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Utility of Multilocus Genotypes for Taxon Assignment in Stands of Closely Related European White Oaks from Switzerland

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- **Background and Aims** European white oaks (*Quercus petraea*, *Q. pubescens*, *Q. robur*) have long puzzled plant biologists owing to disputed species differentiation. Extensive hybridization or shared ancestry have been proposed as alternative hypotheses to explain why genetic differentiation between these oak species is low. Species delimitation is usually weak and often shows gradual transitions in leaf morphology. Hence, individual identification may be difficult, but remains a critical step for both scientific work and practical management.
- **Methods** Multilocus genotype data (five nuclear microsatellites) were used from ten Swiss oak stands for taxon identification without *a priori* grouping of individuals or populations, using model-based Bayesian assignment tests.
- **Key Results** Three groups best structured the data, indicating that the taxonomical signal was stronger than the spatial signal. Most individuals showed high posterior probabilities for either of three genetic groups that were best circumscribed as taxonomical units. The assignment of a subset of trees, whose taxonomic status had been previously characterized in detail, supported this classification scheme.
- **Conclusions** Molecular-genetic assignment tests are useful in the identification of species status in critical taxon complexes such as the European white oaks. Such an approach is of practical importance for forest management, e.g. for stand certification or in seed trade to trace the origin of forest products.

Key words: Assignment test, Bayesian inference, multilocus genotype, nuclear microsatellites, *Quercus* sp., species complex.

INTRODUCTION

Species from the widespread temperate forest tree genus *Quercus* have long been used as model systems for studying hybridization in plants (Stebbins *et al.*, 1947; Kremer *et al.*, 1993; Howard *et al.*, 1997; Craft *et al.*, 2002; Ishida *et al.*, 2003; Petit *et al.*, 2003; Tovar-Sánchez and Oyama, 2004; González-Rodríguez *et al.*, 2005). Particularly in the European white oaks, including *Quercus petraea*, *Q. pubescens* and *Q. robur*, numerous studies have applied morphological (Kremer *et al.*, 2002a), ecophysiological (Ponton *et al.*, 2002), palaeoecological (Brewer *et al.*, 2002), and a broad variety of molecular methods (Zanetto *et al.*, 1994; Bodénès *et al.*, 1997b; Kremer *et al.*, 2002b; Mariette *et al.*, 2002a; Petit *et al.*, 2002; Scotti-Saintagne *et al.*, 2004; Muir and Schlötterer, 2005) to elucidate the evolution of this taxon complex. These studies embraced spatial scales ranging from the continental to the local stand level.

Despite this multitude of studies, it is still debated how relevant natural hybridization and introgression are, and the degree to which these processes affect species differentiation and, hence, taxon identification (Lexer *et al.*, 2006; Muir and Schlötterer, 2006). For the case of *Q. petraea* and *Q. robur*, there are both arguments in favour of extensive hybrid formation leading to nuclear capture (Petit *et al.*, 2003) or of a generally low species differentiation as a consequence of shared ancestral polymorphism (Muir and Schlötterer, 2005). Gugerli *et al.* (2007) argue that

the number of effectively reproductive hybrids within local populations is low owing to small-scale assortative mating, low frequencies of natural interspecific cross-fertilizations, short-distance seed dispersal, and local selection favouring those taxonomically 'pure' seedlings that grow in their maternal habitat.

Molecular studies in *Q. petraea* and *Q. robur* have shown that species differentiation at neutral loci is generally low but heterogeneous. For example, only 2% of 2800 random amplified polymorphic DNA (RAPD) markers showed significant differentiation between the two species (Bodénès *et al.*, 1997a). Likewise, frequency differences in only four out of 155 reliable amplified fragment length polymorphisms (AFLPs) exhibited >10% interspecific genetic differentiation in six mixed populations from across Europe (Mariette *et al.*, 2002b). This suggests that only few genomic regions are involved in species separation, and two recent mapping studies confirmed this assumption (Saintagne *et al.*, 2004; Scotti-Saintagne *et al.*, 2004). Even though no diagnostic molecular marker could be identified to date, it is possible to find distinct taxonomic signals in molecular data sets when choosing either large numbers of nuclear markers or else specifically selected, highly differentiating markers (Muir *et al.*, 2000; Muir and Schlötterer, 2005; Gugerli *et al.*, 2007). For the species pair *Q. petraea* and *Q. pubescens*, studies of isozymes and nuclear microsatellites (simple sequence repeats, nSSRs) generally agree that introgression plays an important role, which is corroborated by leaf morphological analyses and pollination experiments (Müller,

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1999; Bruschi *et al.*, 2000). As such, species identification in the European white oaks is not straightforward and often requires carefully evaluating multiple characters and molecular markers.

To date, studies on European white oaks have relied on a *a priori* taxon assignment based on leaf morphological characters. Here, the reverse approach, namely to test if trees or populations may be reliably assigned to either taxon without prior classification, was used (Craft *et al.*, 2002; Aldrich *et al.*, 2003). Specifically, the potential of identifying the taxonomic composition of European white oak stands was explored using assignment tests based on individual multilocus genotypes. First, we validated whether homogeneity of genetic clusters represents putative species or simply indicates geographical origin. Next, individuals were assigned to one of three clusters based on the posterior probabilities from assignment tests. Finally, an evaluation was carried out to see if the multilocus genotype assignment of the individuals from a mixed stand of *Q. petraea* and *Q. robur* corresponds to their taxonomic identification based on local allele frequencies and leaf morphology as previously characterized by Gugerli *et al.* (2007).

From the results it is concluded that Bayesian assignment methods are valuable for species identification in species complexes, that few nuclear markers may be sufficient for taxon assignment if appropriately selected for differentiation power, and that such a method is also useful for practical purposes such as the certification of forestry products.

MATERIALS AND METHODS

Study species

The three study taxa *Quercus petraea* (Matt.) Liebl., *Q. pubescens* Willd., and *Q. robur* L. of section *Quercus* or white oaks (Nixon, 1993) are important tree species of temperate, low-elevation broadleaved woodlands. *Quercus petraea* and *Q. robur* are widespread in western and central Europe, occurring from Mediterranean to subboreal areas. The natural range of *Q. pubescens* is more restricted, with a centre in southern and south-eastern Europe (Tutin *et al.*, 1993). In accordance with their ecological amplitudes, two or all three species may occur in the same locality.

The taxon pairs *Q. petraea*–*Q. robur* and *Q. petraea*–*Q. pubescens* are considered interfertile (Rushton, 1993; Bacilieri *et al.*, 1996; Müller, 1999; Petit *et al.*, 2003), but the frequency of naturally occurring hybrids is still debated (Bruschi *et al.*, 2000; Petit *et al.*, 2003; Muir and Schlötterer, 2005). The taxa further share the same type of breeding system characterized by monoecy, anemophily and a combination of baleo- and zoochory.

Field sampling

Sampling comprised ten oak stands from Switzerland (Fig. 1, Table 1). The selected stands were previously categorized based on leaf morphological analyses as *Q. petraea* (three stands), *Q. robur* (two), mixed *Q. petraea* and *Q. robur* (two) and *Q. pubescens* (two; Finkeldey and Mátyás, 2003). The morphological assignment of stands

TABLE 1. Taxonomic status and genetic diversity estimates of ten stands of *Quercus* spp. from Switzerland, based on five nuclear microsatellite markers

Stand	n	Taxonomic status		Diversity estimates	
		Leaf morphology	Microsatellites	A _r	H _e
Jussy	31	<i>petraea</i>	<i>petraea</i>	11.8	0.868
Magden	26	<i>petraea</i>	<i>petraea</i>	11.6	0.849
Schoren	31	<i>petraea</i>	<i>petraealrobur</i>	13.1	0.889
Allschwil	32	<i>petraealrobur</i>	<i>petraealpubescens</i>	12.5	0.893
Büren	32	<i>petraealrobur</i>	<i>petraealrobur</i>	13.6	0.838
Lugnez	32	<i>robur</i>	<i>robur</i>	11.8	0.796
Uttwil	32	<i>robur</i>	<i>robur</i>	12.0	0.792
Fully	32	<i>pubescens</i>	<i>petraealrobur</i>	13.2	0.878
Gampel	27	<i>pubescens</i>	<i>pubescens</i>	12.9	0.890
Tamins	30	<i>pubescens</i>	<i>petraealpubescens</i>	11.5	0.876

The taxonomic status of stands was based on leaf morphology (Finkeldey and Mátyás, 2003) or on multilocus genotypes (see Results). n, sample size for nuclear microsatellites, A_r, mean allelic richness, H_e, expected heterozygosity.

was based on 100 leaves randomly collected from the ground across each sampling range. Leaves were measured and data analysed following Kremer *et al.* (2002a) as described in Finkeldey and Mátyás (2003). Within each stand, fresh leaf tissue samples were collected from 32 trees in 2003, respecting a minimum distance of 30 m between sampled individuals. In the mixed stand of Büren (*Q. petraea* and *Q. robur*), where all adult oak trees had already been morphologically assessed and genotyped (Gugerli *et al.*, 2007), a random sample of 32 individuals was drawn from the existing data set. The taxon composition of this subset was representative of the entire stand, i.e. taxon frequencies did not significantly differ (data not shown).

DNA isolation and microsatellite analysis

Following the laboratory procedures described in Gugerli *et al.* (2007), five nuclear dinucleotide microsatellite loci were analysed: QpZAG9, QpZAG104 (Steinkellner *et al.*, 1997), QrZAG30, QrZAG96 (Kampfer *et al.*, 1998) and MSQ13 (Dow *et al.*, 1995). According to Barreneche *et al.* (2004), these loci are on separate linkage groups. The loci proved to be informative in a test on the differentiation potential between *Q. petraea* and *Q. robur* over their entire range (P. Goicoechea *et al.*, Neiker, Vitoria; unpubl. data) and on the local scale (Gugerli *et al.*, 2007). Two multiplex PCRs (QpZAG9, QpZAG104 and MSQ13; QrZAG30 and QrZAG96) were run separately for fragment length analysis on an ABI3100-Avant automated capillary sequencer (Applied Biosystems, Rotkreuz, Switzerland), including an internal size standard ROX400HD (Applied Biosystems). GeneScan 3.1 and Genotyper 2.5 (Applied Biosystems) were used for genotyping.

Statistical analyses

In the few cases where more than two fragments were amplified per locus (cf. Dzialuk *et al.*, 2007), that particular

locus was coded as missing in the respective individual. For analyses, only those individuals were considered for which unambiguous data were obtained for at least three nSSR loci, leaving a total of 305 individuals (Table 1).

Mean allelic richness (A_r , using rarefaction) and mean expected heterozygosity (H_e) were calculated for each stand using FSTAT 2.9.3.2 (Goudet, 1995) to characterize local genetic diversities.

Two types of assignment tests were run with the allelic data (a) to allocate individuals to genetic clusters and (b) to associate the resulting clusters to the three oak taxa. In the first assignment test, for which STRUCTURE 2.2 was used (Pritchard *et al.*, 2000; Falush *et al.*, 2003), an attempt was made to find the optimal grouping of individuals without prior classification. The program was run for $K = 1-10$ expecting $K = 3$ (i.e. three species) to be the optimal cluster number. Chosen for the present study were 50 000 burnin periods and 50 000 Monte Carlo Markov Chain repetitions after burnin, selecting the admixture model option (uniform prior $\alpha = 1.0$ for all populations) with correlated frequencies (prior mean $F_{ST} = 0.1$ equal for all populations) and no *a priori* assignment to local populations (POPINFO off). Five iterations were run at each level of K , using mean assignment probabilities per individual over these runs. From the results of the most likely STRUCTURE run ($K = 3$; see Results), individuals were grouped with a mean assignment probability >0.8 for either of the clusters into arbitrary taxonomic units. Hence, three artificial taxonomic clusters of trees (hereafter termed 'synthetic populations') were obtained, irrespective of their origin. These synthetic populations were considered as representative of any of the three taxa studied (*Q. petraea*, *Q. pubescens* and *Q. robur*), accepting a certain degree of ambiguity in these genetic clusters owing to the relatively low assignment probability threshold (0.8).

The second type of assignment test was performed to confirm that the above clustering indeed represented separate oak species, specifically testing *Q. petraea* and *Q. robur*. This assignment test, implemented in ARLEQUIN 3.11 (Excoffier *et al.*, 2005), calculates the maximum likelihood of multilocus genotypes to belong to either of two entities, given their respective allele frequencies (Paetkau *et al.*, 1997; Waser and Strobeck, 1998). Accordingly, formerly identified individuals from the stand Büren, whose species membership had previously been determined (Gugerli *et al.*, 2007), were assigned to either of the synthetic *Q. petraea* or *Q. robur* populations (see above), excluding the Büren individuals from the respective synthetic populations. Because the majority of Büren individuals had previously been identified as *Q. robur*, all individuals from this stand were *a priori* combined with the *Q. robur* synthetic population for calculating underlying allele frequencies of groups in the test. However, the reverse grouping, i.e. all Büren genotypes associated with *Q. petraea*, did not affect the subsequent assignment results (data not shown). One should note that the Büren individuals showed nearly perfect congruence between the taxon assignments based on leaf morphology and multilocus genotype (Gugerli *et al.*, 2007), precluding circular reasoning.

Analyses of molecular variance (AMOVAs) were also run with ARLEQUIN, with 10 000 permutations for significance testing in order to find the maximum among-group variation (Φ_{CT}), suggesting the biologically most meaningful population grouping in analogy to SAMOVA (Dupanloup *et al.*, 2002). Stands were grouped either as belonging to *Q. petraea*, *Q. pubescens*, *Q. robur*, or being mixed, or only 'pure' stands were classified in three groups. Both types of AMOVAs were run with taxonomic assignment of stands based on leaf morphology (Finkeldey and Mátyás, 2003), based on the above genetic assignment test, respectively (Table 1), or based on their geographic locations (three groups: south-western, north-western and eastern; Fig. 1).

RESULTS

Though not previously applied to *Q. pubescens*, all loci amplified well in all three taxa. Even QrZAG30, despite its complex sequence described for *Q. petraea* and *Q. robur* (Gugerli *et al.*, 2008), revealed electrophoretic patterns that could be readily scored also in *Q. pubescens*. With the five nuclear nSSRs genotyped, a total of 171 alleles was detected, with individual loci revealing 15–50 alleles. Mean allelic richness (A_r) per stand ranged from 11.5 to 13.6, and expected heterozygosities (H_e) were high with values >0.792 (Table 1).

The assignment of individual multilocus genotypes into $K = 1-10$ genetic clusters indicated that $K = 3$ represented the most likely grouping according to the model assumptions and based on the criteria suggested by Pritchard *et al.* (2000) (Fig. 2). These criteria are (a) maximum posterior probability, Ln P(D) and (b) low variation among runs of the same K . The present data nicely fit these requirements for $K = 3$, and convergence of results was visually confirmed. The majority of genotypes (83.9%) had a posterior probability >0.8 to belong to either of these three clusters (Fig. 3). Of the 47 genotypes (15.4%) with missing data at one or two loci, 23.4% showed ambiguous taxon assignment. The 49 trees (16.1%) with individual posterior probability <0.8 were rather equally distributed among the ten stands. When a higher threshold (0.9) was applied, the number of trees with ambiguous assignment increased to 95 (31.1%).

In the case of the mixed stand Büren, all individuals had previously been identified as *Q. petraea*, *Q. robur* or putative hybrids ('unclassified'), based on leaf morphology as well as multilocus genotypes (Gugerli *et al.*, 2007). This information was used to see whether the STRUCTURE clusters had been correctly associated with the respective oak taxa. Therefore, two synthetic populations, comprising all individuals assigned to either *Q. petraea* or *Q. robur* from nine stands, were compiled (see Materials and methods) and these were used for a second assignment test of the 32 Büren individuals randomly selected for the present study. All trees from stand Büren, except for one individual previously identified as *Q. petraea*, were assigned to their respective synthetic populations (Fig. 4). The two previously 'unclassified' trees from stand Büren were both associated with the *Q. robur* group (Fig. 4), which

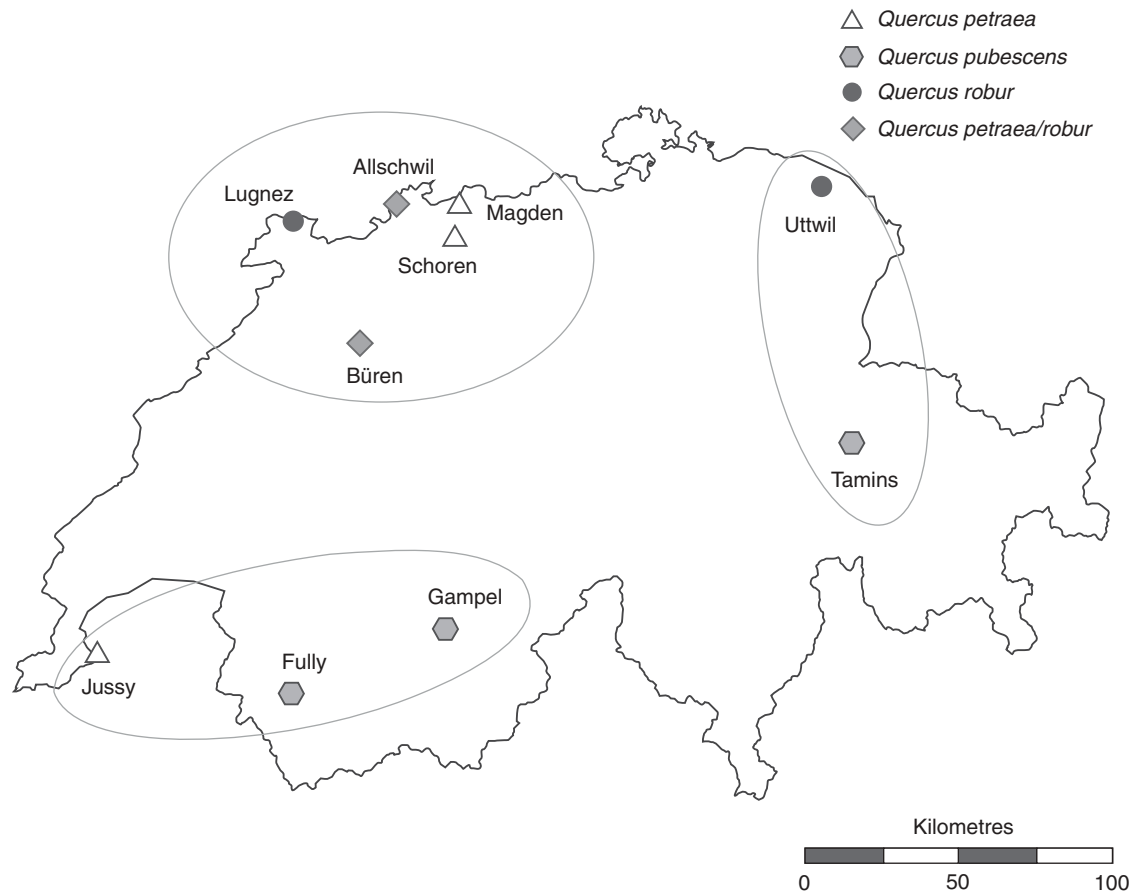


FIG. 1. Location of *Quercus* spp. stands sampled in Switzerland. Symbols refer to *Q. petraea*, *Q. robur*, *Q. pubescens* or *Q. petraea/robur* mixed populations based on leaf morphology (according to Finkeldey and Mátyás, 2003). Encircled stands were grouped as south-western, north-western and eastern in AMOVA (Table 2).

corresponded to the taxon assignment inferred by Gugerli *et al.* (2007).

In all four types of groupings used in the AMOVAs, most of the total genetic variation was assigned to the within-population level (90.9–94.3 %, $P < 0.001$; Table 2). Among-group variation (Φ_{CT}) was higher when considering only pure stands rather than including mixed ones, and groups assigned on the basis of nSSRs differentiated more clearly than when classified according to leaf morphology (Table 2). The highest percentage of among-group genetic variation resulted when only pure stands as identified by multilocus genotype assignment were included (Table 2). When grouping stands according to their geographic locations (Fig. 1), the resulting among-group variation did not significantly differ from zero ($\Phi_{CT} = -0.008$, $P > 0.05$). Values of overall population differentiation (Φ_{ST}) followed the same rank order as the one for Φ_{CT} (Table 2).

The STRUCTURE assignment revealed a taxonomic identification of the ten studied oak stands which sometimes differed from that of the same ten stands as based on leaf morphology (Finkeldey and Mátyás, 2003). For two of the *Q. petraea* populations (Magden and Jussy), the two *Q. robur* populations (Lugnez and Uttwil), one of the *Q. pubescens* populations (Gampel), and the mixed stand Büren, the two assignments corresponded

well (Fig. 3). In contrast, the mixed *Q. petraea/Q. robur* stand Allschwil showed a multilocus genotype pool fitting to *Q. pubescens* with few *Q. petraea* individuals. Likewise, Schoren as a *Q. petraea* population additionally indicated *Q. robur* trees and some influence of *Q. pubescens*, while two of the *Q. pubescens* populations revealed either *Q. petraea* contributions (Tamins) or even dominant *Q. petraea/Q. robur* composition (Fully) (Table 1 and Fig. 3).

DISCUSSION

In the present study on white oak stands of Switzerland (*Quercus petraea*, *Q. pubescens* and *Q. robur*), taxonomic assignment based on multilocus genotypes of nSSRs appeared reliable for a majority of individuals tested, though analysing only five loci. Unlike previous attempts in the literature that deal with genetic differentiation in closely related white oak taxa, the present approach did not rely on *a priori* classification of individuals or stands. Only posterior association of genetic clusters to taxa, based on previous analyses of detailed molecular and leaf morphological data for *Quercus petraea* and *Q. robur* (Gugerli *et al.*, 2007), was used for respective classification, inferring that the third cluster represented *Q. pubescens*.

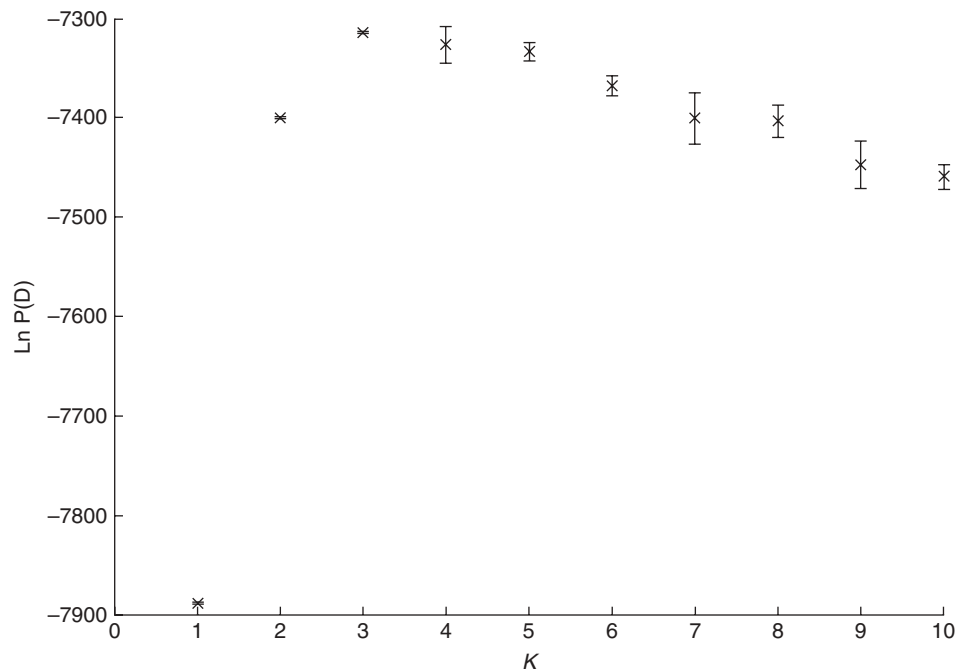


FIG. 2. Mean (from five runs) and standard deviation of posterior probabilities, Ln P(D) , as a function of $K = 1-10$ clusters obtained from STRUCTURE analysis (Pritchard *et al.*, 2000; Falush *et al.*, 2003), using nuclear microsatellite data of ten *Quercus* spp. stands from Switzerland.

It was further demonstrated that five loci may be sufficient to reach good separation of taxa even within a geographically limited area. Despite some limitations in the present study (no individual morphological data, loci selected for discrimination power between two of the three taxa studied), great potential is seen in the application of multi-locus genotype assignments for further clarification of species status in white oaks or in other taxon complexes (Anderson and Thompson, 2002), but also for their use in certification of forestry products.

Several lines of arguments are provided that support the above approach for taxon assignment in the closely related European white oaks: (a) the most likely clustering in STRUCTURE with $K = 3$ corresponded to the number of taxa involved in the analysis; (b) a large majority of individual genotypes showed high posterior probability (>0.8) for either of these three clusters; (c) high success rate of assignment of individuals whose taxon was independently identified based on multivariate leaf morphology and multilocus genotypes; and (d) highest among-group variation when only using pure populations in molecular assignment. These aspects are further elucidated in the subsequent paragraphs.

First, it is shown that selecting three clusters in the STRUCTURE analysis best groups individuals based on their multilocus genotypes (Fig. 2). This finding is in line with the taxonomical rather than spatial association of the oak individuals studied, which is considered as the main pre-assumption to illustrate that the grouping approach is biologically reasonable.

Secondly, the clear assignment of individuals to either of the three clusters, irrespective of their stand of origin, also indicates the relevance of the present results. Since a

general level of posterior probability cannot reasonably be set (Manel *et al.*, 2005), 0.8 was considered as an adequate threshold of assignment (thresholds range between 0.6 and 0.9, depending on the question asked; e.g. Rosenberg *et al.*, 2001; Craft *et al.*, 2002; Werth *et al.*, 2007). Only 16.1 % of the 305 trees analysed fell below this threshold (Fig. 3). Almost one-third of these low-probability assignments concerned individuals of *Q. pubescens* populations, for which the selected loci might not be optimal with regard to species discrimination. In addition, even genotypes with data at only three or four nSSR loci fitted well into one of the three clusters. These figures may indicate a low rate of natural hybridization, at least between *Q. petraea* and *Q. robur*, though five loci is insufficient to provide reliable estimates of hybridization rates (Vähä and Primmer, 2006). On the other hand, the five loci largely provided clear taxon assignment for most trees, even when the posterior probability threshold was increased to 0.9. Given that the chosen loci are not necessarily best suited for discriminating *Q. pubescens* from its congeners because the markers were originally developed for *Q. petraea*, *Q. robur* and *Q. macrocarpa* (Dow *et al.*, 1995; Steinkellner *et al.*, 1997; Kampfer *et al.*, 1998), this is considered a promising resolution of taxonomic identity.

Thirdly, when the present method was tested with the data from individually characterized trees from stand Büren (Gugerli *et al.*, 2007), 31 of the 32 trees were classified as belonging to the same taxon as in the previous study. The multilocus genotype of the single *Q. petraea* individual misclassified showed an only slightly higher likelihood for *Q. robur* than for *Q. petraea* (Fig. 4). Note that individuals of the Büren population were excluded when calculating the allele frequencies of the synthetic

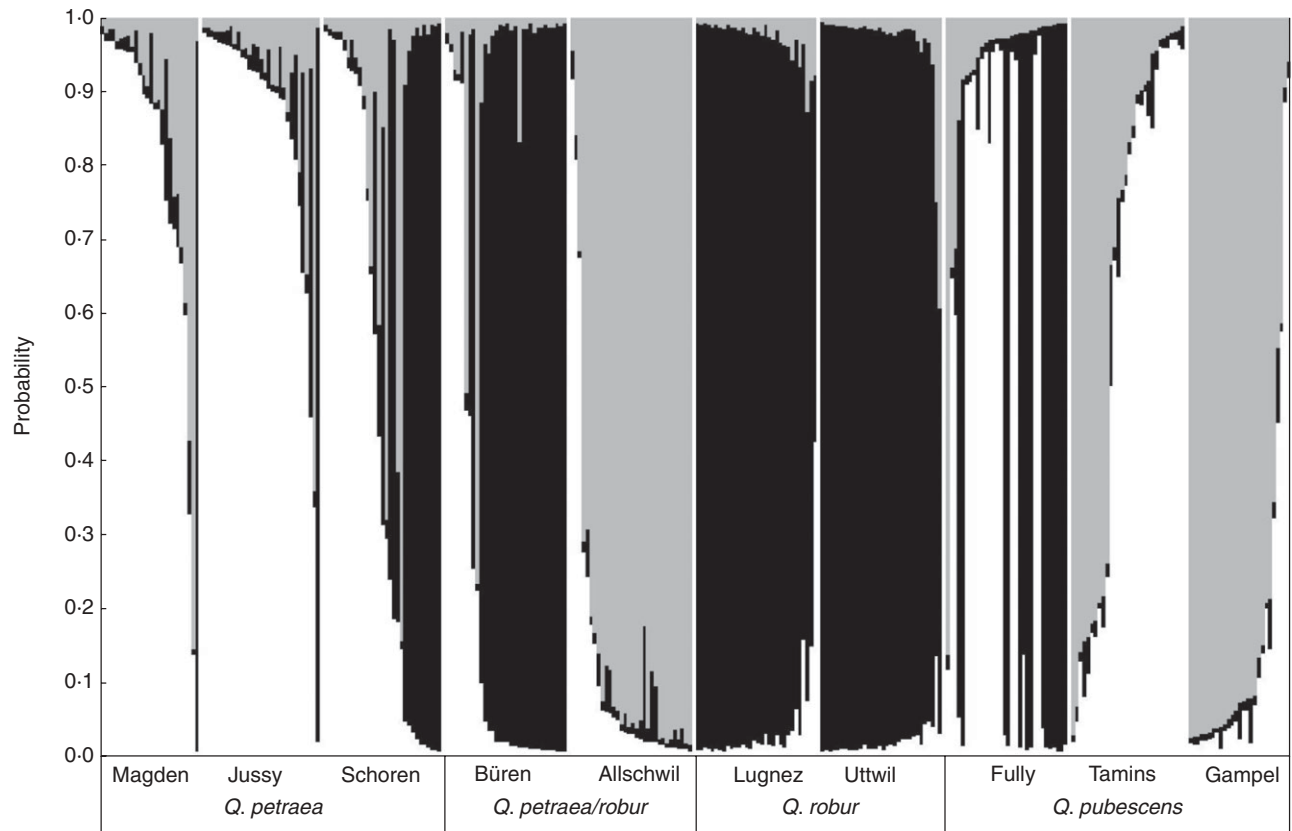


FIG. 3. Results of the assignment test for individuals from ten *Quercus* spp. stands from Switzerland using STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003) with $K = 3$ clusters. Each individual is represented by a vertical bar, with taxon assignment probabilities for *Quercus petraea* (white), *Q. robur* (black) or *Q. pubescens* (grey). Stands are arranged along the x -axis with their taxonomic classification based on leaf morphology by Finkeldey and Mátyás (2003). Within stands, individuals are sorted based on their posterior probabilities.

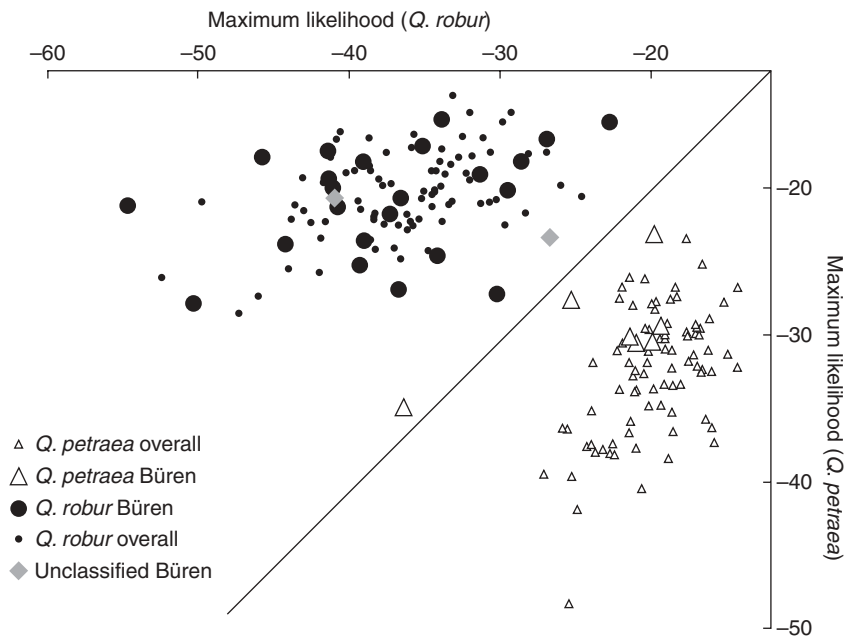


FIG. 4. Assignment of individual trees from the mixed stand Büren (*Quercus petraea*, *Q. robur*) to either of two synthetic populations composed from nine *Quercus* spp. stands from Switzerland (for details see text). Figure amended from (Gugerli *et al.*, 2005).

TABLE 2. Analyses of molecular variance (AMOVAs) based on five nuclear microsatellites of ten Swiss oak stands grouped as pure and mixed stands (*Quercus petraea*, *Q. robur*, *Q. pubescens* and mixed *Q. petraea/robur*) or pure stands only (both groupings were done on the basis of either leaf morphology or microsatellite data)

Grouping	Source of variation	Data basis for taxon assignment					
		Leaf morphology			Microsatellites		
		d.f.	SS	Percentage variation	d.f.	SS	Percentage variation
Pure and mixed stands	Among groups (Φ_{CT})	3	43.2	2.1 n.s.	3	54.2	3.9**
	Among populations within groups	6	42.4	3.6***	6	31.4	2.2***
	Within populations	600	1277.5	94.3***	600	1277.5	93.9***
	Φ_{ST}			0.057***			0.061***
Pure stands	Among groups (Φ_{CT})	2	36.9	3.5*	2	49.6	7.3***
	Among populations within groups	5	28.4	2.6***	3	13.6	1.8***
	Within populations	474	1005.7	93.8***	354	744.2	90.9***
	Φ_{ST}			0.062***			0.091***

Taxonomic assignment of stands to the respective groups follows the one given in Table 1.

d.f., degrees of freedom, SS, sums of squares.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant.

populations to prevent circular reasoning. Of the two previously ‘unclassified’ individuals, that were associated with *Q. robur* by multilocus genotype and spatial analyses in Gugerli *et al.* (2007), both were again assigned to *Q. robur* (Fig. 3). These observations were taken as support for taxonomic clustering of the ten study stands based on the first assignment test, further corroborating the respective association of genetic clusters (synthetic populations) and taxa (Table 1 and Fig. 3).

Fourthly, the clearest separation was obtained in AMOVA, i.e. the highest among-group partitioning of genetic variation (Φ_{CT}), when grouping stands according to the present assignment based on nSSR multilocus genotypes. Alternatively grouping stands according to their geographical proximity resulted in an among-group partitioning of genetic variation that was not significantly different from zero. This evidence shows that the taxon-specific signal of differentiation is stronger than the spatially induced differentiation in the present data set, suggesting that gene flow is lower among taxa than among populations.

Final evidence comes from regional oak occurrence in Switzerland. In several cases, the former taxonomic classification of the study stands, based on leaf morphology (Finkeldey and Mátyás, 2003), did not correspond to the clustering obtained with the assignment of multilocus genotypes in the present study. These discrepancies may be best explained by the distribution of oak species in Switzerland. Schoren is a mesic site in an area for which mixing of *Q. petraea* and *Q. robur* is reported (Brodbeck *et al.*, 1997). The proximity of the Jura mountains, where *Q. pubescens s.l.* occurs, suggests that the *Q. petraea* population Allschwil may well be associated with *Q. pubescens* (Brodbeck *et al.*, 1997). The stand Fully is situated in the central Alpine Rhone valley, where *Q. pubescens* has one of its main areas of distribution in Switzerland. However, both other oak species also occur in this region and intermixing is possible (Gams, 1927; Braun-Blanquet, 1961; Werner, 1988). Finally, Tamins is located in the central Alpine valley of the Rhine, which is at the northern brink of the occurrence

of *Q. pubescens* in Switzerland. It is likely that *Q. petraea*, the most abundant of the three oak species in this area (Müller, 1999), had an introgressive effect on this population. There is even the argument that *Q. pubescens* cannot be found in its pure form north of the Alps (Müller, 1999). Taken together, the present genetic assignment of stands to the three oak species is in accordance with their occurrence in Switzerland and with the literature, though detailed analyses at the individual level would be required to substantiate the coincidence of morphology and molecular data. We further advocate to thoroughly test the applicability of sets of nSSRs to clarify the status of *Q. pubescens* in Switzerland and elsewhere (but see Bruschi *et al.*, 2000). Since it was only possible to associate *Q. petraea* and *Q. robur* to particular genetic clusters based on previous results, the concluding proof that the third cluster represents *Q. pubescens* is also pending.

There were still some individuals with low posterior probabilities for one of the three clusters in the assignment test (Fig. 3). This is taken as an indication that the composition of some genotypes could be the result of hybridization and introgression. However, only diagnostic markers for the three species would unambiguously identify such hybrids and allow us to better characterize species compositions in white oak forests. Unfortunately, such diagnostic molecular markers are not available to date. Furthermore, because of the low intensity of within-genome sampling in the present study it is not possible to infer properly the degree of admixture among the three taxa studied.

From the present study, it is concluded that assignment methods using multilocus genotypes have great potential to identify species composition, genetic admixture and putative hybridization. Given adequate regional sampling and the selection of differentiated loci, single stands or seed lots could be readily characterized. Forest managers are in favour of maintaining pure oak stands, in particular when seed will be harvested for propagation. They argue that hybrid formation in mixed stands may hamper regeneration success – assuming that fitness is reduced in hybrids

compared with offspring from intraspecific crosses. If aiming at selecting pure stands, assignment tests as proposed here provide decision support towards the selection of suitable stands for management purposes. However, one must consider that long-distance gene flow via pollen may still lead to hybrid seeds even in pure stands. Furthermore, the present approach may be useful to identify and certify seed and other forestry products such as wood (Deguilloux *et al.*, 2004).

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