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Herbivore corridors sustain genetic footprint in plant populations: a case for Spanish drove roads

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Habitat fragmentation is one of the greatest threats to biodiversity conservation and productivity, that are mediated by direct human impact. Its consequences include genetic depauperation, including phenomena such as inbreeding depression or reduction in genetic diversity. While the capacity of wild and domestic herbivores to sustain long-distance dispersal has been proven, the impact of herbivore corridors in plant population genetics remains to be observed. We conducted this study in the Conquense Drove Road in Spain, with a sustained use by livestock during centuries where transiting herds passed by twice a year en route to winter and summer pastures. We compared genetic descriptors of *Plantago lagopus* populations in the drove road with populations in the surrounding agricultural matrix, at varying distances from human settlements. We observed significant differences for coefficients of inbreeding between the drove road and the agricultural matrix, as well as significant trends for higher genetic diversity around human settlements. Trends for higher genetic diversity in drove roads may be present, although they were not significant with the available sample size. The resulting functional landscape has human settlements as dispersal hotspots, while the drove road act as a pollinator reservoir and possibly also as a linear structure facilitating long-distance dispersal in an agricultural matrix where local *P. lagopus* populations depend rather on short-distance dispersal. These results highlight the role of herbivore corridors for conserving the migration capacity of plants, and they contribute as well in understanding the role of human dispersal for the spread of invasive species.

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Herbivore corridors sustain genetic footprint in plant populations: a case for Spanish drove roads

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ABSTRACT

Habitat fragmentation is one of the greatest threats to biodiversity conservation and productivity, that are mediated by direct human impact. Its consequences include genetic depauperation, including phenomena such as inbreeding depression or reduction in genetic diversity. While the capacity of wild and domestic herbivores to sustain long-distance dispersal has been proven, the impact of herbivore corridors in plant population genetics remains to be observed. We conducted this study in the Conquense Drove Road in Spain, with a sustained use by livestock during centuries where transiting herds passed by twice a year en route to winter and summer pastures. We compared genetic descriptors of *Plantago lagopus* populations in the drove road with populations in the surrounding agricultural matrix, at varying distances from human settlements. We observed significant differences for coefficients of inbreeding between the drove road and the agricultural matrix, as well as significant trends for higher genetic diversity around human settlements. Trends for higher genetic diversity in drove roads may be present, although they were not significant with the available sample size. The resulting functional landscape has human settlements as dispersal hotspots, while the drove road act as a pollinator reservoir and possibly also as a linear structure facilitating long-distance dispersal in an agricultural matrix where local *P. lagopus* populations depend rather on short-distance dispersal. These results highlight the role of herbivore corridors for conserving the migration capacity of plants, and they contribute as well in understanding the role of human dispersal for the spread of invasive species.

INTRODUCTION

Increased habitat fragmentation has been perceived as a major worldwide threat for ecosystems and biodiversity for the last decades (Fahrig 2003). Threats are very tangible in both developed and developing countries. In developed economies, increased investments in transportation (Benítez-López et al. 2010), exacerbate existing negative consequences. In developing economies, extensive fragmentation occurs due to the expansion of road infrastructure (Ibisch et al. 2016), agricultural development (Herrera et al. 2009), the building of fences (Bartlam-Brooks et al. 2011; Said et al. 2016) and increasing urban sprawl (Said et al. 2016). Developed economies may be able to mitigate negative impacts through very costly yet limited engineering corrections (van Bohemen 1998), but in weaker economies where financial constraints exist, mitigation capacity is severely limited. Affordable mitigation measures reducing fragmentation are problematic because they can have a negative impact on the provision of important ecosystem services to the population (Mitchell et al. 2015). This is well illustrated by the trade-offs posed by limitation of road expansion for the achievement of the Sustainable Development Goals (Ibisch et al. 2016).

Fragmentation limits size and connectivity among populations, leading to genetic drift and reduction in within-population genetic diversity. This, in turn, can hinder evolutionary potential, promote selfing and decrease population viability through inbreeding depression. Such effects impact not only biodiversity conservation (Ouborg et al. 2006), but also ecosystem productivity (Crutsinger et al. 2006) and microevolutionary responses (Matesanz et al. 2017). Although adaptation may arise quickly under such a strong pressure, likely promoting longer-distance dispersal-related traits in the populations, the suite of associated traits selected for may be deleterious in the long run (evolutionary traps, Cheptou et al. 2017), thus compounding the problem.

Dispersal is a central life-history trait that structures the properties of ecosystems (Bonte & Dahirel 2017; Massol et al. 2017). Considering that connectivity by itself does not necessarily promote biodiversity conservation, as in birds (Lopes et al. 2016), the relevance of dispersal becomes paramount in a context of increased fragmentation. For instance, experimental suppression of seed dispersal has shown to have very negative consequences for plant diversity, compromising species existence (Germain et al. 2017). Thus, long-distance dispersal events leave a measurable trace in the functional composition of plants in fragmented grasslands (Janečková et al. 2017). Not surprisingly, in most cases, particularly among fragmented natural habitats, dispersal is a key element for reducing extinction risk, subjected to strong adaptation pressures (Cheptou et al. 2017). Indeed, forecasts for the ongoing climate change include a massive rearrangement of species distributions, which could track their climate niches only if their dispersal is not impeded. This will demand major governance actions to prevent dramatic consequences on human well-being (Pecl et al. 2017). Management actions to promote dispersal and make existing fragmentation elements more porous should pose less problems for the development agenda than suppressing some of those elements (canceling road projects and restoring grasslands from crop fields, among others).

In this context, livestock-mediated dispersal is an important vector for measures targeting facilitation of dispersal. While the ecological importance of large wild herbivores is practically disappearing (Ripple et al. 2014; Smith & Botha-Brink 2014), as are the migratory systems they support (Berger 2004), domestic herbivores have largely overtaken their role to the point that they can even substitute extinct megaherbivores (Pires et al. 2014). The effectiveness of livestock seed dispersal as a cheap and effective management tool for increasing connectivity between isolated grassland habitat patches has been extensively surveyed (Cosyns et al. 2005; Couvreur et al. 2005), showing that the complementarity between different modes of dispersal (endo- and epizoochory) can lead to an almost complete representation of the grassland community in the dispersal spectra (Couvreur et al. 2005).

While roads play an important role in causing fragmentation, their impact may vary depending on their specific characteristics (Ibisch et al. 2016), especially related to their functionality (Ibisch & Selva 2017). Roads can modify habitats by making them more heterogeneous and permeable to dispersal, thereby increasing biodiversity (Zeng et al. 2012). At this stance, drove roads – routes traditionally used for mobile pastoralist livestock – become particularly interesting. Traditional ones are presumed to often derive from ancient migratory routes of wildlife (Manzano Baena & Casas 2010), hence being analogous to other natural roads observed at multiple scales from ants (Azcárate & Peco 2003; Buhl et al. 2009), to elephants (Blake & Inkamba-Nkulu 2004; Shannon et al. 2009). Drove roads have been observed to have a strong effect in increasing multifunctionality of the landscape by working as linear grasslands and increasing heterogeneity at the large scale, translating into an increase of biodiversity in diverse taxonomic groups such as plants (Azcárate et al. 2013a), ants (Azcárate et al. 2013b; Hevia et al. 2013) or even bees, linked with important pollination services (Hevia et al. 2016). Given the proven capacity of livestock in achieving long-distance dispersal along drove roads, both by endozoochory (Manzano et al. 2005) and by epizoochory (Manzano & Malo 2006), mobile pastoralism taking place in drove roads could potentially mitigate isolation of populations, which is a major factor influencing fragmentation-derived viability (Fahrig 2003; Mitchell et al. 2015).

Dispersal is a multi-faceted process where the quantification of every step implies complex measurements that can result in great uncertainties at the landscape scale (Wang & Smith 2002), and estimating its effective footprint is much more feasible through genetic analyses. Also, the study of the genetic structure of the populations is essential to assess their viability in the long term. Fragmentation by isolation leaves a measurable genetic trace (Leblois et al. 2006). With such background, studies in livestock-grazed rangelands have observed that grazed areas display a more homogeneous genetic structure than ungrazed ones (Smith et al. 2009) and a less-than-expected population differentiation in *Anthyllis vulneraria* fragments historically subjected to rotational grazing (Honnay et al. 2006). While (Willerding & Poschlod 2002) were unable to see effects of herd mobility in a rotational context, possibly due to the election of the target species (the anemophilous *Bromus erectus*), Rico & Wagner (2016) recently observed that dispersal of *Dianthus carthusianorum* by rotationally grazing livestock is strong enough to leave a measurable trace in plant populations, reducing genetic structure and increasing the viability of re-introduced grassland plants. It, nevertheless, remains to be seen if migrating livestock, with bigger-sized herds but with a much shorter transit time per area of grasslands, can also achieve a tangible effect on plant genetic structure along the mobility corridors. This hypothesis is particularly relevant in a context of widespread worldwide practice of mobile pastoralism, and of substitution of the ecological functions of wild migratory herbivores.

In this study, we aimed to check whether dispersal processes in drove roads subjected to active use were strong enough to influence the population genetic structure of *Plantago lagopus*, an annual selfing plant species dispersed by livestock endo- and epizoochory along Spanish drove roads (Manzano et al unpublished). These populations were located along the Conquense Drove Road (CDR) in Spain, one of the few drove roads that have been under continuous use since (at least) the Middle Ages until even after the post-industrialization crisis of Spanish transhumance (Manzano Baena & Casas 2010). The CDR connects isolated grasslands inserted in the surrounding agricultural matrix, which act as excellent controls to check for effects of transhumance on landscape connectivity between plant populations. Under this scenario, a landscape genetics approach provides excellent tools to evaluate the effects of anthropogenic factors over the populations of *Plantago lagopus*. In particular, we asked i) Is the CDR activity influencing genetic differentiation among populations? ii) Do the populations in the CDR have similar genetic diversity and/or inbreeding values than those located in the agricultural matrix? and iii) Can other landscape factors (e.g. distance to human settlements, time since last ploughing) modify the genetic structure of the *P. lagopus* populations?

MATERIALS & METHODS

Studied species

Plantago lagopus L. (Plantaginaceae) is a polycarpic annual or biennial forb, abundant in grazed annual-dominated pastures of Central Spain (Peco et al. 2005). This species presents a persistent soil seed bank (Peco et al. 2003) and its seeds are dispersed by cattle endozoochory (Malo & Suárez 1995). Experiments have also shown seeds are largely viable after sheep ingestion (Peco et al. 2006) and remain attached for a long time on sheep coats (de Pablos & Peco 2007), even under real transhumance conditions (Manzano & Malo 2006). This species is self-compatible, with both wind and insects as major pollination agents (Sharma et al. 1993). Annual-dominated pastures in Central Spain are known to present particularly high diaspore availability related to summer drought but translated into massive dispersal by livestock, particularly at the time when transhumant herds are on the move (Manzano 2015a). Such large dispersal, along with the faster dynamics in annual plants, make them particularly suited to test genetic signals of dispersal in fragmented scenarios (Ewers & Didham 2006).

Landscape scenario and population sampling

The study area is located between Quintanar de la Orden, Tomelloso and Villarrobledo municipalities in Castilla-La Mancha, Spain (Coordinates: 39° 18' 03.17'' N and 2°49' 54.50'' W, Figure 1). The area is a plateau (830-900 meters a.s.l.) of sandstones, loams and clay materials under continental Mediterranean climate, with a mean annual rainfall of about 500mm and severe summer droughts. The vegetation is a mosaic of agricultural dry cereal and sunflower croplands, abandoned fields and dry grasslands used by local flocks. The area is crossed by the Conquense Drove Road (hereafter CDR), one of the major road droves (ca. 410 km long) that are still used for transhumant sheep and cattle herds that move every year from the cooler and wetter mountains of Teruel, Cuenca and Guadalajara provinces to the wintering dehesas in Sierra Morena at lower altitude (Oteros-Rozas et al. 2012). The herds cross the study area twice a year (northwards in June and southwards in November), currently comprising about 9,000 heads of sheep and 1,200 of cattle. The size of transhumant herds, dominated by merino sheep, has shrunk from about half a million in the 16th and 17th centuries to 100,000-200,000 in the next two centuries and around 20,000 during the second half of the 20th century (Bacaicoa Salaverri et al. 1993). Productivity of the area is low except for peaks in May and October, because of cold winters and hot dry summers. Consequently, the numbers of transiting nomadic livestock have been historically much higher than those of resident livestock (Manzano Baena & Casas 2010), which only grew in the last centuries (Bacaicoa Salaverri et al. 1993).

In winter 2013 we selected 13 populations of *P. lagopus* (6 in the CDR and 7 in isolated pastures interspersed in the agricultural matrix). Populations located in the agricultural matrix were located at least 5 km from the CDR. All populations were at least 5 km apart from each other (Figure 1, Table 1). In each population, we randomly collected between 20 and 30 flowering individuals of *P. lagopus*, whose leaves were stored under dry conditions until DNA extraction. We deposited some individuals at Real Jardín Botanico de Madrid – CSIC herbarium (reference numbers MA-01-00892341 to MA-01-00892345).

To test for the potential effect of other landscape factors on genetic descriptors, we measured the euclidean distance from the sampling points to the nearest human settlement using Google Maps. At the agricultural matrix points, we also checked the time since last ploughing by inspecting historic aerial photographs (Instituto Geográfico Nacional, Spain). The Conquense Drove Road, as in the case of all other major drove roads in Spain, has been protected from ploughing for the last centuries (Bacaicoa Salaverri et al. 1993).

Microsatellite development and laboratory protocols

We developed specific microsatellite markers for *P. lagopus* in collaboration with the Unidad de Genómica, Parque Científico de Madrid (http://fpcm.es/en/servicios-cientificos/). Briefly, the library was developed from DNA of five individuals of *P. lagopus* extracted using QIAGEN plant Extraction Kit, using manufacturer's protocol. DNA was fragmented with ultrasounds using Bioruptor (Diagenode) and size fragment was selected for an approximated size of 1,2 kb. DNA sequencing was performed using a 454-FLX-Plus Roche sequencer. We obtained approximately 185000 sequences. We used Pal_Finder to select those sequences with more than 5 repetition motifs and a size that ranged from 100 to 300 bp. We used Primer3 (Untergasser et al. 2012) to configure potential primers that amplify those microsatellite regions. Details of the primers used are shown in Table 2.

Genetic descriptors: diversity, Inbreeding, differentiation and structure

We checked for genetic errors and misidentifications of alleles with Micro-checker (van Oousterhout et al, 2004). We estimated four genetic diversity indices: observed and expected heterocigosity (H_O and H_E), the Shannon Information Index (I), as a measure of genetic diversity that considers the number of alleles and its dominance in the population, and the number of private alleles (A_P) using GeneAlEx 6.5 (Peakall & Smouse 2012). Because the number of sampled individuals is different in each population (ranging from 8 to 30, Table 1), we also calculated the genetic diversity using Hp-Rare (Kalinowski 2005) to rarefact the allelic richness and the number of private alleles to the minimum number of individuals sampled in one population (i.e. 8 individuals), hereafter RiqRare and PrivRare, respectively.

We calculated the inbreeding coefficient, F_{IS}, using INEST 2.1. (Chybicki & Burczyk 2009) and the departure from Hardy-Weinberg equilibrium with GeneAlEx 6.5 (Peakall & Smouse 2012) to evaluate the inbreeding status of *P. lagopus* populations. We also assessed the presence of bottlenecks using Bottleneck 1.2.02 (Piry et al. 1999), through a Wilcoxon test with 2000 permutations under the three plausible mutations models, as recommended for microsatellite data. We applied Bonferroni correction for multiple testing.

We estimated genetic differentiation with F_{ST} coefficient calculated with FreeNA (Chapuis & Estoup 2006) to minimize potential biases caused by null alleles or allele dropout. We tested signification between each pair of populations with 999 permutations. We used HIERFSTAT 0.04 (Goudet 2005) to evaluate the F-statistics grouping of the populations according to their location (either within the matrix or in the drove road) with 1000 permutations. To assess the presence of Isolation-by-Distance patterns, we performed a Mantel test with 999 permutations using genetic differentiation, (i.e. F_{ST} coefficients) and Euclidean geographical distance between populations with Vegan package in R (Oksanen et al. 2007).

We assessed genetic structure using the Bayesian clustering method STRUCTURE v. 2.3.4 (Hubisz et al. 2009) with no prior information on populations. We carried out 10 independent runs for each *K* value (i.e. number of groups or clusters), ranging from 1 to 15. Each run comprises a burning period of 105 permutations, followed by 106 MCMC (Markov Chain Monte Carlos) iterations. We assumed correlated allele frequencies and genetic admixture. To assess the most plausible number of clusters (*K*), we used the STRUCTURE Harvester module to obtain the average log probability of the data of each *K* and determine the highest value. We also used the Evanno method (Evanno et al. 2005) with the ad hoc statistic *ΔK* and identified the maximum *ΔK* when plotted against *K*. We also used Clump v.1.1.2 (Jakobsson & Rosenberg 2007) to estimate the membership coefficient of each individual in each cluster and Distruct v 1.1 (Rosenberg 2004) to plot it. We also evaluated genetic structure with an Analysis of Molecular Variance (AMOVA) implemented with GenAlex 6.5. (Peakall & Smouse 2012). With the AMOVA we tested population differentiation between the agricultural matrix and the drove road. We evaluated signification with 999 random permutations.

Relationships of genetic descriptors with environmental factors

We built multiple linear regression models to examine the relationship between genetic descriptors with the population location (agricultural matrix or drove roads), the distance to nearby human settlements (in km), and their interaction. The genetic descriptors used as response variables were the inbreeding coefficient (F_{IS}) and genetic diversity statistics H_E, I and RiqRare. We also tested the correlation of the genetic descriptors with the ploughing history (years since last ploughing) for the populations in the agricultural matrix.

RESULTS

Genetic differentiation and structure

Pairwise genetic differentiation values (F_{ST}) between populations were small (Table 3). On average, LMA14 showed the greatest differentiation, with an average F_{ST} value of 0.08. Hierarchical analysis of the genetic differentiation showed low diferentiation between matrix and drove road locations ($F_{CT} = 0.032$) and a moderate one between populations within each category ($F_{SC} = 0.11$). Both values are significant. Mantel test ($r = 0.13$; $p = 0.22$) did not detect the presence of an isolation-by-distance (IBD) pattern. The genetic structure analyzed with a Bayesian clustering approach (i.e. STRUCTURE) showed two complementary clustering patterns, suggesting that K=2 or K=5 are plausible distributions of the genetic structure. The K=2 scenario highlights the presence of two cluster groups: one cluster includes LMA14 and some LCR09 individuals, while the other populations would have an admixture of both clusters. The K=5 structure also showed a great admixture of the populations with different clusters, with a connection between LMA14 and some individuals of LCR09. (Figure 2). The AMOVA analysis indicated that most of the genetic diversity appears within populations (95%). Very little variance of the genetic diversity was associated with the position inside the drove road or in the agricultural matrix.

Genetic diversity and inbreeding

The values for genetic diversity (I, A_P, H_O, H_E, RiqRare and PrivRare) were similar between populations in the agricultural matrix and populations in the drove road, except for A_P, which was greater in the populations of the agricultural matrix, due to the large number of alleles found in LMA08 (Table 4). The average expected heterozygosity H_E was 0.84 in drove roads populations and 0.87 in matrix populations. However, matrix populations showed greater variability in H_E, with values ranging from 0.93 in LMA13 to 0.75 in LMA14. The same pattern (similar average but different variability) was found in the other genetic diversity indexes (Table 4).

The inbreeding coefficient (F_{IS}) varied greatly among populations and between matrix and drove road locations. Drove road populations showed a mean value of 0.12 (ranging from 0.03 in LCR04 to 0.29 in LCR01), while matrix populations showed a mean value of 0.24 (double the mean value of drove roads), ranging from 0.13 in LMA11 to 0.39 in LMA14 (Table 4). All populations had, at least, one locus that deviated from Hardy-Weinberg (HW) equilibrium. Bottleneck signals were found in some populations under SMM model (LCR09, LMA08 and LMA11), but the results were not significant in all genetic models and applying multi-comparisons corrections (Table 5).

Landscape factors governing genetic descriptors in P. lagopus *populations*

The distance to the nearest settlement had an impact on all the genetic descriptors except for F_{IS} (Table 6). This relationship is independent of the frequency of ploughing, because for the agricultural matrix plots the correlations of years since last ploughing and the residuals of the regressions explaining the genetic descriptors were non-significant. Also, the values of genetic descriptors did not correlate significatively with the number of years since last ploughing (Pearson correlations ranging from -0.38 to -0.13, all $p > 0.05$).
Distance to the nearest settlement showed no overall effect on inbreeding (F_{IS}) because the interaction with population position was significant (Table 6). Thus, F_{IS} increased with distance from human settlements in the agricultural matrix, but remained constant in drove road populations regardless of distance (Figure 3a). For the descriptors of genetic diversity (HE, I and RiqRare), the distance to settlements had a consistent negative and significant effect (p-values < 0.05), and there was no significant effect for population position and its interaction with distance (Table 6). Genetic diversity descriptors between matrix and drove road positions showed a pattern of convergence at short distances and divergence at high distances from human settlements (Figures 3b, c, d).

DISCUSSION

Drove roads have a significant capacity to shape some genetic features of *Plantago lagopus* populations in the study area. This assessment of the population genetics of *P. lagopus* in the study area indicates relevant differences in terms of coefficient of inbreeding between populations and between matrix and drove roads positions. Furthermore, proximity to human settlements appeared to drive the hotspots for genetic diversity in this landscape. Drove roads were able to reduce the inbreeding otherwise observed in the agricultural matrix. Other indicators related to genetic diversity seem to follow the same trend, although they are not significant with the sample size used in this study.

In a self-compatible plant species pollinated both by wind and by insects, such as *P. lagopus*, population inbreeding is mainly determined by the ratio between selfing and outcrossing, where outcrossing is enhanced by pollinator availability. Thus, the smaller inbreeding coefficient observed along the drove road indicates a relevant role played by them in providing pollination services. While a greater coefficient of inbreeding is a genetic indicator that can be related to pollinator limitation (Van Etten et al. 2015), the structural role of drove roads as grassland corridors crossing agricultural landscapes has been proved to be determinant in supporting pollinator services (Hevia et al. 2016). This result goes along other studies that have observed provision of heterogeneity at the landscape level because of the drove road's structural role, translated in higher biodiversity levels (Azcárate et al. 2013a; Hevia et al. 2013). Ploughing does not show any effect or trend on the genetic variables studied, suggesting that structural continuity in space is more important than stability in time to guarantee genetic admixture.

Distance to human settlements showed a consistent effect on genetic diversity, or in inbreeding coefficients when in combination with the effect of the drove road. The resulting functional landscape can be interpreted as settled, high human density areas being hotspots of dispersal that modulate the genetic patterns of diversity and selfing, probably mediated by the commercial livestock exchanges but also by other dispersal types, mainly human-mediated (Auffret 2011; Wichmann et al. 2009). The *P. lagopus* populations on the drove road don't show to be affected by these types of dispersal, likely because their human use is much diluted and human activities are comparatively simplified and less dense, consisting just in accompanying or herding livestock.

The trend of a higher genetic diversity on drove roads compared to the agricultural matrix (Figures 3b, 3c, 3d), even if not significant, may be pointing to a scenario where two types of dispersal processes coexist. On the one hand, populations in the agricultural matrix would receive seeds through the local stock through short-distance dispersal processes. Meanwhile, long-distance dispersal processes favoring greater admixture dominate in the drove roads actively being used by livestock, therefore functioning as a highway for seeds. This would highlight the important role of active livestock movements for preserving the functionality of drove roads and, more generally, herbivore migration corridors. Such effects seem to be too weak to be perceived as significant, given our low sample size and the moderate levels of gene flow that homogenize the genetic diversity between the populations and reduce the occurrence of private alleles. The observable trend, however, may be pointing to a very promising area of research for future studies, especially considering that the long history of drove roads in Spain, with fixed ancient routes coinciding with older herbivore routes, may be showing a sustained contribution to long-distance seed dispersal (Manzano Baena & Casas 2010; Manzano & Malo 2006). Further insights on the persistence of effects may be gained by comparing the CDR with other Spanish drove roads abandoned in the mid-20th century (Manzano Baena & Casas 2010).

Implications for ecosystem function and conservation

Functional herbivore corridors would sustain diversity by reducing the risk of local extinctions due to increased inbreeding and loss of genetic variation in small populations (Caughley 1994). The effect of drove roads on inbreeding observed in our study has relevant implications for conservation, as inbreeding depression is a major concern for the conservation of biodiversity in human-altered fragmented landscapes (Hedrick & Garcia-Dorado 2016). We think that connectivity factors, related to herbivore type and capability of sustaining dispersal corridors for plants, would therefore add to the complexity of factors that influence the relationship between grazing and plant diversity. Such factors include productivity and disturbance (Herrero-Jáuregui & Oesterheld 2018; Olff & Ritchie 1998), historical effects on communities (Cingolani et al. 2005; Osem et al. 2002) or herbivore species and sizes (Bakker et al. 2006; Liu et al. 2015). Functional herbivore corridors would sustain diversity by reducing the risk of local extinctions due to loss of genetic variation in small populations (Caughley 1994).

The non-significant yet observable trend of drove roads in reducing genetic diversity loss of *P. lagopus*, in spite of the rapid transit of transhumance flocks – ca. 15 km/day, or 1.5 km/h when moving (Hevia et al. 2013) is very noteworthy. Such a use, which is intense but nevertheless very restricted in time, would achieve extraordinary dispersal effects due to a “green wave effect” (Merkle et al. 2016), as transhumant flocks use drove road pastures when they are at their maximum productivity (Manzano Baena & Casas 2010) and therefore at their maximum seed production stage. This is a radical contrast with other works studying rotational grazing systems (Rico & Wagner 2016) where the intensity of use per unit of area is much higher and the chances for consolidating dispersal between patches are also higher. Indeed, a confirmation of such trend in future studies would highlight the potential role of herbivore corridors to promote long distance dispersal.

The potential of mobile livestock and the corridors it uses as a major fragmentation mitigation tool, both in developed and in developing countries, faces nevertheless major challenges. Mobile pastoralism is globally threatened by non-conductive policies (Manzano 2017), threatening its important role as a provider of ecosystem function (Leroy et al. 2018). Such risk is aggravated by its potential magnitude, as pastoralism uses cover more than half of the global land (Manzano 2015b). A disruption of dispersal processes due to decreased pastoralist mobility patterns may further contribute to a generalized grassland biodiversity crisis motivated by land use intensification (Gossner et al. 2016) and climate change. Regarding the “green wave effect”, a potential decoupling of herbivore movements from the phenology and seed availability brings also concerns in such climate change context (Berg et al. 2010; Thackeray et al. 2016). The quantification of the effectiveness of such dispersal mechanisms on a landscape scale, to which our study contributes, is urgent in order to orient adequate science-evidenced policy recommendations (Manzano Baena 2012).

The importance of herbivore corridors for plant conservation should not be restricted to livestock, in spite of its ecological importance at the global level (Suárez-Esteban et al. 2013). We obtained our data in a drove road that has experienced a drastic decline in use in the recent centuries, from 600,000 head in the 16th century to less than 10,000 nowadays (Bacaicoa Salaverri et al. 1993; Hevia et al. 2013). Our results suggest that the effect on the genetics of plants must have been much more significant at that time, similar to what they must be in the systems presently steered by hundreds of millions of mobile pastoralists (Manzano & Agarwal 2015), whose capacity to cover wide territories is shown by the fractal structure of their drove roads in different countries (Manzano & Salguero-Herrera 2018). This effect should also be greater in the world’s greatest wild herbivore migrations (Berger 2004).

A further relevant outcome of our study is the important role that human settlements seem to play for seed dispersal. The result is not surprising given that human-mediated dispersal adds to livestock-mediated dispersal mechanisms (Auffret 2011) which are problematic for the spread of invasive species (Abbas et al. 2018). However, it offers insights on how an unnatural over-dispersal that facilitates invasiveness of some plant species (Wilson et al. 2009) may be visible already at small geographical scales such as the one regarded in this study. Genetic analyses that further explore the role of livestock or other dispersal vectors may offer valuable insights on the ecology of potential or confirmed invasive species, especially in relationship with their human-mediated dispersal.

CONCLUSIONS

Our results are relevant for shaping conservation policies that take into account the role of herbivores, be them wild or domestic. We confirm the potential relevance that herbivore corridors and mobile pastoralism have in reducing inbreeding levels in plant populations and in facilitating long-distance plant migration. Such corridors may have to be kept in use in order to preserve full ecological functionality (Starrs 2018) that goes beyond their role as landscape structures. Such potential adds to the already identified needs to reduce fragmentation in the dryland areas occupied by pastoralists, and international conventions that deal with such issues (Durant et al. 2015). Some hotly debated national policies related to drove roads (AA.VV. 2012; Herzog et al. 2005) should as well take note on our results. We also highlight the interest of expanding such studies to other animal-dispersed species in order to quantify the importance of corridors for the maintenance of population genetic diversity levels in a world confronted with global change, and where deleterious effects on plant genetic diversity are already visible (Alsos et al. 2012).

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Table 1(on next page)

Number of individuals sampled per populations considered in the study (N), coordinates (latitude and longitude) and position according to the drove road.

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Population	**N**	**Latitude**	**Longitude**	**Position**
LCR01	30	39.43 N	2.62 W	Drove Road
LCR02	10	39.39 N	2.67 W	Drove Road
LCR04	10	39.34 N	2.77 W	Drove Road
LCR06	13	39.26 N	2.81 W	Drove Road
LCR09	14	39.13 N	2.84 W	Drove Road
LCR10	8	39.08 N	2.84 W	Drove Road
LMA05	10	39.27 N	2.61 W	Agricultural Matrix
LMA08	30	39.16 N	2.99 W	Agricultural Matrix
LMA11	14	39.41 N	2.93 W	Agricultural Matrix
LMA12	10	39.49 N	2.81 W	Agricultural Matrix
LMA13	13	39.26 N	2.76 W	Agricultural Matrix
LMA14	10	39.11 N	2.61 W	Agricultural Matrix
LMA16	14	39.06 N	2.66 W	Agricultural Matrix

1

Table 2(on next page)

Characterization of six microsatellites developed for *Plantago lagopus*, temperature of annealing in PCR amplification (Ta), number of alleles (NA) and GenBank number (GB).

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Locus	Repeat motif	Primer Sequences (5’-3’)	Ta (ºC)	Size range (bp)	N_A	GB
NZ	AAC	F: GCCCCTACGCGTAAAACATG R: TGAAACACACCATGGAATGCA	55	129 - 201	29	MF490249
N3	AC	F: AAAGGCCATCAATCCCAAG R: ATCGTGCTAGGGCTCGACTA	55	160 - 226	38	MF490250
7T	AAC	F: CGTGTTGCTCCTCACATTTC R: GGTCCTAGATGGGAGGCATA	55	297 - 363	27	MF490251
UT	AC	F: TGTACAAATAACAGAGGCACCAA R: AGTGTCACATTCATCTGGATAGC	55	135 - 207	36	MF490252
QL	AC	F: ACTCAAGAGAATGGCGATGA R: TGGTCCATATTGGTTGGAAA	55	167 - 235	39	MF490253
8D	AC	F: GAGGTGCCTAAGGTGGAACA R: TGCTCAACAAAACACCGAAA	55	211 - 293	41	MF490254

Table 3(on next page)

Pairwise F_{ST} values between pairs of studied populations.

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	LCR01	LCR02	LCR04	LCR06	LCR09	LCR10	LMA05	LMA08	LMA11	LMA12	LMA13	LMA14	LMA16
LCR01		0,022	0,006	0,01	0,035	0,034	0,013	0,01	0,008	0,007	0,011	0,066	0,033
LCR02	0,022		0,022	0,014	0,043	0,04	0,02	0,021	0,018	0,017	0,02	0,085	0,029
LCR04	0,006	0,022		0,004	0,045	0,037	0,013	0,013	0,017	0,035	0,006	0,085	0,034
LCR06	0,01	0,014	0,004		0,028	0,028	0,009	0,004	0,005	0,013	0,012	0,084	0,023
LCR09	0,035	0,043	0,045	0,028		0,059	0,045	0,037	0,033	0,037	0,046	0,074	0,063
LCR10	0,034	0,04	0,037	0,028	0,059		0,032	0,031	0,034	0,02	0,02	0,103	0,034
LMA05	0,013	0,02	0,013	0,009	0,045	0,032		0,018	0,022	0,012	0,014	0,096	0,041
LMA08	0,01	0,021	0,013	0,004	0,037	0,031	0,018		-0,007	0,017	0,018	0,087	0,036
LMA11	0,008	0,018	0,017	0,005	0,033	0,034	0,022	-0,007		0,018	0,017	0,082	0,032
LMA12	0,007	0,017	0,035	0,013	0,037	0,02	0,012	0,017	0,018		0,028	0,084	0,028
LMA13	0,011	0,02	0,006	0,012	0,046	0,02	0,014	0,018	0,017	0,028		0,072	0,021
LMA14	0,066	0,085	0,085	0,084	0,074	0,103	0,096	0,087	0,082	0,084	0,072		0,107
LMA16	0,033	0,029	0,034	0,023	0,063	0,034	0,041	0,036	0,032	0,028	0,021	0,107	

Table 4(on next page)

Genetic descriptors for studied populations.

N stands for number of individuals analyzed. Genetic diversity descriptors (*I,* Shannon information index; A_P, total number of private alleles, H_O observed heterocigosity, H_E expected heterozygosity), F_{IS}, inbreeding coefficient and number of alleles deviated from Hardy-Weinberg equilibrium are provided. RiqRare stands for rarefacted allelic richness, and PrivRare for average rarefacted number of private alleles. Average for drove road (LCR) and matrix (LMA) populations are also given.

Population	**N**	***I***	**A_P**	**H_O**	**H_E**	**F_{IS}-HW**	***RiqRare***	***PrivRare***
LCR01	30	2.46	6	0.64	0.88	0.29-4	9.4	0.5
LCR02	10	2.03	1	0.68	0.83	0.10-1	8.8	0.2
LCR04	10	2.29	4	0.81	0.88	0.03-1	10.2	0.7
LCR06	13	2.21	2	0.63	0.84	0.09-3	9.3	0.6
LCR09	14	2.11	2	0.66	0.83	0.09-4	8.6	0.8
LCR10	8	1.88	1	0.67	0.79	0.11-1	8.3	0.6
LCR average (CV)		*2.16 (0.09)*	2.7	*0.68 (0.09)*	*0.84 (0.04)*	*0.12 (0.75)*	*9.1 (0.07)*	*0.6*
LMA05	10	2.21	3	0.70	0.91	0.19-2	9.8	0.5
LMA08	30	2.49	12	0.65	0.90	0.26-4	9.6	0.8
LMA11	14	2.29	1	0.75	0.90	0.14-2	9.4	0.3
LMA12	10	2.04	5	0.65	0.87	0.19-3	8.8	0.8
LMA13	13	2.47	1	0.72	0.93	0.20-3	10.7	0.6
LMA14	10	1.42	1	0.44	0.75	0.39-2	5.1	0.4
LMA16	14	1.99	3	0.58	0.84	0.29-2	7.9	0.4
LMA average (CV)		*2.13 (0.17)*	*4.5*	*0.64 (0.16)*	*0.87 (0.07)*	*0.24(0.3)*	*8.7 (0.21)*	*0.5*

Table 5(on next page)

Bottleneck output for heterozygosity excess (population bottlenecks) in 13 studied populations of *Plantago lagopus.*

The output is provided under three evolutive models: Infinite Allele model (IAM), Two-phase Model (TPM) and Stepwise Mutation Model (SMM). Significant values after Wilcoxon sign-rank test with Bonferroni correction are highlighted in bold.

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	IAM	TPM	SMM
LCR01	0.43	0.84	0.11
LCR02	0.57	0.57	0.84
LCR04	0.15	0.43	1
LCR06	1	0.43	0.15
LCR09	0,15	0,15	**0,01**
LCR10	1	0,84	0,84
LMA05	0,68	1	0,11
LMA08	0,56	0,56	**0,03**
LMA11	0,43	0,56	**0,03**
LMA12	0,43	0,43	1
LMA13	0,56	1	0,84
LMA14	**0,01**	0,11	0,56
LMA16	0,68	0,84	0,15

Table 6(on next page)

Results of linear regression models of genetic descriptors on the population position (drove road / agricultural matrix), the distance to the nearest settlement and their interaction.

The F-statistic, adjusted R^2 and *p*-value for the regressions are given, as well as coefficient estimates (with standard errors) and p-values from a type-II sum of squares for the explanatory variables (note that coefficient for ‘Position’ factor refers to ‘drove road’ level, while the accompanying *p*-value is for the factor as a whole). Significant *p*-values ($p < 0.05$) are highlighted in bold.

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Inbreeding coefficient		Coefficient estimate	*p*
F_{IS}	$F_{3,9}$ = 4.901, R^2 = 49.4%, *p* = **0.027**		
	Intercept	0.179 (0.037)	
	Distance	0.020 (0.008)	0.381
	Position (drove road)	-0.026 (0.067)	**0.015**
	Distance x Position	-0.025 (0.011)	**0.046**
H_E	$F_{3,9}$ = 3.016, R^2 = 33.5%, *p* = 0.087		
	Intercept	0.873 (0.020)	
	Distance	-0.013 (0.005)	**0.025**
	Position (drove road)	-0.0003 (0.036)	0.182
	Distance x Position	-0.008 (0.006)	0.208
I	$F_{3,9}$ = 2.592, R^2 = 28.4%, *p* = 0.117		
	Intercept	2.338 (0.124)	
	Distance	-0.071 (0.028)	**0.033**
	Position (drove road)	0.016 (0.224)	0.205
	Distance x Position	0.044 (0.037)	0.262
RiqRare	$F_{3,9}$ = 3.736, R^2 = 40.6%, *p* = 0.054		
	Intercept	9.873 (0.536)	
	Distance	-0.387 (0.123)	**0.022**
	Position (drove road)	-0.098 (0.968)	0.110
	Distance x Position	0.288 (0.158)	0.102

Figure 1

Location map of the sampled points within Spain and the Castilla-La Mancha region.

Red stars correspond to sampling points in the CDR and green stars correspond to sampling points in the agricultural matrix. Black circles depict nearby human settlements.

Base map by Espanish AlB in Wikimedia Commons - Own work, based on an image of Maps-for-free.com. (Creative Commons Attribution Share Alike 3.0), CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=8398304

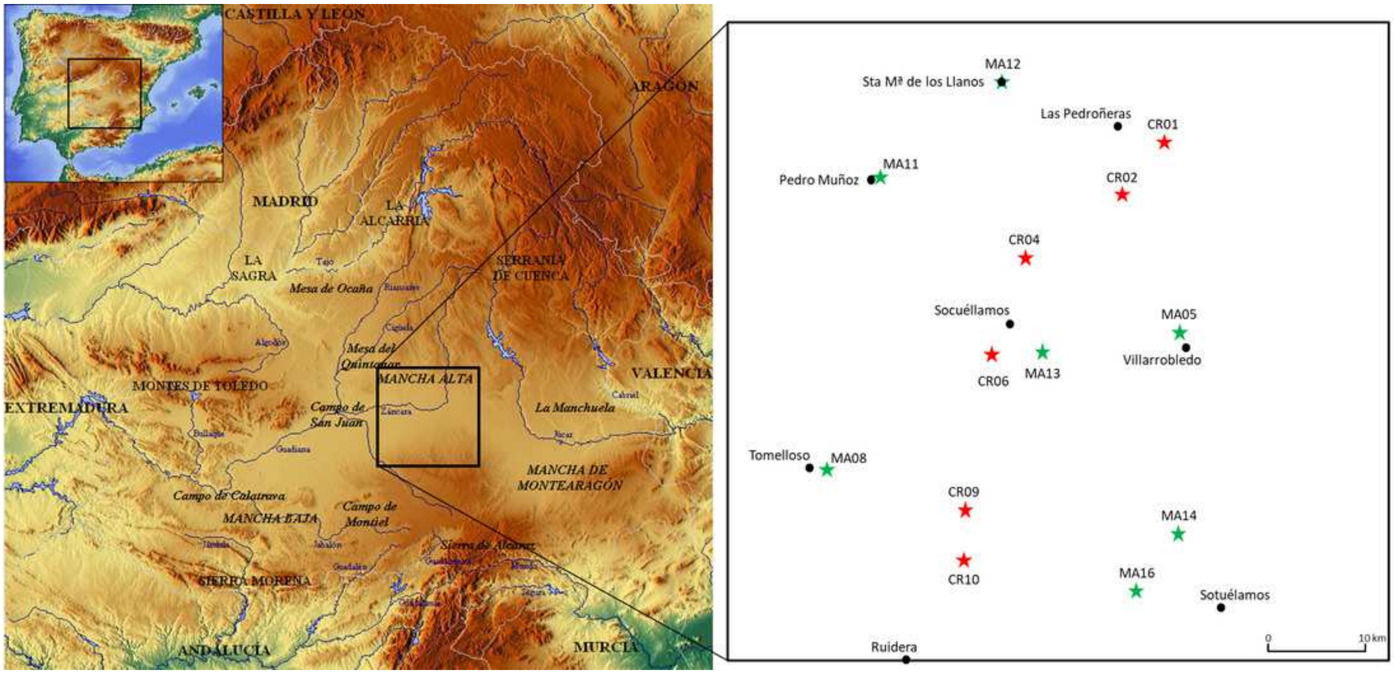


Figure 2

Bayesian genetic structure results obtained with STRUCTURE.

(A) Number of clusters proposed by Evanno method. (B) Genetic structure proposed for *K* = 2. (C) Genetic structure proposed for *K* = 5.

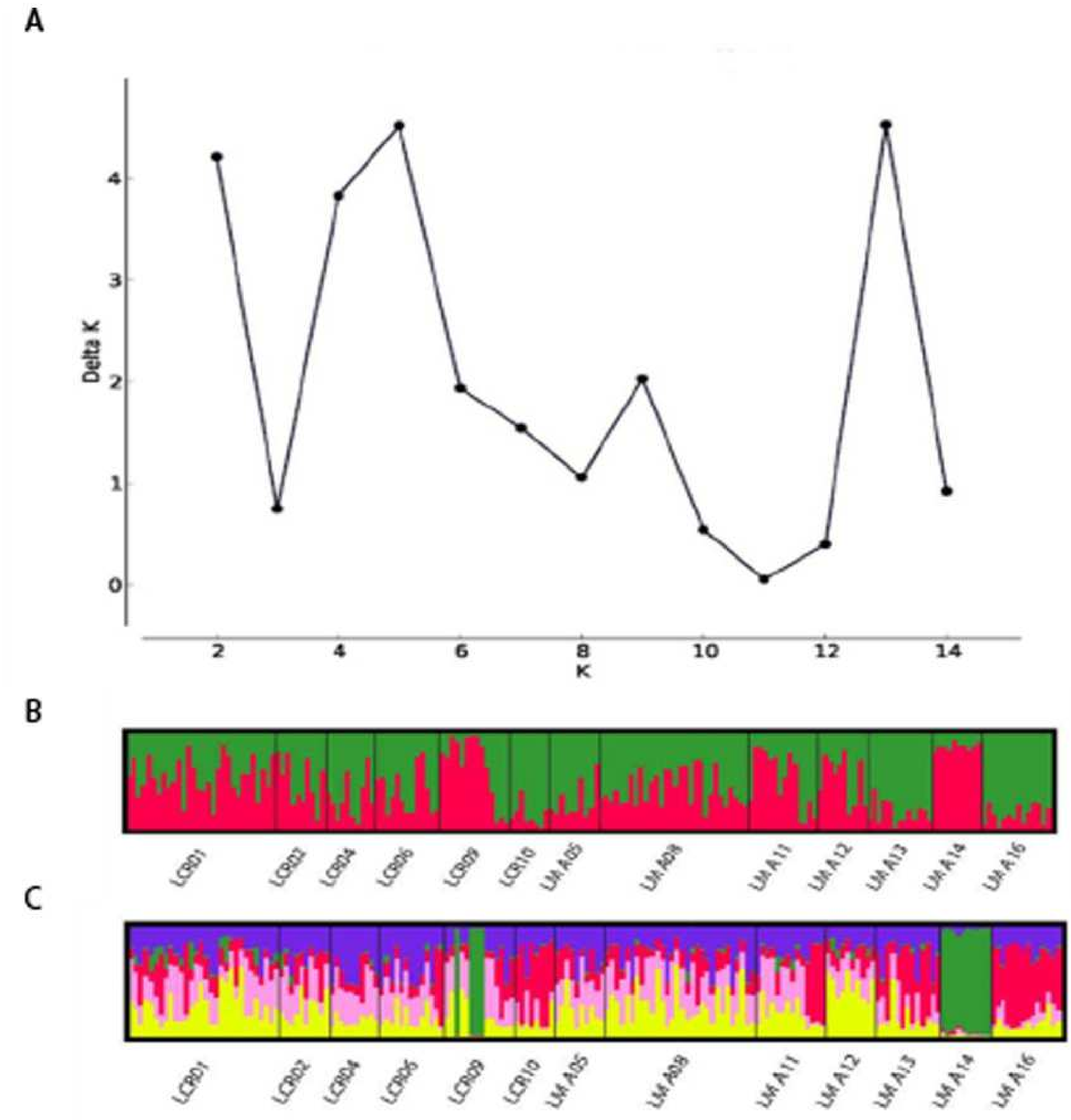
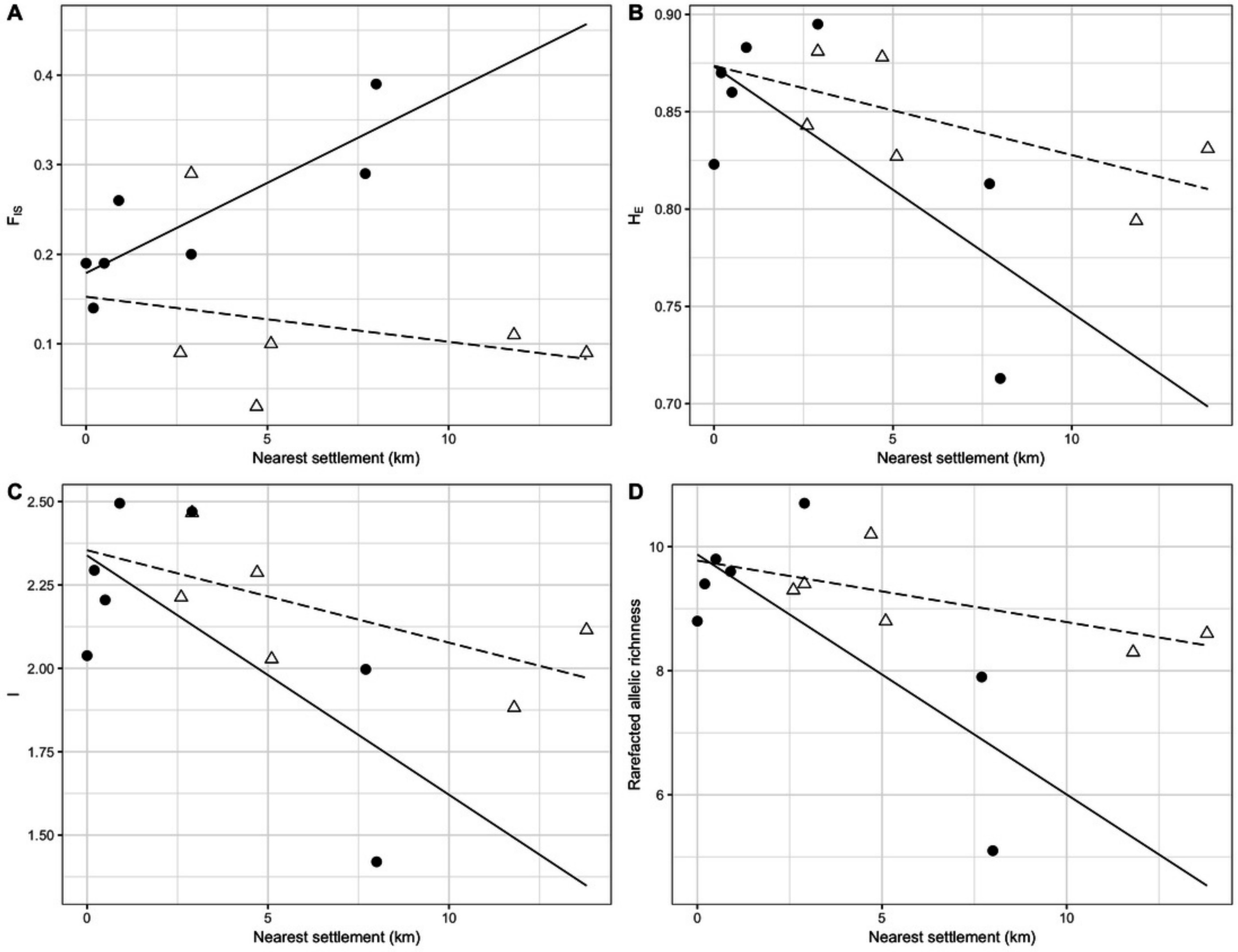


Figure 3

Relationship between genetic and geographic parameters.

Linear regressions are shown between distance to closest settlement for populations in the agricultural matrix (solid circles and solid line) and those in the drove road (open triangles and dashed line), and genetic descriptors: (A) F_{IS} for inbreeding, and (B) H_E, (C) I, and (D) rarefacted allelic richness, for diversity.



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