Theor Appl Genet (2000)101:131–138

F. Balfourier · C. Imbert · G. Charmet

Evidence for phylogeographic structure in *Lolium* species related to the spread of agriculture in Europe. A cpDNA study

Received: 5 November 1999 / Accepted: 24 November 1999

Abstract In order to explain the present distribution area of natural populations of two forage grasses species (*Lolium perenne* and *L. rigidum*), we studied genetic variation for maternally inherited chloroplast DNA (cpDNA) in 447 individual plants from 51 natural populations sampled throughout Europe and the Middle East. The detection of polymorphism by restriction analysis of PCR-amplified cpDNA fragments resulted in the identification of 15 haplotypes. Hierarchical analysis of chloroplastic diversity showed a high level of within-population diversity while, for both species, we found that about 40% of the total diversity still remains among populations. The use of previous isozymes data enabled us to estimate the pollen to seed flow ratio: pollen flow appears to be 3.5 times greater than seed flow for *L. perenne* and 2.2 times higher for *L. rigidum*. A stepwise weighted genetic distance between pairs of populations was calculated using the haplotypes frequencies of populations. A hierarchical clustering of populations clearly divides the two species, while two main clusters of *L. perenne* populations show a strong geographical structure. Different scenario are proposed for explaining the distribution area of the two species. Finally, evidence attesting that these geographical structures are related to the spread of agriculture in Europe are presented and discussed.

Key words Chloroplast DNA · Diversity · Phylogeography · Pollen flow · Population genetics · Seed migration

Communicated by P.M.A. Tigerstedt

F. Balfourier (✉) · C. Imbert · G. Charmet
INRA, Station d'Amélioration des Plantes, 234 avenue du Brézet, F-63039 Clermont-Ferrand Cedex, France
e-mail: balfour@clermont.inra.fr
Fax: +33 04 73 62 44 53

Introduction

In a previous paper (Balfourier et al. 1998), we analysed genetic diversity in natural populations of two forage grass species of the genus *Lolium* (*L. perenne* and *L. rigidum*), using isozymes. These two species are wind-pollinated, self-incompatible outbreeding species. They are still widespread as natural populations: perennial ryegrass (*L. perenne*) is native to most of Europe and part of the Mediterranean and Middle East area, whereas *L. rigidum* is distributed all around the Mediterranean. They were introduced by man in most temperate countries of the world for agriculture and amenity purposes.

Despite a weak genetic differentiation, significant patterns of geographical variation with respect to diversity indices and allele frequencies have been observed in *L. perenne*; in contrast, no spatial organisation of diversity has been detected in *L. rigidum*. Hypotheses on the taxonomic relationships and the genetic and geographical origins of the two species have been proposed: while we can assume that *L. perenne* probably derived from a limited number of *L. rigidum* populations in the Middle East, it is still quite difficult to choose between two different scenario to explain the present distribution area of *L. perenne*. The first scenario would be a re-colonisation of Europe from several southern postglacial refugia. The second scenario would fit historical processes such as the emergence of primitive agriculture in the Middle East and its expansion towards Europe.

The aim of the investigation presented here was to settle the question by means of restriction fragment analysis (RFLP) of chloroplast DNA (cpDNA). As the chloroplast genome is maternally inherited in *Lolium* (Sears 1980; Kiang et al. 1994), it is suitable material for directly studying seed dispersal and also for inferring colonisation routes and reconstructing phylogenies.We interpreted the results in terms of colonisation history of the species and compared them with existing data for nuclear markers (isozymes).

Materials and methods

Plant material

A total of 51 populations belonging to the following five species of the genus *Lolium* (Terrell 1968) were used: 28 populations of *L. perenne* and 14 of *L. rigidum* were chosen from the previous study (Balfourier et al. 1998) in order to have representatives of a wide geographical range – from Denmark to North Africa, and from Ireland to Afghanistan; in addition, four populations of *L. temulentum*, four of *L. persicum* and one of *L. remotum* were sampled in the Middle East and added to this study as outgroup species. Indeed, these last three species, which are self-fertilised species of the genus *Lolium*, are supposed to have differentiated from a common ancestor before the outbreeding species (Charmet et al. 1997).

Most populations were collected as bulked seeds from at least 50 plants taken from an ecologically homogenous area of 100 –1000 m2.

DNA extraction

The CTAB method of Rogers and Bendich (1985) was used for DNA extraction. Total DNA was extracted from 10 individual plants per population of *L. perenne* and *L. rigidum* species and from only 3 individuals for each population of inbred species.

Polymerase chain reaction (PCR)-RFLP procedures

Total DNA of individual plants was used in PCR reaction involving a set of universal primers homologous to the most conserved coding regions of cpDNA and mitochondrial (mt)DNA, allowing the amplification of polymorphic non-coding regions.

In a first step, five pairs of chloroplast primers, one described in Taberlet et al. (1991) and four described in Demesure et al. (1995), and three pairs of mitochondrial primers, also described in Demesure et al. (1995), were used to identify and screen polymorphism on individuals from 5 populations selected for their large distances between geographical origin. Eight PCR products were digested with 20 different restriction endonucleases. This step permitted us to select three combinations of chloroplast-fragment/enzyme that exhibited readable polymorphism at both the intra- and interspecific levels.

In a second step, only these three PCR-fragment/enzyme combinations were used to characterise a total of 447 individuals from 51 populations.

The PCR method used was as described in Demesure et al. (1995). The restriction DNA fragments were loaded after denaturation onto a 5% denaturating polyacrylamide gel (8 *M* urea; 50 cm × 0.4 mm) and electrophoresed at 2,000 V for 3.5 h in 1 × TBE buffer. PCR products were visualised using the silver-nitrate staining method as described by Tixier et al. (1997).

Genetic diversity analysis

Since all polymorphisms are supposed to be completely linked on the chloroplast genome (Chiu and Sears 1985), the study was done at the multi-restriction site level, i.e. each cytotype (haplotype) was considered as one allele at a single haploid locus.

The frequencies of the different haplotypes in each population were used to estimate the total diversity (h_{TC}), the mean within-population diversity (h_{SC}) and the coefficient of differentiation (G_{STC}), following the procedures of Nei (1987), with correction for small sample size.

Estimation of the relative rates of pollen and seed migration among populations was calculated according to Ennos (1993) using isozyme data obtained from a previous study on 12 polymorphic loci (Balfourier et al. 1998) to calculate the same indices at the nuclear level (h_{SN}, h_{TN}, G_{STN}) and to estimate nuclear gene flow. Standard errors of the above measures of diversity and differentiation were estimated by bootstrap resampling over individuals (Efron 1979).

Finally, we calculated a distance between pairs of populations using the frequencies of their different haplotypes. Because of the high number of alleles (haplotypes) at a single locus, we used the stepwise weighted genetic distance D_{sw} (Shriver et al. 1995), which is an extension of Nei's minimum genetic distance (Nei 1972).

$$D_{sw} = \sum\sum_{i\neq j} x_i y_j \delta_{ij} - [\ \sum\sum_{i\neq j} x_i x_j \delta_{ij} + \sum\sum_{i\neq j} y_i y_j \delta_{ij}\ ] / 2$$

where x_i and y_j are the frequencies of the *i*th and *j*th haplotypes in populations *X* and *Y*, and δ_{ij} is a distance between haplotypes *i* and *j*, which is calculated using the matrix of presence/absence of the restriction fragments. The matrix of genetic distance between pairs of populations was then illustrated by means of a UPGMA dendrogram (Sneath and Sokal 1973).

Results

Polymorphism in the *Lolium* chloroplast genome

Table 1 indicates the three chloroplast primers/enzyme combinations retained after the preliminary survey. Several bands were found for each primer/enzyme combination after digestion (Table 2): 4 bands for CS, 6 for HK and 3 for TF. So, considering the three combinations together, a total of 13 bands allowed us to identify 15 different chloroplast haplotypes.

The numbers of individuals and populations sharing the same haplotype are given in Table 3 for the different species. The three inbred species, *L. temulentum*, *L. persicum* and *L. remotum*, harbour the same and unique haplotype, no. 5 which is also present in all *L. rigidum* populations but only in 4 of the *L. perenne* populations. In contrast, *L. perenne* contains a larger number of different haplotypes (12) than *L. rigidum* (6); some of these haplotypes are very frequent (common) in *L. perenne* (haplotypes no. 1, 2, 3, 4), others are rare (haplotypes no. 5, 6 8, 9, 12, 13, 14, 15); a few of them are shared with *L. rigidum* (haplotypes no. 5, 8, 9). If we consider the 15 possible haplotypes, there were 23/28 = 82% polymorphic populations in *L. perenne*, and only 8/14 = 57% in *L. rigidum*.

Table 1 List of the pairs of chloroplast primers/restriction enzymes used in the study

Primer 1	Primer 2	Enzyme	Abbreviation	Reference[a]
*psb*C [psII 44 KD protein]	*trn*S [tRNA-Ser(UGA)]	*Cfo*I	CS	1
*trn*H [tRNA-His (GUG)]	*trnK* [tRNA-Lys(UUU)exon1]	*Alu*I	HK	1
*trn*T [tRNA-Thr (UGU)]	*trnF* [tRNA-Phe (GAA)]	*Mva*I	TF	2

[a] 1, Demesure et al. (1995); 2, Taberlet et al. (1991)

Table 2 Description of the 15 haplotypes identified. Presence (1) or absence (0) of each restriction fragments labelled by decreasing order in fragment size

Haplotype	Polymorphic fragments												
	CS1	CS2	CS3	CS4	HK1	HK2	HK3	HK4	HK5	HK6	TF1	TF2	TF3
1	0	1	1	1	1	0	1	0	0	0	1	0	0
2	0	1	1	1	1	0	1	0	0	0	0	1	0
3	0	1	1	1	1	0	0	1	0	0	1	0	0
4	0	1	1	1	1	0	0	1	0	0	0	1	0
5	0	1	1	1	0	1	0	0	1	1	0	0	1
6	0	1	1	1	0	1	0	0	1	1	1	0	0
7	1	0	0	1	0	1	1	0	0	1	0	0	1
8	1	0	0	1	0	1	0	1	0	1	0	0	1
9	1	0	0	1	0	1	0	0	1	1	0	0	1
10	0	1	1	1	0	1	0	1	0	1	0	0	1
11	0	1	1	1	0	1	1	0	0	1	0	0	1
12	0	1	1	1	0	1	0	0	1	1	0	1	0
13	0	1	1	1	1	0	0	0	1	0	0	1	0
14	0	1	1	1	1	0	0	1	0	0	0	0	1
15	0	1	1	1	1	0	0	0	1	0	0	0	1

Table 3 Number of individuals and *number of populations*[a] in each species with the same haplotype

Haplotype	Species				
	L. perenne	*L. rigidum*	*L. temulentum*	*L. persicum*	*L. remotum*
1	34 (*11*)				
2	72 (*15*)				
3	68 (*18*)				
4	79 (*18*)				
5	9 (*4*)	101 (*14*)	12 (*3*)	12 (*3*)	3 (*1*)
6	1 (*1*)				
7		12 (*3*)			
8	6 (*3*)	21 (*4*)			
9	1 (*1*)	2 (*2*)			
10		3 (*2*)			
11		1 (*1*)			
12	2 (*2*)				
13	1 (*1*)				
14	5 (*3*)				
15	2 (*1*)				
Total	280 (*28*)	140 (*14*)	12 (*3*)	12 (*3*)	3 (*1*)

[a] Number of populations indicated in italics and between parentheses

Table 4 Mean values of genetic diversity indices for chloroplastic and nuclear markers (standard deviations are in parenthesis)

	Species	
	L. perenne	*L. rigidum*
Number of populations	28	14
Number of individuals	280	140
h_{SC}	0.492 (0.053)	0.274 (0.071)
h_{TC}	0.821 (0.019)	0.463 (0.093)
G_{STC}	0.401 (0.066)	0.408 (0.203)
h_{SN}	0.320 (0.008)	0.412 (0.011)
h_{TN}	0.359 (0.011)	0.480 (0.015)
G_{STN}	0.109 (0.035)	0.142 (0.037)
Pollen flow/seed flow	3.5	2.2

Genetic diversity analysis

The results of the hierarchical analysis of chloroplastic diversity (Table 4) confirms this high level of within-population diversity for the two species, since H_{SC}/H_{ST} = 60% of the total diversity resides within populations, and the remaining 40% is distributed among populations. This high level of differentiation of cpDNA (G_{STC} = 40%) contrasts with the situation for nuclear genes (G_{STN} = 11% for *L. perenne* and 14% for *L. rigidum*). If populations are assumed to be at drift/migration equilibrium for these two sets of markers, an estimate of the ratio pollen to seed flow among populations can be calculated; here, pollen flow appears to be 3.5 times greater than seed flow for *L. perenne* and 2.2 times higher for *L. rigidum*.

Phylogeographic analysis

The dendrogram (Fig. 1), based on the stepwise weighted genetic distance D_{sw} between pairs of populations, clearly reveals the genetic divergence of the two outbreeding species, *L. perenne* and *L. rigidum*. Most of the *L. rigidum* populations are grouped with those of the

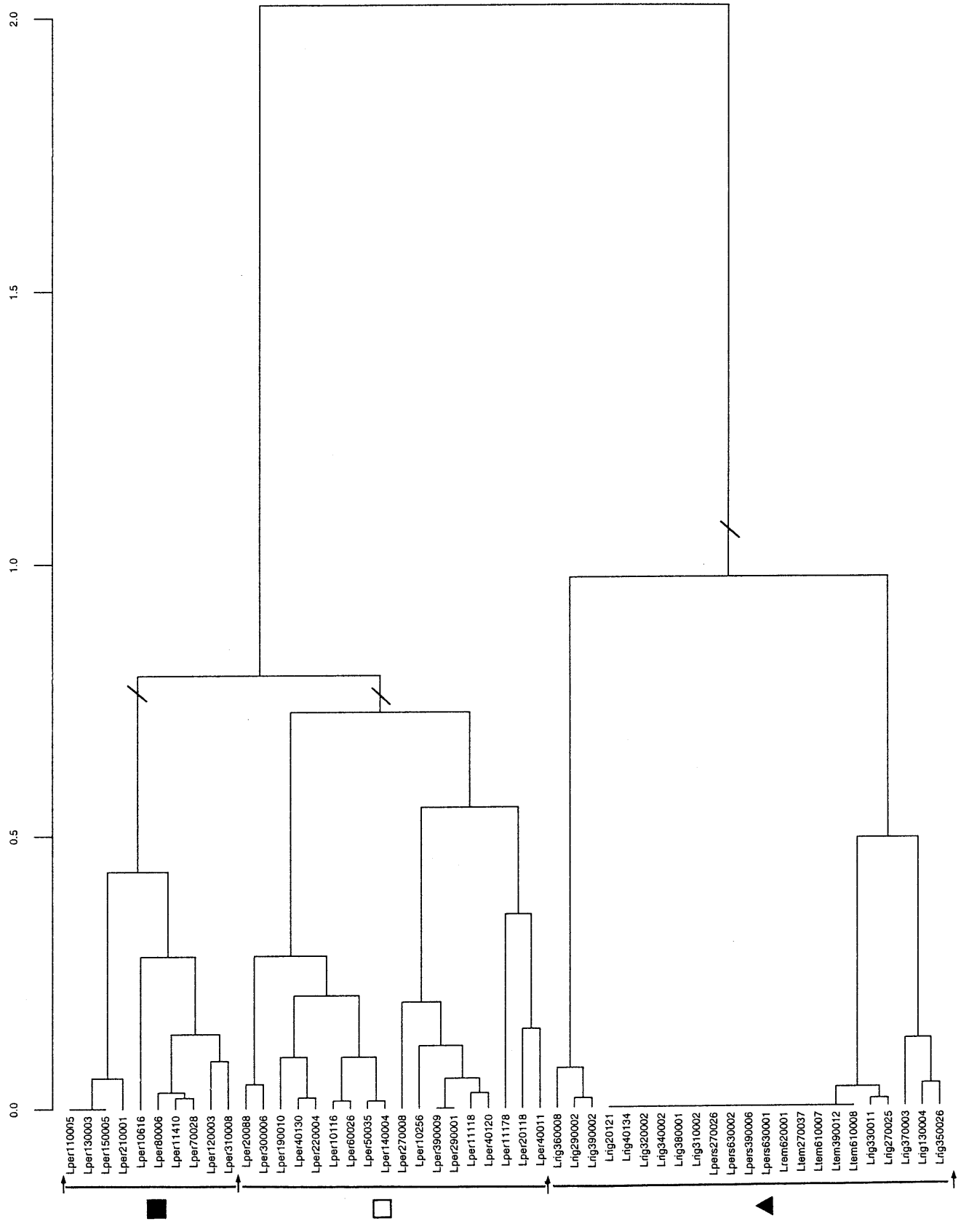

Fig. 1 Dendrogram of the 51 *Lolium* populations based on the UPGMA method using a stepwise weighted genetic distance matrix. Populations are grouped in 3 different clusters: ■, □, ▲

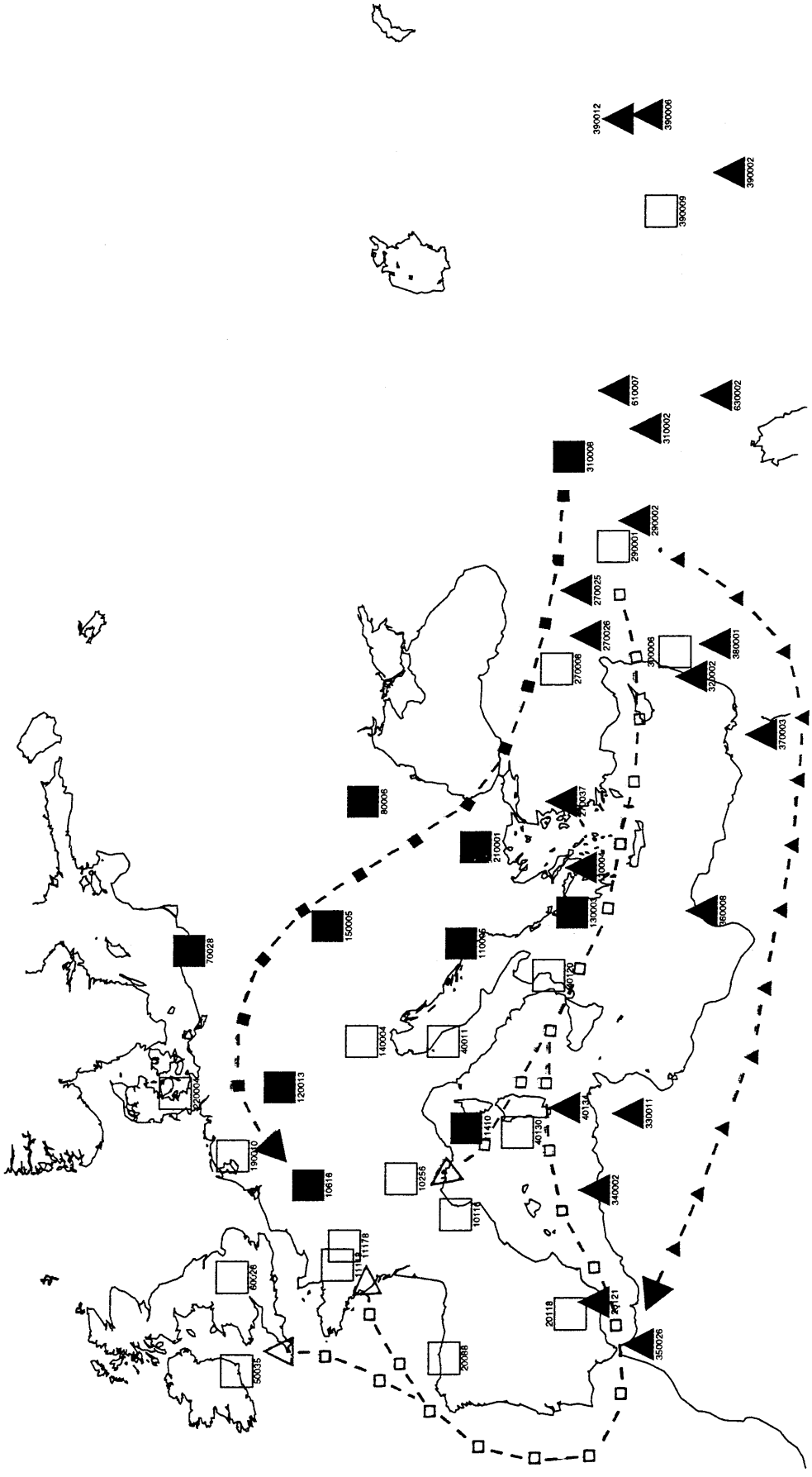

Fig. 2 Map of geographical distribution of the three clusters of populations and possible colonisation routes inferred from historical data on human migration during the spread of agriculture. (■ - - ■ Danubian movement, □ - - □ Mediterranean movement, ▲ - - ▲ North African continental route

three inbreed species (*L. temulentum*, *L. persicum* and *L. remotum*) in a cluster (▲), while the *L. perenne* populations can be divided in two different clusters (■,□).

The geographical distribution of these three clusters is illustrated in Fig. 2. We observe that the distribution of the two *L. perenne* clusters are not geographically randomly distributed. The first cluster (■) is restricted to the Middle East (Iran) and the eastern part of Europe, from Bulgaria, Greece and the former Yugoslavia up to Romania, Slovakia, Poland, Germany and the eastern part of France. A second cluster (□) gathers up all the other populations, from the Middle East (Afghanistan, Iraq, Lebanon, Turkey) and the Mediterranean basin (Italy, France, Spain) up to the western part of Europe (north of Spain, west of France, United Kingdom, Ireland). It is noticeable that all three clusters of populations (and consequently the five different species) are represented in the Middle East. Moreover, all of the 15 different haplotypes are also present in this area. In contrast, populations of the eastern cluster (■) only harbour 4 different haplotypes (nos. 1, 2, 3 and 4), whereas, with 12 different haplotypes, the diversity of the western cluster (□) is more important (results not shown).

Discussion

CpDNA polymorphism

PCR-RFLP analysis of the chloroplast genome has been extensively used in recent studies for phylogenetic purposes as, for instance, on turfgrasses (Davis and Soreng 1993; Yameshita et al. 1993) or *Prunus* (Badenes and Parfitt 1995) (for a review, see Olmstead and Palmer 1994). Similar analyses have also been recently developed for phylogeographical reconstruction on oak (Petit et al. 1993a; Dumolin-Lapegue et al. 1997; Ferris et al. 1998) or on beech (Demesure et al. 1996; Marchelli et al. 1998).

In the present study, we successfully used eight pairs of primers developed by Taberlet et al. (1991) and Demesure et al. (1995), to PCR-amplify homologous fragments. This confirms the universality of the tool and the high degree of conservation of the chloroplast genome. The polymorphism observed with the CS/*Cfo*I combination should perhaps be interpreted as a restriction site change since in this case a large fragment seems to be an alternative to two smaller fragments of complementary sizes. But the very small differences in length found among the polymorphism restriction fragments of the other two combinations (HK/*Alu*I and TF/*Mva*I) makes interpretation quite difficult. And because of inaccurate measurements of the different fragment lengths, our study failed to detect the precise nature of the mutation events. Sequencing the fragments to determine the type and number of mutations would be of great interest and may allow the construction of a phylogenetic tree of the 15 haplotypes. Nevertheless, our study identified 13 restriction fragments which defined 15 different haplotypes (Table 2) in the *Lolium* genus.

This is the first time that such a molecular approach has been used on *Lolium* species to observe the genetic differentiation of maternally inherited cpDNA between and within natural populations. At the interspecific level, the genetic structure of cpDNA in *Lolium* genus appears to be species-dependent; for instance, there is no diversity in the three inbred species, while there are up to 6 different haplotypes in the *L. rigidum* and 12 in *L. perenne* populations with only 3 in common. The three inbred species share the same and unique, very frequent haplotype 5, which is also present in the two outbreeding species. This contrasts with oak where numerous species share the same haplotype and where in most species different haplotypes can be found (Dumolin-Lapegue et al. 1997). Here, differences between inbreeding and outbreeding species could probably be explained by differences in the the reproduction system. The higher number of haplotypes in *L. perenne*, than in *L. rigidum*, and the fact that few of them are shared between the two species are more difficult to explain, As suggested by Pons and Petit (1995), a higher number of *L. rigidum* populations would have allowed the identification of new shared haplotypes in this species and increased the percentage of polymorphic populations. Finally, results on the three inbred species substantiate previous results indicating that these species may have differentiated from a common ancestor before the outbreeding species (Charmet et al. 1997).

Genetic structure

The comparison of hierarchical analyses of both chloroplastic and nuclear diversity (Table 4) is interesting: for both species and for cpDNA markers, there is a high level of within-population diversity (about 60% of the total diversity). This level of within-population diversity constitutes one of the most striking results of the present study as it contrasts with reported values in other species. For instance, on common beech, a wind-pollinated forest species, Demesure et al. (1996) reported a within-population diversity level of 17%, and Dumolin-Lapegue et al. (1997) reported a similar result on white oak.

In the two cross-pollinated *Lolium* species, about 40% of total diversity is distributed among populations, which is higher than for nuclear markers (G_{STN} = 0.109 and 0.142 for *L. perenne* and *L. rigidum*, respectively). In comparison to nuclear genes, this higher degree of population differentiation for maternally inherited organelle genes is well-documented and can be explained by two factors: first, the effective gene flow, which is limited to seed transport for the maternally inherited genome (Petit et al. 1993b), while the nuclear locus can move in both seeds and pollen; second, the drift, which is twice as fast for a haploid genome as for a diploid one.

The estimates of pollen/seed flow ratio (3.5 and 2.2 for *L. perenne* and *L. rigidum*, respectively) should be compared to values obtained in different species: Ennos (1993) found such a ratio ranging from 196 in wind-pol-

linated outbreeding oak, to 4 in a predominantly selfing annual (*Hordeum spontaneum*). Our values are low when compared to those obtained by Petit et al. (1993a) on different oak species, but they are of the same magnitude as those reported for *Silene alba*, an insect-pollinated weedy species. For this species, McCauley (1997) reported a ratio of 3.4 when the estimate was based on a large spatial scale. As for *Silene alba*, the fact that pollen flow in *Lolium* does not contribute to an even larger portion of total gene flow (as compared to oak, for instance) can be due to the weedy nature of *Lolium* populations (Ennos 1994). Another factor to explain this low pollen/seed ratio is to assume a very high level of seed migration, as proposed in the scenario described below.

Phylogeographic structure

Considering the high level of within-population cpDNA diversity in *L. perenne* and *L. rigidum* populations, we chose to look at the phylogeographic structure of the populations, instead of the structure of their haplotypes, in order to infer the colonisation routes of *Lolium* populations in Europe. For that, we used the stepwise weighted genetic distance D_{SW} (Shriver et al. 1995) which takes into account the proximity between haplotypes as estimated by the presence/absence of common restriction fragments (stepwise haplotype approach, Echt et al. 1998).

The dendrogram (Fig. 1) obtained by UPGMA provides a clear separation of the two outbreeding species in two main clusters based on the similarity of their haplotype frequencies. Moreover, at a lower level of clustering, the *L. perenne* populations can be divided in two groups, and the three inbred species appear to be clustered with most of the *L. rigidum* populations, indicating their close relationships with this outbreeding species.

Figure 2 shows a marked geographical structure for the two *L. perenne* clusters that shows some similarity with our previous results obtained with isozyme markers. A plausible explanation for such a geographical differentiation in *L. perenne* is that it derived from a bottleneck of *L. rigidum* populations, that occurred in the Middle East area a few thousand years ago. From that time onwards, two scenario are possible (Balfourier et al. 1998):

The first scenario is a colonisation of Europe from the Middle East before the last glaciation events that led to *Lolium* extinction in most of northern and central Europe. The present distribution area would be the result of recolonisation from several postglacial refugia. Such a scenario of postglacial recolonisation has already been reported for various European trees (Demesure et al. 1996; Dumolin-Lapegue et al. 1997). However, given the present results, this scenario does not seem to be the most convincing. First of all, this scenario supposes a polyphyletic origin of *L. perenne* populations in Europe; such an idea is not in agreement with the dendrogram obtained from the cpDNA polymorphism of populations, which indicates a monophyletic origin of *L. perenne* populations. Moreover, the geographical distribution area of the two *L. perenne* clusters shows that populations from both groups are present in the Middle East (population 310008 in Iran for cluster ■, and populations 270008, 300006, 290001, 390009 for cluster □). This does not fit with two separate southern refugia. Finally, the geographical structure of *L. perenne* populations appears to be more oriented along an east/western cline than the south/northern cline expected in the case of postglacial latitudinal recolonisation.

The second scenario of colonisation of Europe by *L. perenne* populations matches historical processes such as the emergence of agriculture of cereal crops in the Middle East about 10,000 years ago and its spread towards Europe. The fertile crescent is thought to be the centre of origin of the genus *Lolium*, and the three true self-pollinating species (*L. temulentum, L. persicum* and *L. remotum*) are known there only as weeds of cultivated crops. This scenario of colonisation of Europe by populations carried as weeds of cereal crops by the first farmers fits very well with the geographical structure of *L. perenne* populations as revealed in the present study. Based on historical studies of human migration in the Neolithic Period, three different pathways of colonisation from the fertile crescent are known (see Fig. 2): (1) a north-eastern one (Danubian movement) towards Bulgaria, Romania, Slovakia, Poland, Germany and the east of France; (2) a south-western one (Mediterranean movement) towards Italy, the south of France, Spain, then western France, United Kingdom and Ireland; (3) a North African continental route, from the Middle East to Morocco.

The different populations of the two *L. perenne* clusters clearly appear to be distributed along the two first migration routes of the first farmers, while *L. rigidum* populations appear to be distributed all around the Mediterranean road and the third North African continental pathway. In fact, we can imagine that both species were probably transported along the three different pathways. Selection pressure effects after migration might then explain the present distribution area of the two species: *L. rigidum* is an annual species more adapted to the Mediterranean area where drought places the summer survival of perennials at a disadvantage; alternatively, *L. perenne* is a perennial grass well-adapted to northern continental conditions. The lower number of haplotypes observed in the eastern (“Danubian”) cluster of *L. perenne* populations (4 against 12 in the “Mediterranean” cluster) or the lower within-population diversity of *L. rigidum* populations may be explained by successive bottlenecks that may have reduced the diversity during the migration movement. Similar results were reported from simulation studies (Le Corre et al. 1997).

If we sum up all the results concerning the east/western cline, the presence of *L. perenne* populations from the two clusters in the Middle East and the fact that all 12 different haplotypes belonging to *L. perenne* populations in Europe are present in the different species of the restricted fertile crescent area, there is a lot of evidence favouring this sec-

ond scenario. Such a scenario is also in agreement with the low estimate of pollen/seed ratio compared with other wind-pollinated species. If we imagine an important seed migration level due to human transportation (and it was probably the case with the first farmers), we can understand the low pollen/seed ratio estimate.

Finally, it is interesting to observe that in this scenario, French territory is located at the crossroad of two pathways: the "Danubian" movement from north-east part and the "Mediterranean" one from the south. This can be related with geostatistical analysis of isozyme frequencies observed on French perennial ryegrass populations, which will be presented in a subsequent paper.

Clearly the spread of agriculture in the Neolithic Period, characterised by different human transportation pathways of cereal seeds together with *Lolium* species' seeds as weeds, played the greatest role in determining the present geographic pattern of diversity in *L. perenne* and *L. rigidum* species. However, others factors, such as selection pressures due to environmental conditions, cannot be ruled out. Further studies are in progress using geostatistical analysis to determine, at on a smaller regional scale, the role of these different factors in the constituting of the global geographical structure observed.

References

Badenes ML, Parfitt DE (1995) Phylogenetic relationships of cultivated *Prunus* species from an analysis of chloroplast DNA variation. Theor Appl Genet 90: 1035–1041

Balfourier F, Charmet G, Ravel C (1998) Genetic differentiation within and between natural populations of perennial and annual ryegrass (*Lolium perenne* and *L. rigidum*). Heredity 81: 100–110

Charmet G, Ravel C, Balfourier F (1997) Phylogenetic analysis in the Festuca-Lolium complex using molecular markers and ITS rDNA. Theor Appl Genet 94: 1038–1046

Chiu WL, Sears BB (1985) Recombinaison between chloroplast DNAs does not occur in sexual crosses of *Oenothera*. Mol Gen Genet 198: 525–528

Davis JI, Soreng RJ (1993) Phylogenetic structure in the grass family (Poaceae) as inferred from chloroplast DNA restriction site variation. American Journal of Botany 80: 1444–1454

Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol Ecol 4: 129–131

Demesure B, Comps B, Petit RJ (1996) Chloroplast DNA Phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. Evolution 50: 2515–2520

Dumoulin-Lapegue S, Demesure B, Fineschi S, Le Corre V, Petit RJ (1997) Phylogeographic structure of white oaks throughout the european continent. Genetics 146: 1475–1487

Echt CS, De Verno LL, Anzidei M, Vendramin GG (1998) Chloroplast microsatellites reveal population genetic diversity in red pine, *Pinus resinosa* Ait. Mol Ecol 7: 307–317

Efron B (1979) Bootstrap methods: another look at the jackknife. Ann Stat 7: 1–26

Ennos RA (1993) Estimating the relative rates of pollen and seed migration among plant populations. Heredity 72: 250–259

Ferris C, King RA, Vainola R, Hewitt GM, (1998) Chloroplast DNA recognizes three refugial sources of European oaks and suggests independent eastern and western immigrations to Finland. Heredity 80: 584–593

Kiang AS, Connolly V, McConnell DJ, Kavanagh TA (1994) Paternal inheritance of mitochondria and chloroplasts in *Festuca pratensis-Lolium perenne* intergeneric hybrids. Theor Appl Genet 87: 681–688

Le Corre V, Machon N, Petit RJ, Kremer A (1997) Colonisation with long-distance seed dispersal and genetic structure of maternally inherited genes in forest trees: a simulation study. Genet Res 69: 117–125

Marchelli P, Gallo L, Scholz F, Ziegenhagen B (1998) Chloroplast DNA markers reveal a geographical divide across Argentinean southern beech *Nothofagus nervosa* (Phil.) Dim. Et Mil. Distribution area. Theor Appl Genet 97: 642–646

McCauley DE (1997) The relative contributions of seed and pollen movement to the local genetic structure of *Silene alba*. J Hered 88: 257–263

Nei M (1972) Genetic distance between populations. Am Nat 106: 283–292

Nei M (1987) Molecular evolutionary genetics. Colombia University Press, New York

Olmstead RG, Palmer JD (1994) Chloroplast DNA systematics: a review of methods and data analysis. Am J Bot 81: 1205–1224

Petit RJ, Kremer A, Wagner DB, (1993a) Geographic structure of chloroplast DNA polymorphisms in European oaks. Theor Appl Genet 87: 122–128

Petit RJ, Kremer A, Wagner DB, (1993b) Finite island model of organelle and nuclear genes in plants. Heredity 71:630–641

Pons O, Petit RJ (1995) Estimation, variance and optimal sampling of gene diversity. I. Haploid locus. Theor Appl Genet 90: 462–470

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amount of fresh herbarium and mummified plant tissues. Plant Mol Biol 5: 69–76

Sears BB (1980) Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4: 233–255

Shriver MD, Jin L, Boerwinkle E, Deka R, Ferrell RE, Chakraborty R (1995) A novel measure of genetic distance for highly polymorphic tandem repeat loci. Mol Biol Evol 12: 914–920

Sneath PHA, Sokal RR (1973) Numerical taxonomy. WH Freeman, San Francisco

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17: 1105–1109

Terrell E (1968) A taxonomic revision of the genus *Lolium*. Tech Bull US Dep Agric 1392: 1–65

Tixier MH, Sourdille P, Leroy P, Bernard M (1997) Detection of wheat microsatellites using a non radioactive silver-nitrate staining method. J Genet Breed 51: 175–177

Yameshita M, Ohmura T, Sasakuma T, Ogihara Y (1993) Phylogenetic relationships of turfgrasses as revealed by restriction fragment analysis of chloroplast DNA. Theor Appl Genet 87: 129–135