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

Alfredo García-Fernández[1], Pablo Manzano[2 3 4], Javier Seoane[3], Francisco M Azcárate[3], Jose M Iriondo[1], Begoña Peco[3]

[1] Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid, Spain

[2] Commission on Ecosystem Management, International Union for Conservation of Nature, Nairobi, Kenya

[3] Terrestrial Ecology Group - Departamento de Ecología, Centro de Investigación en Biodiversidad y Cambio Global (CIBC), Universidad Autónoma de Madrid, Madrid, Spain

[4] HELSUS, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

Corresponding Author:

Pablo Manzano[2]

P. O Box 68200, Nairobi, 00200, Kenya

Email address: pablo.manzano.baena@gmail.com

ABSTRACT

Habitat fragmentation is one of the greatest threats to biodiversity conservation and ecosystem productivity that are mediated by direct human impact. Its consequences include genetic depauperation, comprising 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 diversity and inbreeding coefficients 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 and population nestedness around human settlements. Trends for higher genetic diversity in drove roads may be present, although they were only marginally significant with the available sample size. The resulting functional landscape has human settlements as dispersal hotspots, while the drove road would confirm its pollinator reservoir role observed in other studies, 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 dispersal related to human activities 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). Fragmentation limits population size and reduces seed and pollen flow among populations, leading to genetic drift and reduction in within-population genetic diversity. This genetic impoverishment can limit evolutionary potential, promote selfing and therefore, 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). Increasing connectivity between isolated vegetation patches is a major conservation strategy to reduce the negative effect of habitat fragmentation (Hodgson et al 2011). Nevertheless, increasing effective dispersal of seeds or pollen among patches (functional connectivity) is not always possible by just increasing structural connectivity, e.g. establishing landscape corridors, because the availability of dispersal vectors or recruitment microsites are needed to sustain functional connectivity (Auffret et al. 2017, Plue et al 2019).

Dispersal is a central life-history trait that structures the properties of ecosystems (Bonte & Dahirel 2017; Massol et al. 2017). Particularly among fragmented natural habitats, dispersal is a key element for reducing extinction risk, and is subjected to strong selective 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. In this context, livestock-mediated seed dispersal is an important vector for increasing functional connectivity. While the ecological importance of large wild herbivores is practically disappearing at the global scale (Ripple et al. 2014; Smith & Botha-Brink 2014; Bar-On et al 2018), as are the migratory systems they support (Berger 2004), domestic herbivores have

73 largely overtaken their role to the point that they can even substitute extinct megaherbivores
74 (Pires et al. 2014). The effectiveness of livestock seed dispersal as a cheap and effective
75 management tool for increasing connectivity between isolated grassland habitat patches has been
76 extensively surveyed (Cosyns et al. 2005; Couvreur et al. 2005; Auffret et al 2012; Emmerson et
77 al 2012), showing that the complementarity between different modes of dispersal (endo- and
78 epizoochory) can lead to an almost complete representation of the grassland community in the
79 dispersal spectra (Couvreur et al. 2005). Herbivory, trampling and nutrient redistribution by
80 livestock also generate microsites favorable to the recruitment of many adapted species (Bullock
81 et al 1994, Olff & Ritchie, 1998), contributing to functional connectivity. Unfortunately,
82 livestock management is changing in many regions of the world for economic and social reasons,
83 and practices such as transhumance and rotational grazing are declining (Poschlod et al 1998,
84 Olea & Mateo Tomás 2009, Auffret et al 2015).
85
86 Drove roads – routes traditionally used for mobile pastoralist livestock in many regions of the
87 world – become particularly interesting to promote functional connectivity between isolated
88 grasslands. Historic ones are presumed to often derive from ancient migratory routes of wildlife
89 (Manzano Baena & Casas 2010) Drove roads have been observed to have a strong effect in
90 increasing multifunctionality of the landscape by working as linear grasslands and increasing
91 heterogeneity at the large scale, translating into an increase of biodiversity in diverse taxonomic
92 groups such as plants (Azcárate et al. 2013a), ants (Azcárate et al. 2013b; Hevia et al. 2013) or
93 even bees, linked with important pollination services (Hevia et al. 2016). Given the proven
94 capacity of livestock in achieving long-distance dispersal along drove roads, both by
95 endozoochory (Manzano et al. 2005) and by epizoochory (Manzano & Malo 2006), mobile
96 pastoralism taking place in drove roads could potentially mitigate the consequences of
97 population isolation, which is a major outcome of fragmentation processes (Fahrig 2003;
98 Mitchell et al. 2015), especially in a grassland context (Pretelli et al 2018).
99
100 Dispersal is a multi-faceted process where the quantification of every step implies complex
101 measurements that can result in great uncertainties at the landscape scale (Wang & Smith 2002),
102 and estimating its effective footprint is much more feasible through genetic analyses. Also, the
103 study of the genetic structure and the gene flow between populations are essential to assess their
104 viability in the long term. Fragmentation by isolation leaves a measurable genetic trace (Leblois
105 et al. 2006). With such background, studies in livestock-grazed rangelands have observed that
106 grazed areas display a more homogeneous genetic structure than ungrazed ones (Smith et al.
107 2009) and a less-than-expected population differentiation in *Anthyllis vulneraria* fragments
108 historically subjected to rotational grazing (Honnay et al. 2006). Willerding & Poschlod (2002)
109 were unable to see effects of herd mobility in a rotational context, possibly due to the election of
110 the target species (the anemophilous *Bromus erectus*). More recently, however, rotational
111 grazing livestock has been observed to genetically link island grassland communities of
112 *Campanula rotundifolia* that are otherwise isolated (Plue et al 2019). Similarly, livestock herded
113 by foot from patch to patch in a rotation scheme has shown to be strong enough to leave a
114 measurable trace in plant populations according to the distance covered, reducing genetic
115 structure and increasing the viability of re-introduced grassland plants for *Dianthus*
116 *carthusianorum* (Rico et al 2014a, Rico et al 2014b, Rico & Wagner 2016) and *Pulsatilla*
117 *vulgaris* (DiLeo et al 2017). While these results highlight the role of livestock as an effective and
118 determinant vector for gene flow across the landscape, the artificial, network-like management of

119 rotational grazing differs from the more corridor-like type of dispersal that is expected from wild
120 migratory herbivores (Berger 2004), with bigger herds, longer distance migrated, shorter transit
121 time per grassland area unit, and routes maintained during centuries, and which is mimicked by
122 transhumant livestock (Manzano Baena & Casas 2010). This perspective is even more relevant
123 considering how widespread transhumance is worldwide, and how livestock can potentially
124 substitute the ecological functions of wild migratory herbivores.
125
126 There are other important gene flow vectors beyond seed dispersal, however. Pollen flow also
127 depends on landscape connectivity, where the structural features of the landscape that harbors
128 pollinator communities (Auffret et al 2017) are more relevant for it than the existence of
129 functional seed-dispersal vectors. This is a factor that seems especially relevant for fragmented
130 agricultural landscapes crossed by livestock corridors, as the latter are reported to harbor a
131 significant pollinator community (Hevia et al 2016). Wind pollination in angiosperms seems to
132 have evolved in cases where biotic pollinators are limited (Culley et al 2002) and long-distance
133 flow would be achieved in less common cases of high wind turbulence and speed, both very
134 dependent on topography (Auffret et al 2017). Persistent seed banks can also alter the effects
135 observed in the genetic structure of plant populations, particularly by delaying or mitigating the
136 effects of fragmentation (Honnay et al 2008, Plue et al 2017).
137
138 In this study, we aimed to check whether dispersal processes in drove roads subjected to active
139 use influence the population genetic structure of *Plantago lagopus*, an annual selfing plant
140 species dispersed by livestock endo- and epizoochory along Spanish drove roads. These
141 populations were located along the Conquense Drove Road (hereafter CDR; Figure 1) in Spain,
142 one of the few drove roads under continuous use since (at least) the Middle Ages until even after
143 the post-industrialization crisis of Spanish transhumance (Manzano Baena & Casas 2010). The
144 CDR connects isolated grasslands inserted in the surrounding agricultural matrix, which act as
145 excellent controls to check for effects of transhumance on landscape connectivity between plant
146 populations. Under this scenario, a landscape genetics approach provides excellent tools to
147 evaluate the effects of anthropogenic factors over the populations of *Plantago lagopus*. In
148 particular, we asked i) Is the CDR activity influencing gene flow among populations? ii) Do the
149 populations in the CDR have similar genetic diversity and/or inbreeding values than those
150 located in the agricultural matrix? and iii) Can other landscape factors (e.g. distance to human
151 settlements, grasslands neighboring the drove road, time since last plowing) modify the genetic
152 structure of the *P. lagopus* populations?
153
154 ## MATERIALS & METHODS
155
156 *Studied species*
157
158 *Plantago lagopus* L. (Plantaginaceae) is a diploid polycarpic annual or biennial forb, abundant in
159 grazed annual-dominated pastures of Central Spain (Peco et al. 2005). This species presents a
160 persistent soil seed bank (Peco et al. 2003), gynodioecous breeding system and its seeds are
161 dispersed by cattle endozoochory (Malo & Suárez 1995). By sheep and under field conditions,
162 epizoochoric dispersal is anecdotal, but endozoochory figures range from 3-7 seeds per g dry
163 weight of manure in late spring when dispersal is highest and flocks are moving northwards, to
164 0.1 seeds in autumn when flocks are moving southwards (P Manzano, 2004-2006, personal

165 observations). This translates into ca. 2 million *P.lagopus* seeds moved northward per
166 transhumance day, or 50,000 seeds moved southward, for a typical flock of 1,000 sheep.
167 Experiments have also shown seeds are largely viable after sheep ingestion (Peco et al. 2006)
168 and remain attached for a long time on sheep coats (de Pablos & Peco 2007), even under real
169 transhumance conditions (Manzano & Malo 2006). This species is self-compatible, with both
170 wind and insects as major pollination agents (Sharma et al. 1993). Annual-dominated pastures in
171 Central Spain are known to present particularly high diaspore availability related to summer
172 drought but translated into massive dispersal by livestock, particularly at the time when
173 transhumant herds are on the move (Manzano 2015a). Such large dispersal, along with the faster
174 dynamics in annual plants (overlapping generations in perennial plants may disturb some genetic
175 descriptors due to the mixture of genotypes), make them particularly suited to test genetic signals
176 of dispersal in fragmented scenarios (Ewers & Didham 2006).
177
178 *Landscape scenario and population sampling*
179
180 The study area is located between Quintanar de la Orden, Tomelloso and Villarrobledo
181 municipalities in Castilla-La Mancha, Spain (coordinates: 39° 18' 03.17'' N and 2°49' 54.50''
182 W, Figure 1). The area is a plateau (830-900 meters a.s.l.) of sandstones, loams and clay
183 materials under continental Mediterranean climate, with a mean annual rainfall of about 500mm
184 and severe summer droughts. The vegetation is a mosaic of agricultural dry cereal and sunflower
185 croplands, abandoned fields and dry grasslands used by local flocks. The area is crossed by the
186 Conquense Drove Road (CDR), one of the major road droves (ca. 410 km long) that are still used
187 for transhumant sheep and cattle herds that move every year from the cooler and wetter
188 mountains of Teruel, Cuenca and Guadalajara provinces to the wintering dehesas in Sierra
189 Morena at lower altitude (Oteros-Rozas et al. 2012). The herds cross the study area twice a year
190 (northwards in June and southwards in November), currently comprising about 9,000 heads of
191 sheep and 1,200 of cattle. The size of transhumant herds, dominated by merino sheep, has shrunk
192 from about half a million in the 16th and 17th centuries to 100,000-200,000 in the next two
193 centuries and around 20,000 during the second half of the 20th century (Bacaicoa Salaverri et al.
194 1993). Productivity of the area is low except for peaks in May and October, because of cold
195 winters and hot dry summers. Consequently, the numbers of transiting nomadic livestock have
196 been historically much higher than those of resident livestock (Manzano Baena & Casas 2010),
197 which only grew in the last centuries (Bacaicoa Salaverri et al. 1993). Such resident livestock
198 herds are, in turn, kept at stables in urban settlements at night and graze grassland patches that
199 are spread across the landscape (Figure 1), overwhelmingly under private tenure. They graze
200 mostly during wetter times in spring and autumn, the time of the year when local vegetation is
201 growing (cf. Manzanares town in Manzano Baena & Casas 2010) and diaspore availability is
202 very low – in spring because plants have not yet produced seeds, and in autumn because rains
203 incorporate the dead plant matter and any remaining diaspores into the soil. At other times of the
204 year they are supplemented with hay, concentrate and water. Drove roads tend to avoid urban
205 settlements and run rather tangentially (Figure 1), so resident livestock do little use of them
206 because of the rather radial pattern of land use, with urban settlements in the center. As a result,
207 management of local resident livestock is likely to have some effect on pollen flow because of
208 available pastures and to cause some short-distance dispersal across them, but it is not likely to
209 have effects on long-distance seed dispersal along the drove roads.
210

211   In winter 2013 we selected 13 populations of *P. lagopus* (6 in the CDR and 7 in isolated pastures
212   interspersed in the agricultural matrix). Populations located in the agricultural matrix were
213   located at least 5 km from the CDR. Each population consisted in a group of plants not further
214   than 100 m from each other, and all populations were at least 5 km apart from each other (Figure
215   1, Table 1). In each population, we randomly collected between 8 and 30 flowering individuals
216   of *P. lagopus*, whose leaves were stored under dry conditions until DNA extraction. Sampling
217   was done without prior permission, in accordance with article 32d of the regional regulating law
218   on drove roads regarding common complementary uses, including educational and formative
219   activities in the environmental and cultural fields (Junta de Comunidades de Castilla la Mancha
220   2003). Authorization is required for such activities when they threaten the protection of sensitive
221   ecosystem, forests with high wildfire risk, and protected flora and fauna species, and
222   infringements are foreseen in its article 41 – but our sampling in the ecosystem was negligible,
223   and *P. lagopus* is neither an endangered nor a protected species. We deposited five individuals at
224   Real Jardín Botanico de Madrid – CSIC herbarium (reference numbers MA-01-00892341 to
225   MA-01-00892345).
226
227   To test for the potential effect of other landscape factors on genetic descriptors, we measured the
228   euclidean distance from the sampling points to the nearest human settlement using Google Maps.
229   At the agricultural matrix points, we also checked the time since last plowing by inspecting
230   historic aerial photographs from the area (Instituto Geográfico Nacional, Spain) – the oldest of
231   which being 70 years old. The Conquense Drove Road, as in the case of all other major drove
232   roads in Spain, has been protected from plowing for the last centuries (Bacaicoa Salaverri et al.
233   1993).
234
235   *Microsatellite development and laboratory protocols*
236
237   We developed specific microsatellite markers for *P. lagopus* in collaboration with the Unidad de
238   Genómica, Parque Científico de Madrid (http://fpcm.es/en/servicios-cientificos/). Briefly, the
239   library was developed from DNA of five individuals of *P. lagopus* extracted using QIAGEN
240   plant Extraction Kit, using manufacturer's protocol. DNA was fragmented with ultrasounds
241   using Bioruptor (Diagenode) and size fragment was selected for an approximated size of 1,2 kb.
242   DNA sequencing was performed using a 454-FLX-Plus Roche sequencer. We obtained
243   approximately 185000 sequences. We used Pal_Finder to select those sequences with more than
244   5 repetition motifs and a size that ranged from 100 to 300 bp. We used Primer3 (Untergasser et
245   al. 2012) to configure potential primers that amplify those microsatellite regions. Details of the
246   primers used are shown in Table S1.
247
248   *Genetic descriptors: diversity, Inbreeding, differentiation and structure*
249
250   We checked the six microsatellites used for genetic errors and misidentifications of alleles with
251   Micro-checker (van Oosterhout et al, 2004). We estimated four genetic diversity indices:
252   observed and expected heterocigosity (H_O and H_E) using INEST 2.1 (Chybicki & Burczyk 2009),
253   the Shannon Information Index (I), as a measure of genetic diversity that considers the number of
254   alleles and its dominance in the population and the number of private alleles (A_P) using
255   GeneAlEx 6.5 (Peakall & Smouse 2012). Because the number of sampled individuals is different
256   in each population (ranging from 8 to 30, Table 1), we also calculated the genetic diversity using

257   Hp-Rare (Kalinowski 2005) to rarefact the allelic richness and the number of private alleles to
258   the minimum number of individuals sampled in one population (i.e. 8 individuals) for better
259   comparisons between populations according to population size, hereafter A_r and PrivRare,
260   respectively. Linkage disequilibrium among loci was assessed with GenAlEx 6.5 (Peakall &
261   Smouse 2012).

263   We calculated the inbreeding coefficient, F_{IS}, using INEST 2.1. (Chybicki & Burczyk 2009) that
264   corrects the effect of null alleles, inbreeding and genotyping failures. DIC values between
265   models with and without inbreeding were compared to evaluate the effect of inbreeding over F_{IS}.
266   Departure from Hardy-Weinberg equilibrium with GeneAlEx 6.5 (Peakall & Smouse 2012) to
267   evaluate the inbreeding status of $P.$ $lagopus$ populations. We also assessed the presence of
268   genetic bottlenecks generated by population dynamics (e.g. colonization events, population size
269   reduction) using Bottleneck 1.2.02 (Piry et al. 1999), through a Wilcoxon test with 2000
270   permutations under the three plausible mutations models, as recommended for microsatellite
271   data. We applied Bonferroni correction for multiple testing.

273   We estimated genetic differentiation with F_{ST} coefficient calculated with FreeNA (Chapuis &
274   Estoup 2006) to minimize potential biases caused by null alleles or allele dropout. We tested
275   signification between each pair of populations with 999 permutations. We used HIERFSTAT
276   0.04 (Goudet 2005) to evaluate the F-statistics grouping of the populations according to their
277   location (either within the matrix or in the drove road) with 1000 permutations. To assess the
278   presence of Isolation-by-Distance patterns, we performed a Mantel test with 999 permutations
279   using genetic differentiation, (i.e. F_{ST} coefficients) and Euclidean geographical distance between
280   populations with Vegan package in R (Oksanen et al. 2007). Mantel tests were also developed
281   considering the location of the populations (Drove Road or Matrix) with the same permutations.
282   Genetic differentiation between patches was compared with the calculation of migration rates or
283   number of migrants. BayesAss (Wilson & Rannala 2003) and Migrate (Beerli 2009) were used to
284   estimate recent and past migration rates respectively, using the default parameters recommended
285   by the manual. This might show differences among ancestral or recent gene flow, given the
286   progressive decrease of livestock movement along the drove road during the last decades. Mantel
287   tests were also developed considering the location of the populations (Drove Road or Matrix)
288   with the same permutations.

290   We assessed genetic structure using the Bayesian clustering method STRUCTURE v. 2.3.4
291   (Hubisz et al. 2009) with prior information on populations. We carried out 10 independent runs
292   for each K value (i.e. number of groups or clusters), ranging from 1 to 15. Each run comprises a
293   burning period of 100,000 permutations, followed by 1,000,000 MCMC (Markov Chain Monte
294   Carlos) iterations. We assumed correlated allele frequencies and genetic admixture. To assess the
295   most plausible number of clusters (K), we used the STRUCTURESelector module (Li & Liu
296   2018) to obtain plausible values of K. We considered the Evanno method (Evanno et al. 2005)
297   with the ad hoc statistic ΔK and identified the maximum ΔK when plotted against K. MedMeaK
298   (median of means), MedMedK (median of medians), MaxMeaK (maximum of means) and
299   MaxMedK (maximum of medians) following Puechmaille (2016) were also estimated (threshold
300   value = 0.6). These estimators are useful to discard spurious clusters and detect substructure in
301   case of uneven sampling (Puechmaille 2016). We also used Clump v.1.1.2 (Jakobsson &

302 Rosenberg 2007) to estimate the membership coefficient of each individual in each cluster and
303 Distruct v 1.1 (Rosenberg 2004).
304
305 *Relationships of genetic descriptors with environmental factors*
306
307 To assess whether movement of transhumant herds along the drove road leaves a footprint, we
308 selected the genetic descriptors that have most commonly been used previously in similar
309 studies, i.e. F_{IS} and A_r (Rico et al 2014a, Rico et al 2014b, Rico & Wagner 2016, DiLeo et al
310 2017, Plue et al 2019). We built multiple linear models fitted with generalized least squares (*gls*
311 function, *nlme* R-package) to examine the relationship between genetic diversity metrics with the
312 population location (agricultural matrix or drove roads), the distance to nearby human
313 settlements (in km) and the interaction location x distance.
314
315 Due to the effect of pollinators activity to crossing system (i.e. selfing, partial selfing or
316 outcrossing, Turner et al 1982, Dutech et al. 2005, Van Etten et al 2015), for the analysis of F_{IS}
317 the amount of grasslands in 500-m radius circles centered in sampling points (in percentage, log-
318 transformed to achieve normality) was also included. The landscape descriptors (distance and
319 percentage of grasslands) were standardized and centered to interpret model coefficients as
320 standardized effects. Spatial autocorrelation was taken into account by building the GLS models
321 with three alternative spatial covariance structures most commonly used to describe spatial
322 correlation (Gaussian, Exponential and Spherical). The presence of nearby grasslands was
323 included for the analysis of F_{IS}. This genetic descriptor is considered to be particularly influenced
324 by pollinator activity, due to its effect over selfing and/or kinship crosses (Rico et al 2014b).
325 Long-distance seed dispersal will rather happen along drove roads and through transhumant
326 livestock that bypass such nearby grasslands, because the resident livestock that use them tend to
327 have short-distance movements around human settlements and do not use the drove roads that
328 run rather tangential to the settlements.
329
330 The differences in genetic diversity among populations may show a nested pattern suggesting
331 that former connections between them have been lost (Aggemyr & Cousins, 2012; Plue et al
332 2019). Nestedness appears when the set of alleles in communities with lower genetic diversity
333 are a subset of those found in communities with higher genetic diversity, which would imply a
334 deterministic loss of alleles. Thus, we explored whether such nested patterns occurred among our
335 study populations and if they could be linked to the presence of the drove road, the distance to
336 settlements and the amount of neighboring grasslands. If nestedness among populations is caused
337 by loss of connectivity, we expect sites within the agricultural matrix and far from settlements to
338 have lower allelic richness, lacking some particular alleles present in the populations of the drove
339 road (in other words, sites within the agricultural matrix would have a higher degree of
340 nestedness). Neighboring grasslands could act as a local source of propagules (seed and pollen)
341 deterring the loss of alleles, and thus the larger the availability of neighboring grasslands the less
342 likely we would find nestedness patterns. To describe nestedness patterns we used the NODF
343 index (Almeida-Neto et al 2008). We tested if nestedness with a suitable null model based on
344 499 randomized matrices (null model 'r2' with package vegan in R which constrains row totals –
345 allelic richness—to equal the observed distribution and samples columns –alleles—with
346 probability proportional to squared incidence frequencies). Then, we modeled the population
347 rank order, from the highest rank of the population with most alleles to the lowest rank of the

348   population with least alleles, with linear models built with generalized least squares as above.
349   The populations differed in the number of individuals analyzed. Then, to balance sampling effort
350   among them we repeated 50 times the above procedure, by randomly selecting the same number
351   of individuals per population. Final NODF, p-values and rank estimates were the mean of the
352   above procedure for these 50 balanced replicates.
353
354   For the three genetic diversity metrics (F_{IS} and A_r) and for nestedness, we did not try to simplify
355   the models but identify the relevant predictors with p-values based on nested log-likelihood ratio
356   tests. The genetic descriptors we used as response variables in the models were the inbreeding
357   coefficient (F_{IS}), the genetic diversity statistic (A_r) and the nestedness estimates. We confirmed
358   nearby grasslands to be only a relevant factor for F_{IS} by comparing models of the three metrics
359   with and without the grassland factor via AICc. Additionally, we tested the correlation of the
360   genetic descriptors with the plowing history (years since last plowing) with base R and *Anova*
361   function, *car* package and just for the populations in the agricultural matrix - the drove road has
362   been protected from plowing for the last centuries.
363
364
365

366   **RESULTS**
367
368   *Genetic diversity and inbreeding*
369
370   The values for genetic diversity (I, A_P, H_O, H_E, A_r and PrivRare) were similar between
371   populations in the agricultural matrix and populations in the drove road, except for A_P, which
372   was greater in the populations of the agricultural matrix, due to the large number of alleles found
373   in LMA08 (Table 1). The average (CV) expected heterozygosity H_E was 0.88 (0.02) in drove
374   roads populations and 0.89 (0.03) in matrix populations. However, matrix populations showed
375   greater variability in H_E, with values ranging from 0.92 in LMA13 to 0.84 in LMA08. The same
376   pattern (similar average but different variability) was found in the other genetic diversity indexes
377   (Table 1).
378
379   The inbreeding coefficient (F_{IS}) varied greatly among populations and between matrix and drove
380   road locations. Drove road populations showed a mean value of 0.08 (ranging from 0.01 in
381   LCR04 to 0.17 in LCR01), while matrix populations showed a mean value of 0.11 ranging from
382   0.06 in LMA05 to 0.17 in LMA14 (Table 1). Inbreeding had an important effect over *Fis* in
383   LCR01, LMA11, LMA14 and LMA16 populations according to INEST model estimation. All
384   populations had, at least, one locus that deviated from Hardy-Weinberg (HW) equilibrium (four
385   loci in LCR01). Bottleneck signals were found in some populations under SMM model (LCR09,
386   LMA08 and LMA11), but the results were not significant in all genetic models and applying
387   multi-comparisons corrections (Table 2). Linkage disequilibrium among loci showed only four
388   significant comparisons, which were non consistent between loci pairs.
389
390   *Genetic differentiation and structure*
391
392   Pairwise genetic differentiation values (F_{ST}) between populations were low (Table S2). On
393   average, LMA14 showed the greatest differentiation, with an average F_{ST} value of 0.08.

394   Hierarchical analysis of the genetic differentiation showed low differentiation between matrix
395   and drove road locations (F_{CT} = 0.032) and a moderate one between populations within each
396   category (F_{SC} = 0.11). Both values are significant. Mantel test (r = 0.13; p = 0.22) did not detect
397   the presence of an isolation-by-distance (IBD) pattern, but results were significant when only
398   analyzing the six populations included in the drove road (r = 1; p < 0.01). The genetic structure
399   analyzed with a Bayesian clustering approach (i.e. STRUCTURE, Figure 2) showed different
400   values depending of the approach consider to estimated K. The estimation of ΔK proposed values
401   of K = 12 and K = 4 as more plausible approaches. MedMedK, MedMeanK and MaxMaxK also
402   pinpoint that K = 4 is the most appropriated distribution of the genetic structure and MaxMedK
403   suggested that K = 5 is the most accurate result. The K=4 distribution showed an independent
404   cluster (green) for LMA14 population, another cluster (purple) for LMA16, LMA13 and LCR10
405   populations and a third cluster (orange). The other populations are assigned to the last cluster,
406   with admixture of the others. The K=12 distribution showed an important population admixture
407   and one cluster assigned to LMA14 population (Figure 2). The results for gene flow (BayesAss
408   and Migrate, Table 3, Figure 3) showed differences for the migration rates estimated, with higher
409   values for past gene flow (i.e. Migrate) than contemporary (i.e BayesAss). The evaluation of the
410   recent gene flow (i.e. BayesAss), focus the main differences in the population LMA08 that
411   received a significant number of migrants and in lesser extent, LMA16 and LCR01. The
412   remaining populations showed similar migrations rates between populations. The ancient gene
413   flow analyzed (i.e. Migrate) showed similar mean values for drove roads and matrix populations
414   but important differences between populations. Although LMA08 maintains high values of gene
415   flow, LCR02 received the highest average migration rate meanwhile LCR10 showed the smallest
416   average value.
417
418   *Landscape factors governing genetic descriptors in* P. lagopus *populations*
419
420   The distance to the nearest settlement had an impact on both genetic descriptors analyzed (Table
421   4). This relationship is independent of the frequency of plowing, because for the agricultural
422   matrix plots the correlations of years since last plowing and the residuals of the regressions
423   explaining the genetic descriptors were non-significant. Also, the values of genetic descriptors
424   did not correlate significantly with the number of years since last plowing (Pearson correlations
425   were 0.07, p=0.89 for F_{IS}; and -0.13, p=0.78 for A$_r$).
426
427   In addition to the distance to the nearest settlement, the interaction with population position
428   showed a significant effect on inbreeding (F_{IS}; Table 4). Thus, F_{IS} increased with distance from
429   human settlements in the agricultural matrix, but followed the opposite trend in drove road
430   populations (Figure 4a). The presence of nearby grasslands also proved to be a significant factor,
431   showing higher rates of inbreeding at higher grassland cover (Table 4).
432
433   For the descriptor of genetic diversity (A$_r$), the distance to settlements had a consistent negative
434   and significant effect, while the effect for population position and its interaction with distance
435   was only marginally significant (Table 4). A$_r$ values between matrix and drove road positions
436   showed a pattern of convergence at short distances and divergence at high distances from human
437   settlements, with the agricultural matrix then showing less diversity (Figure 4b).
438
439   Significant nestedness was found among the *Plantago lagopus* populations (mean observed
440   NODF = 34.7, mean p-value = 0.02, all averaged from 50 random selections of individuals to

441 balance sample size among populations). None of the geographic parameters, however,
442 significantly affected the rank (Table 4).
443
444 **DISCUSSION**
445 Drove roads have a significant capacity to shape some genetic features of *Plantago lagopus*
446 populations in the study area. This assessment of the population genetics of *P. lagopus* in the
447 study area indicates relevant differences both in terms of coefficient of inbreeding and of genetic
448 diversity between populations and between matrix and drove roads positions. Furthermore,
449 proximity to human settlements appeared to drive the hotspots for genetic diversity in this
450 landscape. Drove roads were able to invert the inbreeding that otherwise appears in distant
451 populations from the agricultural matrix. The loss in genetic diversity with increasing distance to
452 human settlements seems weaker at drove roads, although such effect is only marginally
453 significant with the sample size and the molecular markers used in this study. Such a trend
454 would, however, be in line with the one that was observed by other studies involving livestock
455 being moved on hoof (Rico et al 2014a, Rico & Wagner 2016, DiLeo et al 2017), as well as by
456 more artificial transport (boat; Plue et al 2019). The higher estimated migration rates for past
457 events compared to contemporary ones would confirm the declining influence of transhumance
458 in the landscape as transhumant livestock numbers continue to decline. An influence of historical
459 processes of colonization can be discarded, due to the absent effect that plowing history had both
460 on inbreeding and diversity on the populations from the agricultural matrix – the time span
461 considered for plowing history, i.e. 70 years, should also allow for controlling delay effects
462 related to the establishment of a permanent seed bank as discussed by Honnay et al (2008) and
463 Plue et al (2017), of which *P. lagopus* is capable.
464
465 In a self-compatible plant species pollinated both by wind and by insects, such as *P. lagopus,*
466 population inbreeding is mainly determined by the ratio between selfing and outcrossing, where
467 outcrossing is enhanced by pollinator availability. Thus, the smaller selfing coefficient and the
468 reduced influence over F_{IS} observed along the drove road indicates a relevant role played by
469 them in providing pollination services. This relationship is even clearer after controlling for
470 landscape effects, namely the presence of grassland patches in the vicinity of the studied plant
471 populations that could harbor further pollinators. In fact, we found the same potential negative
472 relationship between the presence of such patches and the pollinators, mirroring the results of
473 Rico et al (2014b) and, similarly to the potential explanation there, it could rely on the flowering
474 plants on such patches being continuously grazed during the whole spring by resident sheep
475 flocks. Meanwhile, the transhumant herds graze on the drove road only at the end of the spring,
476 following a "green wave" when the plants have already grained, relatively untouched during the
477 flowering phase. While a greater coefficient of inbreeding is a genetic indicator that can be
478 related to pollinator limitation (Turner et al 1982, Dutech et al. 2005, Van Etten et al. 2015), the
479 structural role of drove roads as grassland corridors crossing agricultural landscapes has been
480 proved to be determinant in supporting pollinator services (Hevia et al. 2016), which our study
481 confirms. This result goes along other studies that have observed provision of heterogeneity at
482 the landscape level because of the drove road's structural role, translated in higher biodiversity
483 levels (Azcárate et al. 2013a; Hevia et al. 2013). Plowing does not show any effect or trend on
484 the genetic variables studied, suggesting that structural continuity in space is more important
485 than stability in time to guarantee genetic admixture.
486

487 Distance to human settlements showed a consistent effect on genetic diversity and inbreeding.
488 The resulting functional landscape can be interpreted as settled, high human density areas being
489 hotspots of dispersal that modulate the genetic patterns of diversity, probably mediated by
490 commercial livestock exchanges but also by other dispersal types, mainly human-mediated
491 (Auffret 2011; Auffret & Cousins 2013; Wichmann et al. 2009). The *P. lagopus* populations on
492 the drove road don't show to be affected by these types of dispersal, likely because their human
493 use is much diluted and human activities are comparatively simplified and less dense, consisting
494 just in accompanying or herding livestock. They also show to be pollinator reserves with a higher
495 relevance than peri-urban grassland patches. Such important role played by human settlements
496 for seed dispersal is not surprising, given that human-mediated dispersal adds to livestock-
497 mediated dispersal mechanisms (Auffret 2011; Auffret & Cousins 2013) which are problematic
498 for the spread of invasive species (Abbas et al. 2018). However, it offers insights on how an
499 unnatural over-dispersal that facilitates invasiveness of some plant species (Wilson et al. 2009)
500 may be visible already at small geographical scales such as the one regarded in this study.
501 Genetic analyses that further explore the role of livestock or other dispersal vectors may offer
502 valuable insights on the ecology of potential or confirmed invasive species, especially in
503 relationship with their human-mediated dispersal.
504
505 The trend of a higher genetic diversity on drove roads compared to the agricultural matrix
506 (Figure 4b), even if only marginally significant and limited by the reduced sample size and the
507 limited number of molecular markers, would be consistent with the determinant role of livestock
508 as seed disperser at the genetic level that has been observed in other studies (Rico et al 2014a,
509 Rico & Wagner 2016, DiLeo et al 2017, Plue et al 2019). It may be pointing to a scenario where
510 two types of dispersal processes coexist. On the one hand, populations in the agricultural matrix
511 would receive seeds through the local stock through short-distance dispersal processes.
512 Meanwhile, long-distance dispersal processes favoring greater admixture dominate in the drove
513 roads actively being used by livestock, therefore functioning as a highway for seeds. This would
514 highlight the important role of active livestock movements for preserving the functionality of
515 drove roads and, more generally, herbivore migration corridors. Such effects seem to be too
516 weak to be perceived as significant, given our low sample size and reduced molecular markers
517 used, on top of the moderate levels of gene flow that homogenize the genetic diversity between
518 the populations. The observable trend and the estimated effects on past and current migration
519 rates, however, may be pointing to a very promising area of research for future studies,
520 especially considering that the long history of drove roads in Spain, with fixed ancient routes
521 coinciding with older herbivore routes, may be showing a sustained contribution to long-distance
522 seed dispersal (Manzano Baena & Casas 2010; Manzano & Malo 2006). Further insights on the
523 persistence of effects may be gained by comparing the CDR with other Spanish drove roads
524 abandoned in the mid-20th century (Manzano Baena & Casas 2010).
525
526 *Implications for ecosystem function and conservation*
527
528 Functional herbivore corridors would sustain diversity by reducing the risk of local extinctions
529 due to increased inbreeding and loss of genetic variation in small populations (Caughley 1994).
530 The effect of drove roads on inbreeding observed in our study has relevant implications for
531 conservation, as inbreeding depression is a major concern for the conservation of biodiversity in
532 human-altered fragmented landscapes (Hedrick & Garcia-Dorado 2016). We think that

533   connectivity factors, related to herbivore type and capability of sustaining dispersal corridors for
534   plants, as well as optimal matching of grazing times with the flowering and seeding phenology of
535   plants, would therefore add to the complexity of factors that influence the relationship between
536   grazing and plant diversity. Such factors include productivity and disturbance (Herrero-Jáuregui
537   & Oesterheld 2018; Olff & Ritchie 1998), historical effects on communities (Cingolani et al.
538   2005; Osem et al. 2002) or herbivore species and sizes (Bakker et al. 2006; Liu et al. 2015).
539   Functional herbivore corridors would sustain diversity by reducing the risk of local extinctions
540   due to loss of genetic variation in small populations (Caughley 1994).
541
542   The marginally significant, yet observable trend of drove roads in reducing genetic diversity loss
543   of *P. lagopus*, in spite of the rapid transit of transhumance flocks – ca. 15 km/day, or 1.5 km/h
544   when moving (Hevia et al. 2013) is very noteworthy. Such a use, which is intense but
545   nevertheless very restricted in time, would achieve extraordinary dispersal effects due to a "green
546   wave effect" (Merkle et al. 2016), as transhumant flocks use drove road pastures when they are
547   at their maximum productivity (Manzano Baena & Casas 2010) and therefore at their maximum
548   seed production stage – which would add to the reduced impact on pollinators we observed that
549   is also related to the "green wave effect". This is a radical contrast with other works studying
550   rotational grazing systems (Rico et al 2014a, Rico et al 2014b, Rico & Wagner 2016, DiLeo et al
551   2017, Plue et al 2019) where the intensity of use per unit of area is much higher and the chances
552   for consolidating dispersal between patches are also higher, and the effects on pollinator
553   community are less clear. Indeed, a confirmation of such trend in future studies that are able to
554   study more populations than we did would highlight the potential role of herbivore corridors to
555   promote long distance dispersal. The higher estimated migration rates in the past than in the
556   present are also an alert on the negative effect that declining transhumant practices may have on
557   ecosystems.
558
559   Mobile pastoralism is globally threatened by non-conductive policies (Manzano 2017),
560   threatening its role as a provider of important ecosystem functions such as habitat provision,
561   nutrient cycling, or bush encroachment/fire control (Leroy et al. 2018). The potential magnitude
562   of the threat is great, for such land use covers more than half of the global land (Manzano
563   2015b). A derived disruption of dispersal processes and the structural role for pollinators played
564   by corridors themselves may further contribute to a generalized grassland biodiversity crisis
565   motivated by land use intensification (Gossner et al. 2016) and climate change – and on the
566   latter, a potential decoupling of herbivore "green wave" movements from the phenology and
567   seed availability brings also concerns (Berg et al. 2010; Thackeray et al. 2016). It is urgent to
568   quantify such dispersal beyond our study in order to orient adequate science-evidenced policy
569   recommendations (Manzano Baena 2012). Furthermore, corridors *per se* are important beyond
570   livestock. We obtained our data in a drove road that has experienced a drastic decline in use in
571   the recent centuries, from 600,000 head in the 16th century to less than 10,000 nowadays
572   (Bacaicoa Salaverri et al. 1993; Hevia et al. 2013). The effect on the genetics of plants must have
573   been much more significant at that time, similar to what they must be in the systems presently
574   steered by hundreds of millions of mobile pastoralists (Manzano & Agarwal 2015), whose
575   capacity to cover wide territories is shown by the fractal structure of their drove roads in
576   different countries (Manzano-Baena & Salguero-Herrera 2018). This effect should also be
577   greater in the world's greatest wild herbivore migrations (Berger 2004).
578

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 relevance that herbivore corridors and mobile pastoralism have in reducing inbreeding levels in plant populations, as well as their potential, within the possibilities of our limited data, in facilitating long-distance plant migration that has been envisioned in seed dispersal studies (Manzano et al 2005, Manzano & Malo 2006). 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. Their potential adds to the already identified needs to reduce fragmentation in the dryland areas occupied by pastoralists (Said et al 2016), 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 exchange 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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Location and 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	Coordinates	N	*I*	A$_P$	*H$_O$*	*H$_E$*	*F$_{IS}$*-HW	*RiqRare*	*PrivRare*
LCR01	39.43 N, 2.62 W	30	2.46	6	0.37	0.93	0.17-4	9.4	0.5
LCR02	39.39 N, 2.67 W	10	2.03	1	0.82	0.88	0.10-1	8.8	0.2
LCR04	39.34 N, 2.77 W	10	2.29	4	0.89	0.88	0.01-1	10.2	0.7
LCR06	39.26 N, 2.81 W	13	2.21	2	0.84	0.88	0.09-3	9.3	0.6
LCR09	39.13 N, 2.84 W	14	2.11	2	0.89	0.87	0.02-4	8.6	0.8
LCR10	39.08 N, 2.84 W	8	1.88	1	0.79	0.86	0.11-1	8.3	0.6
LCR average (CV)			*2.16 (0.09)*	*2.7*	*0.76 (0.24)*	*0.88 (0.02)*	*0.08 (0.73)*	*9.1 (0.07)*	*0.6*
LMA05	39.27 N, 2.61 W	10	2.21	3	0.85	0.89	0.06-2	9.8	0.5
LMA08	39.16 N, 2.99 W	30	2.49	12	0.78	0.84	0.14-4	9.6	0.8
LMA11	39.41 N, 2.93 W	14	2.29	1	0.86	0.89	0.11-2	9.4	0.3
LMA12	39.49 N, 2.81 W	10	2.04	5	0.89	0.89	0.1-3	8.8	0.8
LMA13	39.26 N, 2.76 W	13	2.47	1	0.88	0.92	0.09-3	10.7	0.6
LMA14	39.11 N, 2.61 W	10	1.42	1	0.75	0.86	0.17-2	5.1	0.4
LMA16	39.06 N, 2.66 W	14	1.99	3	0.84	0.91	0.11-2	7.9	0.4
LMA average (CV)			*2.13 (0.17)*	*4.5*	*0.83 (0.06)*	*0.89 (0.03)*	*0.11 (0.35)*	*8.7 (0.21)*	*0.5*

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. Population code are belong into Drove Road (LCR code) or Matrix (LMA code) populations.

	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

Summary of the average rate (± standard deviation) of gene flow received from each population.

The gene flow is estimated with BayesAss (m) and Migrate (N_em) software. Average ± SD for location (drove road and matrix) have been also calculated.

Population	BayesAss	Migrate
LCR01	0.021 ± 0.016	16.77 ± 5.69
LCR02	0.012 ± 0.003	20.23 ± 6.29
LCR04	0.012 ± 0.003	16.96 ± 8.77
LCR06	0.013 ± 0.003	16.58 ± 8.13
LCR09	0.013 ± 0.003	12.58 ± 4.99
LCR10	0.013 ± 0.002	6.95 ± 4.13
LCR average	0.014 ± 0.003	15.01 ± 4.63
LMA05	0.013 ± 0.002	10.64 ± 5.47
LMA08	0.11 ± 0.067	20.03 ± 9.18
LMA11	0.013 ± 0.003	17.04 ± 6.76
LMA12	0.013 ± 0.003	15.58 ± 7.07
LMA13	0.013 ± 0.003	13.08 ± 6.16
LMA14	0.013 ± 0.003	14.91 ± 7.36
LMA16	0.04 ± 0.03	17.36 ± 9.88
LMA average	0.031 ± 0.036	15.52 ± 3.06

Results of linear modeling of the inbreeding depression (F_{IS}), rarefacted allelic richness (A_r) and nestedness on landscape factors.

The linear predictor is built with the population position (drove road / agricultural matrix), the distance to the nearest settlement, the availability of neighboring grasslands (only for F_{IS}; log-transformed) and the interaction between position and distance. Landscape descriptors were standardized and centered. Models were fitted using generalized least squares (GLS) with three alternative spatial correlation structures (Gaussian, exponential, spherical) for the residuals, to account for potential spatial autocorrelation. We show here the best models according to AICc. The log-likelihood ratio test (χ^2) is given after comparison with a null model with the same spatial structure and corresponding p-value, as well as an approximation to R^2 as the squared Pearson correlation between predicted and observed values, and 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.

Genetic descriptors		Coefficient estimate	p
F_{IS}	$\chi^2_5 = 20.82$, $R^2 = 26\%$, Gaussian spatial covariance structure.		**0.001**
	Intercept	0.139 (0.028)	
	Distance to settlement	0.015 (0.032)	**<0.0001**
	Position (drove road)	-0.015 (0.025)	0.280
	Grassland cover	0.037 (0.009)	**<0.0001**
	Distance x Position	-0.106 (0.037)	**0.005**
A_r	$\chi^2_4 = 10.51$, $R^2 = 72\%$, Gaussian spatial covariance structure.		**0.033**
	Intercept	8.056 (0.459)	
	Distance to settlement	-1.724 (0.548)	**0.006**
	Position (drove road)	1.254 (0.666)	0.076
	Distance x Position	1.282 (0.705)	0.070
Nestedness	$\chi^2_4 = 11.68$, $R^2 = 29\%$		**0.019**
	Intercept	5.620 (1.354)	
	Distance to settlement	-3.711 (1.558)	0.416
	Position	2.470 (1.894)	0.404
	Distance x Position	1.094 (2.420)	0.160

Figure 1

Study area.

Left panel: localization map of the sampled points within the Iberian Peninsula. Base map by maps-for-free.com

Right panel: inset with location of sampling points on a simplified landcover map of the study area (elaborated with data compiled between 2009-2011 and provided by the National Geographic Institute at http://www.siose.es). Points were sampled in the drove road (circles, labelled 'CR') and in the agricultural matrix (stars, labelled 'MA'). The drove road ('Cañada Real Conquense'), depicted with broad green line, crosses a landscape formed predominantly by crops (pale yellow) and patches of woodlands (pale green), shrublands (brown) and grasslands (yellow). Urban and non-vegetated areas are shown in red.

Lower panel: Picture of the Conquense Drove Road between La Almarcha and Villar de la Encina in late spring, with transiting transhumant livestock herd. Note the longitudinal pasture with ripe grass, surrounded by agricultural fields. Image courtesy of José Antonio González.
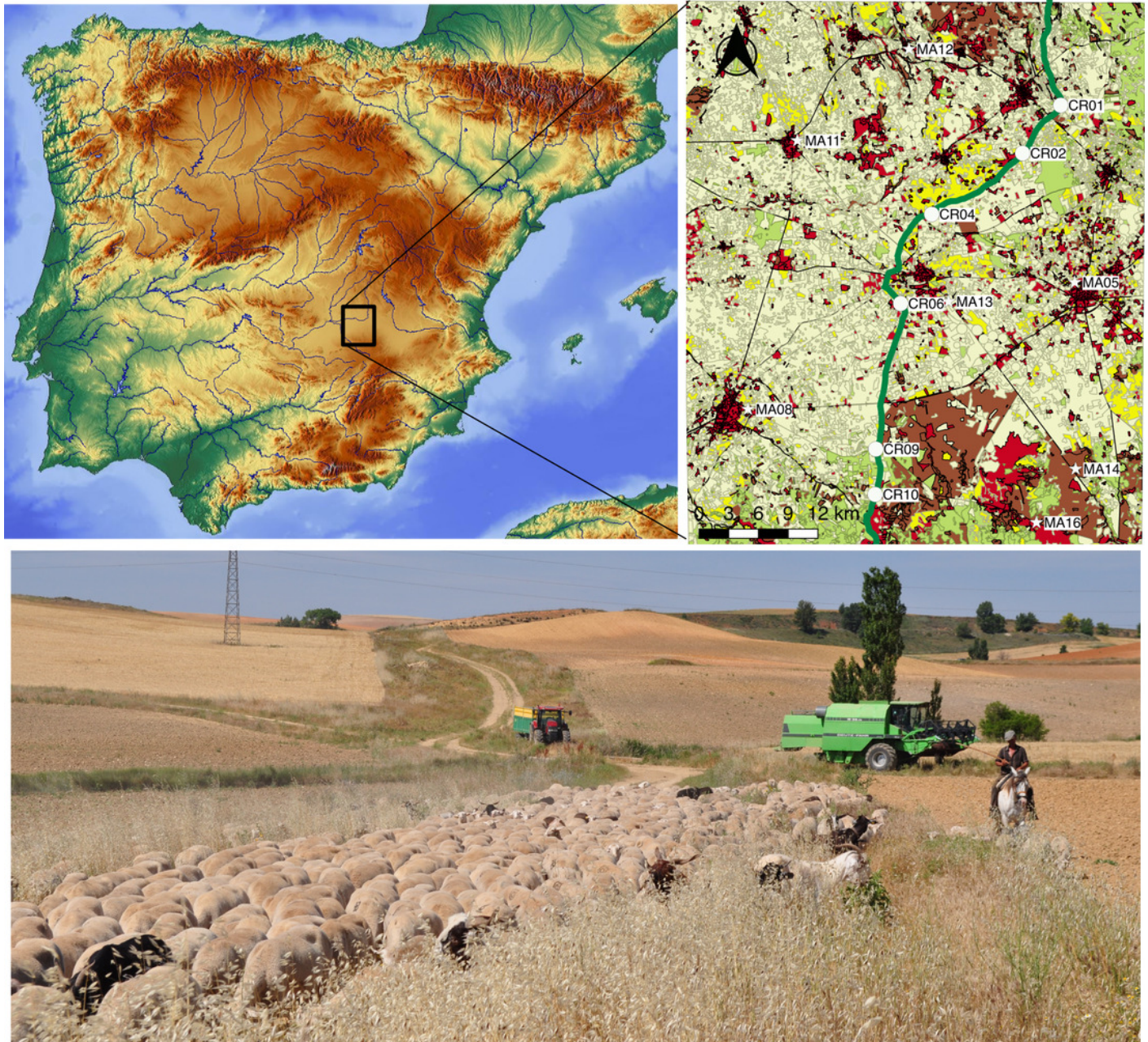
Figure 2

Bayesian genetic structure results obtained with STRUCTURE.

Number of clusters proposed by Evanno method (A). Number of clusters proposed MedMedK (B), MedMeanK (C), MaxMedK (D) and MaxMeanK (E). Genetic structure proposed for *K* = 4 (F). Genetic structure proposed for *K* = 12 (G).

Manuscript to be reviewed
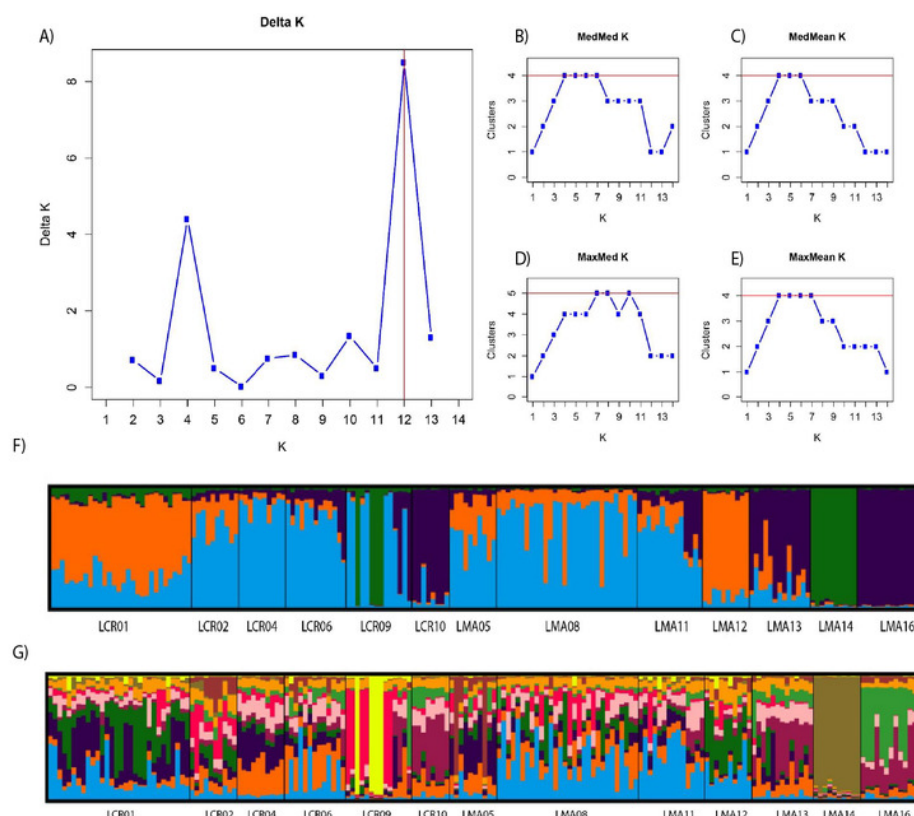
Figure 3

Contemporary (BayesAss) and historical (Migrate) rates of gene flow among the *Plantago lagopus* populations.

Matrix (LMA) populations are represented with red circles, while Cañada (LCR) populations are surrounded by green circles. The width of the edges is proportional to m and N_em values respectively.
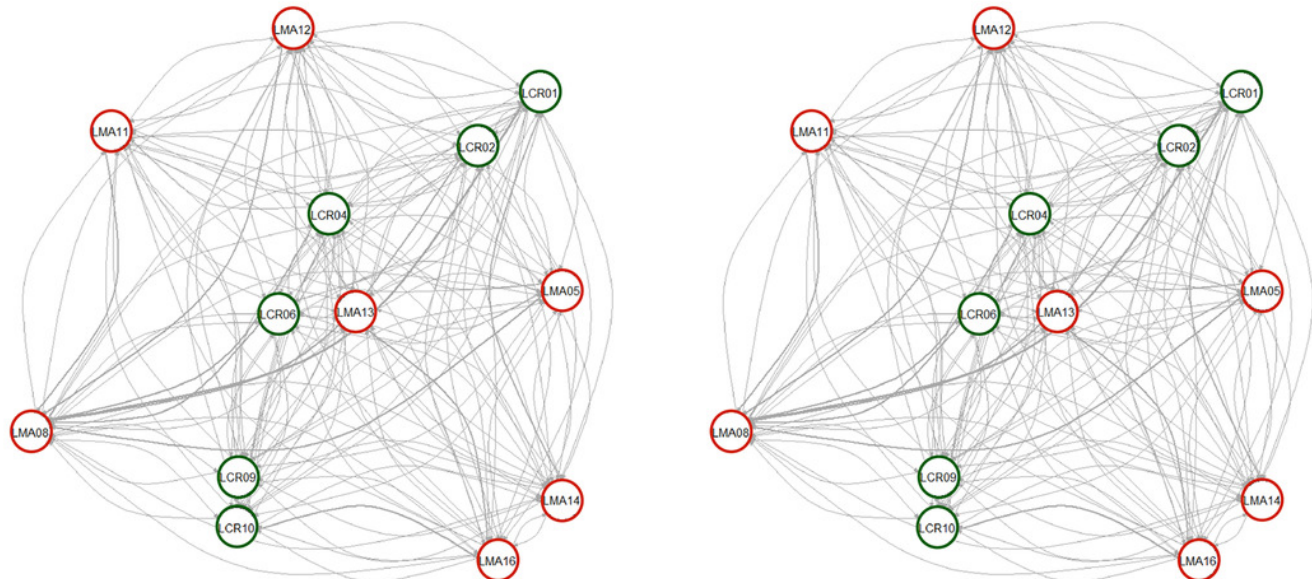
Figure 4

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) A_r (rarefacted allelic richness) for diversity.