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Genetic diversity of *Lotus corniculatus* in relation to habitat type, species composition and species diversity

Eleni M. Abraham [a, *], Ioannis Ganopoulos [b], Panagiota Giagourta [a], Maslin Osathanunkul [d], Irene Bosmali [b], Athanasios Tsaftaris [b], Athanassios Papaioannou [c], Panagiotis Madesis [b, **]

[a] *Laboratory of Range Science (236), Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece*
[b] *Institute of Applied Biosciences, CERTH, 57001, Thessaloniki, Greece*
[c] *Laboratory of Forest Soil, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece*
[d] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand*

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ABSTRACT

The genetic variability of *Lotus corniculatus*, a common and important fodder legume, was studied in relation to habitat type and to species diversity at a local level. The study was conducted in Cholomontas mountain, northern Greece, at altitudes of 760–870 m. Genetic material was selected from four forested, at the edge of forest, and four open grassland sites, and was studied with the aid of ISSR molecular markers. The plant cover at each study site was measured and the floristic composition was estimated. The percentage of graminoides increased in grassland sites with high grazing intensity, while the abundance of legumes, including *L. corniculatus*, forbs and woody species, increased in forested sites with low grazing intensity. Gene diversity H_E within the studied populations of *L. corniculatus* ranged from 0.167 to 0.213 and Shannon index (I) from 0.269 to 0.340. Genetic differentiation was detected between habitats as well as among the populations in each habitat, although it was low, 3% and 7% respectively. However, genetic differentiation was significant within the populations (90%). Genetic diversity of *L. corniculatus* was not correlated with species diversity (richness and evenness) either within or between populations, while was negatively correlated with *L. corniculatus* abundance.

1. Introduction

Ecosystems, species and genes which correspond to the genetic diversity within species are the three fundamental levels of biodiversity, according to Convention on Biological Diversity (CBD; www.cbd.int/convention/text/). The significance of genetic diversity for ecosystem processes, such as primary productivity, ecosystem resistance to disturbance (Hughes and Stachowicz, 2004) and ecosystem recovery (Reusch et al., 2005), has been demonstrated in many studies. Furthermore,

* Corresponding author.
** Corresponding author.
E-mail addresses: eabraham@for.auth.gr (E.M. Abraham), pmadesis@certh.gr (P. Madesis).

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genetic diversity is an important factor for the fitness and persistence of populations which contributes to the preservation of the evolutionary potential of a species. In this regard, the estimation of species genetic diversity is essential for the ecological studies. However, this estimation is laborious and costly, demanding specialized technical skills. For this reason, the estimation of species genetic diversity due to its correlation to species diversity gained interest recently.

In theory, species diversity and genetic diversity are correlated as a result of their response to the same local processes and/or because they interact to one another (Vellend and Geber, 2005). The correlation between genetic diversity at intra-interpopulation level and the species diversity has been investigated in various plant species with contradictory results. Positive relationship has been reported for the herbaceous *Trillium grandiflorum* (Vellend, 2004), *Platango lanceolata* (Odat et al., 2010), legume species (He and Lamont, 2010), the woody *Banksia attenuate* (He et al., 2008), *Euptelea pleiospermum* (Wei et al., 2010), in natural but not in disturbed forests. On the other hand, negative correlation between species diversity and genetic diversity of *Anthoxanthum odoratum* has been reported by Silvertown et al. 2009 in the 150-year-old Park Grass Experiment and for *Lolium perenne* by Nestmann et al. (2011), under experimental conditions. Finally, habitat species richness did not correlate with the genetic diversity of *Ranunculus acris* (Odat et al., 2004) and *Poa alpina* (Rudmann-Maurer et al., 2007) in semi-natural and natural meadows.

It is assumed that genetic diversity at intra-interpopulation level is shaped by the balance between genetic drift, inbreeding, recombination, gene flow, mutation and selection (Loveless and Hamrick, 1984). This balance depends on the mode of reproduction or the life form of the plant species (Hamrick and Godt, 1996), but also on biotic, such as plant communities, and abiotic characteristics of habitats, including anthropogenic factors. Plant communities, in terms of species composition and richness, are shaped by different selective forces of habitats' environmental conditions (Gusmeroli et al., 2013) and in turn they shape the genetic differentiation between local populations (Odat et al., 2004). However, in some cases there is evidence that anthropogenic factors, such as grazing, are the main determinants of species composition (Derner and Hart, 2007) and richness (Oba et al., 2001), especially in grazed ecosystems.

The aim of the present study was to evaluate the genetic diversity in relation to habitat type and to species composition for the common and important fodder legume *Lotus corniculatus*, at a local level, in a mountainous area in Northern Greece. Additionally, the interaction of ecological parameters with human management regimes and their effect on the *L. corniculatus* genetic diversity was detected. This knowledge is essential regarding the *in situ* conservation of genetic diversity, which is the basis for genetic breeding, as natural populations of the species are important gene pools for the development of improved varieties. The populations of *L. corniculatus* were selected from two distinct habitat types with different management history: a), open grasslands and b) forested areas. The following questions were aimed to be addressed: 1) Is there any differentiation in species composition and *L. corniculatus* abundance between the habitat types 2) Is there any genetic differentiation between the populations of *L. corniculatus* from grasslands and from forested areas? 3) Is the genetic diversity of *L. corniculatus* correlated with species composition and species diversity among and within habitats? 4) Is there any effect of grazing on the species composition and the genetic variability of *L. corniculatus*?

2. Materials and methods

2.1. Species description

Lotus corniculatus L. (birdsfoot trefoil) is a perennial cross-pollinated, tetraploid ($2n = 4x = 24$) legume, although diploid populations have also been reported (Grant and Small, 1996). *L. corniculatus* had arisen through autopolyploidy (Larsen, 1954). However, Ross and Jones (1985) supported an allopolyploid origin through hybridization of *L. alpinus* and/or *L. tenuis* (probably as female parent) with *L. uliginosus* (probably as male parent). It is native to Europe and western Asia, but it is also naturalized in Africa, South and North America, Australia, and New Zealand (Steiner and de los Santos, 2001). It is the most agronomically important species of the genus that is used for pasture, hay, and silage production. It has high nutritive value (Escaray et al., 2012), similar to or even higher than *Medicago* spp. and *Trifolium* spp, mainly because of its non bloating features when grazed directly by livestock. It is characterized by good adaptability to different soil and climate conditions, a fact that results in its genetic diversity (Steiner and de los Santos, 2001). Although its centre of diversification has yet to be identified, great diversity of the species has been reported in the Mediterranean basin (Grant and Small, 1996).

2.2. Study sites and plant material

The study was conducted in the area of Taxiarchis in Cholomontas mountain, Chalkidiki prefecture, northern Greece (40°23′N, 23°28′E), at altitudes ranging from 760 to 870 m. The area is situated in the *Quercion confertae* subzone of the *Quercetalia pubescentis* (sub-Mediterranean) zone. The climate of the area is classified as sub-humid Mediterranean, with a mean annual air temperature of 11.1 °C and an annual rainfall of 767 mm. *L. corniculatus* is present in the area, mainly in the open grasslands and at the edge of forest.

Genetic material from four forested, at the edge of forest, and four open grassland sites was used (Table 1). Twenty individual plants at the flowering stage were randomly collected from each of the study sites in August 2011, i.e. a total of 160 plants.

Table 1
General location, habitat characteristics, grazing intensity and soil features recorded in each area and used for the multivariate analyses.

Sites	Altitude (m)	Longitude	Latitude	Dominant herbaceous species	Explanatory variables						
					Habitat	GI	Soil features				
							pH	Or.M (%)	N (%)	P (mg/100gr)	K (cmol/kg)	Na (cmol/kg)
F1	852	23°32′44″	40°26′86″	*Vicia* sp., *Platango* sp.	BF	LG	6.56	3.73	0.16	1.31	0.19	0.52
F2	867	23°30′11″	40°25′94″	*L. corniculatus*, *Cynodon dactylon*	OF	LG	5.18	3.35	0.13	1.20	0.32	0.82
F3	780	23°30′00″	40°24′28″	*Brachypodium* sp., *Trifolium* sp.	OF	LG	5.73	6.38	0.36	1.11	0.55	0.48
F4	815	23°29′83″	40°24′61″	*Trifolium* sp., *Brachypodium* sp.	OF	LG	4.23	3.97	0.21	1.74	0.21	0.51
G1	792	23°29′67″	40°25′96″	*Cynodon dactylon*, *Cynosurus* sp.	G	LG	4.83	6.41	0.27	1.60	0.19	0.61
G2	799	23°29′91″	40°25′98″	*Agrostis* sp., *Cynodon dactylon*	G	LG	5.52	2.98	0.17	1.47	0.15	0.55
G3	765	23°29′06″	40°26′07″	*Agrostis* sp., *Chrysopogon gryllus*	G	HG	6.11	2.75	0.15	1.32	0.12	0.59
G4	812	23°31′87″	40°26′35″	*Cynodon dactylon*, *Hieracium* sp.	G	HG	5.56	3.86	0.25	1.36	0.25	0.49

BF: Beech Forest, OF:Oak Forest, G: Grassland.
GI: Grazing intensity, LG: Low to moderate grazing intensity (<50%), HG: High grazing intensity (˃50%).

Grazing intensity varied from light, moderately grazed (LG: Forage Utilization Percentage < 50%) to heavily grazed (HG: Forage Utilization Percentage > 50%) in the forested and the grassland sites (Table 1). Pairwise, geographical distances between the study sites ranged from 0.6 to 6 km (Fig. 1).

The plant cover at each study site was measured using the line-point method (Cook and Stubbendieck, 1986) in June 2011 and then the floristic composition was estimated (Table 2). Four transect lines 25 m long were used in each study site along the contour lines. Contacts were obtained every 25 cm. Plants were classified in groups of Graminoids (Gr), Legumes (L), Forbs (F) and Woody (W) species. Species richness, species evenness and Morisita–Horn index was calculated using the Past (Version 3) software (Hammer et al., 2001). The Morisita–Horn abundance based similarity index was used to compare species composition between pairs of communities. Soil samples were collected from each site and analysed as described by Parissi et al. (2014).

2.3. DNA isolation

Total genomic DNA was isolated using the procedure described by Doyle (1987). The amount of DNA was quantified by a UV-spectrophotometer (Eppendorf Biophotometer, Hamburg, Germany). Samples were then diluted to a 20 ng/μL working concentration.

Polymerase chain reactions (PCR) for ISSR analysis were performed in a total volume of 25 μL, containing 20 ng total cellular DNA, 200 mM of each dNTP, 2 mM $MgCl_2$, 40 pmol of primers, 2.5 μL 10 × Taq DNA polymerase buffer, and 1U Platinum Taq polymerase (Invitrogen, Carlsbad, CA).

To study inter-simple sequence repeats (ISSRs), seven oligonucleotide primers complementary to simple sequence repeats (M3, M12, M840, M841, UBC811, UBC27 and UBC834) were used for PCR amplification. Binary data points denote the presence/absence of each distinguishable band across all samples for the same primer, in both replicate sets of amplifications. PCR amplifications were performed in a PTC 200 (MJ Research Inc, MA, USA) as follows: an initial step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, for denaturation, 90 s at 45–60 °C (depending on the primer used) for annealing, and 90 s at 72 °C, for elongation. A 5 min step at 72 °C was programmed as the final extension. Amplification products were separated by electrophoresis on 1.5% agarose gel and stained with ethidium bromide. A 100 bp or 1 Kb DNA ladder (Invitrogen, Carlsbad, USA) was used as a size marker. Gel images were placed in a UVItec Transilluminator (UVItec Limited, Cambridge, United Kingdom) and analysed using UVIDoc software UVIDocMw version 99.04 (UVItec, Cambridge, UK).

2.4. Data analysis

ISSRs are dominant markers, each band representing the phenotype at a single biallelic locus. Only bands that could be unambiguously scored were used in the analysis. ISSR amplified bands were scored for band presence (1) or absence (0), and a binary qualitative data matrix was formed. As a measure of population diversity, the binary data matrix was input to POPGENE version 1.32, which assuming Hardy–Weinberg (HW) equilibrium. Furthermore, the Shannon index was calculated as H = -piLog2 pi, in which pi is the frequency of the presence or absence a given ISSR fragment for each population or region

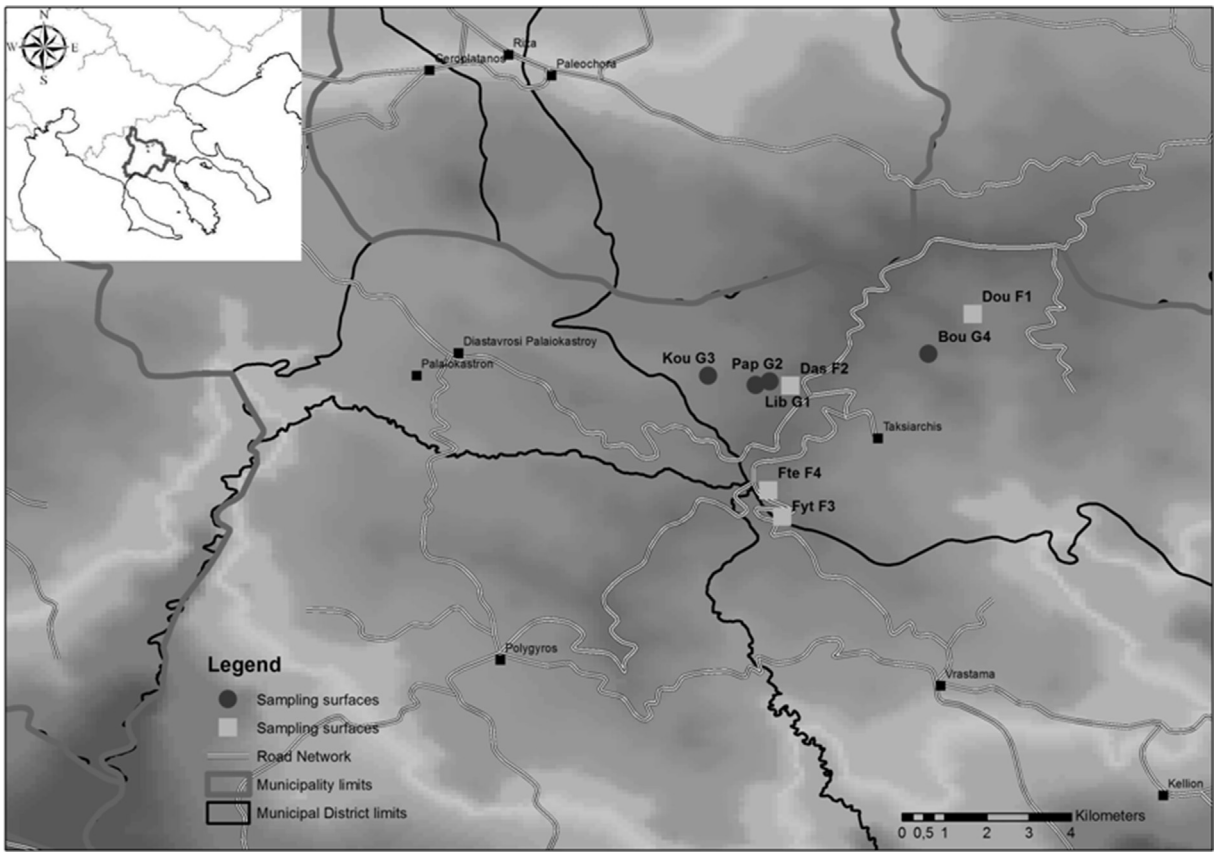

Fig. 1. The map of the four Grassland sites (G:o) and the four Forested sites (F).

Table 2
Floristic composition, floristic diversity and the genetic diversity of *Lotus corniculatus* at the four forested (F) and the four grassland (G) sites.

Sites		Floristic composition (%)					Floristic diversity		Genetic diversity *L. corniculatus*	
	Plant cover (%)	Gr*	F	L	Lo	W	Plant species number	Plant species evenness	H_E	I
F1	77	11.11	44.44	25.49	7.52	11.44	34	0.859	0.183	0.292
F2	70	42.70	29.18	7.47	11.03	9.61	41	0.593	0.167	0.269
F3	70	35.36	19.29	29.29	3.57	12.50	38	0.676	0.189	0.303
F4	72	25.44	24.39	35.89	2.44	11.85	39	0.924	0.187	0.294
F	72b	28.64b	29.33a	24.53a	6.14a	11.35a				
G1	90	65.10	20.22	8.03	3.32	3.32	41	0.883	0.194	0.297
G2	98	66.92	17.98	10.77	2.56	2.56	31	0.696	0.196	0.315
G3	93	50.40	32.35	2.16	0.81	0.81	38	0.850	0.213	0.34
G4	92	36.26	38.46	12.64	2.2	2.20	34	0.653	0.202	0.322
G	93a	54.67a	27.25a	8.40b	2.22a	7.79a				

Gr: Graminoides. F: Forbs, L: Legumes, W: Woody, Lo: *Lotus corniculatus* abundance.
Means in the same column for the same parameter followed by the same letter are not significantly different ($P \geq 0.05$).

(Lewontin, 1972). Shannon's index is less biased since it does not rely on HW equilibrium and was thus used to calculate the total diversity.

Gene diversity (H_E) and Shannon's diversity index (I) was used as a measure of within-population genetic variability. The genetic differentiation between populations of *L. corniculatus* based on ISSRs was calculated according to Nei's (1978) genetic distance. A cluster analysis using an unweighted pair-group method with arithmetic averaging (UPGMA; (Sneath and Sokal, 1973)) was performed using the software popgene 1.32 (Yeh et al., 1999). The individual contributions of habitat and sites on genotypic variability were assessed under the AMOVA framework using GenALEx ver.6.5b5 (Peakall and Smouse, 2006), with sites nested within habitat. Tests for statistical significance were based on 9999 random permutations, followed by sequential Bonferroni correction.

Correlations between within-population gene diversity (H_E) of *L. corniculatus*, species richness and species evenness at each site were tested by Pearson correlation coefficient using XLSTAT 2014 software (Addinsoft, Paris, France). Additionally,

correlations of genetic distances between pairs of populations with the corresponding geographical distances and Moresita–Horn index were investigated by Mantel test.

A Detrended Correspondence Analysis (DCA) was performed in order to select the appropriate canonical ordination. As the gradient of the 1st axis in (DCA) was <3 standard deviation units (SD units) (Ter Braak and Smilauer, 2002), the linear method was more appropriate. Then, two Redundancy Analyses (RDAs) were performed as follows: 1) RDA1 for the ordination of the plant functional groups composition constrained by 8 explanatory variables (Table 1) and 2) RDA2 for genetic diversity by Shannon's index (I) constrained by 5 explanatory variables namely functional group composition (Table 2). The habitat and grazing intensity in RDA1 were included in the analyses as dummy variables. Prior to the DCA and RDA analyses, all data were logarithmically transformed, except for N, K and Na of soil features. The significance of the explanatory variables was tested by an automatic forward selection procedure, using the Monte Carlo test with 999 permutations. The multivariate analyses were carried out by CANOCO v4.5 for Windows (Ter Braak and Smilauer, 2002).

3. Results

3.1. Floristic composition and diversity of the studied sites

The examination of the floristic composition in forested and grassland sites showed that the percentage of the graminoid species on average was significantly higher in grasslands than in forested sites. On the contrary, the percentage of legumes was significantly higher in forested sites than in grasslands (Table 2). The percentage of *L. corniculatus* tended to be higher in forested sites compared to grasslands, however it was not found to be statistically significant (Table 2). Amongst the eight explanatory variables included in the RDA1 (Fig. 2), habitat (F-value 3.59, $P = 0.05$), grazing intensity (F-value 2.74, $P = 0.04$) and Na content of the soil (F-value 3.43, $P = 0.02$) were significant, explaining the 78% of the total variance. The percentage of graminoids (Fig. 2) increased in grassland sites with high grazing intensity and Na soil concentration. On the other hand, the legumes, including *L. corniculatus*, forbs and woody species (Fig. 2), increased in forested sites with low grazing intensity and Na soil concentration.

3.2. Genetic diversity estimated by ISSR markers within and between populations

A total of 92 loci were obtained from the 7 selected ISSR primers. Of all loci analysed, 78.26% were polymorphic and 21.74% were monomorphic within or between populations. Gene diversity (H_E) within the studied populations of *L. corniculatus* ranged from 0.167 to 0.213 and Shannon index (I) from 0.269 to 0.340 (Table 2).

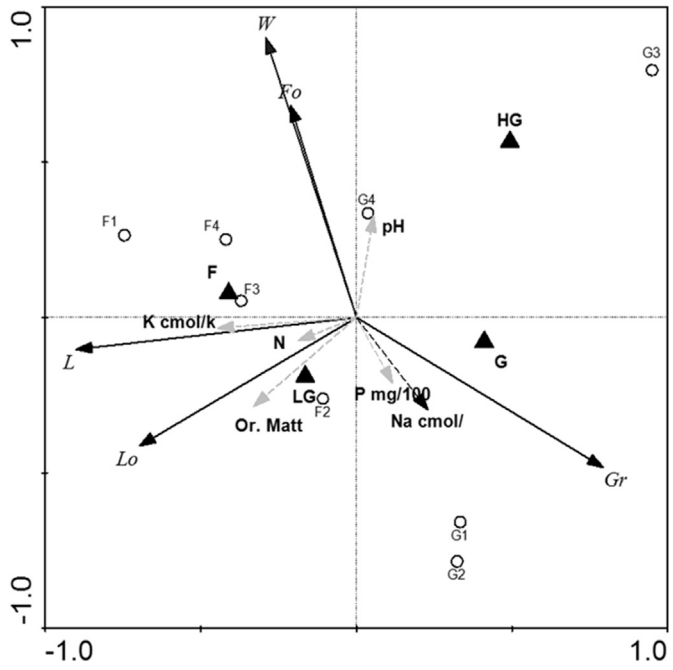

Fig. 2. Biplot from RDA1 showing the position of the following: (i) plant functional groups, (ii) significant (dashed black arrows) and not significant (dashed grey arrows) soil features variables in the four grasslands (G) and the four forested (F) sites. Dummy variables are represented by solid black (significant) and grey (not significant) triangles. Eigenvalues: axis 1 = 0.528, axis 2 = 0.254, axis 3 = 0.170. Cumulative percentage variance: axis 1 = 52.8, axis 2 = 78.2, axis 3 = 95.2. G: Grassland, F:Forest, HG: High grazing intensity, LG: Low grazing intensity, Gr: Graminoids, Fo: Forbs, L: Legumes, Lo: *Lotus corniculatus*, W: Woody.

According to the AMOVA analysis, 91% of the total genetic variation was attributed to differences within populations, and the rest (9%) was attributed to differences among populations. Moreover, according to the nested AMOVA, genetic differentiation was detected between habitat types (i.e. grasslands vs. forested sites) (AMOVA, $P < 0.001$) as well as among the population in each habitat type although in both cases was very low, 3% and 7% respectively. Within-population genetic diversity of *L. corniculatus* steadily tended to be higher in grasslands populations (mean 0.199, SD 0.021) compared to populations from forested sites (mean 0.181, SD 0.018), although the difference was not significant (pooled t-test: $P > 0.2$).

Genetic differentiation [Nei's genetic distance; (Nei, 1973)] between *L. corniculatus* populations ranged from 0.014 (G1–G2 populations) to 0.041 (F1-G4 populations). The UPGMA dendrogram, based on the genetic distances between populations, showed two main clusters of populations (Fig. 3). The first cluster included the F3 and F4 from geographically closed forested sites and the second all the others. However, the second one divided in two main subclusters in which the overgrazed grassland sites G3 and G4 were grouped together. The genetic distances were significantly positively correlated with the corresponding geographical distances (Mantel test; $r = 0.367$, $P < 0.01$) (Table 3), but were significantly negatively correlated with Morisita–Horn index (Mandel's $r = -0.48$, $P < 0.01$).

3.3. Genetic diversity in relation to local plant community composition and diversity

The genetic diversity within population of *L. corniculatus* (Nei's gene diversity; H_E) was not significantly correlated with either species richness (Pearson's $r = -0.23$; $P = 0.57$) or species evenness of the plant communities (Pearson's $r = 0.11$; $P = 0.43$). Amongst the five explanatory variables included in the RDA2 (Fig. 4), only the abundance of *L. corniculatus* (*F*-value 30.76, $P = 0.005$) was significant, explaining the 84% of the total variance. The genetic diversity of *L. corniculatus* according to Shannon's index based on ISSRs was negatively related with the abundance of the species in the studied sites (Fig. 4).

Between populations, genetic differentiation (genetic distances) of *L. corniculatus* was not significantly correlated either with the differences in species evenness (Mantel's $r = -0.53$, $P = 0.42$) or with the differences in species richness (Mantel's $r = -0.23$, $P = 0.29$).

4. Discussion

4.1. Floristic composition and diversity of the studied sites

The two habitat types, open grasslands and edges of forest, were differentiated in species composition. The graminoid species dominated in open grasslands and was positively correlated with high grazing intensity and high Na concentration of the soil. On the other hand, the abundance of *L. corniculatus* tended to be higher at the edges of forest compared to open grasslands and was positively correlated with low grazing intensity. The area was mainly grazed by sheep and goats. Similar increase in grass species in areas grazed by sheep has been also reported by Krahulec et al. (2001). The grass species and especially the tall grasses as *Agrostis* spp. *Chrysopogon gryllus*, and *Cynosurus cristatus* are favoured in the sheep grazing areas, as short species of intermediate toughness are selected more often by sheep (Cingolani et al., 2005). Moreover, the high Na concentration of the soil in open grasslands is also related with the high grazing intensity (Yates et al., 2000).

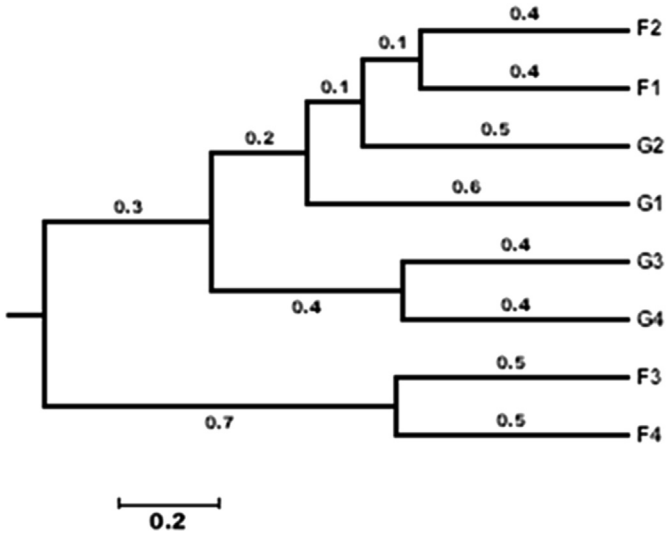

Fig. 3. UPGMA dendrogram based on Nei's genetic distance (Nei, 1978) of *Lotus corniculatus* populations of agriculturally improved and semi-natural habitats. *Scale bar show genetic distance.**Numbers on the nodes indicate bootstrap values generated after 1000 replications.

Table 3
Pairwise genetic distances (below diagonal) and geographical distances (in km, above diagonal) between the populations of *Lotus corniculatus* studied.

Population	F1	F2	F3	F4	G1	G2	G3	G4
F1	0.000	3.710	5.900	5.560	4.250	3.930	4.990	1.240
F2	0.017	0.000	3.080	2.430	0.620	0.290	1.510	2.590
F3	0.021	0.016	0.000	0.660	3.160	3.160	3.580	4.660
F4	0.031	0.028	0.015	0.000	2.510	2.540	2.910	4.330
G1	0.017	0.017	0.022	0.018	0.000	0.33	0.880	3.200
G2	0.022	0.015	0.020	0.025	0.014	0.000	0.880	3.190
G3	0.035	0.026	0.030	0.032	0.025	0.015	0.000	4.010
G4	0.041	0.031	0.035	0.035	0.026	0.024	0.016	0.000

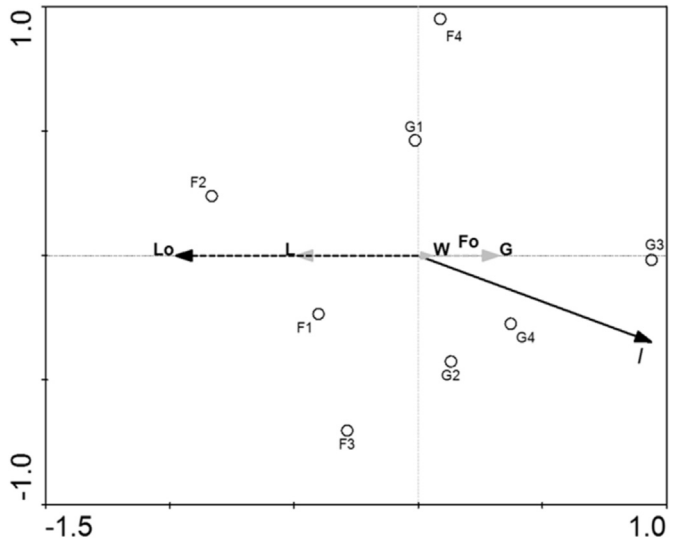

Fig. 4. Biplot from RDA2 showing the position of the following: (i) Shannon's index based on ISSRs, (ii) significant (dashed black arrows) and not significant (dashed grey arrows) functional groups variables in the four grasslands (G) and the four forested (F) sites. Eigenvalues: axis 1 = 0.425, axis 2 = 0.315, axis 3 = 0.182. Cumulative percentage variance: axis 1 = 42.5, axis 2 = 74.0, axis 3 = 92.2. I: Shannon's index, Gr: Graminoids, Fo: Forbs, L: Legumes, Lo: *Lotus corniculatus*, W: Woody.

The decrease of *L. corniculatus* abundance in grasslands could be attributed to the direct and indirect effect of grazing. Its growth is affected directly, as it is legume species and it is preferred by sheep. At the same time, indirectly, the increase of grasses, which are favoured by this grazing behaviour depresses its growth, as it is less competitive compared to them (Wurst and van Beersum, 2009).

4.2. Genetic diversity estimated by ISSR markers within and between populations

There is limited information about genetic diversity of natural population of *L. corniculatus*. According to Savo Sardaro et al. (2008), the gene diversity of populations from central Italy based on SSRs ranged from 0.253 to 0.437. However, it is not possible to compare it with the present study, where marker of different nature was used. The observed high proportion (91%) of ISSRs genetic variation within rather than between populations of *L. corniculatus* is expected for a common, geographically widespread, cross-pollinated perennial plant species (Nybom et al., 1990). On the contrary, higher genetic variation of ALFPs and SSRs among than within population of *L. corniculatus* has been reported by Savo Sardaro et al. (2008), for large scale collections in Italy. These contrasting results could probably be attributed to the strong positive effect of geographic distance between sampled populations on the among-population diversity (Nybom, 2004).

A slight genetic differentiation was observed between habitat types (Forested vs Grasslands) as the study was restricted to a local level and the distances among the plots were small. Generally, genetic differentiation can be contributed to the effect of both abiotic and biotic environmental factors on distribution patterns and populations genetic structures, as measured by random dominant markers (Odat et al., 2004). The habitats in the present study differ from one another in regard to abiotic environmental factors, such as nutrient availability, light and land use (grazing) as well as to biotic ones, such as species composition. Nestmann et al. (2011) reported that species composition of experimental grasslands had effect on genetic differentiation of *L. perenne* populations. According to their study, species composition modulated the population sizes of *L. perenne* as result of differences in interspecific competition. Additionally, Prati and Schmid (2000) referred that the

competition with grasses produced genetic differentiation among *Ranunculus reptans* populations. As the differentiation of habitats in species composition was mainly related with the abundance of grasses, the genetic differentiation of *L. corniculatus* could be the result of its competition with grasses.

Regardless of the habitat type, the genetic differentiation among the populations was positively correlated to the geographic distances, indicating that there was reduced gene flow among distant populations. Gene flow in natural populations of *L. corniculatus*, an insect pollinated plant, is mainly affected by the pollinators and their movements through the populations (Rasmussen and Brødsgaard, 1992). The majority of its pollination is carried out by bumblebees, which generally have a foraging radius under a kilometre. The maximum observed range for *Bombus terrestris*, which is the most common *Bombus* species in Greece, was 758 m (Knight et al., 2005). Thus, it is reasonable to expect the populations to be partly isolated. Similarly, phenotypic differentiation among regional British natural populations of *L. corniculatus* was reported (Smith et al., 2009). However, the G3 and G4 populations with a geographical distance of around 4 km, were clustered together in the dendrogram based on the genetic distances. In this case, seed dispersal, which is aided by animal transfer, may have also contributed to gene flow (Rasmussen and Brødsgaard, 1992), as in G3 and G4 sites the grazing intensity was high.

4.3. Genetic diversity in relation to local plant community composition and diversity

Species diversity (richness and evenness) was not correlated with genetic diversity of *L. corniculatus*, either within or between populations at the local scale of the study. These results could be attributed to the effect of habitat characteristics on the abundance and genetic diversity of the studied species, as well as on the species diversity. According to Odat et al. (2010) hypothesis, relationship between species and genetic diversity had been indirect and mediated by differences in habitat characteristics. In their study, Odat et al. (2010) reported positive correlation between *Platango lanceolata* diversity and species diversity. However, the relationship of the *P. lanceolata* abundance disappeared when the effect of abiotic habitat characteristics was controlled using partial correlations. Furthermore, according to Vellend and Geber (2005), single species models of genetic diversity, which predict positive relationships between diversity and heterogeneity, might fail when individual fitness depends strongly on community context. The abundance of *L. corniculatus* in the present study was affected by grazing intensity and species composition of the habitat.

The genetic diversity of *L. corniculatus* was negatively correlated with its abundance, according to the results of RDA2. This is probably related to its more or less prostrate growth habit. Additionally, although it mainly reproduces by seeds, a limited capacity for vegetative reproduction also exists (Grime, 2006). Thus, it produces spots and its abundance increases in the less competitive environment of the edge of forest, yet, this increases the possibility of pollinators visiting relatives, resulting in the decrease of genetic diversity of the population.

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