

# Impact of different agricultural practices on the genetic structure of *Lumbricus terrestris*, *Arion lusitanicus* and *Microtus arvalis*

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## Abstract

*Impact of different agricultural practices on genetic structure of Lumbricus terrestris, Arion lusitanicus and Microtus arvalis.*— Little attention has been given to date to the potential influence of agricultural land use methods or farming practice on the genetic variability of native species. In the present study, we measured the genetic structure of three model species —*Microtus arvalis*, *Arion lusitanicus* and *Lumbricus terrestris*— in an agricultural landscape with a diversity of land use types and farming practices. The aim of the study was to investigate whether different management strategies such as the method of land use or type of farming practice (conventional and ecological farming) have an impact on the species' genetic structure. We used RAPD markers and multilocus DNA fingerprints as genetic tools. Genetic similarity was based on the presence or absence of bands, which revealed a wide range of variability within and between the analysed populations for each model species. Cluster analysis and Mantel tests (isolation by distance) showed different genetic structures in the populations of *M. arvalis* from sampling sites with different land use. However, the main factors influencing the genetic variability of these vole populations were geographic distances and isolation barriers. The genetic variability observed in *A. lusitanicus* populations correlated with geographic distance and the type of land use method, but no correlation was found with different farming practices. Our preliminary results suggest that the genetic structure of *L. terrestris* populations is influenced by the agricultural land use method used at the different sampling sites but not by the geographic distance.

Key words: *Arion lusitanicus*, Genetic structure, Land use, *Lumbricus terrestris*, *Microtus arvalis*, DNA fingerprinting.

## Resumen

*La influencia de distintas prácticas agrícolas en la estructura genética de Lumbricus terrestris, Arion lusitanicus y Microtus arvalis.*— Hasta la fecha se ha prestado poca atención a la influencia potencial de las distintas formas de uso del suelo o de las prácticas agrícolas en relación a la variabilidad genética de las especies autóctonas. En el presente estudio se analizó la estructura genética de tres especies representativas —*Microtus arvalis*, *Arion lusitanicus* y *Lumbricus terrestris*— en suelos agrícolas sometidos a distintos usos del suelo y prácticas agrícolas. El objetivo de este estudio es evaluar si las distintas estrategias de gestión tales como el método de cultivo o el tipo de práctica agrícola empleada (convencional o ecológica) pueden influir en la estructura genética de las especies. Como herramienta de análisis genético se aplicaron las técnicas RAPD (RAPD markers) y de las huellas genéticas multilocus del DNA (multilocus DNA fingerprinting). La semejanza genética fue evaluada en base a la presencia o ausencia de bandas, que reveló una amplia variabilidad dentro y entre las poblaciones analizadas de cada especie modelo. A través del análisis de conglomerados y del test de Mantel (aislamiento por la distancia) se comprobó que las poblaciones de *M. arvalis* procedentes de muestreos en suelos con distintos usos presentaban distintas estructuras genéticas. Sin embargo, la distancia geográfica y el aislamiento por barreras fueron los principales factores influyentes sobre la variabilidad genética de estas poblaciones de topillo de campo. En el caso de *A. lusitanicus* se pudo observar que la variabilidad genética de sus poblaciones estaba correlacionada con las distintas formas de uso del suelo y la distancia geográfica, pero no se halló correlación alguna con las distintas prácticas agrícolas. Nuestros resultados preliminares

sugieren que la estructura genética de las poblaciones de *L. terrestris* se ve influida por el tipo de uso del suelo de los distintos lugares de muestreo, pero no por la distancia geográfica.

Palabras clave: *Arion lusitanicus*, Estructura genética, Usos del suelo, *Lumbricus terrestris*, *Microtus arvalis*, DNA fingerprinting.

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## Introduction

Over the last decades, numerous agrarian ecological studies have focused on the impact of land use on animal populations (Hurd & Fagan, 1992; Müller, 1995; Jacob & Hempel, 2003; Gehring & Swihart, 2003; Dauber & Wolter, 2004). The authors of these studies suggest that population dynamics of mainly depend on land use methods and farming systems. Although studies in conservation genetics have been carried out for over 20 years (Soulé, 1980; Frankel & Soulé, 1981; Schonewald-Cox et al., 1983), limited attention has been given to the potential influences of agricultural land use methods and farming practice on the genetic variability of native species living therein. This is of particular interest, for example, to determine whether pest organisms develop genetic resistance to synthetic chemical pesticides (Hawksworth, 1991; Avise & Hamrick, 1995; Dickson & Whitman, 1996; Pons et al., 1998; Nevo, 2001; Pearman, 2001; Weibull et al., 2003).

Native species often face habitat loss and fragmentation in landscapes which have been modified by humans for agriculture (Cale, 2003). Such changes may include a decline in the size of habitat patches and an increase in their spatial and genetic isolation (Saunders et al., 1991). However, it is generally considered essential that levels of genetic diversity remain constant in order to maintain long-term conservation of populations (Frankel & Soulé, 1981; Simberloff, 1988; Opdam, 1990). Decreased levels of genetic variation can lead to inbreeding depression, reducing a population's ability to adapt to short-term environmental disturbances (Allendorf & Leary, 1986; O'Brien & Evermann, 1988; Milligan et al., 1994), hindering adaptation to long-term environmental changes and possibly leading to extinction (Gilpin & Soulé, 1986; Lacy, 1997). Fragmentation or destruction of habitats, through agriculture for example, is a major cause of the decline of genetic variability and also genetic exchange among populations (Lande & Shannon, 1996; Frankham, 1995; Bjornstad et al., 1998).

The earthworm *Lumbricus terrestris* (Linnaeus 1758) plays a valuable role in nutrient cycles and energy flows in terrestrial ecosystems. Due to their biology, earthworm populations can indicate the structural, microclimatic, nutritive and toxic situation in soils (Christensen, 1988; Edwards & Bohlen, 1996; Edwards, 1998). They are therefore a suitable model species as their genetic variability should be strongly influenced by diverse agricultural practices such as soil tillage, pesticide use, fertilization and crop rotations (Piffner, 2000).

The Spanish slug *Arion lusitanicus* (Mabille 1868) was originally a local species in the Western Iberian peninsula (Chichester & Getz, 1969). Distributed by man, it is now introduced in all of Europe. Due to its high reproduction rate and great genetic adaptability to changing environmental conditions, *A. lusitanicus* is an ideal indi-

cator to detect the effects of short-term land use on genetic structure, particularly because its life expectancy is usually only one year.

Rodents are very suitable as indicators of land use effects as their reproduction rates and ecological valence are generally high. Land management can affect population density, survival and breeding (Jacob & Hempel, 2003). The Common vole *Microtus arvalis* (Pallas, 1779) commonly uses agroecosystems as its habitat; farming practices such as the use of pesticides and fertilizers, and the removal of shelter, food nesting sites and overwintering sites may cause the animal considerable stress (Jacob & Brown, 2000). In small isolated populations, genetic diversity may decrease due to genetic drift or inbreeding (Van Treuren et al., 1993). This can seriously diminish their potential to adapt to changing environments, decrease average individual fitness and consequently increase the extinction risk of populations (Hedrick et al., 1996; Bijlma, 1994, 2000).

In the present study, we measured the genetic variability of the three selected model species (*M. arvalis*, *A. lusitanicus* and *L. terrestris*) in an agricultural landscape which included several types of land use types and different farming practices. Our objective was to analyse whether diverse agricultural management strategies lead to different genetic structure in the selected taxa. As genetic tools, we used RAPD markers to analyse the populations of *A. lusitanicus* and *L. terrestris* and multilocus DNA fingerprinting to analyse the genetic structure of *M. arvalis* because the screened RAPD primers revealed no polymorphic and reproducible RAPD marker.

## Materials and methods

### Site descriptions

This study was carried out in two agricultural landscapes in western Germany (fig. 1), one site in northern Saarland (Wahlen) and the other in western Rhineland-Palatinate (Herl/Trier). The sampling sites for the three model species were selected based on different agricultural land types such as arable land (conventional maize, conventional, integrated and ecological barley, forage), meadow and fallow land. The geographic distance between the two sampling sites was about 33 km (the geographic distance between the different sampling locations at each sampling site was 0.1 to 1.8 km). We created digitalized land use maps (years 1999–2001) on the basis of the official cadastral maps for both sites.

### Sampling design

The sampling design for the analyzed populations was generally based on two different levels. First, we analyzed populations from geographically separated sites. Second, we analyzed within each site

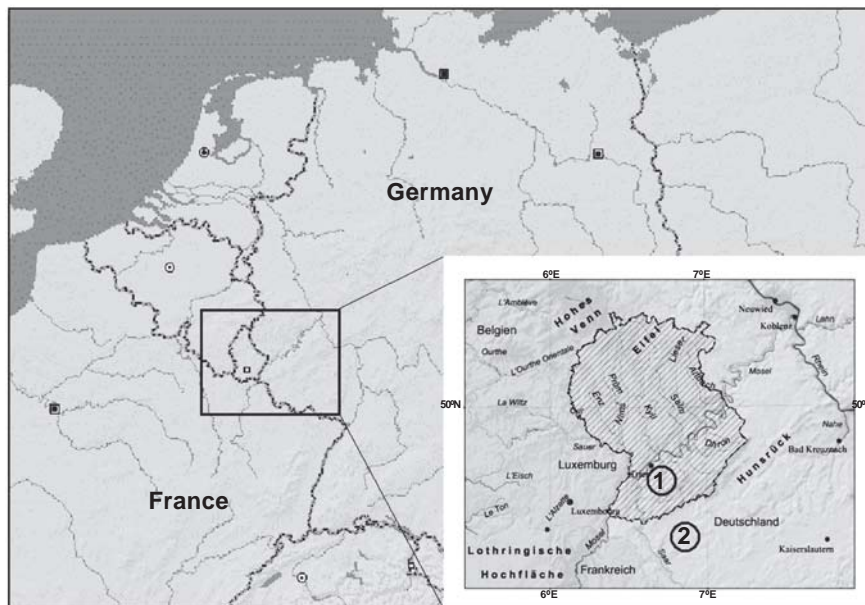


Fig. 1. Sampling sites: 1. Herl, near Trier; 2. Wahlen.

Fig. 1. Localidades de muestreo: 1. Herl, cerca de Tréveris; 2. Wahlen.

populations from sampling locations with different land use types (see table 1). Between the two geographically separated sites, Herl and Wahlen, there is no possibility of human-mediated dispersal of the three model species (there is no human-mediated transfer of soil, plants, eggs or organisms between the two sites). Species sampling was carried out during April and October 2000. Seventeen individuals of *L. terrestris* were collected from the soil using electrical sampling technique according to a standard operation procedure guideline (Klein & Paulus, 1995). Nineteen individuals of *A. lusitanicus* were collected by hand from 10 different sampling sites (190 slugs in total) and 12–14 individuals of *M. arvalis* were trapped in live capture traps in 5 different fields (65 voles in total). Muscle tissue samples of all collected individuals were stored at  $-20^{\circ}\text{C}$ .

#### DNA-techniques

**Multilocus DNA fingerprinting:** all eukaryotic genomes contain many polymorphic loci known as variable number of tandem repeats (VNTRs). Polymorphisms at such loci are the result of variations in the number of tandem repeats of a short core sequence. DNA probes comprising tandem repeats of a core sequence are used to hybridize multiple variable DNA fragments, and produce an individual-specific multilocus DNA fingerprint (Jeffreys et al., 1985; Epplen et al., 1991; Wan & Fang, 2003). This can be performed by the appli-

cation of a mixture of single locus probes or application of a single probe that identifies multiple similar sequence polymorphisms. In the latter case, one is detecting unidentified fragments of DNA and the result is therefore a DNA phenotype rather than a genotype.

**RAPD-PCR fingerprinting:** a single 10-base oligonucleotide primer is used to amplify genomic DNA for the RAPD-PCR technique (Williams et al., 1990; Welsh & McClelland, 1990). A DNA amplification product is generated for each genomic region that happens to be flanked by a pair of 10-base priming sites (in the appropriate orientation), which are within about 5,000 base pairs of each other. Amplification products are analysed by gel electrophoresis. Genomic DNA from two different individuals often produces different amplification fragment patterns. A particular DNA fragment, which is generated for one individual but not for another, represents a DNA polymorphism and can be used as a genetic marker.

#### DNA extraction

Genomic DNA from muscle tissue was extracted with a modified salt-chloroform method (Müllenbach et al., 1989). The frozen tissue (10–20 mg) was ground in liquid nitrogen, transferred to a sterile Eppendorf tube with 0.5 ml of extraction buffer (160 mM Saccharose, 80 mM EDTA, 100 mM Tris/HCl, pH 8.0), 20  $\mu\text{l}$  Proteinase K (20 mg/ml) and incubated for 12–18 h at

Table 1. Analysed species, number of analysed individuals (N), and description of the sampling sites (Ss: H. Herl; W. Wahlen) and locations including land use (Sl: Cm. Conventional maize; Eb. Ecological barley; Ef. Ecological forage; Fl. Fallow land [FIH. Fallow land Hohberg; FIZ. Fallow land Zill]; lb. Integrated barley; Cb. Conventional barley; M. Meadow).

Table 1. Especies analizadas, número de individuos analizados (N) y descripción de los lugares de muestreo (Ss: H. Herl; W. Wahlen) y su localización incluyendo el uso del suelo (Sl: Cm. Maíz convencional; Eb. Cebada ecológica; Ef. Forraje ecológico; Fl. Tierra de barbecho; [FIH. Barbecho Hohberg; FIZ. Barbecho Zill]; Cb. Cebada convencional; M. Prado).

Species	Ss	Sl	N
<i>L. terrestris</i>	H	Cm	17
		Eb	17
		Fl	17
	W	Cm	17
<i>A. lusitanicus</i>	H	Eb 01	19
		Ef 02	19
		Ef 03	19
		Eb 04	19
		lb 05	19
		Cb 06	19
		Cb 07	19
		Fl 08	19
		M 09	19
		W	M10
<i>M. arvalis</i>	H	Cb	13
		Eb	14
		Fl	14
	W	FIH	12
		FIZ	12

65°C. After the addition of 180 µl 6 M NaCl, proteins and lipids were removed by two extraction steps with 500 µl phenol–chloroform–isoamyl alcohol (25:24:1). DNA was precipitated by the addition of a double volume of cold ethanol. A DNA pellet was recovered by centrifugation, washed in 70% ethanol, dried and dissolved in 300 µl of sterile water.

### RAPD profiling

Several oligonucleotide primers (Roth GmbH) were surveyed and the most intense and reproducible bands for each species were selected (table 2). Amplifications were carried out in 25 µl volumes containing 2 µl of template DNA (~100 ng), 18 µl of sterile H<sub>2</sub>O, 2.5 µl of 10 x PCR buffer (DNAzyme™ II), 0.5 Units (0.25 µl) DNAzyme™ II Polymerase (FINNZYMES), 2.5 µl of 10 mM primer and 0.5 µl of 10 mM dNTPs (Amresco). The DNA amplification was performed in a thermal cycler (TGradient, Biometra) programmed for an initial denaturation of 120s at 94°C, followed by 45 cycles of 30s at 94°C, 60s at 38°C, and 120s at 72°C. The final primer extension step was extended to 10 min at 72°C. Polymerase chain reaction (PCR) products were analysed by electrophoresis on 1.4% agarose gels in 1 x TBE buffer (0.089 M Tris–borate, 2 mM EDTA, pH 8.0) for 4 hours at 70 V (55 mA), visualized by staining with ethidium bromide and photographed under UV light with a Polaroid type 667 film (Polaroid Corp.). Precautions were taken to ensure PCR reproducibility. PCR conditions were optimized following Bielawski et al. (1995), excluding influence of different concentration of genomic DNA. Concentrations of 50 ng/µl genomic DNA were used. Additionally, one randomly chosen sample was amplified with each PCR as reference and one sample was amplified twice in the same PCR. RAPD profiles were replicated by at least two PCR amplifications for each individual genotype so that irregularities could be detected immediately in amplification and electrophoresis conditions.

### Multilocus DNA fingerprinting

The DNA (50 mg) was digested to completion with 100 units of the restriction enzyme HinfI. DNA fragments were resolved on a 0.8% agarose gel, stained with ethidium bromide, in TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA) for 24h at 1.0 V/cm. After electrophoresis, the DNA fragments were transferred to nylon membranes (Zetaprobe, Bio–Rad) by vacuum blotting and baked at 80°C for 2h. Preblocking, prehybridization, hybridization and blocking as well as detection of the hybridization signal were performed according to the manufacturer's protocol (Roche Diagnostics GmbH). The digoxigeninated multilocus probes (GACA)<sub>4</sub> and (GTG)<sub>5</sub>, (Roche Diagnostics GmbH) were hybridized to the nylon membran at 38°C for (GACA)<sub>4</sub> and 40°C for (GTG)<sub>5</sub> for 5h.

### Statistical analysis

The repeatability of the RAPD amplification was checked first, and only fragments with 100% repeatability in amplification reactions were included in further analysis.

Each reproducible band in the RAPD profiles was treated as an independent locus with two



Table 2. Characteristics of the RAPD–primers, oligonucleotide probe and level of polymorphism for the RAPD and multilocus fingerprint markers: Pn. Primer name (Carl Roth GmbH & Co., Karlsruhe, Germany); S(5'–3'). Sequence (5'–3'); G+C. DNA content of Guanine and Cytosine; Fs. Fragment size; Nb. Number of bands; Pb. Polymorphic bands; Re. Restriction enzyme; Op. Oligonucleotide probe.

Table 2. Características de los cebadores (primers) RAPD, la sonda de oligonucleótidos y el nivel de polimorfismo de los marcadores de huellas genéticas multilocus y RAPD: Pn. Nombre del cebador (Carl Roth GmbH & Co., Karlsruhe, Germany); S(5'–3'). Secuencia (5'–3'); G+C. Contenido de Citosina y Guanina en el ADN; Fs. Tamaño del fragmento; Nb. Número de bandas; Pb. Bandas polimórficas; Re. Enzima de restricción; Op. Prueba del oligonucleótido.

Species	Pn	S(5'–3')	G+C (%)	Fs (bp)	Nb	Pb (%)
<i>L. terrestris</i>	180–08	CGCCCTCAGC	80	320–1500	25	23 (92.0)
<i>L. terrestris</i>	A–10	GTGATCGCAG	60	290–1470	18	16 (88.9)
<i>L. terrestris</i>	B–10	CTGCTGGGAC	70	390–1610	17	16 (94.1)
<i>A. lusitanicus</i>	270–05	GCCCTCTTCG	70	460–1860	26	25 (96.2)
<i>A. lusitanicus</i>	380–3	GGCCCCATCG	80	530–1840	19	18 (94.7)
<i>A. lusitanicus</i>	480–4	CGCCACGAGC	80	510–1860	25	25 (100.0)
<i>A. lusitanicus</i>	Arion lus.	GTAGTCTCGC	60	380–1940	24	24 (100.0)
Species	Re	Op		Fs (kbp)	Nb	Pb (%)
<i>M. arvalis</i>	Hinf I	(GACA) <sub>4</sub>		1.0–23.5	134	134 (100.0)

alleles, presence or absence of a band. RAPD markers (ranging from 0.1 to 2.3 kb) were scored for presence (1) or absence (0) and entered into a binary matrix representing the RAPD phenotype of each individual genotype. The statistical analyses (similarity indices and genetic distances) were calculated according to the methods of Lynch (1991) and Lynch & Milligan (1994). We further investigated genetic relationships within and between populations by cluster analysis based on Euclidean distances (Statistica 5.0 for Windows, StatSoft, Inc.) using UPGMA (unweighted pair group method using arithmetic average, Sneath & Sokal, 1973). Mantel tests (Mantel, 1967) were performed in order to correlate the matrix of genetic distance and the geographical distance (analysis of isolation by distance, GENEPOP software, version 3.1, Slatkin, 1993; Raymond & Rousset, 1995). The significance of matrix correlation was evaluated by comparing the observed Mantel test statistic, Z, with its random distribution obtained after 1,000 permutations. All 10mer oligonucleotide primers for RAPD–PCR (ROTH Kit 170, 180, 270, 280 and Kit A through D, 120 primers in total) were tested on two different individuals of *L. terrestris* and *A. lusitanicus* respectively. The primers used for statistics were selected by the same method as described by Bowditch et al. (1993) and Allegrucci et al. (1995). For the statistical analyses, all markers of the selected primers were combined as suggested by Williams et al. (1993).

#### Multilocus DNA fingerprinting

Presence (1) or absence (0) of a band at a particular position in the multilocus DNA fingerprint (ranging from 2.5 to 23 kb) was treated as a discrete character, and banding patterns were converted

Table 3. Mean  $S_{ab}$  (based on 60 RAPD markers) within (*italic*) and between the analysed populations of *L. terrestris* from the different sampling locations. (For abbreviations see table 1.)

Table 3.  $S_{ab}$  media (basada en 60 marcadores RAPD) en cada población (*cursiva*) y entre las poblaciones analizadas de *L. terrestris* de diferentes localidades de muestreo. (Para las abreviaturas ver tabla 1.)

	Cm(H)	FI(H)	Eb(H)	Cm(W)
Cm(H)	0,735	0,492	0,533	0,537
FI(H)		0,644	0,504	0,482
Eb(Herl)			0,667	0,508
Cm(W)				0,605

into binary matrices. The total banding number of each isolate and the number of bands shared by each pair of isolates were counted. The similarity index ( $S_{ab}$ ) was calculated according to the formula:  $S_{ab} = 2 \times n_{ab} / (n_a + n_b)$ , where  $n_a$  and  $n_b$  represent the total number of bands present in the DNA fingerprint patterns of sample a and b, respectively, and  $n_{ab}$  is the number of bands shared by a and b (Nei & Li, 1979; Lynch, 1991). Subsequently, pairwise genetic distance ( $D_{ab}$ ) between sample a and b was calculated according to the method of Lynch (1991). The resulting genetic distance values were used as the basis of cluster analysis (UPGMA), and Mantel tests were performed to test isolation by distance.

### Results and discussion

The selection of primers used for the statistical analysis was based solely on the repeatability of patterns, not on the degree of polymorphism displayed by a primer. The seven informative primers chosen for the analysis of *L. terrestris* (three primers) and *A. lusitanicus* (four primers) are listed in table 2. In total, 147 polymorphic bands out of 154 unambiguous and reproducible products were generated with the selected primers, corresponding to 95.5% polymorphism. The primers used in the present study shared no specific motifs and the high percentage of polymorphic bands demonstrate, that the selected primers have generated predominantly independent RAPD markers.

#### *Lumbricus terrestris*

Based on the reproducibility of the amplified RAPD markers, three primers were chosen for all samples of *L. terrestris* (table 2). The analysed PCR products ranged from 290 to 1610 bp. In the 68 individuals of the 4 earthworm populations studied, the three RAPD primers amplified a total of 60 scorable fragments; of those, 55 (91.7%) of which were polymorphic. Within all populations of *L. terrestris* analysed, the similarity indices ( $S_{ab}$ , see table 3) were of a relatively similar high value (range between 0.605 and 0.735). Among the populations,  $S_{ab}$  were much lower (between 0.482 and 0.537). Using Lynch & Milligan's (1994) correction for RAPD loci, Nei's distances (1972) between all pairs of samples were smallest between the earthworm populations of the two maize fields (0.085), although the geographic distances were highest. This finding was confirmed by a UPGMA cluster analysis based on the genetic distances. The UPGMA tree (fig. 2), based on Euclidean distances between the genetic distances  $D_{ij}$  of all analysed populations, revealed two main clusters, one of the two populations from the maize fields in Herl and Wahlen and one from the two other fields (fallow and barley,  $D_{ij} = 0.112$ ) in Herl. To test isolation by distance, the results from genetic distance measures were entered

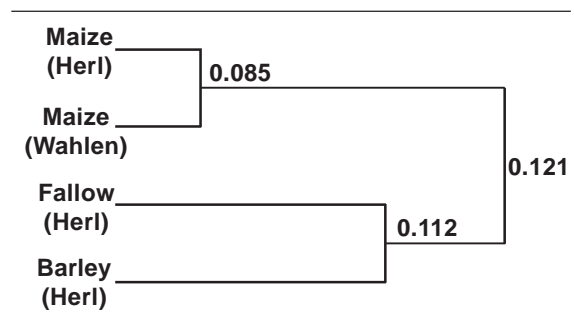


Fig. 2. UPGMA dendrogram based on genetic distances  $D_{ij}$  for the four populations of *L. terrestris* from the different farming fields.

Fig. 2. Dendrograma UPGMA basado en las distancias genéticas  $D_{ij}$  para las cuatro poblaciones de *L. terrestris* de los diferentes terrenos agrícolas.

into a Mantel test (1,000 permutations) with geographic distance (fig. 3A). The results from this test revealed no significant correlation between geographic and genetic distance in these populations ( $D_{ij}$  vs. geographical distance  $r = -0.564$ ,  $P = 0.958$ ). This confirmed the finding obtained from cluster analysis.

The results for earthworms in this study agree with previously published Lumbricides' findings. Using RAPD PCR of *Aporrectodea* spp., Dyer et al. (1998) in Australia showed with that the analysed populations also exhibit a high degree of homogeneity. Stille et al. (1980) found a small amount of genetic variability with enzyme investigations of *Aporrectodea tuberculata* based only on geographical separation. Several authors (e.g. Brooks et al., 1995; Pfiffner & Mäder, 1997; Blakemore, 2000) have likewise shown that earthworms populations are strongly influenced by diverse cultural practices, such as soil tillage, use of pesticides, fertilisation and crop rotations (crop residues). Finally, by means of enzyme investigations of *L. rubellus* in the Faroe Islands, Enckell et al. (1986) determined that geographical barriers or distances have only a slight, or no influence on genetic variation between different populations. In summary, our results coincide with previously published findings reporting that the genetic structure of *L. terrestris* populations is first dependent on the farming practice and only secondary affected through isolation by distance.

#### *Arion lusitanicus*

For the populations of *A. lusitanicus*, the four oligonucleotide primers (Roth GmbH, see table 2) were selected for their intense and reproducible bands.

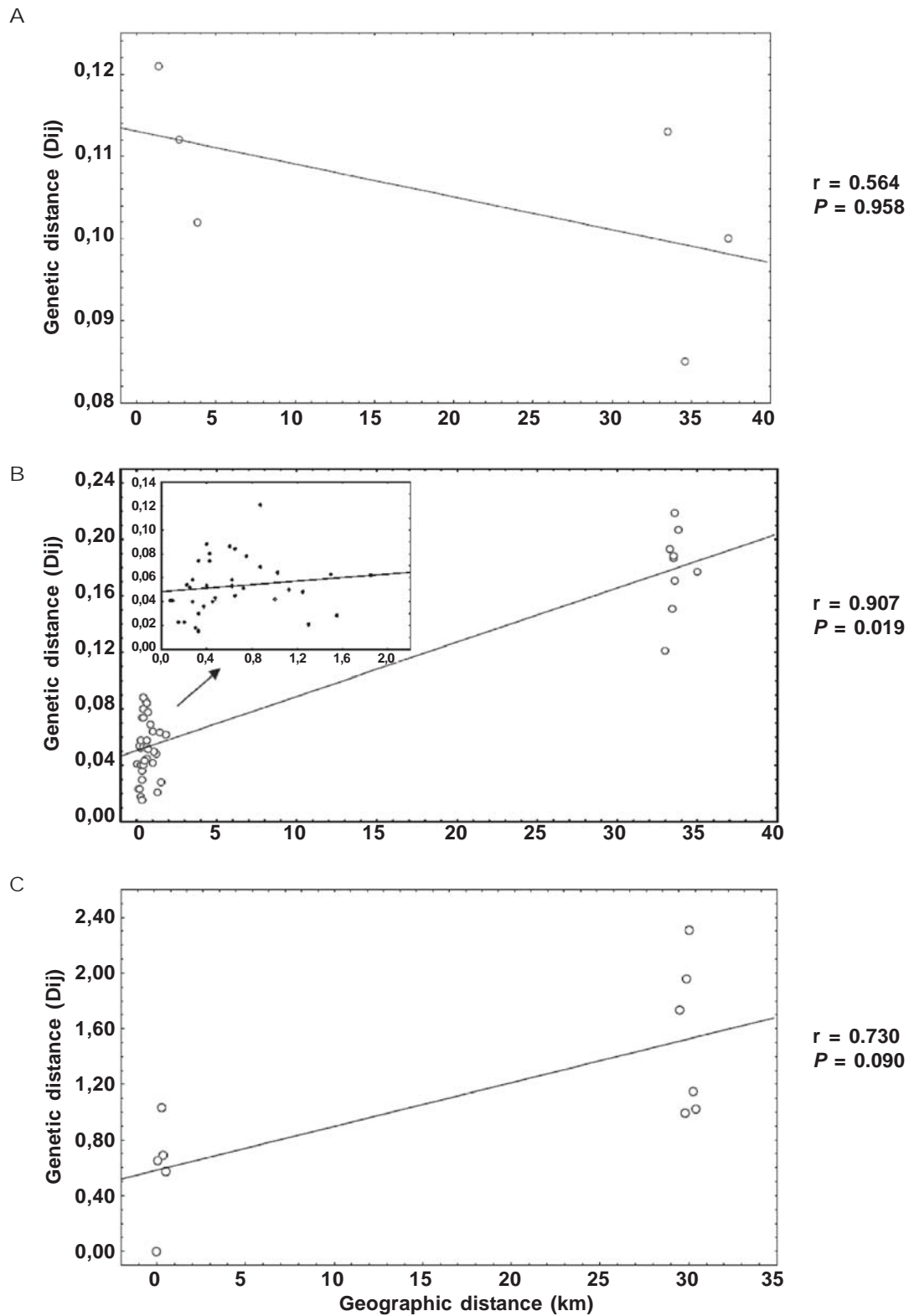


Fig. 3. Relationship (isolation by distance) between genetic distance  $D_{ij}$  (Lynch, 1991) and geographic distance of the analyzed populations: A. *L. terrestris*; B. *A. lusitanicus*; C. *M. arvalis*.

Fig. 3. Relación (aislamiento por la distancia) entre la distancia genética  $D_{ij}$  (Lynch, 1991) y la distancia geográfica de las poblaciones analizadas: A. *L. terrestris*; B. *A. lusitanicus*; C. *M. arvalis*.



Table 4. Mean  $S_{ab}$  (based on 94 RAPD markers) within (*italic*) and between the analysed populations of *A. lusitanicus*. (For abbreviations see table 1.)

Table 4.  $S_{ab}$  media (basada en 94 marcadores RAPD) en cada población (*cursiva*) y entre las poblaciones analizadas de *A. lusitanicus*. (Para las abreviaturas ver tabla 1.)

	Sampling locations									
	Ib H05	Cb H06	Cb H07	Eb H01	Ef H02	Ef H03	Eb H04	FI H08	M H09	M W10
Ib H05	<i>0.710</i>									
Cb H06	0.686	<i>0.736</i>								
Cb H07	0.713	0.733	<i>0.784</i>							
Eb H01	0.660	0.672	0.693	<i>0.708</i>						
Ef H02	0.684	0.709	0.730	0.680	<i>0.735</i>					
Ef H03	0.686	0.725	0.732	0.675	0.715	<i>0.729</i>				
Eb H04	0.685	0.720	0.745	0.637	0.706	0.717	<i>0.747</i>			
FI H08	0.668	0.645	0.660	0.632	0.649	0.638	0.638	<i>0.718</i>		
M H09	0.674	0.683	0.706	0.637	0.663	0.685	0.685	0.643	<i>0.685</i>	
M W10	0.614	0.624	0.637	0.623	0.614	0.617	0.654	0.600	0.608	<i>0.721</i>

The statistically evaluated RAPD markers ranged in size from 380 to 1940 bp. In the 190 individuals of the ten populations of *A. lusitanicus* studied, the primers yielded a total of reproducible 94 fragments. Of those, 92 (97.9%) were polymorphic. Within all analysed populations, the similarity indices ( $S_{ab}$ , see table 4) were of a relatively similar high value (range between 0.685 and 0.784). Between the populations  $S_{ab}$  are lower (between 0.632 and 0.733). Values of genetic distance were measured following Lynch (1991).

The highest genetic distances (range from 0.121 to 0.219) can be detected between the geographically most isolated sites from Herl and Wahlen. However, no clear genetic separation between the different farming fields in Herl was seen (values on a relatively low level ranged from 0.015 to 0.088). This result was confirmed by a cluster analysis based on the genetic distances. The UPGMA tree (fig. 4), based on Euclidean distances between the genetic distances  $D_{ij}$  of all analysed populations, revealed one main cluster (all sampling sites in Herl) and one exterior branch (the population of Wahlen). The main cluster can be subdivided into the slug populations of the arable fields (forage and barley sites) and the two analyzed meadow and fallow populations. Pfenninger (2002) suggested that the population structure of the terrestrial snail *Pomatias elegans* is mainly a function of the habitat quality and of the spatial arrangement of the habitat network in the landscape and not solely a function of the geographic distance.

To test isolation by distance in our study, the results from genetic distance measures were entered into a Mantel test (1,000 permutations) with geographic distance (fig. 3B). The results suggest a significant isolation by distance in these populations. ( $D_{ij}$  vs. geographical distance  $r = 0.907$ ,  $P = 0.019$ ). This agrees with the study of Ross (1999) where genetic distances of the lowa Pleistocene snail (*Discus macclintocki*) were strongly related to the geographical distance between all populations; the relationship between genetic distance and watershed distance was especially significant ( $P = 0.0196$ ). This isolation by distance is also consistent with a study by Pfenninger et al. (1996) with RAPD markers, which found that genetic distance and geographical distance were highly correlated in a similarly sized snail, *Trochoidea geyeri* (Mantel test:  $r = 0.567$ ,  $P < 0.0001$ , 1,000 permutations). Preliminary results on mtDNA variation in *Helix aspersa* (Guiller et al., 2001) showed that estimated pairwise correlations between sets of genetic, molecular, morphometric and spatial measurements in northern African colonies indicate that anatomical, and especially biochemical variation is significantly associated with spatial position of sampling localities. The correlation between geographical and Nei's distance was  $r = 0.72$  (Madec et al., 1996), while  $r = 0.50$  between geographical and molecular (Kimura 2-parameter) distance. In conclusion, consistent with the literature, we also found an isolation by distance system for the slugs analysed, as well as a distinct influence of the different land use types.

*Microtus arvalis*

The structure and amount of genetic variation within and between populations of the common vole *M. arvalis* were assessed by multilocus DNA fingerprinting. To obtain informative fingerprints, a suitable combination of multilocus minisatellite probes and restriction enzymes had to be found in order to produce the best compromise between a large number of detected bands and minimize the proportions of shared band patterns. As probes we tested (CA)<sub>8</sub>, (GTG)<sub>5</sub>, (GACA)<sub>4</sub> and (GATA)<sub>4</sub>, and as restriction enzymes we used EcoR1, Hae III, Hind III and Hinf I. The best variable multibanded pattern was obtained by the combination of Hinf I and (GACA)<sub>4</sub>. Scorable bands were found ranging from 1.0 to 23.5 kb in all populations. A total of 134 informative bands were detected for all individuals (n = 65), and the mean number of bands per individual was 14.3 ± 0.5. All multilocus fingerprint markers were polymorphic. The genetic similarity within all populations was found relatively low (table 5). Similarity indices within the fallow populations (0.344–0.447) were significantly higher than in the two barley populations (0.152 and 0.202). This result might reflect a smaller effective population size (N<sub>e</sub>) in the barley fields in relation to the fallow land. The calculation of mean similarity indices between all analyzed populations was significantly lower (Mann–Whitney U–test, P = 0.005) than values within populations (table 5). Only the mean similarity index between the two

Table 5. Comparison of the mean similarity indices (S<sub>ab</sub>) detected by multilocus DNA fingerprinting within (*italic*) and between *M. arvalis* populations from the different sampling locations. (For abbreviations see table 1.)

Table 5. Comparación de los índices de similitud media (S<sub>ab</sub>) detectados por huellas genéticas multilocus del DNA en cada población (*cursiva*) y entre las poblaciones de *M. arvalis* de diferentes localidades de muestreo. (Para las abreviaturas ver tabla 1.)

	FIZ(W)	FIH(W)	Eb(H)	Cb(H)	FI(H)
FIZ(W)	<i>0.447</i>	0.264	0.111	0.037	0.069
FIH(W)		<i>0.426</i>	0.093	0.092	0.038
Eb(H)			<i>0.202</i>	0.091	0.094
Cb(H)				<i>0.152</i>	0.115
F(H)					<i>0.344</i>

fallow populations of Wahlen (0.264) showed a relatively high value. The low similarity indices analyzed in this study agree with values detected in unrelated individuals of pine voles (*M. pinetorum*, Marfori et al., 1997). These results suggest a high

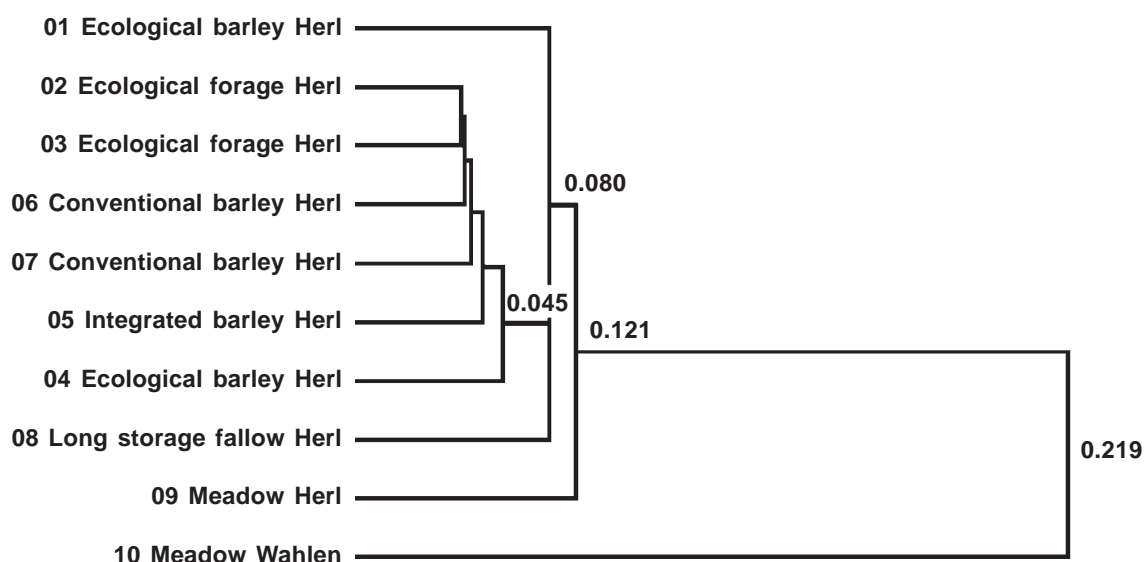


Fig. 4. UPGMA dendrogram based on genetic distances  $D_{ij}$  for the ten populations of *A. lusitanicus* from different land use and farming types.

Fig. 4. Dendrograma UPGMA basado en las distancias genéticas  $D_{ij}$  de las diez poblaciones de *A. lusitanicus* procedentes de distintos usos del suelo y prácticas agrícolas.

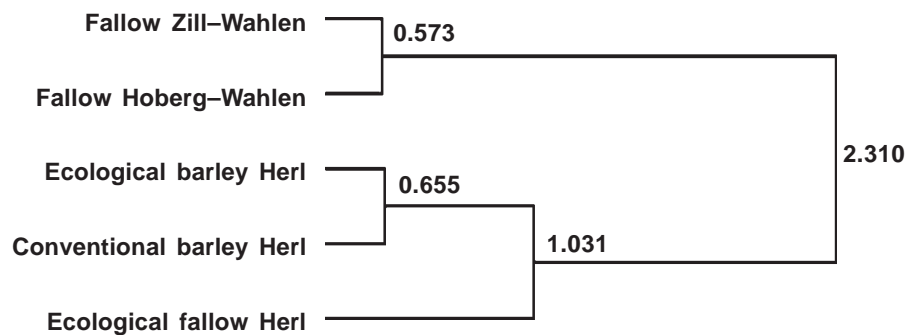


Fig. 5. Genetic relationship (UPGMA tree) between the populations of *Microtus arvalis* based on genetic distances according Lynch (1991) revealed by multilocus DNA fingerprinting.

Fig 5. Relación genética (árbol UPGMA) entre las poblaciones de *Microtus arvalis*, basada en las distancias genéticas según Lynch (1991), reveladas por la técnica de las huellas genéticas multilocus del DNA.

level of genetic variability of common voles within populations as well as between geographically separated populations.

Additional insights on genetic structure are offered by a cluster analysis, based on Euclidean distances between the genetic distances (Lynch, 1991) of all populations. The UPGMA tree (fig. 5) revealed two main clusters. The first division of the dendrogram separated the two populations of Wahlen from the sampling sites in Herl according to their geographic distance. The second division of the tree subdivides the different land use types (barley and fallow land) of the sampling site Herl. However, a possible influence of farming practice (ecological or conventional farming) on the genetic structure of *M. arvalis* cannot be detected. The result observed in this study contrasts with that found for the spatial behavior of common voles in relation to different farming practices (Jacob & Hempel, 2003). However, the results of the cluster analysis are confirmed by the analysis of isolation by distance (fig. 3C). Increased genetic distance was associated with increased geographical distance but the Mantel test revealed no significant correlation ( $r = 0.730$ ,  $P = 0.090$ , 1,000 permutations). A comparison of our results with previously published data might be difficult because no other multilocus DNA fingerprinting findings of *M. arvalis* are available. Van de Zande et al. (2000) also observed a high level of genetic variability within root vole (*M. oeconomus*) populations revealed by microsatellite markers. In summary, the relatively low genetic similarity of *M. arvalis* observed in our study agrees with other vole studies. In contrast with other findings, we did not find a clear influence of different farming practices on vole genetics.

In conclusion, populations of *M. arvalis* from sampling sites with different land use show different genetic structures; however, geographic distances and isolation barriers are the main factors influencing the genetic variability of *M. arvalis* populations. The genetic variability in *A. lusitanicus* populations is correlated with geographic distance as well as with different land use methods (i.e. fallow land, meadow land or barley). However, different farming forms (conventionally or ecologically) show no significant influence on the slug genetics. Our results suggest that the genetic structure of *L. terrestris* populations is influenced by the agricultural land use method practiced on the different sampling sites but not by geographical distance. Although *L. terrestris* and *A. lusitanicus* are hermaphrodite species, self-fertilisation could not be detected in this study because no identical RAPD patterns for two individuals were revealed. Additionally, the level of polymorphism in all three analysed species showed comparably high values, near 100%, so that in spite of the different genetic methods used the statistical results were at least distantly related. For conservation management strategies in agriculturally used landscapes it would be relevant to determine what kind of organism should be protected. Depending on the species analysed, the type of farming practice or changes in land use can cause a severe impact on the genetic structure of populations. The three model organisms analysed in the present study showed high reproduction rates and therefore high effective population sizes so that within each sampling site a high genetic diversity can be observed. However, the loss of genetic variability due to intensive agricultural land use can significantly undermine the viability of populations, particularly for some long-lived species with lower reproductive rates.

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