



Genetic variability in populations of the terrestrial isopod *Armadillidium vulgare* living in soils with different land uses

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ARTICLE INFO

Keywords:

Land-use change

Soil fauna

Genetic variability

Population genetic structure

Armadillidium vulgare

ABSTRACT

The Argentine Pampas region is recognized by its high productivity and fertility, which make its soils suitable for agricultural use. However, the intensification of agricultural activities over the last forty years has led to an intense disturbance regime, characterized by an increase in the soil degradation rate. Soil degradation and its relationship with soil fauna communities are crucial issues in resource management. In this study, we investigated the effects of land-use change on the genetic variability of the terrestrial isopod *Armadillidium vulgare*, chosen as a biological model. The diversity and population genetic structure of this species were analyzed using inter-simple sequence repeat (ISSR)-PCR markers, in three land uses in two localities of Luján, Buenos Aires, Argentina. Genetic variability was high in natural grassland populations and lower in agricultural land uses. Both conventional F_{ST} analysis and Bayesian approach for dominant markers showed significant genetic differences between land uses within each locality. The loss of genetic variability and the population genetic structure can be used as indicators of system disturbance. Thus, in the soils studied, the degree of genetic variability of representative populations of the soil fauna can be a good indicator of the disturbance degree.

1. Introduction

The Argentine Pampas has suffered a marked process of agriculturalization characterized by a strong and continuous increase in the land area dedicated to crop production, displacing pastures and cattle (Manuel-Navarrete et al., 2009). The substitution of the natural vegetation cover to establish intensive production systems results in soil deterioration, reduction of the agroecosystem productivity, and loss of biodiversity and its functions (Barrios, 2007). Moreover, the increase in the culture of soybean (*Glycine max*) in the last decades has abruptly increased the demand for phytosanitary products (Aizen et al., 2009; Viglizzo et al., 2011). Pesticides used to control plagues also affect non-target and even beneficial organisms such as parasitoids, pest predators and wildlife associated with different soil ecological functions (Decaëns et al., 2002).

The important role of invertebrates in the soil is well known. These organisms process and decompose organic matter, enhance the formation of stable soil aggregates and improve the population stability of other soil-inhabiting organisms (Coleman et al., 2004). The diversity and functions of soil invertebrates are sensitive to stress and environmental changes associated with tillage, fertilizer and pesticide application, and other agriculture practices (Bedano et al., 2006a). The loss of soil fauna abundance and diversity in the Pampas has been recorded in several studies (Bedano et al., 2006a, 2006b; Arolfo et al., 2010; Domínguez et al., 2010; Díaz Porres et al., 2014; Falco et al., 2015; Bedano et al., 2016). This phenomenon occurs together with soil physical and chemical degradation (Duhour et al., 2009).

Within soil invertebrates, isopods are abundant, with several species, and considered as potential bioindicators of the soil quality in agroecosystems. Terrestrial isopods inhabit the litter layer and affect

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<https://doi.org/10.1016/j.pedobi.2018.07.001>

Received 24 January 2018; Received in revised form 16 July 2018; Accepted 23 July 2018

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decomposition and mineralization processes by feeding on a wide range of organic sources (Špaldoňová and Frouz, 2014; Jia et al., 2015). They are widespread and, in most cases, the dominant component of detritivores in temperate regions (Coleman et al., 2004; Paoletti et al., 2007; Hornung et al., 2015). In the Argentine Pampas, *Armadillidium vulgare* (Latreille, 1804; Isopoda: Oniscidea) is one of the most abundant isopod species in both natural and managed systems (Díaz Porres et al., 2014), a fact that reflects its broad tolerance and invasive nature. Although this species is native to Europe it has been dispersed worldwide by humans and has been frequently recorded in the agricultural systems of South America (Araujo et al., 1996; Martínez et al., 2014), including Argentina, especially in the province of Buenos Aires (Faber et al., 2014). *A. vulgare* strongly depends on the quality of the edaphic environment, where it needs to find not only food but also the necessary moisture to avoid dehydration (Warburg, 1968). *A. vulgare* is characterized by a short dispersal distance, of 1–13 m per day (Paris, 1963), and has an inherent tendency to aggregate, which may limit its dispersal (Homor et al., 2003); these characteristics can be important for its quality as a bioindicator because of their presence and abundance reflect the local conditions. Land-use intensification can affect the survival and behavior of *A. vulgare*, changing its population densities and, eventually, its genetic pool.

Population genetic structure is the distribution of genotypes in space and time, and is determined by both historical and current evolutionary processes involving gene flow, genetic drift and selection (Slatkin, 1987). In addition, ongoing evolutionary processes may be affected by more recent events such as environmental stressors (e.g. land-use change, persistent soil contamination). The effects of environmental stressors on the population genetic structure depend on the way evolutionary processes are affected. For instance, stressed and non-stressed populations may be genetically differentiated due to selection of a particular character and/or to the increase in genetic drift when the environmental stressor leads to a decrease in the population size (Costa et al., 2013). At population level, habitat fragmentation and land-use change could result in declining levels of gene flow and in a reduction in the population size, which may in turn lead to a greater level of inbreeding, increasing the importance of genetic drift (Hartl and Clark, 2007). It is known that environmental factors, such as tillage or pesticide application, affect the allelic frequencies and population genetic structure of earthworm species (Peles et al., 2003; Kautenburger, 2006); unfortunately, it is not yet clear how these environmental factors affect the genetic structure of *A. vulgare* (Homor et al., 2003).

In Argentina, there are few data available on how different agricultural practices affect the genetic variability and differentiation of the soil fauna. In other parts of the world, similar effects have been studied in invertebrates such as Hemiptera, Diptera (Abbot, 2001), earthworms (Enckell et al., 1986; Peles et al., 2003; Kautenburger, 2006) and Lepidoptera (Roux et al., 2007; Palma et al., 2015).

The objective of this study was to analyze the genetic diversity and population genetic structure of *Armadillidium vulgare* in Argentine Pampas soils under three different land uses. To this end, we investigated whether anonymous DNA molecular markers considered highly polymorphic in most species could detect population subdivisions according to land-use change. We used inter-simple sequence repeat (ISSR-PCR), a technique that has proved effective in detecting genetic structure at the intra-specific level in different invertebrate species (Abbot, 2001; Vijayan et al., 2006; Roux et al., 2007; de Aranzamendi et al., 2008, 2014; Palma et al., 2015). In comparison with other molecular markers of similar sensitivity, ISSRs do not require an initial investment in DNA sequencing and primer design, produce highly reproducible band patterns, and reveal high levels of polymorphism, thus increasing the potential to detect genetic differentiation (Zietkiewicz et al., 1994; Wolfe, 2005).

2. Materials and methods

2.1. Study area and sampling

The study was conducted in two localities of Luján, Buenos Aires province, Argentina. The climate in the study area is humid temperate with a mean annual precipitation of 1000 mm and a mean annual temperature of 17 °C. The soil in the fields studied is Typic Argiudoll with silt loam texture in the surface horizon (Soil Survey Staff, 2010). The work was carried out in the experimental field of the Universidad Nacional de Luján (U) (34°34'S, 59°04'W) and in a private rural property, Etchegoyen (E) (34°26'S; 59°04'W); the distance between the two sites is about 15 km. Samples were taken in three land uses in each site: conventional farming with agrochemical use and conventional tillage (A), pasture with cattle raising (C), and natural grasslands (N). This gave a total of six sampling sites: AU, AE, CU, CE, NU and NE. The latter, which have been undisturbed for the last 50 years, were included in the study as reference sites. Each sampling site had an area ranging from 8 to 12 ha.

In each sampling site, three sampling points were defined every 10 m along a transect with a random starting point. In each sampling point, the soil macrofauna was sampled by digging a soil monolith of 0.25 × 0.25 × 0.1 m in depth, according to the TSBF ("Tropical Soil Biology and Fertility") method (Anderson and Ingram, 1993). Isopods were separated and kept alive in the laboratory where they were taxonomically identified using keys (Araujo et al., 1996; Dindal, 1990; Pérez-Schultheiss, 2010) and checking their identity by detailed descriptions (Cruz-Suárez, 1993). Sex were separated using external anatomy (telson and appendices dimorphism) and their DNA were extracted.

2.2. DNA extraction and ISSR-PCR amplification

A total of 215 adult males of *A. vulgare* were analyzed at molecular level. Total genomic DNA was isolated by dissecting specimens using the CTAB-based protocol, according to von der Schulenburg et al. (2001), with minor modifications. Individual tissue was ground and digested overnight at 65 °C in 700 µl of CTAB buffer (2% w/v). DNA was extracted with 400 µl of chloroform:isoamylalcohol (24:1 v/v), precipitated with 600 µl of isopropanol and 300 µl of ammonium acetate 5 M, and centrifuged at 10,000 rpm at 4 °C for 10 min. The DNA pellet was washed with 1 ml of 70% ethanol and air-dried. Finally, the DNA was dissolved in 100 µl TE (0.01 M Tris-HCl pH 8.0, 0.001 M EDTA) and stored at –20 °C. DNA concentrations were determined by comparison to a molecular weight marker (Lambda, digested with *EcoRI* and *HindIII*, Promega) on 1% agarose gels, stained with SYBR® Safe and visualized under UV light.

Twelve anchored ISSR primers were tested for the PCR amplification reactions. Four of them, which showed a high number of clear, polymorphic bands, were selected for the analyses: (CA)₇AC, (CA)₇AG, (AC)₈G and (AG)₈T (Table 1).

Each PCR mixture contained 1 × PCR buffer minus Mg, 1.75 mM of MgCl₂, 0.25 mM of each dNTP (Invitrogen®), 3 µM of primer, 1 mg/ml of bovine serum albumin (Sigma-Aldrich, USA), 1.5 U of *Taq* DNA polymerase (*Pegasus*, PB-L®), 5 µl of 1/10 diluted DNA template, and sterile Millipore water to a final volume of 20 µl. PCR amplification was performed in an Eppendorf® Mastercycler programmed for an initial denaturalization cycle of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at 44 °C (for primers (CA)₇AC and (CA)₇AG), 53 °C (for primer (AC)₈G) and 51 °C (for primer (AG)₈T), and 2 min at 72 °C. A post-treatment of 10 min at 72 °C and final cooling at 4 °C were performed. Negative controls were included in each PCR to verify the repeatability of ISSR results. For each amplification, replicate individuals (positive controls) were also included.

Amplification products were separated in 1.5% agarose gels using 1X TBE buffer. Then, 5 µl of 100-bp ladder plus (Dongsheng Biotech®)

Table 1

Primer sequences and annealing temperature used in the inter-simple sequence repeat (ISSR) analyses and range of molecular weight in base pairs (bp) and number of polymorphic bands per primer amplified by ISSR-PCR for 215 individuals of *Armadillidium vulgare*.

Primer sequence (5' – 3')	Annealing temperature (°C)	Size-range of polymorphic bands (bp)	Polymorphic bands scored	PIC ^a
(CA) ₇ AC	44	250–3000	21	0.33
(CA) ₇ AG	44	200–2000	19	0.30
(AC) ₈ G	53	200–2000	19	0.28
(AG) ₈ T	51	150–1200	18	0.31
		Total:	77	

^a PIC: Polymorphic Information Content.

was run for fragment size reference within each gel. Gels were run at 90 V for 2 h and stained using SYBR[®] Safe (Invitrogen), following the manufacturer's recommendations. The ISSR banding patterns were visualized using a UV transilluminator and recorded by digital photography.

2.3. Data analysis

ISSR bands were scored as present (1) or absent (0), and transformed into a 0/1 binary character matrix. Only reproducible fragments were considered to generate the ISSR matrix dataset. For each primer, the Polymorphic Information Content (PIC) was calculated using the formula $PIC_i = 2f_i(1-f_i)$, where f_i is the relative frequency of the amplified marker (band present) and $(1-f_i)$ is the relative frequency of the null marker (band absent) of marker i (de Aranzamendi et al., 2014). The number of scored bands and the percentage of polymorphic loci (P) per land use were obtained. The genetic variability of each land use was analyzed by the mean Shannon's information index (I) and the diversity index (h), with GenAlEx 6.5 (Peakall and Smouse, 2012). Shannon's information index was estimated as $I = -1 * (p * \ln(p) + q * \ln(q))$, whereas the diversity index was estimated as $h = 1 - (p^2 + q^2)$, where p is the marker frequency (band present) and $q = 1 - p$. Since the data did not have a normal distribution, I and h mean values were compared between land uses using the non-parametric Kruskal–Wallis test.

To visualize the genetic relationships among individuals, a Neighbor-joining (NJ) tree was constructed based on Nei and Li (1979) genetic distances, using the NEIGHBOR and CONSENSE programs of the PHYLIP package version 3.66 (Felsenstein, 2004), with 1000 bootstraps. Nei and Li distances were computed with the InfoGen© software (Balzarini and Di Rienzo, 2014).

Existence of isolation by distance was assessed using the Mantel test (Mantel, 1967) between genetic and geographic distance matrices, using 1000 permutations with GenAlEx 6.5 software (Peakall and Smouse, 2012). A matrix of geographic distances between land-use pairs in each site was obtained using Google Earth (Google Corporation, 2016). The coefficient of determination (r^2) indicating how well data points fit a linear regression model was calculated.

To measure genetic differentiation between land-use pairs, the F_{ST} estimator for dominant markers (Lynch and Milligan, 1994) was calculated. F_{ST} is an estimate of the proportion of genetic diversity among samples: when F_{ST} is 0, there is no differentiation, whereas when it is 1, the populations are fixed for different markers (Hartl and Clark, 2007). The F_{ST} values were determined with the AFLP-Surv program (Vekemans et al., 2002). Pairwise values were estimated through a bootstrap of 1000 replicates. Marker frequencies were estimated by implementing the method of Zhivotovsky (1999), assuming Hardy–Weinberg equilibrium. The significance of F_{ST} values was obtained using the formula $\chi^2 = 2NF_{ST}(k-1)$, with degrees of freedom equal to the number of subpopulations (land uses) minus 1 (de Aranzamendi

et al., 2008).

Population differentiation was also analyzed by a Bayesian approach, which requires no prior knowledge of the inbreeding level within populations, using Hickory version 1.1 (Holsinger and Lewis, 2003). This package estimates θ^{II} , which is the Bayesian analog of F_{ST} , and f is the analogous coefficient of F_{IS} through the use of Markov chain Monte-Carlo simulations. Data were analyzed under three different models of population structure: (i) $\theta^{II} \neq 0$ and $f \neq 0$ (full model); (ii) $f = 0$ and $\theta^{II} \neq 0$ (no inbreeding within populations); and (iii) $\theta^{II} = 0$ and $f \neq 0$ (no differentiation between populations). The program was run using the following parameters: burn-in = 100,000, number of samples = 1,000,000, and thinning = 50. The mean estimates of θ^{II} were analyzed and compared under the different models, by using the Deviant Information Criterion (DIC). Models with the smaller DIC are preferred (Holsinger et al., 2002).

To further infer the genetic structure of the dataset, the program STRUCTURE version 2.3.1 adapted for dominant markers was used (Pritchard et al., 2000; Falush et al., 2007). This program uses a Bayesian model-based clustering algorithm to estimate the likelihood of the model given the data and which individuals are most likely to belong to each cluster (the membership of each individual is estimated as q , which varies between 0 and 1, with the latter indicating full membership; Falush et al., 2007). To determine the optimal number of groups (K), the STRUCTURE program was run with different values of K and with three independent runs using Markov Chain Monte Carlo of 10,000 generations (burn-in 1000). The posterior probability of the model was estimated using the maximal average value of $\ln P(D)$ as an ad hoc guidance. Using dominant markers the minimum number of bands required depends, among other factors, on the analyses being performed. Based on the FSTs, as few as 30 markers will yield acceptable results but STRUCTURE will require a minimum of 60 polymorphic loci, for small sample size as recommended by Nelson and Anderson (2013).

3. Results

The four primers used yielded a total of 77 ISSR markers, ranging in size from 150 to 3000 bp, for the 215 individuals of *A. vulgare* studied. All loci were polymorphic at 95% level. The PIC values ranged from 0.28 to 0.33 (Table 1). The population with the highest percentage of polymorphism was NU (98.7%), while those with the lowest percentages were AU and AE (90.9% and 92.2%, respectively), with a mean within population polymorphism rate of 94.4% in the experimental field of the Universidad Nacional de Luján and 95.7% in Etchegoyen (Table 2). The Shannon's information index and the parameter of genetic diversity were higher in the field of the Universidad Nacional de

Table 2

Genetic diversity of *Armadillidium vulgare* between the land uses studied.

	n	BS	P (%)	I (SD)	h (SD)
NU	37	76	98.7	0.57 (0.14) a	0.39 (0.12) a
CU	47	72	93.5	0.51 (0.20) ab	0.35 (0.16) ab
AU	33	70	90.9	0.47 (0.22) b	0.31 (0.16) b
Mean			94.4 (2.4)	0.52 (0.00)	0.35 (0.00)
NE	33	75	97.4	0.52 (0.19) n.s.	0.35 (0.15) n.s.
CE	34	75	97.4	0.53 (0.19) n.s.	0.36 (0.15) n.s.
AE	31	71	92.2	0.48 (0.22) n.s.	0.33 (0.17) n.s.
Mean			95.7 (1.6)	0.51 (0.00)	0.34 (0.00)

n : total number of individuals per land use; BS: number of polymorphic bands scored; P : percentage of polymorphic loci; I : Shannon's information index; h : genetic diversity. SD: Standard deviation. Different letters indicate statistically significant differences between land uses ($p < 0.05$). n.s.: non-significant. NU: Natural grassland Universidad Nacional de Luján; CU: Cattle raising Universidad Nacional de Luján; AU: Agriculture Universidad Nacional de Luján; NE: Natural grassland Etchegoyen; CE: Cattle raising Etchegoyen; AE: Agriculture Etchegoyen.

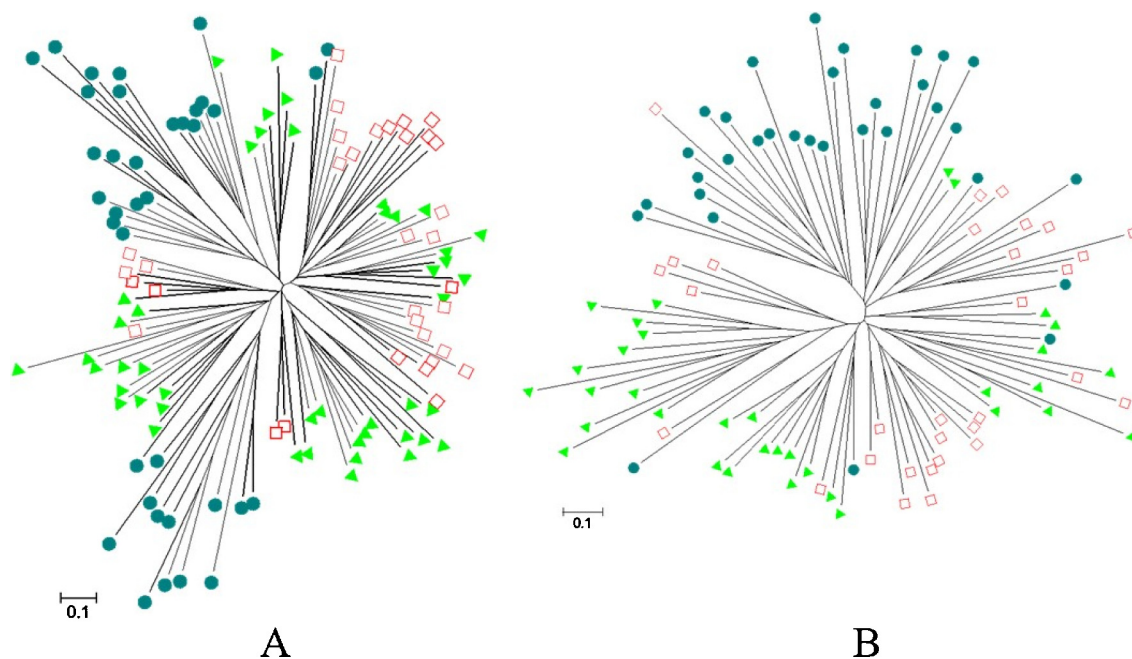


Fig. 1. Neighbor-joining tree based on Nei and Li (1979) genetic distances between pairwise individuals of *Armadillidium vulgare* in Universidad Nacional de Luján (A) and Etchegoyen (B). Symbols represent individuals according to their land uses of origin. ●: Natural grassland; ▼: Cattle raising; □: Agriculture.

Luján than in Etchegoyen. The population NU showed the highest genetic diversity with $I = 0.57$ and $h = 0.39$, while AU showed the lowest genetic diversity, with $I = 0.47$ and $h = 0.31$ (Table 2).

The NJ tree (Fig. 1) shows that the individuals belonging to the same land use were grouped together in the same clade. There was a weak but significant correlation between genetic and geographic distances in both sites (Universidad Nacional de Luján ($r^2 = 0.37$, $p = 0.001$); Etchegoyen ($r^2 = 0.22$, $p = 0.001$)).

Assuming Hardy–Weinberg equilibrium, pairwise values of F_{ST} were used to estimate the genetic differentiation between land uses within sites. F_{ST} values indicated a significant genetic divergence between NU and the other two land uses in Universidad Nacional de Luján, while CE and AE showed no genetic differentiation in Etchegoyen (Table 3). The genetic differentiation in Universidad Nacional de Luján was higher than in Etchegoyen ($F_{ST} = 0.066$, $p < 0.0001$ and $F_{ST} = 0.041$; $p = 0.0004$, respectively).

Bayesian analysis using Hickory showed genetic differentiation within each site. The three models indicated a significant genetic differentiation for all pairwise comparisons (Table 4). In these comparisons, the values of deviance were a little different between the full model and the $f = 0$ model, indicating that there is no reason to prefer the full model to the one with $f = 0$. Therefore, there is no evidence of

Table 3
Genetic differentiation levels of *Armadillidium vulgare* between land uses within localities, estimated using F_{ST} (Lynch & Milligan).

	F_{ST}	p
UNLu	0.0659	< 0.0001
NU – CU	0.0607	0.0014
NU – AU	0.0882	0.0004
CU – AU	0.0471	0.0060
Etchegoyen	0.0405	0.0004
NE – CE	0.0542	0.0070
NE – AE	0.0415	0.0212
CE – AE	0.0244	0.0749 n.s.

n.s.: non-significant. UNLu: Universidad Nacional de Luján. NU: Natural grassland UNLu; CU: Cattle raising UNLu; AU: Agriculture UNLu; NE: Natural grassland Etchegoyen; CE: Cattle raising Etchegoyen; AE: Agriculture Etchegoyen.

Table 4
Genetic differentiation of *A. vulgare* between land uses within localities, estimated using θ^{II} . Mean (95% credible intervals). Deviant Information Criterion (DIC).

	Model	θ^{II}	DIC
UNLu	Full	0.1028 (0.0734 – 0.1336)	1233.05
	$f = 0$	0.0759 (0.0577 – 0.0978)	1249.50
	$\theta^{II} = 0$	–	1772.14
NU–CU	Full	0.1001 (0.0639 – 0.1437)	855.45
	$f = 0$	0.0751 (0.0511 – 0.1066)	866.36
	$\theta^{II} = 0$	–	1155.89
NU–AU	Full	0.1353 (0.0892 – 0.1918)	810.81
	$f = 0$	0.1033 (0.0713 – 0.1442)	823.77
	$\theta^{II} = 0$	–	1152.96
CU–AU	Full	0.0709 (0.0427 – 0.1082)	799.20
	$f = 0$	0.0544 (0.0345 – 0.0808)	810.09
	$\theta^{II} = 0$	–	975.08
Etchegoyen	Full	0.0737 (0.0503 – 0.1022)	1180.93
	$f = 0$	0.0579 (0.0425 – 0.0771)	1192.59
	$\theta^{II} = 0$	–	1512.74
NE–CE	Full	0.0949 (0.0589 – 0.1401)	803.38
	$f = 0$	0.0722 (0.0478 – 0.1039)	816.81
	$\theta^{II} = 0$	–	1027.16
NE–AE	Full	0.0806 (0.0490 – 0.1219)	780.19
	$f = 0$	0.0626 (0.0402 – 0.0924)	790.18
	$\theta^{II} = 0$	–	961.36
CE–AE	Full	0.0553 (0.0322 – 0.0867)	779.10
	$f = 0$	0.0434 (0.0262 – 0.0666)	786.77
	$\theta^{II} = 0$	–	894.66

Bold type values show the best-fit model. UNLu: Universidad Nacional de Luján. NU: Natural grassland UNLu; CU: Cattle raising UNLu; AU: Agriculture UNLu; NE: Natural grassland Etchegoyen; CE: Cattle raising Etchegoyen; AE: Agriculture Etchegoyen.

inbreeding in these populations. The $f = 0$ model is, however, strongly preferred to the $\theta^{II} = 0$ model, supporting the existence of a significant level of differentiation between pairs of populations. The θ^{II} value (full model) was higher in Universidad Nacional de Luján (0.1028) than in Etchegoyen (0.0737). The θ^{II} values estimated between land-use pairs within each site showed that populations from the natural grassland are genetically different from those from the cattle-raising and agricultural land uses. Differences among pair comparisons were lower in

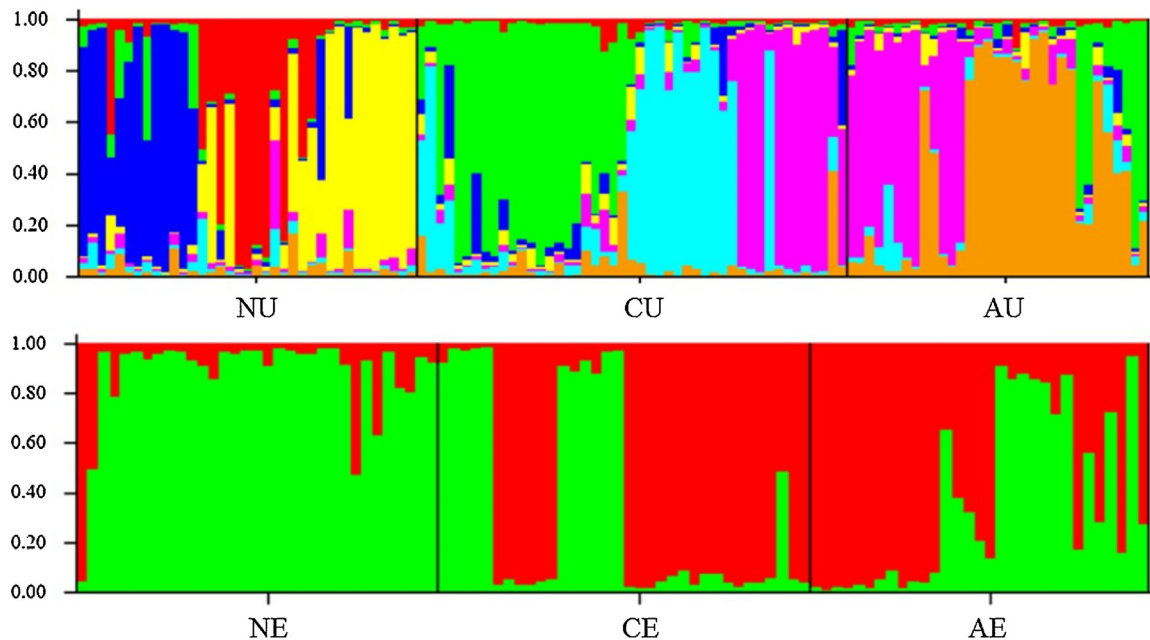


Fig. 2. Patterns of genetic differentiation observed in *Armadillidium vulgare* in Universidad Nacional de Luján (top; $K = 7$) and Etchegoyen (down; $K = 2$) through the Bayesian structuring analysis. Each vertical line represents one individual. The length of each line reflects the probability of membership of each individual for each cluster. NU: Natural grassland Universidad Nacional de Luján; CU: Cattle raising Universidad Nacional de Luján; AU: Agriculture Universidad Nacional de Luján; NE: Natural grassland Etchegoyen; CE: Cattle raising Etchegoyen; AE: Agriculture Etchegoyen.

Etchegoyen than in Universidad Nacional de Luján (Table 4).

The population structure detected by Bayesian clustering showed that the maximal average value of $\ln P(D)$ was obtained with $K = 7$ in Universidad Nacional de Luján and with $K = 2$ in Etchegoyen. These values indicate an *A. vulgare* structure of seven groups in Universidad Nacional de Luján (Fig. 2) (three exclusive groups for the natural grasslands (blue, red and yellow), one exclusive group for the cattle-raising land use (light blue), one exclusive group for the agricultural land use (orange), and two groups shared by the cattle-raising and agricultural land uses (green and violet)), and an *A. vulgare* structure of only two groups shared by the three land uses in Etchegoyen. This suggests a greater exchange of genetic information between groups in Etchegoyen.

4. Discussion

In this work, we showed that the soils with productive uses (cattle raising and agriculture) had less genetic variability of the isopod *A. vulgare* than the natural grasslands. Our analysis showed that the genetic diversity of *A. vulgare* in sites under agricultural uses was significantly lower than that in the other land uses. This is true for the percentage of polymorphic loci, number of bands, Shannon's information index and genetic diversity. The significant decrease in genetic diversity in the agricultural land uses indicates that these populations probably belonged to smaller ones, affected by high disturbances, which could increase the importance of genetic drift (Hartl and Clark, 2007). The level of genetic diversity in the different populations could be explained by several factors, such as the population size, gene flow among the populations, and dispersal of individuals.

The genetic variation in the colonizing areas of a species generally represents a portion of that in the original population. The low level of genetic variability detected in the agricultural populations studied might indicate that these populations correspond to a more recently colonized area (Sesarini and Remis, 2008). Given the limited mobility level of *A. vulgare*, it is possible that, when populations are reduced by disturbances, recolonizing processes are established. Another alternative explanation that could reduce heterozygosity is the possible

directional selection on specific genotypes. However, this explanation does not seem to be general to all analyzed ISSRs and environments.

When the genetic distances were analyzed using the NJ tree, fragmented clusters appeared, especially in the more intensive uses in Etchegoyen (Fig. 1B), while the individuals from the natural grassland remained almost all in the same root. In Universidad Nacional de Luján (Fig. 1A), subgroups appeared in all land uses, although the division was more marked in those under intensive use (the natural grassland was subdivided into only two branches). This information supports the idea of a metapopulation effect. In that case, the subgroups observed could be associated with individuals collected on different sampling dates. Since *A. vulgare* has an annual reproduction cycle and actively moves in search of favorable habitats, different genetic groups may be recolonizing land uses each year from neighboring patches. This effect would be stronger for the most disturbed uses.

Under isolation by distance model (Wright, 1943; Slatkin, 1987), a continuous increase in the genetic distance between populations as a function of an increase in the geographic distance is expected. The population genetic structure may be due to genetic drift and dispersion (Wright, 1951). The distance isolation model examines the relationship of gene flow between pairs of populations, and is related to the fact that genetic differentiation increases at a greater distance. In this case, for the Mantel test, although the correlation between genetic distance and geographic distance is weak (low slope and high dispersion), it is significant. This suggests that distances between land uses are in the species dispersion range, although there is evidence of isolation by distance.

The pairwise F_{ST} values obtained for *A. vulgare* were significant in seven out of eight cases. The associated levels of differentiation, according to the criteria established by Hartl and Clark (2007), indicate low to moderate genetic differentiation between land-use pairs (pairwise F_{ST} varying between 0 and 0.10). The genetic differentiation in Universidad Nacional de Luján was higher than that in Etchegoyen: in Universidad Nacional de Luján, there was moderate but significant within-site genetic differentiation, while, in Etchegoyen, there was low within-site genetic differentiation.

The pattern of differences was not the same for the two localities.

Genetic divergence was higher between the natural grassland and the agricultural land use in Universidad Nacional de Luján, and between the natural grassland and the cattle-raising land use in Etchegoyen, while no genetic differentiation was observed between the cattle-raising and the agricultural land-use populations in Etchegoyen. The significant genetic differentiation between land uses in Universidad Nacional de Luján, specifically between the natural grassland and the agricultural land use, could suggest the importance of bottlenecks in this site or the combined effects of genetic drift and migration (Sesarini and Remis, 2008). Meanwhile, the lower genetic differentiation between land uses in Etchegoyen and between the agricultural and cattle-raising land uses in Universidad Nacional de Luján can be attributed to the shared ancestral polymorphisms or to the greater gene flow among the groups, benefited by the smaller geographic distance between them (less than 350 m).

Both the conventional F_{ST} analysis and the Bayesian approach for dominant markers showed that there were significant genetic differences between land uses within Universidad Nacional de Luján, showing that the observed genetic differentiation can be explained by distance isolation. The three models indicated a significant genetic differentiation for all pairwise comparisons, but suggested higher gene flow in the populations from Etchegoyen. The low genetic differentiation in Etchegoyen may be also considered as a result of a most recently agricultural management in this site.

The NJ tree revealed intermixed individuals, which formed groups by land-use origin or proximity. Furthermore, the Bayesian assignment analysis using STRUCTURE showed $K = 7$ in Universidad Nacional de Luján as the most likely number of genetic entities in the sample, and the individuals were significantly assigned to any genetic group. Indeed, Universidad Nacional de Luján supported more diversity of genetic groups that were more specific of the land use: three exclusive groups for the natural grassland, one exclusive group for the agricultural land use and one for the cattle-raising land use, and two groups shared by the agricultural and the cattle-raising land uses. In contrast, in Etchegoyen, there were only two genetic groups and they were shared among the three land-uses. We can suggest that Universidad Nacional de Luján is an environment with higher total genetic variability. This may be related to the effect caused by higher spatial heterogeneity.

The largest number of exclusive groups in the natural grassland in Universidad Nacional de Luján could be due to their greater geographic distance with the other two land uses (about 950 m), which were adjacent and that, consistently, shared groups. The homogeneity observed in Etchegoyen could be explained either by a more recent isolation (Hoelzel, 1998) or by a high genetic flow between the groups (Shaklee & Samollow, 1984), favoured by the proximity between the land uses.

Armadillidium vulgare could be considered an indicator species for the study of the impact of the change in soil uses on the edaphic fauna, since the level of polymorphism shows that it depends on many factors, including biotic ones. However, *A. vulgare* appears to be highly variable at the molecular level. In a study carried out in Europe and North America using enzymes, Garthwaite et al. (1995) reported a high level of genetic variability of *A. vulgare* within and between populations, especially in Europe. Other authors found similar results at smaller spatial scales, using mitochondrial DNA genetic markers (Rigaud et al., 1999) and RAPD (Homor et al., 2003). Additional evidence is provided by the huge list of detrimental effects of human activities on natural populations. The Pampean region of Argentina is no exception; more than 40 years of intensive agricultural and cattle-raising activity can isolate populations of natural grasslands in a smaller spatial scale. One possible explanation for this, although not the only one, is population isolation. Many background processes may explain the differences in the genetic makeup of populations. Genetic markers are useful because they can provide high levels of polymorphism but, in general, background processes remain uncertain.

In short, this study shows that the genetic diversity of *A. vulgare* was

lower in lands under agricultural use than in the other land uses in both sites studied. Thus, the loss of genetic variability, differentiation and structure in this species can be a good indicator of system disturbance.

Acknowledgments

This study was supported by PRH 19 of Agencia Nacional de Promoción Científica y Tecnológica and Universidad Nacional de Luján, Instituto de Ciencias (Universidad Nacional de General Sarmiento), Instituto de Ecología y Desarrollo Sustentable (INEDES – CONICET), and 15/E725 of Universidad Nacional de Mar del Plata, Argentina.

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