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Title

Landscape resistance affects individual habitat selection but not genetic relatedness in a reintroduced desert ungulate

Abstract

The long-term success of species reintroductions is strongly dependent on the availability of large areas of suitable habitat and the genetic make-up of the population. If available habitat is poorly connected this can hinder gene flow and lead to genetic fragmentation of the population, potentially increasing its extinction risk. We employed a conservation genomics approach in which we combined analyses of genetic structure with testing for potential landscape effects on habitat selection and gene flow in reintroduced Asiatic wild ass *Equus hemionus ssp.* in the Israeli Negev desert. Genetic structure and pairwise relatedness were first investigated followed by examination of landscape effects on individual habitat selection using records of GPS collared individuals. We then built habitat resistance surfaces and used electrical circuit theory to test for landscape effects on genetic relatedness. We detected weak genetic structuring, yet low spatial coherence among individuals from the same genetic cluster. Landscape variables had a significant impact on individual habitat selection, with wild ass avoiding steep slopes and habitats of low suitability as predicted by a species distribution model. However, the landscape genetic analysis revealed no effect of habitat resistance on genetic relatedness. These results suggest that gene flow in the reintroduced population is not impacted by landscape resistance. Indeed, the high mobility of the species may increase its resistance to the genetic effects of habitat fragmentation, at least over a small number of generations. We discuss other potential causes for the observed genetic structure including a behavioural effect. Our study highlights the importance of understanding species-habitat interactions for the long-term success of reintroductions.

Keywords

Landscape resistance, habitat selection, genetic structure, reintroduction, *Equus hemionus*, circuit theory

1. Introduction

Reintroductions are one of the most powerful conservation tools for reinforcing and re-establishing populations of threatened species, but success rates are often low. The most important determinants of the long-term success of a reintroduction are i) the availability of large areas of suitable habitat and ii) the genetic makeup of the reintroduced population (Wolf et al. 1998; Armstrong & Seddon 2008; Baguette et al. 2013). Genetic makeup is important since many reintroductions are based on a small number of founders. The resulting small population size during the early stages of the reintroduction can lead to increased genetic drift and inbreeding, causing the loss of genetic diversity and adaptive flexibility in the established population (Frankham et al. 2002; Templeton 2017). These negative effects are further exacerbated if the reintroduced population is fragmented. Resulting genetic isolation of subpopulations can make these population fragments more vulnerable to extinction due to inbreeding and stochastic genetic and demographic processes (Saccheri et al. 1998; Bozzuto et al. 2019).

Large connected areas of suitable habitat are also crucial to facilitate sufficient demographic growth of the reintroduced population (Armstrong & Seddon 2008). In contrast, habitats with low functional connectivity (whereby the landscape impedes individual movement) can hinder range expansion and prevent reintroduced populations from successfully colonising the available habitat (Templeton et al. 2011; Neuwald & Templeton 2013; Ziółkowska et al. 2016). Furthermore, low connectivity can also limit gene flow between occupied patches, resulting in spatial sub-structuring of the population (Manel et al. 2003; Bergl & Vigilant 2007). This may explain observations of within-population genetic structure in reintroduced populations, with genetic clusters centring around release sites (Howell et al. 2016; Grauer et al. 2017; Moraes et al. 2017). In order to avoid the problem of genetic isolation, individuals must be able to disperse between occupied patches into new suitable territory at a rate that facilitates sufficient gene flow (Mills & Allendorf 1996).

Gene flow is limited by factors restricting individual dispersal, here defined as the movement between habitat patches or subpopulations (Benton & Bowler 2012). In terrestrial mammals, dispersal ability is usually affected by landscape structure, climatic and anthropogenic factors, or specific combinations of these (Howell et al. 2016). Major landscape features (e.g., roads, mountain ridges) may act as physical barriers completely preventing movement across them, but areas of less preferred habitat may also reduce gene flow (Storfer et al. 2007). For example, in female-philopatric mountain goats, male habitat selection best predicted gene flow and relatedness across the landscape (Shafer et al. 2012). However, for many reintroduced populations, information on habitat use and preference is limited, especially when the species has been absent from the area for a long time or when it is replaced by a closely-related group (e.g., a different subspecies) which makes prediction of resource use and dispersal more difficult (Seddon & Soorae 1999). Therefore, directly assessing habitat connectivity and gene flow and the factors impacting them is an important measure to optimise population management to enhance chances of long-term population persistence.

The Asiatic wild ass *Equus hemionus ssp.* (Pallas, 1775) reintroduced to Israel, presents an ideal opportunity for furthering our understanding of the environmental effects on the dispersal and genetic structure in small, reintroduced populations. *Equus hemionus ssp.* was reintroduced in Israel after the local subspecies (Syrian wild ass *E.h.hemippus)* became extinct (Saltz & Rubenstein 1995). A captive breeding facility was established by the Israeli Nature and Parks Authority (INPA) in 1968 from individuals of two subspecies; Iranian onager *E.h.onager* and Turkmen kulan *E.h.kulan* (Saltz et al. 2000). From this breeding facility, 38 individuals were released into the Negev desert between 1982 and 1993 at two release sites (Fig. 1) (Saltz & Rubenstein 1995). The population has since expanded its spatial distribution across the highly heterogenous landscape and is currently estimated at >250 individuals (Gueta et al. 2014; Renan et al. 2018).

Asiatic wild ass have a highly flexible fission-fusion social structure and a resource defence polygyny mating system (Boyd et al. 2016; Renan et al. 2018). Dominant males defend territories near permanent water sources, while females form unstable social groups with individual associations based on reproductive status rather than relatedness (Rubenstein 1994; Saltz et al. 2000; Wallach et al. 2007; Altman 2016). Previous analyses based on mitochondrial DNA haplotypes and nuclear microsatellite markers identified a weak spatial genetic structure in the established population (Gueta et al. 2014; Renan 2014). The authors suggested a combined effect of range expansion and low habitat connectivity between colonised areas to be the underlying cause (Gueta et al. 2014). This possibility is supported by previous studies which identified resource distribution and topography as the main predictors for wild ass presence and pathway usage (Davidson et al. 2013; Nezer et al. 2017). In the Negev, patches of suitable habitat appear to be separated by areas of low resource availability and challenging topography such as steep cliffs and canyons that could act as barriers to wild ass movement, hence limiting gene flow between patches. Since the recently established population in Israel is geographically isolated with no opportunity for external migrants from neighbouring countries, it is particularly vulnerable to the negative effects of genetic drift (Frankham et al. 2002). Further spatial subdivision would be a severe threat to this recently established population and could jeopardise the long-term success of the reintroduction (With & King 1999; Wang et al. 2017; Pelletier et al. 2019).

The aim of the present study was to investigate potential landscape effects that may cause genetic structuring of the reintroduced population. First, we assessed genetic clustering of the population using a panel of 1645 genome-wide single nucleotide polymorphisms (SNPs). Then, we analysed individual GPS collar data and investigated habitat selection with respect to slope and habitat suitability based on a species distribution model (SDM). Finally, we created landscape resistance surfaces from habitat selection data and applied electrical circuit theory to test for an effect of habitat resistance on genetic relatedness. Based on wild ass ecology and previous studies of the population, we predict: i) the population in Israel is genetically structured into spatially distinct clusters ii) individuals avoid areas of low habitat suitability (based on the SDM) and steep slope, as reported for wild ass in other populations (e.g. Sharma *et al.*, 2004), and iii) steep cliffs form a complete barrier to wild ass movement and hence we predict a stronger effect of slope-based landscape resistance than suitability-based landscape resistance on genetic relatedness in the population.

2. Materials and Methods

2.1 Study site

The Negev is a hyper-arid desert that extends throughout Southern Israel. The landscape is defined by a steep gradient in elevation ranging from the Negev Highlands in the Northwest (>1000m above sea level) decreasing towards the Arava valley and the Dead Sea in the East (<300m below sea level) (Stern et al. 1986). This elevation gradient coincides with a gradient in mean maximum annual temperature and precipitation, ranging from 22.6°C and 150mm in the Negev Highlands to 31.1°C and 30mm in the hotter and more arid Arava (Israel Meteorological Service). This climatic gradient also causes differences in vegetation, with shrub-steppes in the

Negev highlands giving way to sand and desert savannoid vegetation types in the Arava (Danin 1999). The topography of the Negev is complex and characterised by steep cliffs and levelled floodplains. Vegetation is mostly limited to ephemeral streambeds and floodplains. Permanent water sources are scarce, however, flash floods occurring after heavy rainfall in the winter fill up natural rock pools which retain water for several months (Nezer et al. 2017). In addition, there are three artificial water sources which are maintained throughout the year by the INPA to provide wildlife with water, which have also become activity centres of the wild ass population (Gueta et al. 2014; Nezer et al. 2017).

2.2 DNA sample collection and sequencing

DNA samples were collected opportunistically by rangers and veterinarians of the INPA across seasons, between 2010 and 2017. Blood or tissue samples were taken from individuals that were killed in road traffic accidents, from injured individuals receiving veterinary treatment or during the fitting of global positioning system (GPS) collars. Precise geographical locations were available for all samples (Fig. 1). Whole blood samples were stored in EDTA tubes (not heparinized; BD Vacutainer K2E 18.0mg, Vacuette K3E 3mg), tissue samples were either stored in 70% ethanol or untreated in paper envelopes. All blood and tissue samples were stored frozen (at -20°C or -80°C). We purified DNA from samples using commercial silica spin column-based extraction kits (QIAGEN DNeasy Blood and Tissue Kit, Thermo Scientific GeneJET Genomic DNA Purification Kit), following manufacturers protocol. We sequenced samples using double digest restriction-site associated DNA marker sequencing (ddRADseq) methods and the high-fidelity versions of the enzymes *Eco*RI and *Sbf*I (R3101S and R3642L, respectively; New England Biolabs). We prepared libraries following a protocol adapted from Peterson et al. (2012) and sequenced them on a single flow cell lane of an Illumina HiSeq 4000 system. Over 400 million raw paired-end sequence reads were produced with a mean read length of 300bp. We assessed the quality of raw reads using the FastQC tool (Andrews 2010). A mean Phred+33 quality score >30 was recorded for all bases. We processed raw sequences in the STACKS 2.0 pipeline (Catchen et al. 2013) and assembled loci *de novo* using the denovo_map wrapper program in STACKS and identified optimal parameter settings using an approach adapted from Paris et al. (2017) and SNP error rates, calculated using seven replicate pairs of individuals included in the data set. To avoid linkage between markers we retained only the first SNP on a locus using the *--write-single-snp* function in the population program in STACKS. Subsequently, we filtered called SNPs in 3 steps in the vcftools programme (Danecek et al. 2011) using site and individual filtering options (minimum mean individual coverage ≥35x, minor allele count ≥3, SNPs present in minimum of 80% of individuals). Finally, SNPs that deviated from Hardy-Weinberg equilibrium as defined by p-value threshold >0.05 were removed.

2.3 Genetic structure analysis

Initially, we explored the data using Principal Component Analysis (PCA), which fits orthogonal Principal Components (PCs) that summarise overall variability between individuals. Subsequently, we investigated genetic structure in more detail using two different approaches: A discriminant analysis of principal components (DAPC; Jombart, 2008) and a Bayesian cluster analysis implemented in the program STRUCTURE (Pritchard et al. 2000).

DAPC is a multivariate approach which performs a PCA in a first step and then subjects the PC scores to a discriminant function analysis (DFA). Unlike PCA, DFA fits orthogonal discriminant functions that maximise between group relative to within-group variation. Therefore, it is suited to differentiating between genetic groups (Jombart et al. 2010). A K-means clustering approach can be applied to assess the number and composition of genetic clusters (K) in the data. The best supported model is identified using the Bayesian Information Criterion (BIC), where the lowest BIC, which is often indicated by an elbow in the curve, is preferred. We performed PCA and DAPC in the ‘adegenet' package (Jombart 2008) in R (R 3.5.3, R core team 2020). In both analyses we retained the first 10 PCs, which explained 54.96% of the total variance.

We ran the program STRUCTURE with the admixture model and correlated allele frequencies, for K=1-10, with 10 repetitions for each K. The runs were performed with $1x10^6$ iterations of the Markov Chain Monte Carlo (MCMC) chain preceded by $1x10^5$ burn-in iterations. We assessed STRUCTURE outputs for the optimal value of K using the log likelihood (Pritchard et al. 2000) and the Evanno method (Evanno et al. 2005) in the web-based version of STRUCTURE HARVESTER (Earl & vonHoldt 2012). Pritchard et al. (2000) suggest that a value of K which maximizes the model log likelihood Ln(PD) is optimal. However, Ln(PD) often plateaus or continues to increase after reaching the optimal K-value and so Evanno et al. (2005) proposed an improved method to estimating optimal K, based on the second order rate of change of the likelihood function. We produced ancestry bar plots in STRUCTURE HARVESTER. As GPS data from collared individuals suggested fidelity to a smaller range during the breeding season (Supplementary material, Fig. A1), population genetic differentiation may be easier to detect at this time. We therefore repeated our analyses on the 15 individuals sampled during the breeding season.

2.4 Individual habitat selection

To investigate a potential effect of landscape characteristics on gene flow we used individual movement as a proxy for gene flow and investigated factors that restrict individual movement using high resolution movement data from GPS collars. Previous studies have highlighted two habitat characteristics impacting wild ass distribution: Species-specific habitat suitability and topography (Davidson et al. 2013; Gueta et al. 2014; Nezer et al. 2017). To verify the impact of these characteristics on wild ass movement, we first performed a compositional analysis of habitat selection (Aebischer et al. 1993). The analysis compares the relative abundance of a specific habitat type with its relative use by individuals. This way, habitat types that are avoided by individuals and potentially represent barrier to movement can be identified.

We investigated habitat selection with respect to habitat suitability, based on a previously developed species distribution model (SDM, Nezer et al., 2017). The model, which covered most of the area of the present study (Fig.1), was created using indirect observations and a data set of nine habitat variables from different categories relevant to wild ass biology (water, topography, biotic conditions, climate, anthropogenic disturbance). Since the model was based on faecal mount surveys rather than direct observations of wild ass, we tested the predictive power of the model using the high resolutions GPS-collar data. We used the model output, a probabilistic distribution map that represents the probability of wild ass distribution in the area with values ranging from 0 (low probability) to 99 (high probability), as an indicator for habitat suitability (habitat suitability index). Specifically, low probability values indicate low habitat suitability and high probability values indicate high habitat suitability. Since the SDM from which habitat suitability was derived did not cover the entire study area, the analysis was restricted to the part of the study area covered by the SDM. The SDM did not consider seasonal differences in habitat suitability, and potential seasonal patterns in wild ass natal dispersal are unknown. Hence, the analysis did not account for seasonality.

Previous studies have reported topography as one of the most important physical constraints to wild ass movement, with steep slopes (> 30°) being avoided entirely (Sharma et al. 2004; Henley et al. 2007; Davidson et al. 2013; Nezer et al. 2017). Therefore, we decided to also investigate habitat selection with respect to topography as a habitat measure directly linked to movement ability. The same slope layer from the SDM was used, which was generated from a contour dataset retrieved from the Survey of Israel (MAPI; for further details see Nezer et al. 2017).

To investigate individual habitat selection with respect to habitat suitability and slope, we used movement records from 7 GPS collared individuals. Between 2012 and 2013, five individuals (4 males, 1 female) of the reintroduced population were fitted with GPS collars (African Wildlife Tracking company) (Giotto et al. 2015) and an additional 2 females were collared in 2015. Collars were set to record the location every hour and animals wore collars between 10–25 months resulting in a minimum of 2937 records per individual within the reduced study area (Supplementary material, Table A1).

Slope and habitat suitability raster layers had a resolution of 100m and we extracted the grid cell values for each GPS record from the collared individuals using the extract values to points function in ARCGIS (ESRI 2011). Subsequently, we divided extracted data for each variable into categories, to investigate proportional habitat use. For habitat suitability index we divided the range from 0–99 equally, rendering a low (0–33), intermediate (34–66) and high (67–99) suitability category. Based on previous studies (Sharma et al. 2004; Davidson et al. 2013) we set a threshold for steep slopes at 30 degrees and divided the slope into low (0–15°), intermediate (16–30°) and steep slope, containing all records >30°.

We performed a compositional analysis of habitat selection on the defined habitat categories using the compana function in the 'adehabitatHS' package in R (Calenge 2006). The analysis first tests for significance of habitat selection using Wilks lambda and subsequently produces a ranking matrix indicating whether a specific habitat type is used significantly more or less than another. P-values were estimated by randomisation tests (999 permutations of the data). Aebischer et al. (1993) recommend using a minimum of 6 individuals, therefore, we pooled males and females for the analysis. We analysed habitat use relative to habitat availability within the entire habitat area, corresponding to third order selection as described by Johnson et al. (1980). We did not limit the analysis to habitat available within an individual's home range, since gene flow is mediated by long-distance dispersal movements extending beyond home range boundaries. Finally, compositional analysis assumes no individual variation in habitat selection, and we tested this assumption by eigenanalysis of selection ratios with the eisra function.

2.5 Landscape genetic analysis

2.5.1 Resistance surfaces

After assessing the impact of habitat characteristics on individual movement, we created habitat resistance surfaces. This approach assigns resistance values to each cell in a habitat grid, reflecting the relative cost inflicted on an individual moving through it (Spear et al. 2010). We created three habitat resistance surfaces based on habitat suitability index, slope, and geographic distance. First, we inverted the habitat suitability map using the raster calculator in ARCGIS. To grid cells with a probability of 0 we assigned a marginally positive value 0.01 to comply with input requirements of downstream analysis. The resulting habitat resistance map based on habitat suitability ranged from 0.01 (low resistance) to 99 (high resistance). We parameterised the second resistance surface based on slope, so that grid cells with a slope of 1°–30° were assigned a resistance value of 1–30 respectively. We set a threshold by assigning grid cells with a slope >30° a resistance value of 99. Grid cells with a slope of 0° we assigned a resistance of 0.01. Additionally, we created a control resistance surface based solely on geographic distance, by assigning all grid cells of the resistance surface a value of 1. All resistance surfaces had a spatial resolution of 100m and were produced with ARCGIS (Fig. 2).

2.5.2 Pairwise distances

Since the landscape genetic analysis was restricted to the part of the study area covered by the SDM, we excluded three individuals which fell outside the SDM area from the analysis (Fig. 1). We used the programme CIRCUITSCAPE (version 4.0, McRae et al. 2013) to calculate pairwise resistance distances for the remaining 27 individuals for the three resistance surfaces. Circuitscape applies algorithms from electronic circuit theory to estimate resistances to current flow between nodes. The program was run in pairwise mode with individuals set as nodes, connected to all eight neighbouring cells surrounding a node. Pairwise genetic distance was expressed through a relatedness coefficient, which is effectively a measure of the genetic distance between two individuals. We estimated pairwise relatedness coefficients in the 'related' R package (Pew et al. 2015) using the corrected Wang (2002) estimator, which has been shown to achieve high accuracy with small sample sizes (Wang, 2017).

2.5.3 Distance-based redundancy analysis

To test for a potential relationship between habitat resistance distance and genetic distance we performed a distance-based redundancy analysis (dbRDA) using the *capsscale* function in the 'vegan' R package (Oksanen et al. 2010). dbRDA is an extension of multivariate regression which accepts distance matrices as response variables. The response matrix is transformed into synthetic variables which are then regressed on multiple explanatory variables (Legendre et al. 1999; Buttigieg & Ramette 2014). First, we transformed the pairwise habitat resistance matrices to generate one-dimensional explanatory variables for the dbRDA. For this purpose, we performed principal coordinate analyses using the *pcoa* function in the 'ape' R package (Paradis & Schliep 2018) with a Lingoes correction for negative eigenvalues to preserve all variation of the landscape resistance matrices. Subsequently, we used a Broken Stick model to estimate the number of significant principal coordinates (PCos) (MacArthur, 1957; Supplementary material, Fig. A2). For all three resistance variables only the first or first and second PCos explained more variation than expected under the Broken Stick model. However, since this accounted for only ~35% of variation in each variable, we repeated the analysis with the first 4 PCos retained which accounted for >50% of variation (Supplementary material, Table A2).

We tested a total of seven models, once with the first 4 PCos and once with only the first PCo retained (Table 1). We ran three models that tested for landscape resistance effects on gene flow by setting the pairwise relatedness matrix as the response variable and one of the three transformed habitat resistance matrices (based on either geographic distance, slope, SDM) as explanatory variables. Additionally, we tested four partial models that controlled for an effect of geographic distance on habitat resistance and the reciprocal. We tested for significance with permutation tests using the *anova.cca* function with 9999 permutations. Since GPS data from collared individuals suggested fidelity to a smaller range during the breeding season (Supplementary material, Fig. A1), a potential landscape genetic effect may be easier to detect during the breeding season. Hence, we repeated our analyses on the 14 individuals sampled during the breeding season.

3. Results

3.1 Sequencing and summary statistics

Illumina sequencing produced a total of 803,092,446 raw sequence reads. The de novo assembly with optimal parameter settings (m3N0M4n4) produced 2,639 polymorphic loci with an average of 2.27 SNPs per locus and a mean(±SD) SNP error rate of 1.08±0.31%. After SNP filtering the final data set contained 1496 SNPs and 30 individuals. Initial population genetic analysis revealed no significant difference between the mean (±SD) expected heterozygosity (He=0.344 ± 0.128) and observed heterozygosity (Ho=0.345 ±0.144; Paired Student's t-test: t(1495)=-0.440, p-value=0.660) of the population. The inbreeding coefficient indicated no population level inbreeding (Fis=-0.002).

3.2 Genetic structure analyses

The variation explained by the first two principle components of the PCA was low (PC1 9.86%, PC2 7.65%) and no prominent genetic clusters could be identified (Fig. 3a). Also, the BIC plot of the DAPC indicated K=1 as optimal (Fig. 3c). This suggested no meaningful genetic clustering in the population. In contrast, for the STRUCTURE analysis, the Evanno method identified a clear peak of delta(K) for K=4 (delta(K)=57.07; Fig. 3d). However, the Evanno method cannot identify an optimum of K=1 and may indicate peaks at higher values of K even in the absence of any genetic structure (Evanno et al. 2005). The mean Ln P(D) across different values of K remains consistent with no distinct maximum value or plateau (Fig. 3d), suggesting that there may be only a very weak signal of genetic structure. The STRUCTURE ancestry plot highlights 4 clusters with high admixture levels in some individuals (Fig. 4b).

Since the two approaches gave slightly different results, we assessed their robustness by comparing the individual assignments to the four clusters between the multivariate and the Bayesian approach. Based on the results of the Evanno method, we ran the DAPC with predefined K=4. Three out of the four described clusters were differentiated along the first PC while the fourth cluster was differentiated more strongly by the second PC (Supplementary material, Fig. A3). Subsequently, we compared individual assignments from DAPC and STRUCTURE. In the DAPC analysis all individuals had assignment probabilities of 1, whereas in STRUCTURE, 12 individuals could not be assigned clearly to a single ancestral population (q-values <0.7) and these individuals were excluded from the comparison. Of the 18 remaining individuals, 16 clustered together in groups consistent between STRUCTURE and DAPC analyses (Fig. 4). However, these clusters were geographically dispersed. Six individuals assigned to the same cluster were located in close proximity near an artificial water source (Fig. 4). However, most individuals were dispersed across the study area with no clear spatial segregation between genetic clusters. Repeating the genetic structure analysis using only samples collected during the breeding season did not impact these findings (Supplementary material, B1).

3.3 Individual habitat selection

Compositional analysis of habitat categories revealed that individual habitat selection differed significantly from random with respect to habitat suitability index (Lambda=0.013, p=0.012, by randomisation) and slope (Lambda=0.064, p=0.021, by randomisation). The ranking matrix highlighted a clear preference for low slope and high suitability habitats (Supplementary material, Table A3). Wild ass used more low slope and more intermediate and high suitability habitat than proportionally available (Fig. 5). The analysis using GPS-collar data therefore indicated that both habitat suitability index and slope are relevant variables affecting habitat selection in wild ass. Eigenanalysis of selection ratios indicated no difference in habitat selection between individuals (Supplementary material, Fig. A4).

3.4 Landscape genetic analysis

None of the tested models of the distance-based redundancy analysis returned significant results and the explained variance was close to zero for all predictor variables (Table 1). Habitat suitability and slope explained negligible variation in genetic relatedness between individuals of the population. This was also true for models controlling for geographic distance and resistance distances, respectively (Table 1). The results were consistent across models that retained only the first PCo or the first 4 PCos of the explanatory variables, hence, the models appear to be insensitive to these minor variations, indicating robustness of the results. Repeating the landscape genetic analysis using only samples collected during the breeding season did not affect the results (Supplementary material, B2).

4. Discussion

The analysis revealed some genetic structuring in the reintroduced population of wild ass in Israel. However, inconsistencies in the optimal number of clusters and individual assignment between the different methods highlight that the genetic differentiation is weak. These results are consistent with a previous study on the same population using lower resolution genetic data (eight microsatellite markers) which demonstrated weak yet significant genetic differentiation between four a priori defined subpopulations (Renan 2014). Taken together, these results suggest a weak genetic structure within the reintroduced population. Nevertheless, our new analyses of landscape resistance to individual movement does not support this as being a cause. The analysis of GPS data showed that landscape resistance affected wild ass habitat selection, with individuals clearly avoiding low suitability habitats and steep slopes. However, the landscape genetic analysis gave no support for an effect of landscape resistance on genetic relatedness.

The present study demonstrates that the Asiatic wild ass clearly avoid certain habitats, yet functional connectivity across the study area appears to be retained. Although large proportions of the habitat have low suitability, these are interwoven by a network of low resistance paths, which likely facilitate individual movement across the study area (Fig. 2). In contrast to our expectations, habitat resistance based on slope was found to have no negative association with relatedness. Slopes above 30° account for only a very small proportion of the habitat in the Negev, nonetheless, they occur in the form of steep cliffs extending over large geographical areas and are expected to form true barriers to wild ass movement. However, wild ass are large-bodied, highly mobile mammals which have been reported to range long distances, and it is likely that even if individuals are unable to climb these cliffs, they can circumvent them (Nandintsetseg et al. 2016; Nezer et al. 2017). In fact, the GPS data showed some long-distance movements by two females, which support the findings that even high resistance habitat does not prevent movement across the landscape in the Negev population (Supplementary material, Fig. A1). Therefore, despite being important for individual habitat selection, it currently appears that slope and habitat suitability have no to little effect on gene flow. These are promising findings for the reintroduced population of wild ass in Israel and potentially for other equid populations in heterogenous habitats.

The results contradict our expectations and underlines the importance of testing believed landscape barriers to gene flow, as assumptions based on movement behaviour or habitat selection may be misleading. Similarly, other studies have reported differential effects of roads on gene flow, even in cooccurring mammals of similar size and mobility (Frantz et al. 2012). In this study we investigated generic movement from GPS records and found that wild ass appeared to avoid low suitability habitats. However, we did not detect any dispersal movements and it is possible that dispersing individuals may be willing to cross low suitability habitats which are otherwise avoided during routine movements (Fey et al. 2016; Keeley et al. 2017). Other studies have found such patterns, for example radio-tracking of red squirrels identified that dispersing individuals frequently crossed roads, which were otherwise avoided during routine movements (Fey et al. 2016). Finally, little is known about the natal dispersal of Asiatic wild ass with regard to seasonality or sex bias. Consequently, in our habitat selection analysis we did not differentiate between sexes nor test for seasonal effects. However, if such a bias in natal dispersal existed, it is possible that an existing landscape genetic effect was obscured (Shafer et al. 2012). Long-term data sets from GPS movement records could provide information on wild ass natal dispersal, which could be used to parameterise dispersal-specific resistance layers and improve landscape genetic analysis.

Despite the apparent lack of a landscape effect on gene flow, the present study identified a weak genetic structure in the population, which is likely caused by factors that have not been measured here. Three potential causes for genetic structuring are related to the population's demographic history. First, at the onset of the reintroduction, a captive breeding core was created from individuals of two different subspecies (Saltz & Rubenstein 1995). Differences in the effective niche of these two subspecies may result in divergent habitat preferences and lead to spatial separation and limited interbreeding, ultimately promoting the rise of genetic substructure (McDonald et al. 2019). However, an analysis investigating spatial autocorrelation based on individual hybrid indices found no support for spatial segregation based on subspecies ancestry (unpublished results). A second possible reason for genetic structuring in our study population is that it could be the signature of the multiple release events during establishment of the wild population. Individuals were released at two reintroduction sites, from which they dispersed across the habitat. Founder effects and genetic drift experienced by the population during early stages of establishment could have resulted in the weak genetic differentiation. Other studies have described a genetic signature of release events in translocated populations (Williams et al.

2000; Biebach & Keller 2009; Puckett et al. 2014; Moraes et al. 2017). For example, Grauer et al. (2017) reported unique patterns of genetic structure caused by serial release events of individuals from different sources, in a reintroduced population of American Marten. Finally, a behavioural effect related to the resource-defence-polygyny of the Asiatic wild ass could be the cause for the observed genetic clustering (Renan 2014). Male wild ass defend territories around permanent water sources. Increased resource requirements restrict females to the vicinity of these permanent water sources during the foaling and breeding season in the summer (Saltz et al. 2000; Wallach et al. 2007; Boyd et al. 2016). The GPS records of radio collared individuals reflected these behavioural patterns: Males remained close to a water source all year round, while females extended their movement range in the winter, yet returned to the same area of the permanent water source in the summer when mating occurs (Supplementary material, Fig. A1). This seasonal range contraction and the resulting highly localised breeding activity could result in a genetic differentiation between individuals from different activity centres (Renan 2014; Giotto et al. 2015). This could explain the presence of a fine-scale weak genetic structure despite high mobility of the species. A similar effect has been observed in feral horses in Nevada: during the hot summer, when most of the mating occurred, herds were unable to disperse from the limited water sources, which resulted in a weak genetic differentiation between subgroups from different water sources, despite their overlapping winter ranges (Ashley 2004).

While the current analysis failed to identify an effect of habitat on gene flow, it is important to consider the short lag time since the initial release of individuals which was less than five generations ago (given a generation time of 7.5 years; Ransom et al. 2016). Landscape resistance may have an impact on gene flow, however, not enough time has passed for the signal to become established (Landguth et al. 2010). At this point it is not possible to determine with certainty what is causing the observed weak genetic differentiation. If it is due to the release events and range expansion combined with genetic drift during the establishment phase, it is expected to diminish over time due to continued gene flow (Short & Petren 2011). However, if it is caused by a behavioural or a (not yet detectable) landscape effect, then it is likely to persist or even intensify over time.

Some restriction to gene flow can increase the potential for retaining genetic diversity and is therefore beneficial (Chesser 1991; Chesser et al. 1993). However, intensification of the genetic structure may lead to population fragmentation and genetic isolation of subpopulations, which could increase the populations extinction risk (With & King 1999; Wang et al. 2017). In an isolated population of woodland caribou, Rangifer tarandus caribou, reduced gene flow has caused the rise of genetic substructure over a short time period (15 years) (Pelletier et al. 2019). The authors believe that this fragmentation is severely threatening the populations long-term persistence, as a 53% reduction in the population's inbreeding effective size has been recorded over a timespan of only two generations. To avoid the risks of genetic isolation, management of the Asiatic wild ass population should aim to prevent any further reinforcement of the observed structure. Specifically, creating additional permanent water sources is expected to increase the number of activity centres, minimise distances between these and possibly encourage more dispersal movements. Furthermore, additional permanent water sources provide more high-quality territories for Asiatic wild ass, thereby enabling a greater number of males to contribute to the gene pool (Greenbaum et al. 2018; Renan et al. 2018).

5. Conclusions

Here we presented an investigation into landscape barriers to gene flow in a reintroduced population by combining GPS movement records and genetic samples. The results demonstrate the importance of genetic analysis to test presumed landscape barriers to gene flow. Particularly, large-bodied highly mobile species may likely be able to maintain gene flow even across unsuitable habitat. Further, the present study highlights the importance for long-term genetic monitoring of reintroduced populations. Genetic structure may develop even after successful establishment of a growing population (Neuwald & Templeton 2013), and in the absence of obvious landscape barriers. While this may be simply a transient phenomenon caused by a founder effect, it may have other underlying causes. If a genetic differentiation persists and intensifies, it can reduce reintroduction success even long after initial release of individuals and hence should be considered in conservation management protocols (Kramer-Schadt et al. 2004).

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Tables and Figures

Table 1 Distance based redundancy analysis models tested and their total variance (Inertia), the % variation explained (R2) and adjusted % variation explained (adjusted R2), the degrees of freedom (df), F-statistic (F) and p-value of the permutation tests (9999 permutations). Partial models controlling for a third variable are indicated with |.

Variable	Inertia	%Variation (constrained Inertia or R2)	Adjusted % variation explained (adjustedR2)	df	F	p
only first PCo retained						
SDM resistance	4.47	3.74%	<1%	1	0.972	0.610
Slope resistance	4.47	3.86%	<1%	1	1.003	0.497
Geographic distance	4.47	3.64%	<1%	1	0.943	0.698
SDM resistance | geographic distance	4.47	3.96%	<1%	1	1.028	0.407
Slope resistance | geographic distance	4.47	3.23%	<1%	1	0.833	0.915
Geographic distance | SDM resistance	4.47	3.85%	<1%	1	1.000	0.495
Geographic distance | slope resistance	4.47	3.01%	<1%	1	0.776	0.961
first 4 PCos retained						
SDM resistance	4.47	15.05%	<1%	4	0.975	0.682
Slope resistance	4.47	15.98%	<1%	4	1.046	0.204
Geographic distance	4.47	14.48%	<1%	4	0.932	0.881
SDM resistance | geographic distance	4.47	15.84%	<1%	4	1.023	0.415
Slope resistance | geographic distance	4.47	15.12%	<1%	4	0.967	0.655
Geographic distance | SDM resistance	4.47	15.27%	<1%	4	0.986	0.577
Geographic distance | slope resistance	4.47	13.63%	<1%	4	0.871	0.918

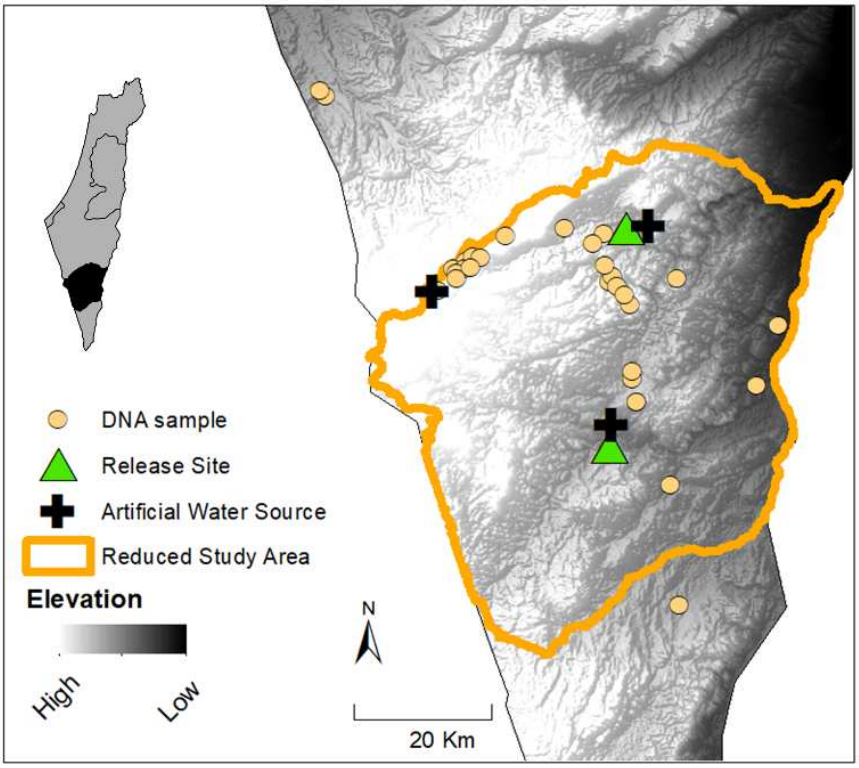

Fig. 1 Map of the study area in Southern Israel, depicting locations of *Equus hemionus ssp*. DNA sample collection (n=30), release sites of the reintroduction and location of three artificial water sources. The orange outline indicates the area of the species distribution model created by Nezer et al. (2017) and the study area for the landscape genetic analysis

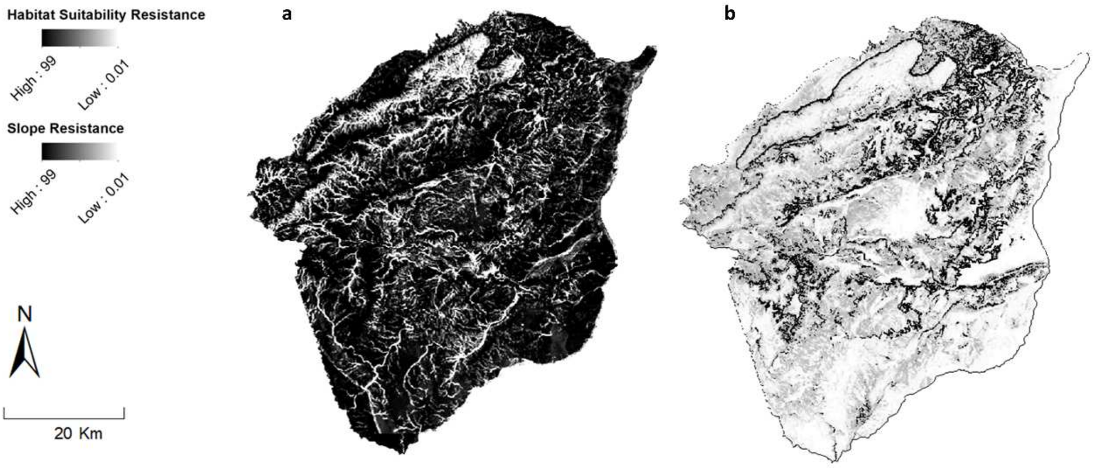

Fig. 2 Habitat resistance surfaces for the study area in Southern Israel, based on (a) habitat suitability index and (b) slope. Shading indicates resistance value.

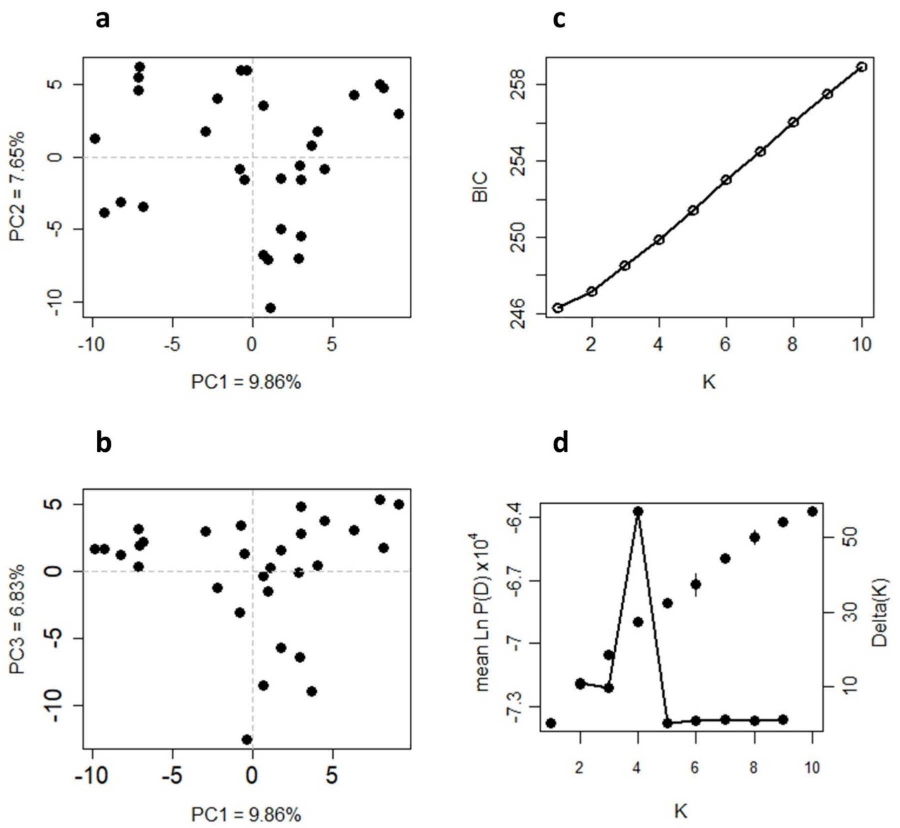

Fig. 3 Optimal number of genetic clusters in the reintroduced population of *Equus hemionus ssp*. in Southern Israel. Initial exploration using principle component analysis indicates no distinct clustering along (a) the first and second and (b) along the first and third principle components. (c) The Bayesian information criterion (DAPC analysis) is lowest for K=1 indicating no genetic clustering. (d) The Evanno method (STRUCTURE analysis) indicates a clear peak in Delta(K) for K=4, while the mean Ln P(D), in dots, does not reach a plateau.

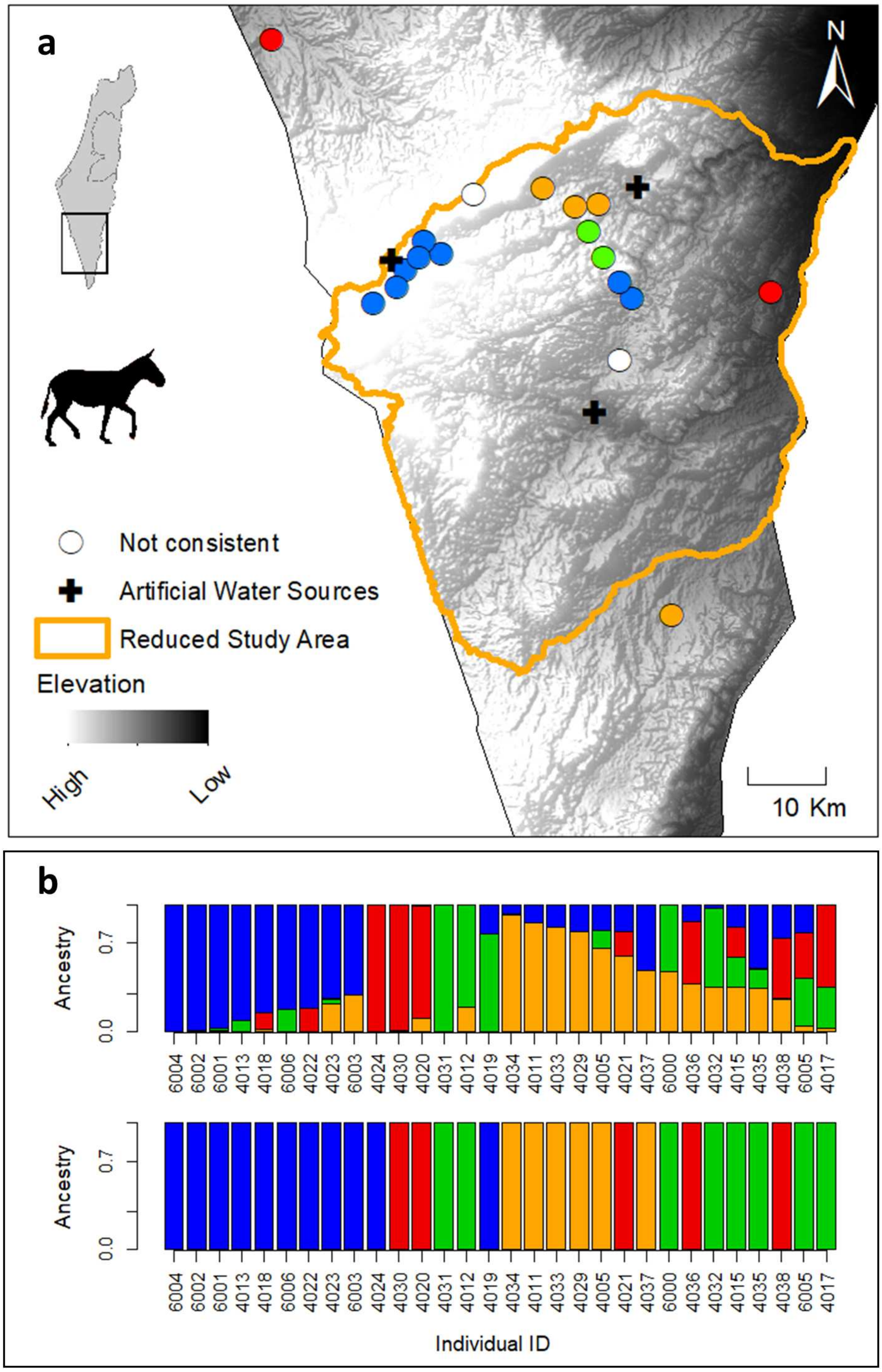

Fig. 4 Genetic structure analysis of reintroduced *Equus hemionus ssp.* in Southern Israel. (a) Spatial distribution of sampling locations for individuals consistently assigned to the same cluster by both STRUCTURE and a discriminant analysis of principle components (DAPC). Only individuals with a high assignment probability (>=0.7) to a single genetic cluster are displayed. Colours indicate 4 genetic clusters (blue, green, red, orange). White points indicate individuals not assigned consistently by the two analyses. (b) Proportional ancestry of all individuals (n=30) for K=4 as estimated by STRUCTURE (top) and DAPC (bottom).

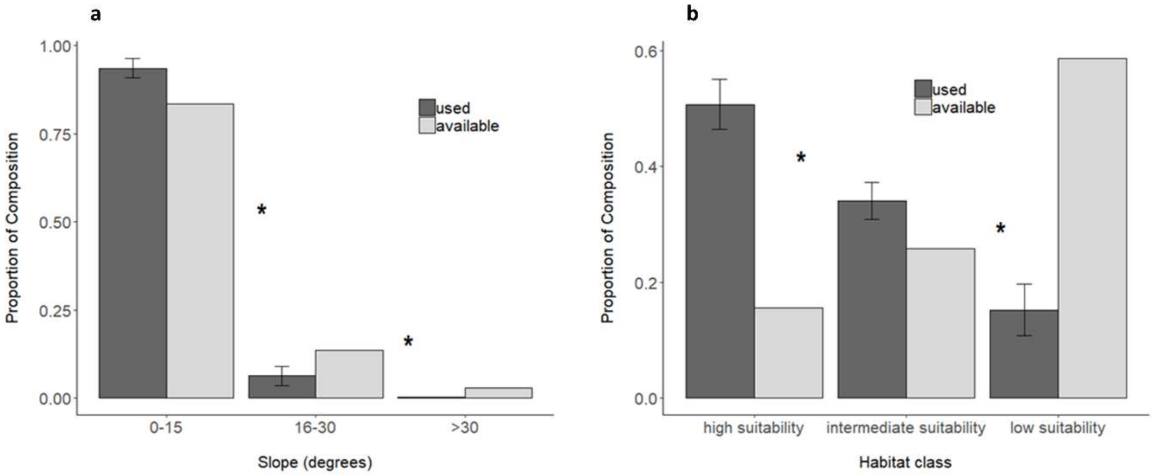

Fig. 5 Proportional habitat use by 7 *E.hemionus ssp.* individuals in Southern Israel between 2013-2017 based on GPS record data. Habitat is classified based on (a) slope and (b) suitability index. Dark bars indicate mean (+-SD) proportional usage by individuals and light bars indicate proportional availability in the study area of each habitat class. "*" indicates significance by permutation of differences in mean proportional habitat use between categories.

Supplementary material

Appendix A

Table A1 Location records collected for different time intervals for 7 individuals equipped with GPS collars recording at hourly intervals

ID	Name	Sex	Start date	End date	Total time	Total number of records	Number of records within reduced study area
6000	Ktsoutsy	Male	16.10.2012	05.12.2014	25 months	15712	5323
6001	Short tail	Male	08.06.2013	18.04.2014	10 months	7786	4011
6002	Nahum Tacum	Male	12.07.2013	31.12.2014	17 months	14101	7898
6003	Gila	Female	07.08.2013	08.02.2015	18 months	14980	5547
6004	Idan	Male	08.08.2013	18.01.2015	17 months	14901	2937
6005	Alona	Female	08.07.2015	22.06.2017	24 months	16700	10718
6006	Ariela	Female	17.07.2015	18.02.2017	19 months	14442	3827

Table A2 Percentage of variation explained by the retained principle coordinates (PCos) of different habitat resistance variables

Variable	Variance explained by retained principle coordinate
only first PCo retained	
Habitat suitability resistance	34.92%
Slope resistance	37.07%
Geographic distance	35.18%
first 4 PCos retained	
Habitat suitability resistance	61.96%
Slope resistance	63.29%
Geographic distance	59.41%

Table A3 Simplified ranking matrix comparing proportional habitat use with overall habitat availability in the study area for a) different categories of habitat slope and b) different categories of habitat suitability. "+" indicates the habitat in the row is used more than the habitat in the column, "-" indicates the opposite. "+++" and "---" indicate that the difference is significant at p<0.05.

a)	**Habitat slope**			
	0°-15° slope	16° -30° slope	>30° slope	Rank
0°-15° slope		+++	+++	2
16°-30° slope	---		+++	1
>30° slope	---	---		0

b)	Habitat suitability			
	High suitability	Intermediate suitability	Low suitability	Rank
High suitability		+++	+++	2
Intermediate suitability	---		+++	1
Low suitability	---	---		0

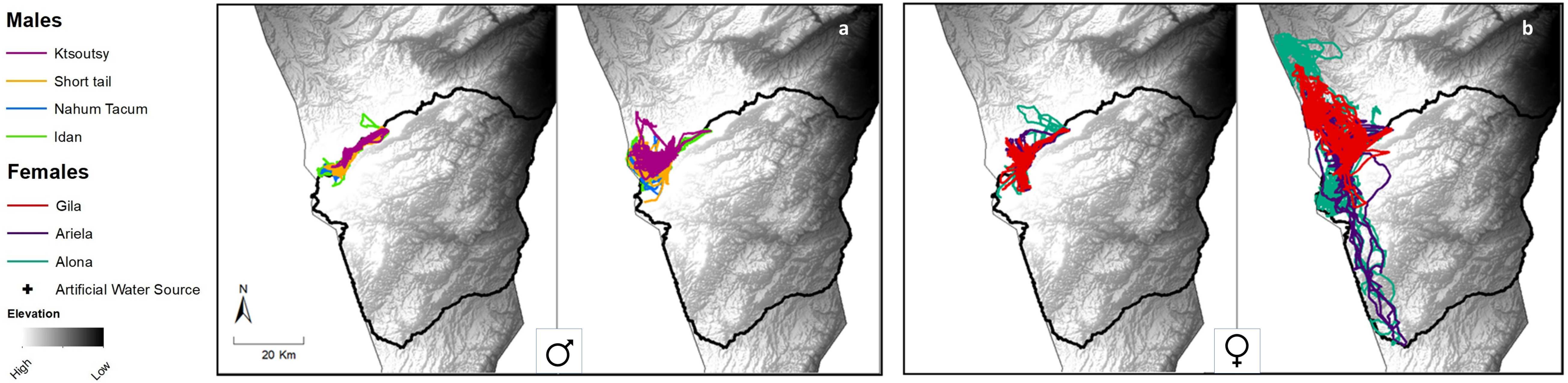

Fig. A1 Individual movement tracks for four males (left) and three females (right). Data represent hourly records obtained from GPS collars over a minimum period of 10 months. Left panels indicated movements recorded during the breeding season (June-August), right panels represent movements during non-breeding season (October-May). For three individuals (Nahum Tacum, Alona, Ariela) data were obtained for two consecutive breeding seasons. Two females (Alona, Ariela) which displayed long-distance movements during the non-breeding seasons, returned to the area near the permanent water source during breeding season in two consecutive years.

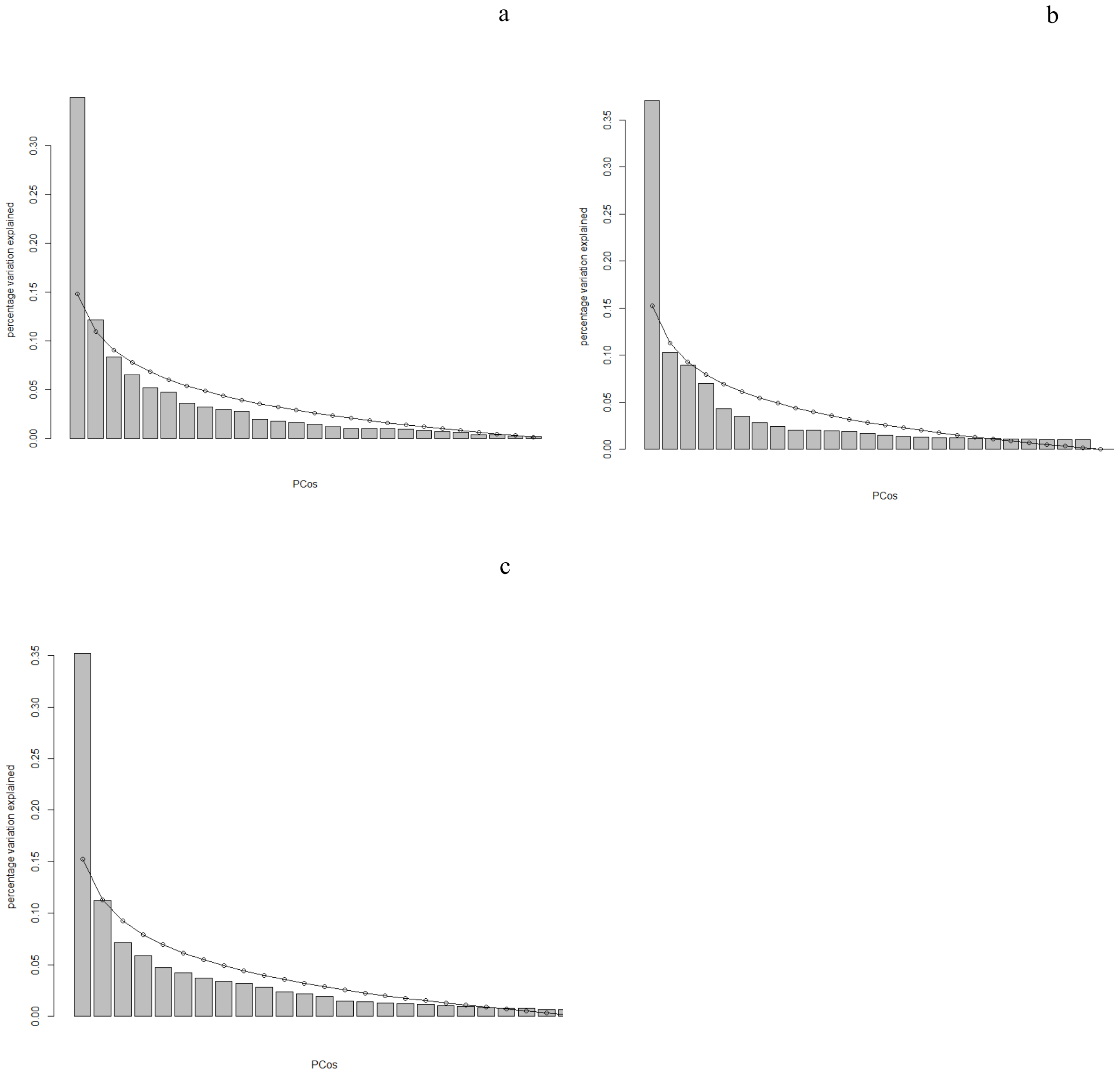

Fig. A2 Percentage variation explained by the principle coordinates of the pairwise resistance matrices based on a) the species distribution model, b) slope and c) geographic distance. Connected dots are indicating the variation explained as expected under a broken stick model. Only the first (b, c) or first and second (a) principle coordinates explain more variation than expected

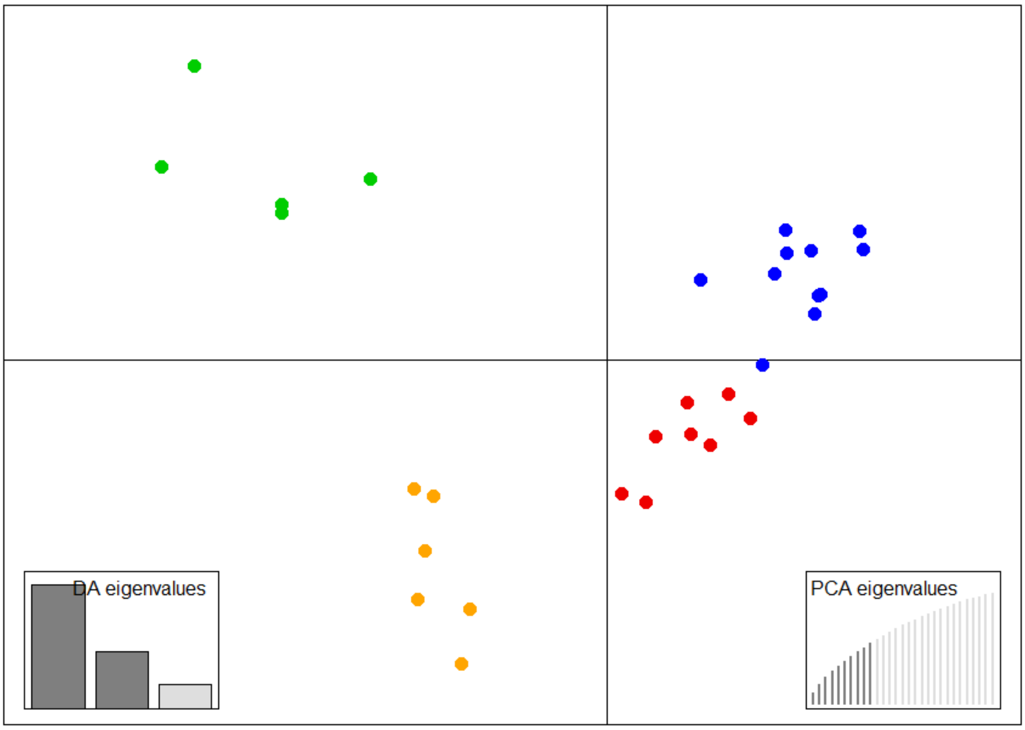

Fig. A3 DAPC plot of the reintroduced *Equus hemionus ssp*. population in Southern Israel for predefined K=4. Right inset shows a bar chart of eigenvalues of the PCA with dark retained eigenvalues. Left inset shows a bar chart of DA eigenvalues with dark corresponding components.

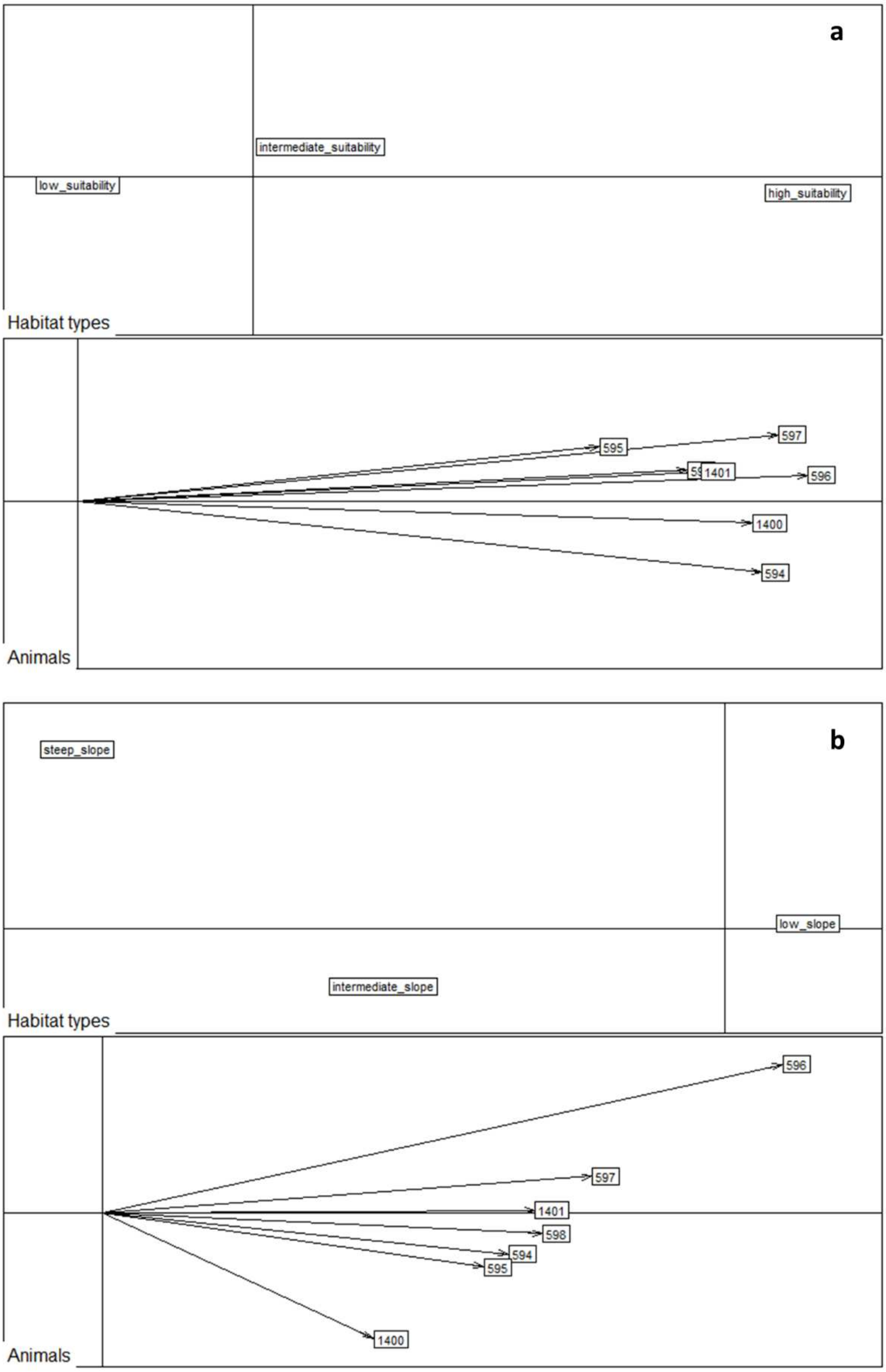

Fig. A4 Results of the eigenanalysis of selection ratios to evaluate habitat selection by 7 GPS-collared Asiatic wild ass *E.h.ssp* with respect to a) habitat suitability and b) habitat slope. Top figures show the habitat types, bottom figures show habitat preference of each individual.

Appendix B

B1. Repeated genetic clustering analysis using only samples (N=15) collected during the breeding season (June-August)

The variation explained by the first two principle components of the PCA was low (PC1 7.62%, PC2 6.54%) and no prominent genetic clusters could be identified (Fig. B1.1a, B1.1b). Also, the BIC plot of the DAPC did not display a clear minimum value after which the BIC rises again, which would indicate the optimal number of clusters (Fig. B1.1c). This suggested no meaningful genetic clustering in the population. In contrast, for the STRUCTURE analysis, the Evanno method identified a clear peak of delta(K) for K=2 (delta(K)=118.78; Fig. B1.1d). However, the Evanno method cannot identify an optimum of K=1 and may indicate peaks at higher values of K even in the absence of any genetic structure (Evanno et al. 2005). The mean Ln P(D) across different values of K displays a plateau between K=2 – K=4 (Fig. B1.1d), supporting the results of the Evanno method.

To conclude, the genetic structure analysis based on the reduced data set also offers support for the presence of a weak genetic structure in the populations. The genetic cluster analysis in Structure suggested that the population may be differentiated into fewer genetic clusters (Best K by Evanno, K=2). However, this is somewhat expected, given the reduced number of samples.

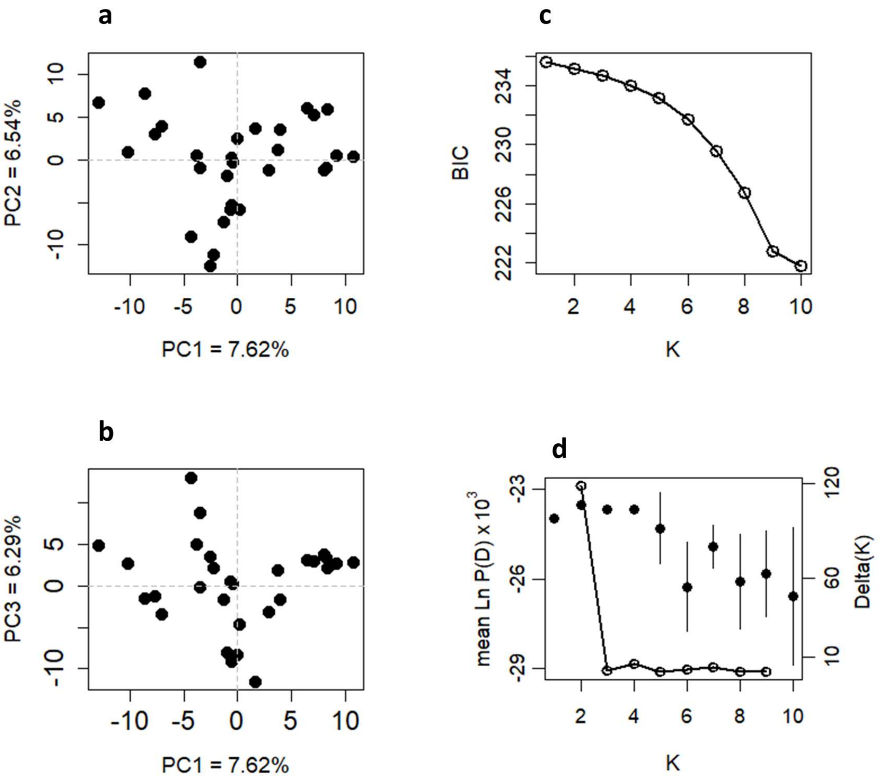

Fig. B1.1 Optimal number of genetic clusters in the reintroduced population of *Equus hemionus ssp*. in Southern Israel based on the reduced data (N=15) set including only samples collected during the breeding season (June-August). Initial exploration using principle component analysis indicates no distinct clustering along (a) the first and second and (b) along the first and third principle components. (c) The Bayesian information criterion (DAPC analysis) does not clearly identify an optimal number of clusters. (d) The Evanno method (STRUCTURE analysis) indicates a clear peak in Delta(K) for K=2, which is supported by the mean Ln P(D), in dots, which reaches a plateau between K=2–K=4.

B2. Repeated landscape genetic analysis using only samples (N=14) collected during the breeding season (June-August)

For all three resistance variables only the first or first and second PCos explained more variation than expected under the Broken Stick model (Fig B2.1). However, since this accounted for only ~40% of variation in each variable, we repeated the analysis with the first 4 PCos retained which accounted for ~70% of variation (Table B2.1). None of the tested models of the distance-based redundancy analysis returned significant results and the explained variance was very low (<3%) for all predictor variables (Table B2.2). Habitat suitability and slope explained negligible variation in genetic relatedness between individuals of the population. This was also true for models controlling for geographic distance and resistance distances, respectively (Table B2.2).

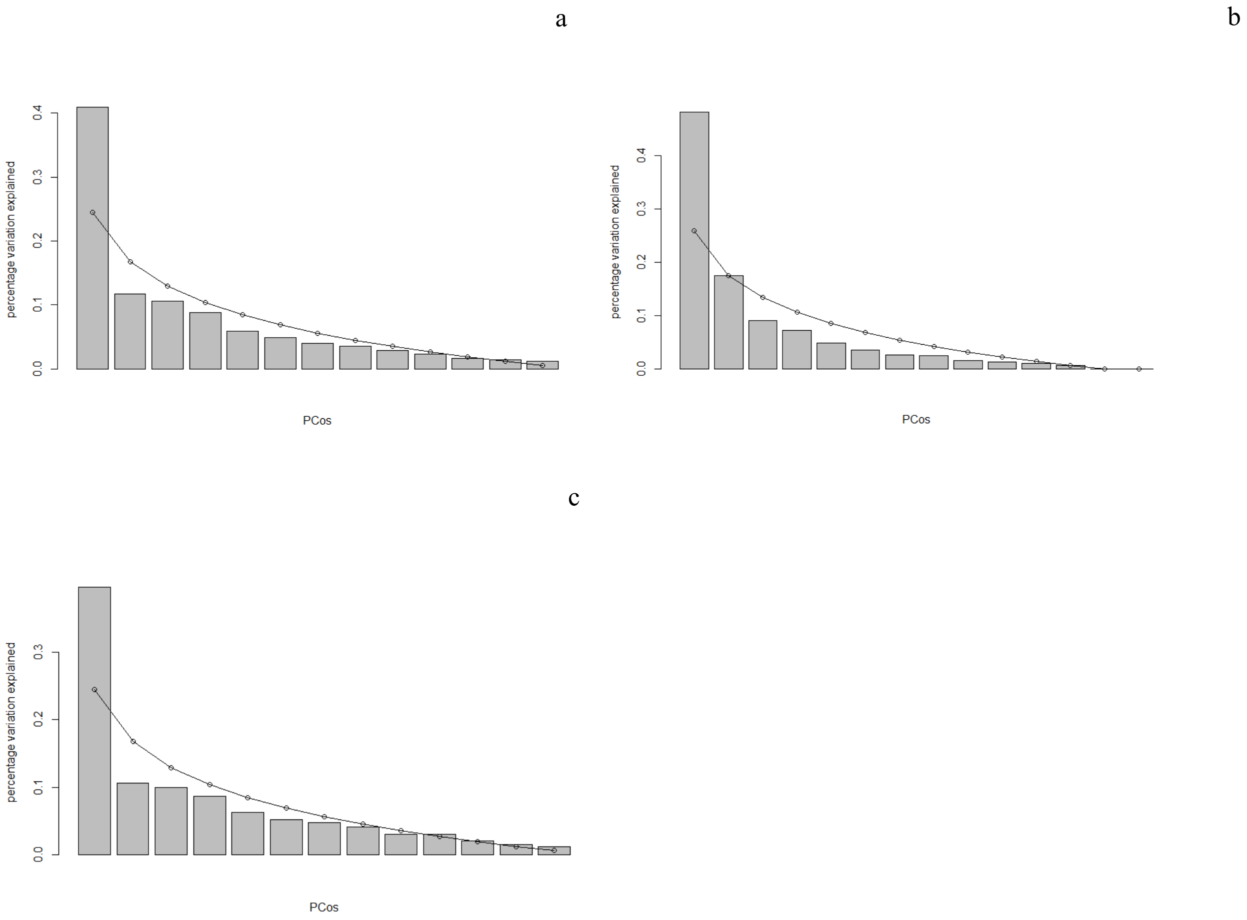

Fig. B2.1 Percentage variation explained by the principle coordinates of the pairwise resistance matrices based on a) the species distribution model, b) slope and c) geographic distance. Connected dots are indicating the variation explained as expected under a broken stick model. Only the first (b, c) or first and second (a) principle coordinates explain more variation than expected

Table B2.1 Percentage of variation explained by the retained principle coordinates (PCos) of different habitat resistance variables

Variable	Variance explained by retained principle coordinate
only first PCo retained	
Habitat suitability resistance	40.88%
Slope resistance	48.11%
Geographic distance	39.65%
first 4 PCos retained	
Habitat suitability resistance	72.01%
Slope resistance	81.92%
Geographic distance	69.03%

Table B2.2 Distance based redundancy analysis models tested for samples (N=14) collected during the breeding season (June-August). Total variance (Inertia), the % variation explained (R2) and adjusted % variation explained (adjusted R2), the degrees of freedom (df), F-statistic (F) and p-value of the permutation tests (9999 permutations). Partial models controlling for a third variable are indicated with |.

Variable	Inertia	%Variation (constrained Inertia or R2)	Adjusted % variation explained (adjustedR2)	df	F	p
only first PCo retained						
SDM resistance	0.74	9.58%	2.04%	1	1.271	0.175
Slope resistance	0.74	8.71%	1.10%	1	1.145	0.290
Geographic distance	0.74	9.53%	1.99%	1	1.264	0.179
SDM resistance | geographic distance	0.74	7.98%	<1%	1	1.063	0.389
Slope resistance | geographic distance	0.74	6.01%	<1%	1	0.782	0.773
Geographic distance | SDM resistance	0.74	7.93%	<1%	1	1.057	0.401
Geographic distance | slope resistance	0.74	6.83%	<1%	1	0.889	0.624
first 4 PCos retained						
SDM resistance	0.74	28.87%	<1%	4	0.913	0.726
Slope resistance	0.74	31.59%	1.19%	4	1.039	0.393
Geographic distance	0.74	29.14%	<1%	4	0.926	0.690
SDM resistance | geographic distance	0.74	31.23%	<1%	4	0.985	0.520
Slope resistance | geographic distance	0.74	32.47%	2.56%	4	1.058	0.407
Geographic distance | SDM resistance	0.74	31.51%	<1%	4	0.994	0.514
Geographic distance | slope resistance	0.74	30.03%	<1%	4	0.978	0.526