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RESEARCH ARTICLE

Landscape genetics matches with behavioral ecology and brings new insight on the functional connectivity in Mediterranean mouflon

Elodie Portanier · Jeremy Larroque · Mathieu Garel · Pascal Marchand · Daniel Maillard · Gilles Bourgoin · Sébastien Devillard

Abstract

Context In natural populations, gene flow often represents a key factor in determining and maintaining genetic diversity. In a worldwide context of habitat fragmentation, assessing the relative contribution of landscape features to gene flow thus appears crucial for sustainable management of species.

Objective We addressed this issue in Mediterranean mouflon (*Ovis gmelini musimon × Ovis* sp.) by combining previous knowledge on behavioral ecology with landscape genetics. We also assessed how sex-specific behavioral differences translated in term of functional connectivity in both sexes.

Methods We relied on 239 individuals genotyped at 16 microsatellite markers. We applied a model optimization approach in a causal modeling framework of landscape genetics to test for the effects on gene flow of habitat types and linear landscape features previously identified as important for movements and habitat selection in both sexes. Five resistance values were alternately assigned to these landscape characteristics leading to a comprehensive set of resistance surfaces.

Results Isolation by resistance shaped female gene flow, supporting the central role of linear landscape features as behavioral barriers for animal movements. Conversely, no isolation by resistance was detected in males. Although a lack of statistical power cannot be discarded to explain this result, it tended to confirm that males are less influenced by landscape structures during the mating period.

Conclusions Combining previous knowledge on behavioral ecology with results from landscape genetics was decisive in assessing functional landscape connectivity in both sexes. These results highlighted the need to perform sex-specific studies for management and conservation of dimorphic species.

Keywords Causal modeling · *Ovis* · Functional landscape connectivity · Landscape genetics · Mammals · Mediterranean mouflon

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Introduction

Animal movements are linked to various fundamental biological processes (e.g. foraging, dispersal, mating, Zeller et al. 2012) and determine in turn individual performance, gene flow and population dynamics. At the inter- (e.g. metapopulations, sensu Moilanen and Hanski 1998) as well as at the intra-population scale, maintaining connectivity between demes, habitat patches and individuals makes it possible to avoid strong impacts from stochastic processes (demographic, environmental and genetic) and thus extinction vortices (Crooks and Sanjayan 2006). Among the different elements that can impact and influence individual movements, landscape plays a crucial role (e.g. Harris and Reed 2002; Cozzi et al. 2013; Seidler et al. 2014; Zimmermann et al. 2014). Understanding the potential impact of landscape on animal movements and its consequences on individual performance, gene flow and population dynamics is thus of primary importance for conservation and sustainable management of many species and populations in the current context of habitat loss and fragmentation (Lande 1998; Fahrig 2003).

Landscape impacts on movements depend on several factors (e.g. landscape composition, structure) and among them, a fundamental element is landscape connectivity. Landscape connectivity was originally defined as "the degree to which the landscape facilitates or impedes movement among resource patches" (Taylor et al. 1993). More recently, landscape ecologists have asserted that there are actually two fundamental types of connectivity: structural and functional. Structural connectivity refers only to physical characteristics of landscapes, while functional connectivity considers the behavioral responses of organisms to this physical landscape structure (Taylor et al. 2006; Baguette and Van Dyck 2007). Indeed, physically connected habitats are not necessarily functionally connected and vice versa (e.g. unused corridors, Taylor et al. 2006; Imong et al. 2014). Landscape functional connectivity can vary between species, classes of individuals and individuals, since their behaviors and movements can be drastically different. For instance, in polygynous mammal species, males and females may exhibit very different dispersal movements (natal or reproductive) (Greenwood 1980 but see Clutton-Brock and Lukas 2012) resulting in different functional landscape connectivity values between sexes (e.g. Larroque et al. 2016b). Accordingly, in conservation and management problematics, studies on landscape impacts on movement must consider the functional connectivity of landscape for individual classes (e.g. sex).

Assessing functional landscape connectivity is commonly achieved by identifying "permeable" landscape elements that favor movement and "resistant" elements that impede it (Ray et al. 2002; Beyer et al. 2016; Panzacchi et al. 2016). The costs of moving across different landscape elements are assessed based on biological data to establish resistance surfaces (Zeller et al. 2012, 2016). Among the different biological data types that can be used to estimate the resistance of each landscape element (see Zeller et al. 2012 for a review), genetic data makes it possible to focus on estimating the resistance imposed by the landscape on gene flow. The study of gene flow is of primary concern for populations, since genetic diversity has implications for population adaptability and viability, and hence conservation (Frankham et al. 2004, Segelbacher et al. 2010). Due to the increasing need for landscape connectivity assessment and restoration, the landscape genetics approach, combining population genetics, landscape ecology and spatial statistics (Manel et al. 2003) and enabling the direct measurement of functional connectivity in landscapes, has become a central topic for researchers (Richardson et al. 2016). Unlike classic population genetics, landscape genetics explicitly accounts for landscape resistance to movements and a common approach is to relate genetic distances between individuals, groups or populations to distances that account for landscape resistance to movements (e.g. least-cost distances). Based on this framework, the impacts of landscape elements on gene flow have been illustrated in different species (e.g. Coulon et al. 2004 in roe deer (*Capreolus capreolus*), Cushman et al. 2006 in black bear (*Ursus americanus*), Larroque et al. 2016a in European pine marten (*Martes martes*), Barros et al. 2016 in Egyptian mongoose (*Herpestes ichneumon*), Olah et al. 2017 in scarlet macaws (*Ara macao*)).

A commonly used approach for obtaining a final resistance surface best describing resistance of landscape to gene flow is to define several alternative resistance surfaces and then determine which one best explains the spatial pattern of genetic variation observed ("Two-stage empirical approach", see

Zeller et al. 2012). The definition of such alternative resistance surfaces requires defining (i) the landscape characteristics and elements to consider (e.g. those impacting movements) and (ii) the resistance values for each of these landscape elements. The development of GPS monitoring, which enables the recording of fine-scale data on animal movements (Nathan et al. 2008) provides a unique opportunity to shed light on the two previous points by objectively quantifying the most important factors influencing movements (e.g. habitat suitability/selection or step selection studies). Nonetheless, since they do not focus on dispersal only, habitat suitability or preferences are not systematically related to landscape resistance on gene flow (e.g. Wasserman et al. 2010; Reding et al. 2013; Mateo-Sanchez et al. 2015; Roffler et al. 2016; Keeley et al. 2017) and as such might not be the most suitable way to define landscape element resistance values. As an alternative to approaches using a priori information, approaches making no a priori on resistance values are promising. However, testing every possible combination of resistance values for each of the landscape elements considered exponentially increases the number of resistance surfaces to test and concurrently the computation time. So far, and to our knowledge, few studies have used these approaches and tested all possible combinations of resistance values of all environmental parameters (but see Larroque et al. 2016a, b).

Numerous landscape elements, such as linear landscape features (e.g. Kuehn et al. 2007; Hepenstrick et al. 2012; Robinson et al. 2012; Parks et al. 2015; Wilson et al. 2015) or habitat types and topography (e.g. Epps et al. 2007; Perez-Espona et al. 2008; Shirk et al. 2010; Robinson et al. 2012; Parks et al. 2015; Roffler et al. 2016; Creech et al. 2017; Gubili et al. 2017) have been shown to influence gene flow in large herbivores and wild sheep. Elevation has even been found to shape genetic diversity and extinction risks in desert bighorn sheep (*Ovis Canadensis nelson*, Epps et al. 2004, 2006) supporting the idea that studying impacts of landscape on gene flow in wild sheep could be particularly relevant in this group of species for which multiple conservation (e.g. Corsican mouflon populations, Shackleton & IUCN/SSC Caprinae Specialist Group 1997, Cypriot mouflon, (*Ovis orientalis ophion*), Valdez 2008) and management purposes exist (e.g. trophy hunting; Harris and Pletscher 2002; Hofer 2002; detrimental

effects on native animal and plant species Chapuis et al. 1994; Nogales et al. 2006; Bertolino et al. 2009). Here, we proposed to gain better knowledge on functional connectivity in these species by studying, in a population of Mediterranean mouflon (*Ovis gmelini musimon* × *Ovis* sp.), the impacts on gene flow of several landscape characteristics known to influence habitat selection and animal movements. In the studied population, animals have been shown to have marked habitat preferences determined by land cover and topography all year round, with marked differences between sexes (Marchand et al. 2015). Additionally, natural and anthropogenic linear landscape features acted as behavioral barriers and influenced movements and home-range selection of individuals (Marchand et al. 2017a). Accordingly, we evaluated the impacts on genetic differentiation in the study population of the linear landscape features and land-cover classes known to impact habitat selection and movements. We expected sex-specific effects of landscape elements on gene flow, in accordance with the sex-specific space-use observed in this dimorphic species exhibiting strong sexual segregation (Ruckstuhl and Neuhaus 2006; Marchand et al. 2015, 2017a; Bourgoin et al. 2018). More specifically, male gene flow should be much less impacted by landscape features than female, as gene flow is thought to be mostly insured by male reproductive dispersal in this population (reproductive excursions, Marchand et al. unpublished data, Portanier et al. 2017) in which females are philopatric (Dubois et al. 1992, 1994; Dupuis et al. 2002) and natal dispersal would be limited in males (Dubois et al. 1993, 1996; King and Brooks 2003). Furthermore, lower spatial and genetic structures and weaker impacts of linear landscape elements on movements have been observed in males than in females, in particular during the mating period (Dubois et al. 1993, 1996; Marchand et al. 2017a; Portanier et al. 2017) when males exhibit very different behavior as compared to the rest of the year (e.g. hypophagia, Pelletier et al. 2009, increased movements, Karns et al. 2011, Jarnemo 2011) and use coursing as a mating strategy (Bon et al. 1992; Dubois et al. 1993, 1996; Marchand et al. 2015). Since linear landscape features have been shown to have much higher impacts on movements than land cover (Marchand et al. 2017a), we also expected these landscape elements to be the principal driver of gene flow for females.

Based on 16 microsatellite loci, we tested a comprehensive set of resistance maps by using five possible resistance values for each of the nine landscape elements known to impact mouflon movements and representing natural or anthropogenic linear features, resource and/or refuge areas. We then selected for each sex the resistance surface best describing the landscape genetic resistance using a model optimization framework modified from the univariate optimization procedure of Shirk et al. (2010) and Larroque et al. (2016a, b). Then, to determine if there was a spatial genetic structure and to disentangle between isolation-by-distance (IBD, when only geographic distance plays a role) and isolation-by-resistance (IBR, when landscape resistance impacts gene flow, McRae 2006) patterns of genetic variation, we used a causal modeling framework

(Cushman et al. 2006, 2013), assessing if least-cost distances were correlated to levels of genetic differentiation between individuals.

Material and methods

Study population, data collection and species

Data were collected in the low mountain Caroux-Espinouse massif (43°38′N, 2°58′E, 17,000 ha, 130-1,124 m asl, southern France, Fig. 1a). The studied Mediterranean mouflon population grew from the release of nineteen individuals between 1956 and 1960 (Garel et al. 2005; Portanier et al. 2017) and inhabits a National Hunting and Wildlife Reserve (1658 ha, 532-1,124 m above sea level; hereafter
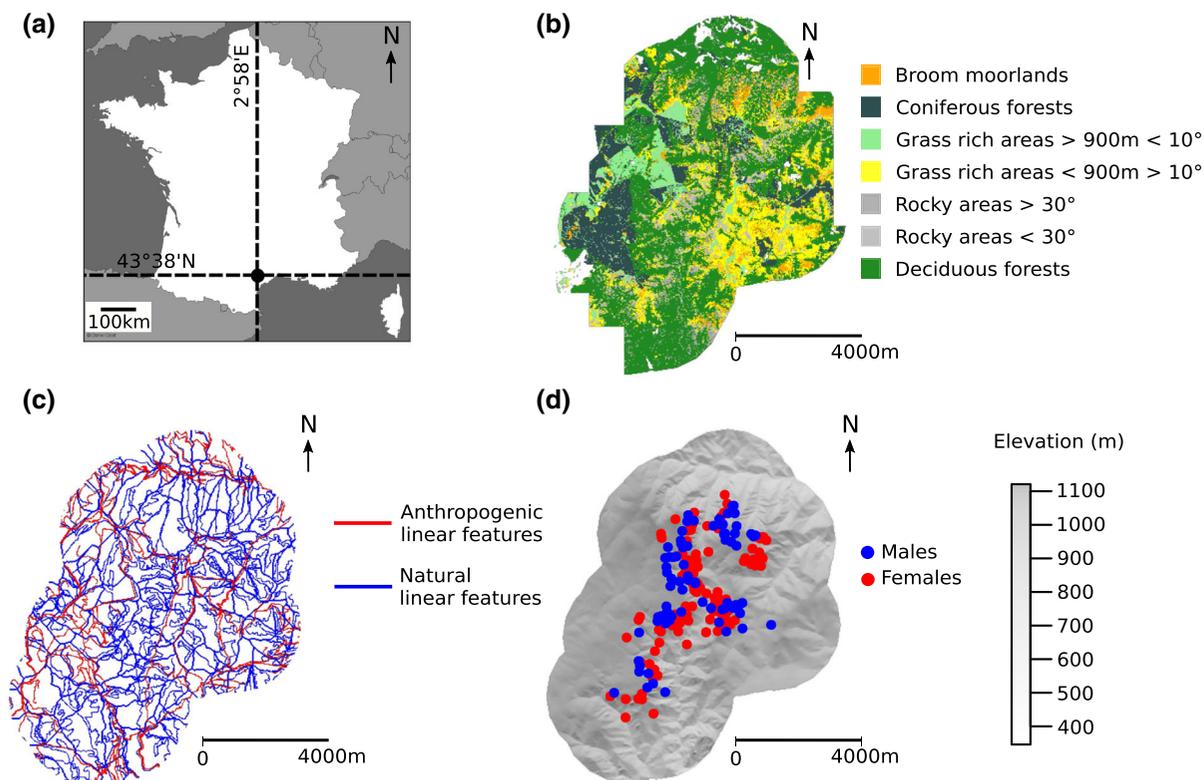
Fig. 1 Description of the Caroux-Espinouse massif where landscape resistance was investigated for Mediterranean mouflon. **a** Geographic location of the Caroux-Espinouse massif in France. **b** Land-cover classes considered in landscape genetics analyses. Grass-rich areas were classified in two classes according to elevation and slope (on plateaus with slope < 10°, elevation > 900 m or on slopes with slope > 10°, elevation < 900 m). Rocky areas were classified according to slopes

(gently-sloped rocky areas if slope was < 30° and steeply-sloped rocky areas if slope was > 30°). **c** Linear landscape features considered in landscape genetics analyses. Anthropogenic linear features included roads, tracks, hiking trails, while natural linear features included ridges, talwegs, forest edges. **d** Digital elevation model represented with spatial locations of individuals as defined in the main text

Table 1 Description of the nine landscape elements used in landscape genetics analyses (reproduced with permission from Marchand et al. 2015, 2017a; see also Fig. 1b, c)

Landscape variable	Description
Deciduous forests	Deciduous trees: beech, *Fagus sylvatica*, chestnut tree, *Castanea sativa*, holm oak, *Quercus ilex*, and mixed deciduous patches at altitude < 900 m
Coniferous forests	Coniferous trees at altitude > 900 m
Gently-sloped rocky areas	Rocky areas with slope < 30°
Steeply-sloped rocky areas	Rocky areas with slope > 30°
Grass-rich areas in slopes	Heather and blueberry moorlands, pastures, meadows and artificial cultures devoted to wildlife with slope > 10° and altitude < 900 m
Grass-rich areas on plateaus	Heather and blueberry moorlands, pastures, meadows and artificial cultures devoted to wildlife with slope < 10° and altitude > 900 m above sea level
Broom and fern moorland	Broom and fern
Anthropogenic linear features	Roads, tracks and hiking trails
Natural linear features	Ridges, talwegs (lines of lowest elevation within a valley) and forests edges

called "reserve"). Habitat is characterized by deep valleys indenting plateaus and creating a mosaic of ridges and talwegs (Marchand et al. 2017a, Fig. 1c, d). Vegetation is an irregular mosaic of deciduous and coniferous forests with open areas dominated by rocky areas and broom moorlands (Marchand et al. 2015, Table 1, Fig. 1b). Within the reserve, hunting is forbidden and recreational activities are restricted to hiking on a few main trails (Marchand et al. 2014a) but in surrounding unprotected areas, both sexes are harvested each year since 1973 (Garel et al. 2007).

Since 1974, individuals have been annually captured in the reserve. Animals are marked and biometric measurements and hair sampling are performed. Marked animals are visually monitored year-round and spatially located. Since 2003, some individuals have also been equipped with GPS collars (see Marchand et al. 2014b, 2015 for more details). All captures, handling, sampling and collaring were done according to the appropriate national laws for animal welfare and procedures were approved by the pertinent administration.

In the study population, philopatric (Dubois et al. 1992, 1994; Dupuis et al. 2002) Mediterranean mouflon ewes are sexually mature from 1.5 years of age and monotocous (twinning rate is < 3%; Garel et al. 2005). Although probably sexually mature at 2 years old (see Festa-Bianchet 2012 in bighorn sheep (*Ovis canadensis*)), only rams three or more years old have been observed involved in rutting activities (Bon et al. 1992, 1995). Mature males and females segregate most of the year but less during the rut as males join females to mate (Bon and Campan 1989; Bon et al. 1992; Dubois et al. 1993; Le Pendu et al. 1996; Cransac et al. 1998; Bourgoin et al. 2018). The mating system is thought to be polygynous with a few large males achieving most of the mating (see Geist 1971 for Dall's sheep (*Ovis dalli*), see Jarman 1983, Hogg 1987 for bighorn sheep).

As other wild sheep species, Mediterranean mouflon are known to be poor dispersers with expected small dispersal distances, especially for females (Geist 1971; Dubois et al. 1994; Gross et al. 2000; Worley et al. 2004; Marchand et al. unpublished data). Males are nevertheless more mobile than females, with both natal dispersers and philopatric individuals observed (unknown proportions, see Dubois et al. 1993, 1996; King and Brooks 2003). Additionally, rams have been shown to perform excursions (temporary movements outside an established home range) during the rutting period (October–December, Bon et al. 1992; Marchand et al. unpublished data) that are thought to insure gene flows (Portanier et al. 2017). Both faithful to their rutting site and unfaithful males have been observed

(Dubois et al. 1993, 1996; Dupuis et al. 2002; Martins et al. 2002).

Mediterranean mouflon is a wild grazer ungulate species (Cransac et al. 1997; Cazau et al. 2011; Marchand et al. 2013). Sex-specific trade-offs between habitats providing food and cover have been found in this population (Marchand et al. 2015). Typically, safe habitats (rocky or cover areas) are favored over food-rich habitats during rutting/hunting (September–February) by both sexes, and also during lambing (March–June, Bon et al. 1993) by females. Females switched to the best foraging (grass-rich areas on plateaus) habitats in summer when lambs were weaned. Additionally, linear landscape features have been found to be even more determinants for animal movements than land cover, with mouflon avoiding crossing both anthropogenic (i.e. roads, tracks and hiking trails) and natural landscape features (i.e. ridges, talwegs and forest edges, Marchand et al. 2017a). No seasonal migration has been reported in this population (mean overlap of home ranges through the year for males: $89.8 \pm 2.5\%$ and for females: $88.4 \pm 4.5\%$; Marchand et al. 2014b and unpublished data).

Microsatellites genotyping

We used hair samples from 262 individuals trapped between 2010 and 2014, i.e. within a time period inferior to generation time of mouflon (4.21 years, Hamel et al. 2016), which should limit temporal genetic structure. Genotyping was performed by the Antagene laboratory (Limonest, France, www.antagene.com) following the procedure presented in Portanier et al. (2017). Each DNA sample was typed at 16 microsatellites markers (see Portanier et al. 2017). Analyses were performed on individuals of both sexes expected to have a fixed home range outside the rutting period, i.e. females two or more years old and males four or more years old (e.g. Dubois et al. 1992, 1993; Dupuis et al. 2002). Information about genetic diversity and Hardy–Weinberg equilibrium in the population are available in Portanier et al. (2017).

Among the genotyped samples, two outliers and five pairs of twins were identified using a correspondence analysis (CA) and the matching option in GenAlex v.6.501 (Peakall and Smouse 2006, 2012), respectively. Outliers, and one of the two twins for each pair, were randomly deleted to prevent bias in subsequent analyses. Among the remaining individuals, we considered in the following analyses 239 individuals (77 males and 162 females) with fixed home ranges, at least 13 successfully genotyped genetic markers and for which spatial locations were available. Comparison of the observed genotypes with the distribution of randomized genotypes generated with the program MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004) revealed that there were no null alleles in the data set.

Landscape genetics analyses

We used a causal modeling framework (Legendre and Troussellier 1988; Legendre 1993; Cushman et al. 2006, 2013) to distinguish between isolation by distance (IBD) and isolation by resistance (IBR) as spatial drivers of genetic differentiation in our population.

Genetic distances

Since it was especially designed to estimate genetic differentiation among individuals in continuous populations and was shown to perform well in landscape genetics studies (Shirk et al. 2017), the $\hat{a}_r$ (Rousset 2000) pairwise genetic distance was calculated using SPAGeDi 1.5 software (Hardy and Vekemans 2002).

Spatial locations of mouflon

We considered genotyped individuals for which at least five spatial locations were available. GPS spatial locations (85 females and 32 males: from 5 to 8570 locations) or visual resighting (104 females and 45 males without GPS collars: from 5 to 73 locations) were averaged to assign each individual at the centroid of all its spatial locations (Fig. 1d). This centroid value was used as spatial location for each individual.

IBD model

The IBD model predicts that genetic distance between individuals increases with increasing geographic (Euclidean) distance. In the IBD model, pairwise Euclidean geographic distances (straight line, hereafter EuD) were calculated. These distance were topographically corrected (i.e. taking into account elevation changes between pixels, digital elevation

model with a 25 m resolution, BD ALTI©, French National Institute of Geography, www.ign.fr, see Fig. 1d) following the method reported in Larroque et al. (2016a).

IBR models definition and optimization

The IBR model predicts that genetic distance between individuals increases with increasing resistance of the landscape between them. In IBR models, the resistance distance was calculated using least-cost pathways between each pair of individuals.

Seven land-cover classes and two types of linear structures (natural and anthropogenic) known to impact habitat selection, home range selection and movements of mouflon were considered in the different resistance surfaces modeled (Fig. 1b, c, Table 1). These habitat categories explicitly took into account slope and elevation parameters (Fig. 1b, Table 1, see Marchand et al. 2015, 2017a for details). Habitat map was derived from a SPOT satellite image and field validation in a 25 × 25 m grid (Tronchot 2008), and the digital elevation model was used to derive slope (see Marchand et al. 2015). Each pixel was characterized by the dominant habitat type (Fig. 1b). Linear landscape features were derived in accordance with Marchand et al. (2017a). Natural linear landscape features referred to ridges, talwegs and forest edges and were extracted from the digital elevation model and the BD FORÊT© (French National Institute of Geography, http://www.ign.fr). Anthropogenic linear landscape features referred to roads, tracks and hiking trails and were extracted from the BD CARTO© (French National Institute of Geography, http://www.ign.fr). A 15 m buffer was applied to linear landscape features to reinforce their size and avoid raster breaks. In order to avoid artificial map boundary effects that might affect directional choice of the least-cost algorithm and bias resistance values estimations (see Koen et al. 2010), we buffered 2 km around individual locations to define study area limits (Fig. 1d). In our case, two kilometers made it possible to obtain a circular area of 1250 ha around each individual, which is largely representative of the expected maximum area of a mouflon home range (1000 ha, see Marchand 2013). In the final map, all habitat classes were represented in similar proportions ($< 10\%$) with the exception of deciduous forests and natural linear landscape features, which are largely more

represented (24 and 23% respectively, see Fig. 1b, Online Resource 1). Missing data in the final map (see Online Resource 1) are mostly located in the external range of the study area and are due to the fact that the precise habitat map had lower extent than extents obtained after buffering of 2 km (see Fig. 1b, d).

Five varying resistance values were alternatively assigned to each of the nine landscape elements leading to 1,953,125 different possible combinations of resistance values (scenarios hereafter). Values were chosen to illustrate the case where the landscape element was not resistant to movements (resistance of 1), weakly and moderately resistant (25 and 50 respectively) and highly and totally resistant (75 and 100 respectively). In order to identify which scenario best described genetic data, we calculated least-cost distance matrices (LCD) measuring the cumulative cost (as recommended by Etherington and Holland 2013) under each scenario using the *costDistance* function in *gdistance* R package (van Etten 2017).

For each sex, we then evaluated each of the 1,953,125 scenarios based on the correlation between genetic distances $\hat{a}_r$ (G) and the log transformed LCD using partial mantel tests, partialling out the log transformed Euclidean distance (G $\sim$ log(LCD)$_i$|-log(EuD) for the ith scenario). In accordance with the model optimization procedures of Shirk et al. (2010) and Larroque et al. (2016a, b), the top scenario was identified by a unimodal peak of support in partial Mantel r values (LCD$_{top}$). When no unimodal peak of support was identified, we selected significant (two-tailed p *values*) scenarios having a positive Mantel r and calculated the Relative Support index (RS, Cushman et al. 2013). RS is the difference in Mantel r of the models (i) G $\sim$ log(LCD)$_i$|log(EuD) and (ii) G $\sim$ log(EuD)|log(LCD)$_i$ and was introduced to prevent the high type I error rates of Mantel tests. We ranked models according to their RS and selected the scenario having the highest RS (LCD$_{top}$ hereafter). In keeping with the recommendations of Cushman et al. (2013) to counterbalance the high type I error rates and to decrease the risks of finding spurious support for IBD null models while IBR is the true process, we used an alpha level of 0.01 to evaluate the significance of Mantel tests. It is especially recommended when highly correlated alternative hypotheses are confronted, like IBD and IBR are in the present study (see Online Resource 2), because Mantel-based

methods are less reliable (Cushman et al. [2013](#); Zeller et al. [2016](#)).

According to this lower discriminant power of causal modeling when alternative scenarios are highly correlated, we assessed the sensitivity of our results to the number of selected scenarios by calculating averaged scenarios across the first n models (see Table S3, Online Resource 3). Averaging resistance values allowed us to construct an averaged resistance surface based on the n first models and to calculate least-cost distances under this averaged model (LCD_{av}).

All Mantel and partial Mantel tests were performed using the *ecodist* package in R (Goslee and Urban [2007](#)) and 9999 permutations.

Principal driver of the genetic differentiation

Once we identified the top or the averaged scenarios among all the alternative resistance models, we performed diagnostic tests of causal modeling (Cushman et al. [2006](#), [2013](#)) to distinguish between the three possible drivers of genetic differentiation in each sex (IBD, IBR and no structure). We first tested for correlations between genetic and Euclidean and resistance distances alone using simple Mantel tests (G ~ log(EuD) and G ~ log(LCD), tests "t_1" and "t_2" hereafter, respectively). Causal modeling then predicts that for IBR to be recognized as the principal driver of genetic differentiation, partial Mantel tests between genetic and resistance distances must be significant for G ~ log(LCD)|log(EuD) and non-significant for G ~ log(EuD)|log(LCD) (tests "t_3" and "t_4" hereafter, respectively).

Results

IBR model optimization and driver of the genetic differentiation

No IBD patterns were detected (tests t_1, Table [2](#)) for both sexes indicating that genetic differentiation is not determined by Euclidean distances. No unimodal peak of support for scenarios was observed in Mantel r values, neither for females nor for males (Online Resource 4). In females, we calculated the RS for the 4906 scenarios having a significant positive Mantel r ($p < 0.01$, mean Mantel $r = 0.08$, mean RS = 0.15) and selected the best one to perform causal modeling. We also performed causal modeling on the averaged scenarios (LCD_{av}, see Table S4, Online Resource 3) and obtained qualitatively the same results indicating that results were robust. As expected, IBR was identified as the principal driver of the genetic structure since the test t_3 was significant and had positive relative support (RS = 0.16), while the test t_4 was non-significant (Table [2](#)). As we expected, model optimization indicated very high resistance values of linear landscape features (natural as well as anthropogenic), coniferous forests and grass rich areas on slopes (Fig. [2a](#)). These resistance values mean that these landscape elements are between 75 and 100 times more resistant than any landscape element having a resistance of 1. Deciduous forests and broom moorlands had relatively low resistance values while gently- and steeply-sloped rocky areas and grass-rich areas on plateaus did not oppose any resistance to female gene flow (Fig. [2a](#)). In addition, the resistance values for steeply-sloped rocky areas and linear landscape features changed only slightly between the

Table 2 IBD (EuD), IBR (LCD$_{top}$) and causal modeling tests performed for females and males Mediterranean mouflon

Test		Females		Males	
		Mantel r	P value	Mantel r	P value
t_1	G ~ log(EuD)	0.016	0.562	− 0.0095	0.775
t_2	G ~ log(LCD$_{top}$)	**0.068**	**0.003**	0.0865	0.047
t_3	G ~ log(LCD)$_{top}$|log(EuD)	**0.095**	**0.007**	0.1444	0.011
t_4	G ~ log(EuD)|log(LCD)$_{top}$	− 0.069	0.069	− 0.1165	0.019

G refers to genetic (â$_r$), EuD to Euclidean and LCD$_{top}$ to least-cost distances matrices. Bold values indicate significant ($p < 0.01$) simple (G ~ D$_1$) or partial Mantel tests (G ~ D$_1$| D$_2$, with D$_1$ and D$_2$ standing for IBD or IBR). For females IBR appeared as the principal driver of genetic structure (t_3 significant and t_4 non-significant) while for males no genetic structure was detected (all tests non-significant)
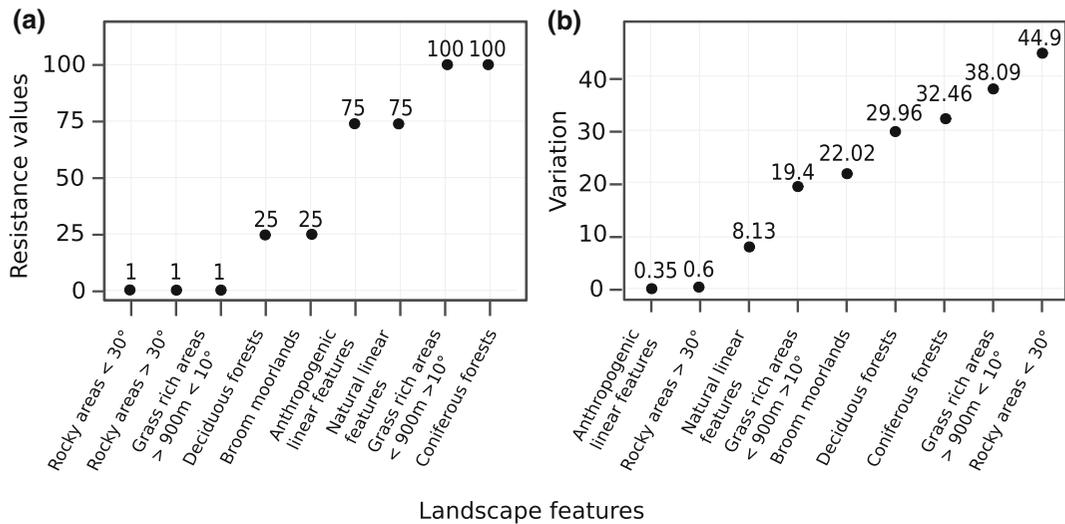
Fig. 2 Resistance values obtained in the best scenario for each of the landscape elements considered in landscape genetics analyses for females (**a**). A resistance value of 100 refers to totally resistant landscape element while a value of 1 refers to totally permeable landscape elements. **b** Variation of resistance values between the first top scenario (highest RS) and the resistance values obtained after averaging across 4906 best scenarios for females Mediterranean mouflon. Variation was calculated as the absolute value of the difference: $v_1 - v_{av}$

first top scenario and averaged values over 4906 scenarios (difference < 10%, see Fig. 2b) indicating the marked robustness of these results as compared to other habitat types and supporting the expected preeminent role of linear landscape features on female gene flow.

In males, only one scenario had a significant positive Mantel r ($p < 0.01$, Mantel $r = 0.14$, RS = 0.26) and accordingly no scenario averaging was performed. However, no conclusion can be drawn from resistance values observed (see Online Resource 3, Table S5) in the top scenario since the conditions to conclude an effect of landscape on gene flow were not met (t_3 and t_4 were both non-significant, Table 2). Although relative support is positive (RS = 0.26), these results supported the expectation that landscape features had much lower effects on gene flow in males than in females.

Discussion

Landscape genetics revealed sex-specific effects of landscape on gene flow in the studied Mediterranean mouflon population. In females, the significant IBR detected revealed that the role of the landscape on genetic differentiation was mostly related to the high

resistance of linear landscape features and unsafe habitats (e.g. coniferous forests) to gene flow, and the higher permeability of the habitats perceived as safe (e.g. deciduous forests and rocky areas). These results are thus in accordance with previous studies on movements and habitat selection (Marchand et al. 2015, 2017a). In contrast, no IBR was detected in males, suggesting that landscape features had limited impact on gene flow, and confirming previous results showing that landscape characteristics had less influence on movements of males during the rutting period and consequently on population spatial genetic structure (Dubois et al. 1993, 1996; Marchand et al. 2017a; Portanier et al. 2017). Combining previous knowledge on movements and habitat selection with the results from this landscape genetics approach was hence decisive in characterizing functional connectivity of both sexes in the studied population.

Landscape impacts on female movements and gene flow

In numerous animal species (e.g. Ehrlich 1961 in insects, Harris and Reed 2002 in non-migratory birds, Frantz et al. 2010 in badgers (*Meles meles*), Zimmermann et al. 2014 in large carnivores), linear landscape features (natural and anthropogenic) have been shown

to impact movements. In the study population, these linear features are crossed less than expected all year round, although they can easily be (i.e. are behavioral barriers instead of physical barriers), with consequences on the design of individual home range (Marchand et al. 2017a). In addition, in Mediterranean mouflon as in numerous ungulate species, habitat selection is driven by a pervasive food/cover trade-off (Festa-Bianchet 1988; Dussault et al. 2005; Ciuti et al. 2009; Mabille et al. 2012; Marchand et al. 2015). During the rutting period, habitats perceived as unsafe (plateaus) are thus avoided by females while habitats perceived as safe and/or providing resources are selected (i.e. deciduous forests or rocky areas on slopes providing high visibility and low accessibility for predators, see Marchand et al. 2015). Here, we showed that these behavioral characteristics translated into consequences on gene flow and spatial genetic structure in females. Indeed, both natural and anthropogenic linear landscape features had very high resistance values, with a very strong impact on female gene flow and movements, both here and in studies of habitat selection. Furthermore, resistance values for linear features were highly consistent across scenarios, highlighting the preeminent role of such features for female movements. Similarly, unsafe habitats such as coniferous forests appeared as totally resistant while deciduous forests and rocky areas appeared as weakly and not resistant to gene flow, respectively, in the present study. Resistance values observed for grass rich areas on plateaus were nevertheless unexpected since this habitat type is avoided during the rutting period (see Marchand et al. 2015). The low resistance value observed for steeply-sloped rocky areas was almost the same across all scenarios, suggesting that this landscape feature is among the principal drivers of gene flow in females. In accordance with observations made in other wild sheep species (see Epps et al. 2007; Roffler et al. 2016; Creech et al. 2017), land cover and topography also determined how and through which habitat types female gene flow occurred in the Mediterranean mouflon population under study.

In other large herbivores species, linear landscape features have also been shown to impact movements (e.g. bison (*Bison bison*), Bruggeman et al. 2006, 2007, pronghorn (*Antilocapra Americana*), Seidler et al. 2014, red deer (*Cervus elaphus*), Prokopenko et al. 2017). However, most studies focused on physical barriers. While ridges imposed

directional dispersal movements (individuals follow the ridges, Long et al. 2010) in male white-tailed deer (*Odocoileus virginianus*), to our knowledge no other study investigated the effects of such natural behavioral barriers on gene flow in large herbivores. Similarly, although anthropogenic linear landscape features have been studied much more and shown to impact gene flow in numerous ungulate species (e.g. red deer, Frantz et al. 2012, roe deer (*Capreolus capreolus*), Breyne et al. 2014, bighorn sheep, Creech et al. 2017), most studies referring to such features have considered highways or wide, frequently traveled roads representing physical barriers (e.g. Epps et al. 2005; Wilson et al. 2015). It was only recently shown that even unpaved roads with little traffic could impact the presence probability and space use of animals (e.g. red deer and wild boars (*Sus scrofa*), D'Amico et al. 2016, Mt. Graham red squirrels (*Tamiasciurus hudsonicus grahamensis*), Chen and Koprowski 2016). Anthropogenic linear features considered in our study were composed of one small remote road, tracks and hiking trails with relatively low human use. They nevertheless imposed a very high resistance to female gene flow, providing evidence that the behavioral limitations imposed by these elements impacted movements during the mating season.

One mechanism involved in avoidance of linear landscape features can be an increased perceived risk near these features ("landscape of fear", Laundré et al. 2001). Indeed, females favored safe environments during the rutting period, and linear landscape features could appear as unsafe due to vegetation gaps (gap avoidance, e.g. D'Amico et al. 2016; Chen and Koprowski 2016). In addition, despite a low rate of human activity (0.40 tourist groups per hour, Martinetto et al. 1998) and the absence of hunting in the protected area (but occurring outside), behavioral responses to human disturbances and hunting have been observed in the protected area during the rutting period (Benoist et al. 2013; Marchand et al. 2014a). Human activities could thus participate in the landscape of fear (e.g. increased vigilance in moose (*Alces alces*) along roads, Ciuti et al. 2012) and females might avoid crossing linear landscape elements to decrease contacts with humans.

Marchand et al. (2017a) also hypothesized that linear landscape features could represent visual landmarks in cognitive maps driving animal navigation and, showed their role in the definition of the

boundaries of individual home range. Since females are highly philopatric and less mobile than males (Dubois et al. 1992; Dupuis et al. 2002), the cognitive map may be another mechanism in accordance with our expectations, explaining linear landscape features avoidance. It is worth noting that in numerous ungulate species, including Mediterranean mouflon, females exhibit a marked socio-spatial structure and live in spatially separated groups (e.g. Geist 1971; Festa-Bianchet 1991; Garel et al. 2007; Portanier et al. 2017) for which boundaries can rely on linear landscape features (e.g. Laurian et al. 2008; Bartzke et al. 2015). These groups can be differentiated in terms of behaviors, body mass and fitness (e.g. Coulson et al. 1999; Pettorelli et al. 2001, 2003; Focardi et al. 2002; Garel et al. 2007), sanitary status (e.g. Altizer et al. 2003; Marchand et al. 2017b), or genetics (e.g. Nussey et al. 2005; Podgórski et al. 2014; Noble et al. 2016; Portanier et al. 2017), highlighting that behavioral separations imposed by linear landscape features between groups can have implications for populations in numerous aspects and reinforce spatial genetic structure.

Behavioral ecology and landscape genetics are complementary for males

Regarding males, no impact of landscape was detected on gene flow. A lack of statistical power due to a lower sample size for males than for females cannot be discarded to explain this result. Indeed, performing a power analysis (i.e. applying landscape genetics analyses on five randomly selected female sub-samples of 70 individuals), we evidenced that IBR signal is not systematically detected when considering male's sample size (see Online Resource 5). On the other hand, the absence of a landscape genetic signal was expected in males and had a strong biological support. Indeed, in numerous ungulate species, males exhibit seasonal movement patterns (e.g. Pépin et al. 2009; Unterthiner et al. 2012; White et al. 2012) with increased movements during the mating period (e.g. Karns et al. 2011; Jarnemo 2011). In Mediterranean mouflon, during the rutting period, males are more mobile and prone to move toward unfamiliar areas than females (Dubois et al. 1993, 1996; Dupuis et al. 2002; Marchand et al. 2017a). They are also thought to insure most of the gene flow through reproductive excursions (Portanier et al. 2017; Marchand et al.

unpublished data) leading to lower levels of spatial genetic structure (Portanier et al. 2017). Accordingly, the absence of landscape effect on gene flow in males was biologically meaningful and tended to confirm that landscape had less impact on male reproductive movements than on females.

Although habitat preferences have been highlighted for males during autumn, they are the same as female preferences (Marchand et al. 2015) and authors hypothesized that habitat selection by males during the rutting period is the consequence of female choices since *Ovis* species and Mediterranean mouflon use coursing as mating strategies, with males following females (Hogg 1984; Hogg and Forbes 1997; Bon et al. 1992; Coltman et al. 2002). In addition, Marchand et al. (2017a) demonstrated impacts of some linear landscape features on male movements during the rutting period, but they did not account for excursions outside home ranges since they only considered movement steps included in the 95% home range. These excursions might constitute reproductive excursions and resistance of linear landscape features measured within home ranges might therefore over-estimate the genetic resistance of these landscape elements. Recent studies have indeed shown that the behavioral state (e.g. foraging, dispersing) is an important factor to take into account when studying landscape connectivity to avoid potentially erroneous conclusions about isolation of patches (Abrahms et al. 2017; Blazquez-Cabrera et al. 2016; Maiorano et al. 2017). For instance, focusing on 'inside home range' locations instead of on 'outside home range' locations (dispersal and exploratory movements) could lead to underestimating landscape functional connectivity (Blazquez-Cabrera et al. 2016). Our results might illustrate this case since the linear landscape features having an impact on male movements seemed to have no impact on male gene flow. Genetic connectivity and connectivity linked to 'inside home range' movements (e.g. when foraging) can be different since habitat selection aims at maximizing individuals' fitness on their home range while reproductive dispersal movements achieve a different goal and lead individuals to modify their behavior (Larroque 2015). Mating may thus be a sufficient motivation to overcome landscape constraints to movements.

Alternatively, landscape effects on male gene flow might not yet be strong enough to lead to a detectable IBR pattern. Landguth et al. (2010)

evidenced that when too few generations elapsed, landscape effects on genetic differentiation are not detectable by Mantel tests. In the Caroux-Espinouse population, only 14 generations elapsed since introduction (Garel et al. 2005). Due to higher philopatry and spatial stability (Dubois et al. 1992, 1994; Martins et al. 2002; Marchand et al. 2017a), landscape effects were expected to be stronger for females than for males, which might also explain why IBR pattern was already detectable for females while not for males.

Conclusions and perspectives

In the current context of global changes, investigating processes (e.g. gene flow) and not only patterns (e.g. spatial structure) is necessary to better predict consequences of global changes on populations and species (Plard et al. 2015; Moran et al. 2016). As illustrated here, combining previous knowledge of movements and habitat selection with the results from a landscape genetics approach focusing on gene flow was decisive to better understand functional connectivity in the studied population. Spatial approaches allowed us to focus on landscape elements known to be important for Mediterranean mouflon movements and habitat selection and the landscape genetics approach allowed us to accurately describe landscape functional connectivity.

Our findings showed that males and females can present very different resistance surfaces, highlighting the necessity of performing sex-specific landscape connectivity studies, especially in polygynous mammals exhibiting sex-biased dispersal. Our results also showed strong impacts of behavioral barriers on gene flow and spatial genetic structure and illustrated the importance of also accounting for such types of barriers when studying functional connectivity. More specifically, more studies on impacts of natural linear landscape features acting as behavioral barriers are needed. In the current context of habitat loss and fragmentation (Fahrig 2003) reducing wildlife corridors and isolating populations (Crooks and Sanjayan 2006), our results can serve to better understand spatial genetic patterns of other mouflon populations, especially endangered ones (e.g. Corsican mouflon *Ovis gmelini musimon var. corsicana*).

Some landscape factors constantly appeared as a constraint to gene flow in wild sheep landscape genetic studies (e.g. slope, Epps et al. 2007; Roffler et al. 2016; Creech et al. 2017, present study), but landscape resistance in one study area does not systematically correspond to what happens in another study area (e.g. Larroque et al. 2016a, Short-Bull et al. 2011). More research is thus needed to improve our understanding of landscape impacts on wild sheep gene flow. Another important point here is that, although natal dispersal of males is expected to be limited (Dubois et al. 1993, 1996; King and Brooks 2003), it can occur, and more studies on natal dispersal will be needed to fully understand gene flow in Mediterranean mouflon. In addition, using the least-cost path approach we assumed that individuals knew ahead which path will be optimal (Balkenhol et al. 2016). This hypothesis is not unrealistic for Mediterranean mouflon given their abilities to recognize familiar areas and move away from linear landscape elements before being in direct contact with (see Marchand et al. 2017a). Circuit-theory has nevertheless been applied in landscape genetics to release this assumption and could represent an interesting complementary approach to the present study. Furthermore, although being the most used (Zeller et al. 2016), landscape genetics Mantel based methods have been criticized due to inflated type I error (e.g. Legendre et al. 2015) and low discrimination power between highly correlated alternative hypotheses (Zeller et al. 2016). Here we used Mantel r and relative support (see Cushman et al. 2013) and performed scenarios' averaging to avoid these biases. While no clear consensus has yet been reached about the best method in landscape genetics (Balkenhol et al. 2016), alternative methods to Mantel tests are promising (see Shirk et al. 2018) and applying such approaches in Mediterranean mouflon would allow to confirm our results.

Finally, landscape functional connectivity encompasses several components among which genetic connectivity but also for instance demographic or behavioral connectivity (see Lowe and Allendorf 2010). The data type which should be used to estimate landscape functional connectivity depends on the question that is addressed. For example, genetic connectivity can overlook most of the host movements potentially leading to parasite transmission across the landscape since it will not only be determined by reproductive movements but instead by several movement types (e.g. migratory movements, Conner and Miller 2004). Management strategies should thus

account for all functional connectivity components, and several data types enabling the measurement of different connectivity components are thus needed to fully understand impacts of landscape on populations.

Data availability Microsatellite and spatial data sets will be available from the Dryad Digital Repository.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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