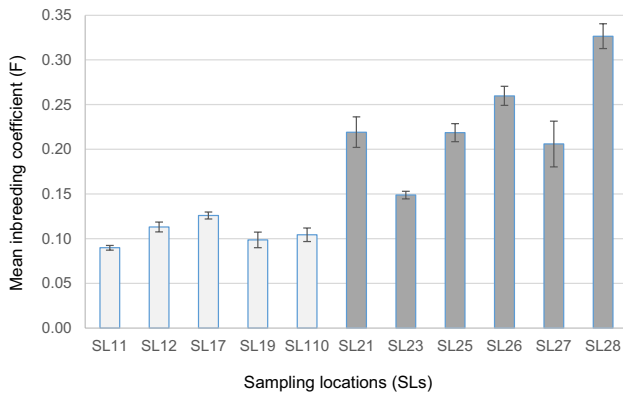


Fig. 2 Mean (with standard error) of hazel dormouse individual inbreeding coefficient (F) in each sampling location (SL) pertaining to the SLR continuous sampling population (light grey) and to the VTH fragmented sampling population (dark grey)

block (SLR population). Bani et al. (2017) already highlighted that, as opposed to the continuous landscape, the genetic divergence in the fragmented landscape could not be explained in terms of IBD. In this paper, we investigated

the causes of this divergence and clarified it through the one-tailed partial Mantel tests performed for both populations, by which the pairwise genetic distances between SLs (i.e. subpopulations) were related to the ecological distances, controlling for the Euclidean distances (IBR effect). Among the four buffer-radii (50, 100, 150 and 200 m) used to calculate the fractional cover of suitable habitat for the hazel dormouse, only the 150 and 200 m radii led to the development of resistance maps from which the calculated ecological distance was significantly correlated with the genetic distances between each pair of subpopulations within the VTH population. This suggested that, when a map representing the spatial distribution of habitats must be translated into a map of habitat suitability for the hazel dormouse in order to investigate dispersal processes, the suitability values should be calculated within a buffer with a radius of at least 150 m. This is probably due to the suitability of forest edge habitats for the hazel dormouse (Ramakers et al. 2014) and to the possibility of individuals easily moving out of the edge for at least 150–200 m (Bright 1998; Büchner 2008). Thus, not considering a sufficiently wide buffer around suitable patches could lead to a

Table 3 Inbreeding landscape determinants of the hazel dormouse in Central Italy. Dredge procedure applied to linear mixed regression models

(Intercept)	scl(FRAG)	scl(log(PATCH))	SPOP	df	logLik	AICc	Delta	Top models	Weight
0.228	-0.016		+	6	212.9	-413.4	-	*	0.278
0.240	-0.012	-0.015		6	212.7	-413.0	0.47	*	0.220
0.240		-0.022		5	211.4	-412.6	0.87	*	0.180
0.241	-0.018			5	211.0	-411.6	1.79	*	0.113
0.230	-0.015	-0.002	+	7	212.9	-411.3	2.13		0.096
0.244		-0.026	+	6	211.5	-410.6	2.87		0.066
0.224			+	5	209.9	-409.5	3.97		0.038
0.243				4	207.3	-406.5	6.95		0.009

scl(FRAG) scaled number of neighbouring fragments within 1000 m from each sampled patch, connected by continuous hedgerows, scl(log(PATCH)) scaled log₁₀ of patch size, SPOP categorical variable indicating the sampling population where each individual was captured. Values indicate the estimates of the regression coefficients, df degree of freedom, AICc corrected Akaike Information Criterion; delta model's difference in the AICc respect to the model with the lowest AICc; Top models: *Indicates if a model was included in the top model set, having a delta AICc < 2; Weight: Akaike weight of the model

+ Indicates if the sampling population variable was included in a model

Table 4 Inbreeding landscape determinants of the hazel dormouse in Central Italy. Coefficients of the conditional-averaged model obtained from the four top models

	Estimate	Std. error	Adjusted SE	z	Pr(> z)	Importance
(Intercept)	0.236	0.009	0.009	24.95	<0.001	-
scl(FRAG)	-0.015	0.007	0.007	2.05	0.040	0.77
SPOPVTH	0.029	0.013	0.013	2.20	0.028	0.35
scl(log(PATCH))	-0.018	0.008	0.008	2.41	0.016	0.51

scl(FRAG) scaled number of neighbouring fragments within 1000 m from each sampled patch, connected by continuous hedgerows, scl(log(PATCH)) scaled log₁₀ of patch size, SPOP categorical variable indicating the sampling population where each individual was captured; Estimate—conditional-averaged coefficients of covariates, Std. error standard error of estimates, Adjusted SE adjusted error of estimates, z Wald statistic for testing the hypothesis that the corresponding estimate is equal to zero (null hypothesis), Pr(>|z|) probability that the null hypothesis is true, Importance Relative variable importance

Table 5 Effective number of alleles of the hazel dormouse per sampling location (SL) calculated for the Selva del Lamone Regional Reserve (SLR) and Viterbo Hills (VTH) sampling population

Sampling population	Sampling location (SL)	N	Na	Ne
SLR	SL11	47.57 (0.20)	6.57 (0.65)	3.96 (0.40)
	SL12	16	5.29 (0.94)	3.53 (0.55)
	SL17	40	6.86 (0.74)	4.07 (0.63)
	SL19	10	5.00 (0.62)	3.57 (0.59)
	SL110	11.86 (0.14)	5.14 (0.59)	3.45 (0.47)
VTH	SL21	8.86 (0.14)	3.71 (0.47)	2.96 (0.37)
	SL23	28.57 (0.43)	4.86 (0.59)	3.32 (0.49)
	SL25	14.29 (0.71)	3.71 (0.42)	2.63 (0.34)
	SL26	16.43 (0.43)	3.29 (0.47)	2.48 (0.35)
	SL27	4.71 (0.18)	3.71 (0.52)	2.70 (0.38)
	SL28	11.71 (0.18)	3.71 (0.29)	2.19 (0.15)

N mean sample genotyped over all the loci, Na number of different alleles, Ne number of effective alleles. Standard deviation in brackets

misleading representation of habitat suitability, in terms of habitat permeability, for the hazel dormouse. Anyhow, we have to acknowledge the simplicity of the modelling strategy adopted herein, which only took into account the physical continuity of the habitat, and not its structural features. These have been proved to be crucial in affecting the actual suitability for the hazel dormouse (Ehlers 2012; Mortelliti 2013; Dondina et al. 2016a).

Between the two hypothesized relationships describing the effect of habitat amount on dormice suitability, the linear relationship was found to have the best performance in the 150-m buffer maps. This suggests that habitat suitability for the hazel dormouse, in terms of habitat permeability, increases proportionally to the amount of habitat, and not logarithmically. As a logarithmic relationship between habitat amount and permeability is typical of species with a dispersal that can benefit from the presence of stepping stones, we can state that the hazel dormouse cannot effectively disperse through these small landscape elements.

Table 6 Genetic drift (expressed as across all loci number of effective alleles) landscape determinants of the hazel dormouse in Central Italy: Dredge procedure applied to linear mixed regression models

(Intercept)	scl(FRAG)	scl(log(PATCH))	SPOP	df	logLik	AICc	Delta	Top models	Weight
3.581	0.301		+	5	3.470	15.1	–	*	0.368
3.169		0.558		4	–0.565	15.8	0.74	*	0.255
3.202	0.311			4	–0.644	16.0	0.89	*	0.236
3.169	0.210	0.431		5	2.120	17.8	2.70		0.096
3.716			+	4	–2.835	20.3	5.28		0.026
3.211				3	–6.380	22.2	7.13		0.010
3.220		0.512	+	5	–0.539	23.1	8.02		0.007
3.489	0.273	0.108	+	6	3.635	25.7	10.67		0.002

scl(FRAG) scaled number of neighbouring fragments within 1000 m from each sampled patch, connected by continuous hedgerows, scl(log(PATCH)) scaled log₁₀ of patch size, SPOP categorical variable indicating the sampling population where each individual was captured. Values indicate the estimates of the regression coefficients, df degree of freedom, AICc corrected Akaike Information Criterion, delta model’s difference in the AICc respect to the model with the lowest AICc; Top models: *Indicates if a model was included in the top model set, having a delta AICc < 2; Weight: Akaike weight of the model

+ Indicates if the sampling population variable was included in a modelm

Table 7 Genetic drift (expressed as across all loci number of effective alleles) landscape determinants of the hazel dormouse in Central Italy: Coefficients of the conditional-averaged model obtained from the three top models

	Estimate	Std. error	Adjusted SE	z	Pr(> z)	Importance
(Intercept)	3.355	0.250	0.272	12.35	<0.001	–
scl(FRAG)	0.305	0.065	0.079	3.84	<0.001	0.70
SPOPVTH	–0.755	0.118	0.148	5.11	<0.001	0.43
scl(log(PATCH))	0.577	0.081	0.097	5.74	<0.001	0.30

scl(FRAG) scaled number of neighbouring fragments within 1000 m from each sampled patch, connected by continuous hedgerows, scl(log(PATCH)) scaled log₁₀ of patch size, SPOP categorical variable indicating the sampling population where each individual was captured; Estimate conditional-averaged coefficients of covariates, Std. error standard error of estimates, Adjusted SE adjusted error of estimates, z Wald statistic for testing the hypothesis that the corresponding estimate is equal to zero (null hypothesis); Pr(>|z|) probability that the null hypothesis is true, Importance Relative variable importance

This finding could be crucial for future planning of conservation strategies for this species in fragmented landscapes.

The results also suggested that the dispersal of the hazel dormouse is strongly influenced by the presence of barriers, i.e. by the presence and configuration of landscape elements characterized by a resistance disproportionately high compared with the other land-covers (Zeller et al. 2012).

Considering as a resistance model the one developed using the 150-m buffer, the linear function, and the presence of a barrier-effect, the partial Mantel test did not show any relationship between the genetic distance between subpopulations and their ecological distance within the continuous SLR landscape. Thus, the SLR population seemed not to suffer from fragmentation and appeared to be more similar to a population in which genetic differences are mainly due to the IBD effect, as stated in Bani et al. (2017), and confirmed in this study by the high correlation value observed between ecological and Euclidean distances (Table S2).

Conversely, the ecological distance between subpopulations of the VTH population did not correlate with the log-transformed Euclidean distance, and we found a significant relationship between genetic distance and the partialized ecological distance (Table S2). This suggests that dispersal does not occur along straight lines between subpopulations within the fragmented landscape, and that it probably occurs along more tortuous permeable routes, where suitable habitats for the dormouse, such as hedgerows or small woodlots, are still present.

However, the analyses of private alleles and inbreeding highlighted that the ecological connectivity supported by the network of small forest patches and hedgerows was poorly effective in sustaining dispersal flows in the VTH landscape.

Bani et al. (2017) observed a strong structure of SLs within the VTH sampling population, due to the strong effect of IBR found in the present research. The consequent genetic differentiation is further reflected in the relatively large number of loci with private alleles found in four out of five SLs in the fragmented landscape. Overall, these results support the hypothesis of a general strong isolation of SLs in the VTH fragmented landscape. Here, the exchange of genes between subpopulations was actually highly restricted, as confirmed by two independent analyses performed to estimate the migration rates per generation among SLs. Indeed, a very small overall migration rate (about one migrant per generation) was found among SLs of the VTH sampling population through the private alleles' analysis, and only one marginally significant unidirectional migration was found from SL23 to SL21 through the Bayesian approach. However, the latter result is quite relevant in our opinion, since it refers to a one-generation dispersal of about 4 km. This distance seems to be quite

large for the hazel dormouse, compared with the values reported in literature (Juškaitis 1997; but see Schulze 1987), and could probably be considered as an extreme event, even considering dispersal distances occurring within suitable habitats. In our case, the dispersal movement was probably supported by the presence of a hedgerow, which was apparently well structured and with few short gaps (features deduced from remote images taken from Google Earth), which allowed the animal to cross a mainly agricultural area.

Gene flow is an important force for the maintenance of genetic diversity of spatially-separated populations. One between-subpopulation mating per generation could prevent an increase in differentiation in those populations in which distance or any geographical feature does not influence gene flow (Slatkin 1987; Allendorf et al. 2012; Greenbaum et al. 2014). In the VTH landscape the number of between-subpopulation migrants is probably lower than this minimal threshold, considering the very low number of estimated migrants. However, these migrant rates might have been underestimated because of the low number of investigated loci. In addition, we could not exclude the presence of other migrants coming from other small habitat fragments which were not investigated. In fact, the analyses of inbreeding and genetic drift landscape determinants showed the great importance of the number of surrounding connected fragments in limiting the isolation effect in VTH fragmented landscape.

Even though the main concerns regarded the VTH fragmented sampling population, we discovered a non-negligible degree of inbreeding in the continuous SLR landscape, too. However, in this population the number of individuals with a relatively high coefficient of inbreeding was small. The degree of inbreeding showed by the hazel dormouse in SLR population was similar to that showed by other species with similar ecological requirements in continuous habitats (Ralls and Ballou 1982). In the SLR landscape, the observed inbreeding values were probably due to the general philopatry (Berg and Berg 1999) and the low mobility of the species, which disperses over short distances even within continuous habitats (250 m mean dispersal distance in suitable habitat; Juškaitis 1997). This research showed the presence of a feeble degree of inbreeding, while Bani et al. (2017) previously found a weak excess of heterozygosity in the SLR population. These two aspects could prove a light increase of ecological resistance or some recent population declines (Cornuet and Luikart 1996; Peery et al. 2012), respectively, possibly due to forestry management (e.g. coppice turnover and the extent of the clearcutting areas) put in place in this protected area (see Sozio et al. 2016). Indeed, forest management practices may determine a patchwork of forest with different developing stages, with a different degree of suitability, as well as permeability for

forest species, thus increasing the inbreeding degree even in continuous contexts.

The evaluation of the genetic drift effect produced on genetic diversity showed lower values of the number of all loci effective alleles in SLs pertaining to the VTH fragmented landscape compared with those of SLR continuous landscape. This is another obvious clue of the isolation of VTH subpopulations.

The analyses of the landscape features acting as inbreeding, and genetic drift drivers highlighted a negative, and positive, relationship with habitat patch size and the number of neighbouring connected patches. As expected, these results confirmed the postulates of the Small Population Paradigm (Caughley 1994) and the importance of the Genetic Rescue Effect (Brown and Kodric-Brown 1977). Indeed, the maintenance of wide patches is crucial to sustain and conserve large populations, where the negative effect of inbreeding and genetic drift are restrained. On the other hand, in landscapes characterized by small patches, such as the VTH landscape where fragments are often smaller than 10 ha, the spatially-separated populations can be preserved over long period as metapopulations (Dondina et al. 2016b). In particular, our results suggested that, in order to ensure the maintenance of a functional metapopulation for the hazel dormouse, it is crucial to maintain a network composed of an archipelago of habitat fragments ecologically connected by hedgerows. However, the low number of migrants in the VTH landscape also suggests that dispersal processes in this landscape are probably severely hampered by the presence of barriers and by a not sufficiently suitable structure of the existing network of hedgerows.

For conservation purposes, it would be important to reduce the resistance opposed by barriers, defragmenting them in strategic sites, in order to increase the landscape connectivity by generating new ecological corridors (McRae et al. 2012).

As stated above, the existing hedgerow network also shows some concerns, since hedgerows in VTH landscape are too often flawed, with several important gaps. Discontinuous hedgerows have been proved to be not only unsuitable, but also detrimental (acting as ecological traps) for the hazel dormouse (Bright 1998), making the existing network largely ineffective for the conservation of the species. Important actions focusing on the enhancement of functional connectivity should thus be implemented to improve the internal characteristics of existing hedgerows. The hedgerow network needs a restoration of its physical continuity (Büchner 2008; Dondina et al. 2016a) and hedgerows need to be managed in order to obtain a well-developed and diversified shrub layer, which provides shelters, suitable sites for nesting and a continuous succession of food sources. These factors can increase the propensity of the

species to use hedgerows (Bright and Morris 1990, 2005; Ehlers 2012; Dondina et al. 2016a).

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