

Research review

Hybridization as a mechanism of invasion in oaks

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Summary

Key words: asymmetric introgression, chloroplast DNA, cytoplasmic capture, *Quercus petraea*, *Quercus robur*, seed dispersal, succession.

We review here our own research and related work on hybridization between two widespread and largely sympatric European oak species (*Quercus petraea* and *Q. robur*). There is a near total lack of local differentiation in chloroplast DNA markers between them. A model is proposed to account for this lack of differentiation: invasion by one species of the range occupied by the other through pollen swamping. In support of this model, ecological, palaeoecological and population genetic studies indicate that one species (*Q. robur*) disperses its seeds better than the other one, that pollen flow is much more efficient than seed flow in oaks and that hybridization and introgression are asymmetric, hence reinforcing the ecological dynamics by facilitating the dispersal of *Q. petraea* in regions already colonized by *Q. robur*. 'Resurrection' of *Q. petraea* following this wave of hybridization appears to be rapid. More generally, available evidence indicates that hybridization could constitute an important mechanism of dispersal in both natural and human-induced plant invasions.

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Introduction

The reasons why hybridization and introgression have raised (and are still raising) special difficulties for evolutionary theory are clear: these processes are operating at the interface between macro- and microevolution and hence the relevant level(s) of selection are not obvious. After disregarding hybridization as a dead end, an accident of nature, it became clear, especially with plants, that hybridization often plays a significant role in evolution. The most frequently cited consequences of hybridization are the apparition of new hybrid taxa, the demise of one or both parental taxa or (more rarely) the reinforcement of the parental taxa, all processes

related to speciation (Grant, 1981). Less attention has been paid to the seemingly less interesting case where the two parental taxa remain distinct (i.e. do not merge and do not produce new taxa) despite some level of gene flow between them. Indeed, the role of regular gene flow between genetically differentiated taxa has not been of central interest in hybridization studies, even in modern treatments of the topic. For instance, although Arnold (1997) emphasized the fact that hybridization has the potential to be evolutionary significant in its own right, he mostly stresses its creative role in producing new lineages. This view underscores other roles for hybridization in the functioning of 'species complexes'. This latter term had been used by Pernès (1984) to describe

species linked by evolutionary adjusted gene flow: 'Compartments [of a species complex] are created by disruptive selection, it is an adaptive response genetically coordinated. When the control of these exchanges is made by a reproductive barrier, this barrier is not passive but is actively adjusted, in an evolutionary context that makes it desirable, by selecting within the available genetic diversity' (our translation). Within such species complexes, interspecific gene flow is fully acknowledged as being part of the evolving system and both proximate and ultimate explanations for the degree of control of these exchanges are called for. Furthermore, such gene exchanges are not necessarily connected with particular stages of the speciation process.

Here, we would like to examine one of the 'ancillary' roles of hybridization, but one which might turn out to be more important than generally assumed, namely hybridization as a mechanism of dispersal (Potts & Reid, 1988, 1990; Petit, 1992; Petit *et al.*, 1997). As we will show, this requests that we consider species ranges as dynamic entities. The relation between invasion and hybridization is a classic of plant evolutionary studies (Anderson, 1949; Abbott, 1992), but the emphasis has been on the new genetic combinations generated by hybrids. The so-called 'hybrid soup', by masking deleterious genes or transferring favourable ones, may indeed help trigger invasions (Abbott, 1992; Petit *et al.*, 2003), but hybridization may also boost invasion independently of the transfer of adaptive traits, as it is a form of gene flow. Here, we show that colonization of newly available territories by oaks (for instance following successive ice ages) is facilitated by interspecific gene exchanges, allowing some species to bypass colonization by seed.

Oaks and the species question

Oaks have long been one of the favourite groups of plants to address the issue of the nature of species. They are abundant, often dominant species in the temperate or Mediterranean parts of the northern hemisphere, and hence a very visible and familiar part of the human environment in these regions. The last checklist of the genus *Quercus* includes 531 species (Govaerts & Frodin, 1998), which compares with some 300 in the first comprehensive list established by de Candolle (1862). In Chapter 2 of the sixth edition of *The origin of species by means of natural selection* Darwin describes the refined principles used by de Candolle to delineate oak species (Darwin, 1872). Somewhat paradoxically and ironically, such careful analyses do not result in greater taxonomic clarity but instead result in greater confusion. This led him to reject the idea of the fixity of species and the very reference to an ideal type postulated by creationists. The early attempts by Darwin to show that species limits were often fuzzy made full sense in the scientific context of the time. The question of whether species constitute real natural biological objects could probably only develop after biological evolution had become

widely accepted. Oaks then continued to be the focus of intense research and have appeared in all major discussions on plant evolution, largely because of their propensity to intercross (Stebbins, 1950; Burger, 1975; Grant, 1981). Among the species concepts that flourished during the twentieth century, one (the ecological species concept) was developed with reference to oaks (Van Valen, 1977).

Cytoplasmic sharing in oaks and its interpretation

Sharing of cytoplasm among sympatric oak species

In Europe, the two species that have been the object of most attention are pedunculate oak (*Quercus robur* L.) and sessile oak (*Q. petraea* (Matt.) Liebl.). These oaks differ in many respects and have been selected by Camus (1936–1954) to represent the types of two different subsections of the section *Lepidobalanus*. They are largely sympatric: the range of *Q. petraea* lies nearly completely within that of *Q. robur* (Fig. 1). Furthermore, they are often mixed: in northern and western Germany, detailed morphological analyses of over 1400 stands showed that only 18% consisted of a single oak species (Kleinschmit & Kleinschmit, 2000).

Despite innumerable studies, it turns out that no single morphological trait can distinguish these two species unambiguously. With the arrival of DNA technology, there were hopes that clear-cut specific differences would be readily identified. Instead, the first studies revealed that most chloroplast DNA (cpDNA) variants were shared across the two species (Kremer *et al.*, 1991; Petit, 1992). Similar findings were obtained simultaneously for the American white oaks (Whittemore & Schaal, 1991). Kremer & Petit (1993) summarized how nuclear and organelle diversity are partitioned within oak species complexes, pointing to the low differentiation between species. Subsequent studies demonstrated that the lack of differentiation at organelle markers was not only obvious at a broad scale but also at a much finer geographical scale (Petit *et al.*, 1997). This implied that interspecific exchanges had occurred after the last ice age, either during or after postglacial colonization, only a few thousand years ago. The propensity of different oak species to share similar cpDNA variants when in sympatry has now been quantified and tested statistically. One recent survey, based on 274 mixed-species French populations, indicates that one *Q. robur* individual tree is equally likely to share the same cpDNA variant with a nearby conspecific ($P = 0.80$) than with a nearby *Q. petraea* tree ($P = 0.81$) (Table 1; Petit *et al.*, 2002c). Mitochondrial markers, which are cotransmitted with cpDNA markers through the female gamete in oaks (Dumolin *et al.*, 1995), display a similar absence of differentiation between species (Dumolin-Lapègue *et al.*, 1998). Contrary to cytoplasmic markers, some nuclear markers show more or less important differences in allelic frequencies between species (Bodénès *et al.*, 1997a). Levels of nucleotide

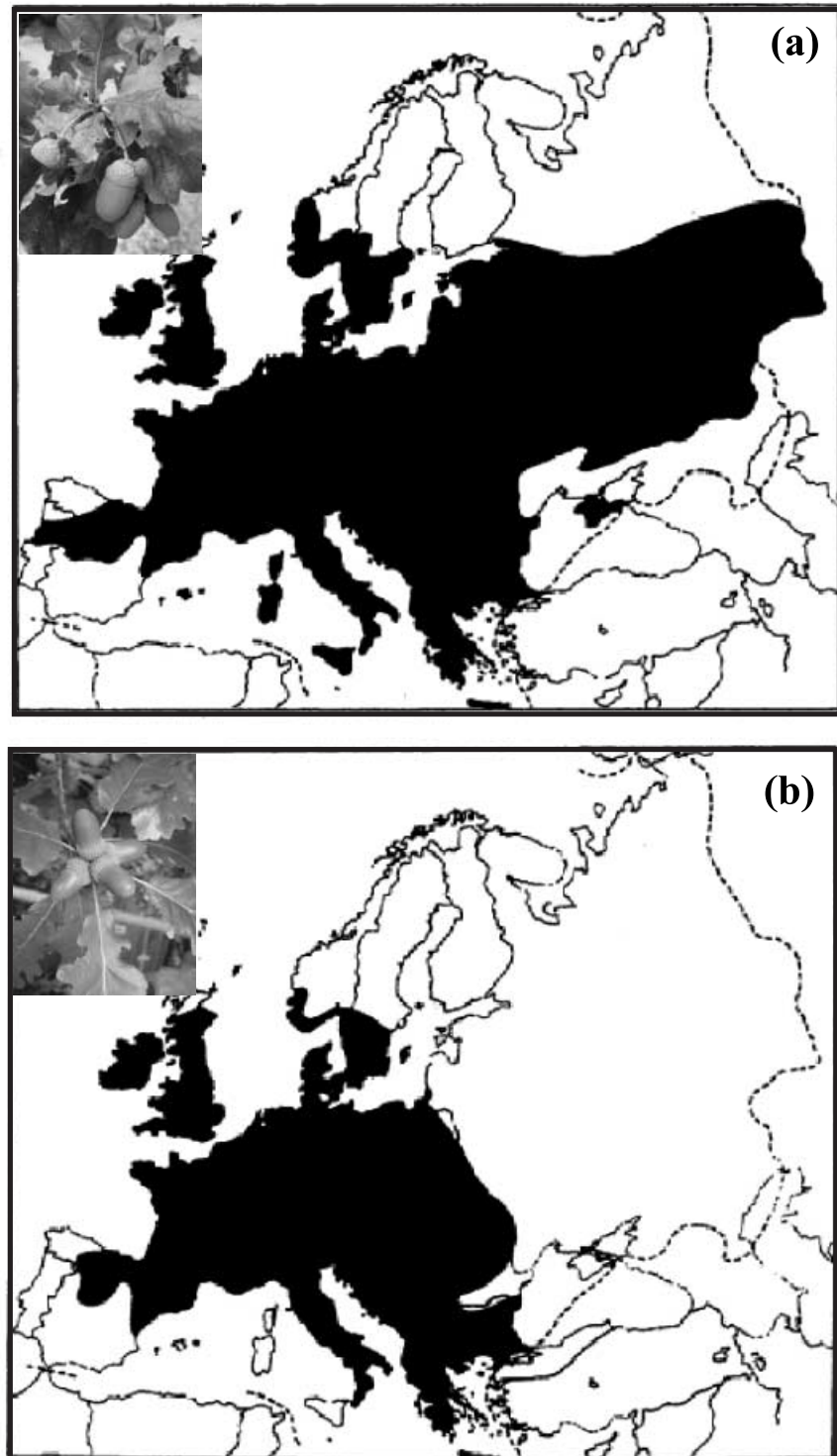


Fig. 1 Simplified distribution ranges of *Quercus robur* (a) and *Q. petraea* (b) in Europe. From Meusel *et al.* (1965). Note that the range of *Q. petraea* is nearly completely included within that of *Q. robur*. The presence or absence of peduncle is one of the most distinctive features distinguishing these two species.

divergence between the two species are generally low (0.5% on average), but may reach up to 3.3% in the most differentiated chromosomal regions. Other studies indicate that species are broadly consistent across the range, both in morphology (Kremer *et al.*, 2002; see Fig. 2) and at molecular

markers. When interspecific differentiation was partitioned into two components ('general' and 'local' differentiation), it could be shown that general interspecific differentiation was predominant over local interspecific differentiation at most nuclear loci investigated (Zanetto *et al.*, 1994; Bodénès *et al.*,

Table 1 Mean inter vs intraspecific cpDNA identities for oak (*Quercus*) individuals from the same population in France

Species	Number of populations	Interspecific identity	Intraspecific identities
<i>Q. robur</i> / <i>Q. petraea</i>	274	0.81	
<i>Q. robur</i>	155		0.80
<i>Q. petraea</i>	146		0.88

Identity: probability that two individuals share the same chloroplast haplotype. From Petit *et al.* (2002c).

1997b). Hence, there is a lack of cytoplasmic differentiation between these two species despite real consistent genetic differences at nuclear markers and at adaptive traits.

Interpretation of cytoplasmic sharing: invasion by the pollen parent

In the first detailed review on the introgression of organelle genes across species (Rieseberg & Soltis, 1991), numerous cases of plants were found to have the nuclear genome of one species and the cytoplasmic genome of another. This phenomenon was labelled 'cytoplasmic capture', implying that cytoplasmic exchange had occurred in the absence of significant gene flow, the 'native' cytoplasm being completely displaced by an alien one. Because hybrids and their offspring are often male-sterile, they could in principle transmit their organelle genomes without diffusing too many nuclear genes, thereby incorporating alien cytoplasm in a given population

without altering the local nuclear gene frequencies much. Our scenario considers instead that species often colonize a site already occupied by a related species through pollen rather than through seeds (i.e. through pollen swamping, following hybridization) (Petit, 1992; Bacilieri *et al.*, 1996a; Petit *et al.*, 1997, Fig. 3). Indeed, once a given species has established itself, a large number of female flowers are produced, which are partly receptive to the pollen of the other species. Given the frequent asymmetry of pollen and seed flow, this would result in hybrids, and eventually in backcrosses and other hybrid-derivatives characterized by the original (maternal) cpDNA structure but an increasing proportion of the nuclear genome of the second species. The species expanding later relies therefore not on seeds but on pollen for its dispersal into new environments, as in the model proposed for *Eucalyptus* in Tasmania (Potts & Reid, 1988). For these authors, 'where seed migration is limited, a moving front may be heralded by a wave of hybridization'.

Because seed flow is low (implying that maternal lineages are nearly static) and pollen flow extensive in this model, the terms 'nuclear capture' or 'pollen swamping' would better reflect the actual process (i.e. repeated backcrosses with incoming pollen). In fact, 'cytoplasmic capture' is a term coined in the context of phylogenetic reconstruction, which has gained popularity and is now being used in another context (population biology) where it appears misleading. Strictly speaking, the only case of chloroplast 'capture' by a resident nucleus has been described in kiwifruit, where gynogenetic plants receive their chloroplasts from the father without concomitant transfer of paternal nuclear DNA (Chat *et al.*, 2003).

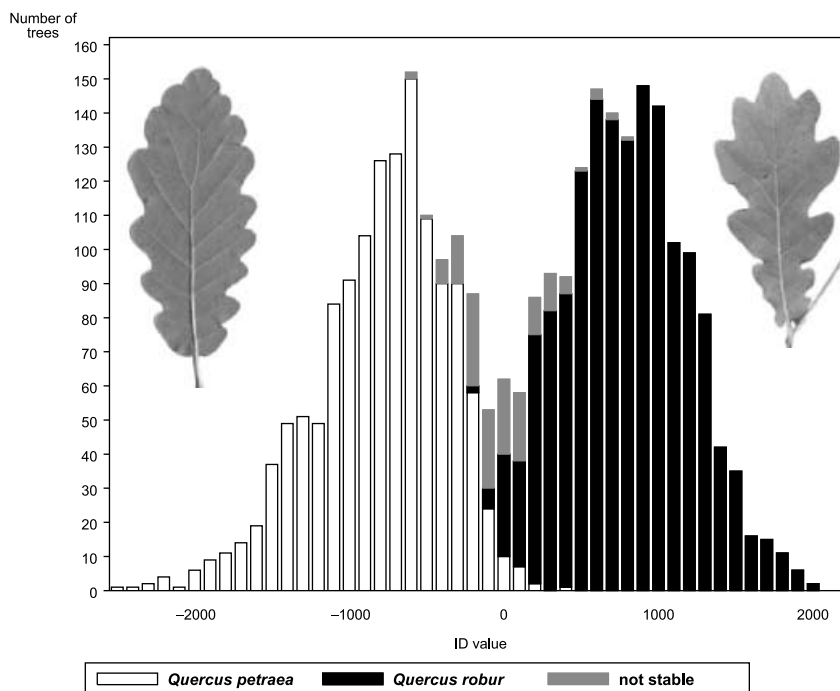
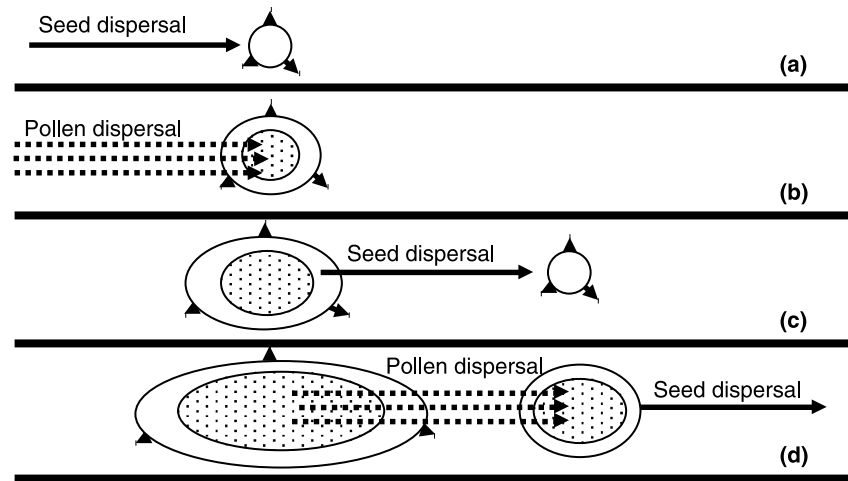


Fig. 2 Discrimination of leaves of *Quercus robur* and *Q. petraea* across Europe. First axis of a multivariate analysis based on several morphological characters. From Kremer *et al.* (2002), with permission.

Fig. 3 Model of invasion in the *Quercus robur*/*Q. petraea* complex. (a) Long distance dispersal of an acorn of *Q. robur* establishes an isolated pioneer population of that species. (b) Long-distance pollen flow of *Q. petraea* allows this latter species to invade through hybridization with the already established *Q. robur*. Backcrosses of these hybrids with *Q. petraea* and selection in favour of this late-successional species then allow the emergence of *Q. petraea* types in few generations. *Q. petraea* trees are located in the densest parts of the new population which is bordered by *Q. robur*. This latter species extends further by diffusion. (c) and (d) This process continues, first involving long-distance seed-dispersal of *Q. robur*, then interspecific pollen flow into the newly formed population.



Below, we describe the arguments that support the scenario of pollen swamping (resulting in the dispersal of the pollen parent) for these two particular oak species. We first show that seed dispersal abilities differ between the two oak species, using information from the ecological and palaeoecological literature and from population genetics surveys. We then summarise evidence indicating that pollen flow is much more prevalent than seed flow in these species, and show that there is both an ecological and a genetic component to succession, since the direction of introgression favours the spread of the genes of the later successional species.

Gene flow within each species

Relative dispersal abilities of the two oak species: ecological and palaeoecological evidence

Despite nearly complete cytoplasmic sharing between the two species, their ecology suggests that their seed dispersal ability should differ. First, oaks growing in open habitats or at the edge of a forest (which are more likely to belong to *Q. robur*) bear acorns earlier and in greater quantities (Jones, 1959). Second, several factors may contribute to the preferential dispersal of *Q. robur* acorns by jays. Acorns are eaten by a great variety of animals, but jays, and to a smaller extent other birds, are the principal long-distance dispersers (Ducousso *et al.*, 1993). Bossema (1979) has clearly shown the close coevolution of oaks and the European jay (*Garrulus glandarius* L.). This bird relies on oaks year round, because even after germination the cotyledons retained by seedlings are collected by the jay. A single bird can scatterhoard some 4600 acorns each year (Schuster, 1950), and a minimum of 50% of oaks were planted by jays in Bossema's study area, as shown by bill marks on the cotyledons of young seedlings. Seeds are transported in the oesophagus and in the bill (up to nine per trip) at distances of up to 8 kilometres from the mother tree

(Schuster, 1950). This behaviour should favour oak dispersal because not all caches are recovered and 14 days after germination cotyledon removal does not hinder seedling growth anymore (García-Cebrián *et al.*, 2003). Bossema (1979) experiments indicate that acorns are selected by the birds according to their weight and shape: they prefer long, slim acorns that can be easily swallowed. The average width : length ratio and weight of acorns differ significantly between the two oaks, and acorns of *Q. robur* much more closely match the jays' preferred ideotype (Jones, 1959; Bossema, 1979; Dupouey & Le Bouler, 1989, see Fig. 4).

Also, jays collect acorns still hanging on the trees in September/October before their dehiscence (Bossema, 1979), and, because 'the acorn of *Q. robur* is rendered much more conspicuous than that of *Q. petraea* when on the tree by its long peduncle and relatively thin foliage' (Jones, 1959, p. 194), the birds are likely to visit *Q. robur* trees preferentially. One of the most conspicuous differences between the two oak species is indeed the peduncle that bears the cup in *Q. robur* (hence its name 'pedunculate' oak), which consists of the greatly elongated inflorescence rachis (Fig. 1). The pattern of fruit presentation is thereby considerably modified in this particular species, providing a visual clue for the bird.

In addition, radicle emergence is often delayed after the winter for *Q. robur* whereas acorns of *Q. petraea* generally germinate quickly in autumn (Ciumac, 1966). Jones (1959) also noticed that acorns of *Q. petraea* are often viviparous. The seeds of *Q. robur* rapidly lose viability on the soil surface, and the act of burial by birds may be vital for the regeneration of this species in some habitats (Watt, 1919). By contrast, *Q. petraea* acorns can survive the loss of an appreciably higher proportion of water than those of *Q. robur* (Jones, 1959). *Q. petraea* germination appears optimum under a thin litter cover and lowest when buried in the soil (Shaw, 1968). Initial growth, largely because of strong maternal effects (Krahl-Urban, 1959), is faster for *Q. robur* than for *Q. petraea* by a

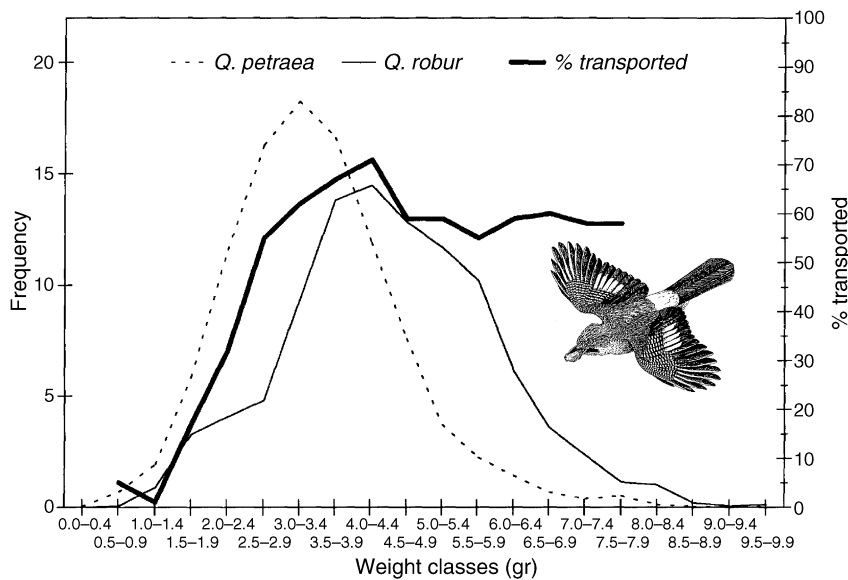


Fig. 4 Selection of acorns by jays according to weight, showing that acorns of *Quercus robur* will be preferentially collected by the bird, as their size better match its preferences. After Bossema (1979) and Dupouey & Le Boulter (1989).

factor of two when light is not limiting (Steinhoff, 1998). In summary, it appears that *Q. robur* acorns should be dispersed more frequently by jays over longer distances and should then be able to establish more readily.

Palaeoecological data should allow retrospective test of the presumed differences in colonization ability between the two species. Unfortunately, most available data from palynological studies deal with deciduous species of *Quercus* indistinctly since the identification of pollen grains is difficult. Recolonization was rapid, up to 500 meters per year in England where oaks had completely disappeared during the last glaciation (Huntley & Birks, 1983). A careful pollen-analytical study of the range expansions of several tree taxa in Norfolk reveals two distinct phases of expansion separated by approx. 700 yr for *Quercus* spp. (Bennett, 1983). The rate of increase during the two phases differed. Populations doubling time was estimated to be 60–73 yr for the first phase and 127–290 yr for the second phase. Bennett (1983) suggested that the more opportunist *Q. robur* formed the earlier, faster phase, and *Q. petraea* the second, slower phase. *Q. robur* may also have arrived first in southern Scandinavia, about 8500 yr ago, while *Q. petraea* only arrived some 4000 yr ago (von Post, 1924). This result was obtained by relying on slight differences (mostly based on size) between the pollen of both species. Using a similar approach, Planchais (1970) found a good correlation of *Q. robur* pollen-type with *Alnus* pollen and of *Q. petraea* pollen-type with *Fagus* pollen, in agreement with the ecology and successional role of these species. The percentage of *Q. petraea* pollen-type was strongly affected by deforestation and recovered more slowly afterwards. These early studies should now be critically evaluated using other methods. Although leaf or fruit remains (more readily identified to the species level than pollen grains) are rare, wood macrofossils could be used along with pollen data to obtain information on species-specific

Table 2 Comparison of genetic differentiation at cpDNA markers between *Quercus robur* and *Q. petraea*

	<i>Q. robur</i>		<i>Q. petraea</i>	
	N ¹	G _{ST} (se)	N ¹	G _{ST} (se)
Alpine region	168	0.749 (0.036)	121	0.892 (0.029)*
France	305	0.755 (0.022)	170	0.835 (0.026)*
Central Europe	213	0.671 (0.026)	169	0.799 (0.026)*
Great Britain	93	0.651 (0.045)	84	0.845 (0.040)*
North Balkans	82	0.718 (0.042)	97	0.825 (0.033)*
Northern Europe	116	0.864 (0.026)	27	0.873 (0.042)
All	984	0.781 (0.010)	650	0.856 (0.011)*

¹N, number of populations. *, $P < 0.01$.

colonization patterns after the last ice-age (T. Litt, pers. comm.). Indeed, careful morphological and anatomical studies (Feuillat *et al.*, 1997) indicate that identification of these wood remains could be attempted.

Relative dispersal abilities of the two oak species: genetic evidence

Contrasted seed dispersal abilities should result in different levels of differentiation at maternally inherited genes. A recent extensive European survey of cpDNA variation of oaks in Europe has allowed testing of this prediction: in all six regions investigated, *Q. robur* had lower level of differentiation than *Q. petraea*, the difference being significant in five out of six cases (Table 2).

At nuclear genes, the local (within stand) spatial genetic structure (due in large part to the maternal – i.e. seed – component of gene flow) should also differ between species. Studies in France using both isozymes and microsatellites (Streiff

Table 3 Comparison of genetic differentiation at nuclear markers between *Quercus robur* and *Q. petraea*

Study	Region studied	Marker	Nb of loci	Nb of pairs of populations	G_{ST} <i>Q. robur</i>	<i>Q. petraea</i>
Coart <i>et al.</i> (2002)	Belgium	AFLP	170	4	0.021	0.021
Mariette <i>et al.</i> (2002)	Europe	AFLP	155	7	0.030	0.044
		Microsatellites	6		0.020	0.023
Müller-Starck <i>et al.</i> (1993) ¹	Germany	Isozymes	13	5	0.017	0.037
Zanetto <i>et al.</i> (1994)	Europe	Isozymes	13	7	0.024	0.032
Mean					0.022	0.031

¹Computations after Petit (1992).

et al., 1998) and in England using microsatellites (Cottrell *et al.*, 2003) have confirmed this prediction: small-scale spatial genetic structure is stronger in *Q. petraea* than in *Q. robur*. At broader scales as well, genetic differentiation is higher for *Q. petraea*. In five out of six cases, *Q. robur* displays lower genetic differentiation among populations, pointing to higher gene flow, regardless of the type of nuclear markers considered (Table 3).

What is remarkable is the consistently lower level of differentiation for nuclear compared to cpDNA markers: 0.02 compared to 0.78 for *Q. robur* and 0.03 compared to 0.86 for *Q. petraea*. This suggests that pollen flow is much larger than seed flow in these two species: 150 times greater in *Q. robur* and 180 times greater in *Q. petraea* (Petit, 1992), if we accept the very strong assumptions of the underlying model (Oddou-Muratorio *et al.*, 2001).

Recent parentage analyses in American oaks using hyper-variable microsatellite nuclear markers also indicate extremely high levels of long-distance pollination and more limited acorn dispersal (Dow & Ashley, 1996). Currently, direct estimates of pollen and seed dispersal are being gathered in several laboratories across Europe (through paternity and maternity analyses) that should considerably refine our knowledge on gene flow in *Q. robur* and *Q. petraea*. However, the relative importance of seed and pollen flow is likely to be scale-dependant and results obtained at a local scale might in fact underestimate the relative contribution of pollen flow at larger distances (McCauley, 1997).

Ecological and genetic mechanisms of oak invasion and succession

Ecological evidence: succession and ecological specialization

We have seen that *Q. robur* better disperses its seeds than *Q. petraea*. Numerous results have now shown that these oaks also differ in other ecological attributes related to their more or less pioneer character. Duhamel (1984) has calculated the width growth of the stem as well as a 'crown concurrence index' for the two species in 124 mature, mixed populations

in north-west France. The concurrence index (percentage of the crown which is in contact with other trees) was 86% for *Q. petraea* and 78% for *Q. robur* in mixed populations, and *Q. petraea* had, on average, a 46% greater width growth (Fig. 5). Common garden experiments indicate that some differences observed *in situ* between these oaks species have a genetic basis. For instance, in various Danish field trials established between 1900 and 1988, provenances of *Q. petraea* were growing more rapidly than provenances of *Q. robur* on sandy locations and produced fewer epicormic branches (Jensen, 2000). Similarly, under reduced light, *Q. petraea* achieves better relative growth and survival (Plaisance, 1955). Further studies suggest that both abiotic and biotic mechanisms account for the advantage of *Q. petraea* in mixed stands, leading to the mechanism of succession, including both greater drought tolerance of *Q. petraea* and greater sensitivity of *Q. robur* to diseases (Table 4) or to herbivores. However, *Q. robur* appears to be better tolerant to soil anoxia. Therefore, in mixed stands, succession towards *Q. petraea* would be the rule except under permanently humid conditions. The process of succession could help understand not only the respective timing of the invasion by the two species following ice retreat but also their dynamics once range expansion was achieved. Perturbations, whether natural or induced by man, mean that oak species differing by their successional status should persist in the landscape, as a consequence of trade-offs between traits, namely allocation of resources to seed production and dispersal vs resistance to density through increased drought and disease resistance.

Genetic evidence: the direction of interspecific gene flow

If they occurred in the same direction than ecological succession, interspecific gene exchanges could reinforce succession (Petit *et al.*, 1997). Under natural conditions, mating system studies based on isozymes have shown that the contribution of *Q. petraea* to *Q. robur* progenies can vary from 17% to 48%. They also demonstrated that ovules of *Q. petraea* trees are preferentially fertilized by 'extreme' *Q. petraea* genotypes, resulting in progenies more different from *Q. robur* across



Fig. 5 Crown architecture and interspecific competition between *Quercus robur* (left, forefront) and *Q. petraea* (tallest tree on the right). The presence of epicormic branches on the trunk of the *Q. robur* individual illustrates the stress experienced by this tree. Picture taken after thinning of a denser mixed stand.

generations (Bacilieri *et al.*, 1996a). This suggests that backcrosses are occurring and that they are also asymmetric. In the mixed oak stand investigated, *Q. petraea* represented 47% of the adult trees but at least 63% of the seedlings (Bacilieri *et al.*, 1996a). More than half of this increase was caused by directional hybridization or backcrossing, the rest being attributed to the ecological dynamics.

Morphological markers have also been employed to analyse hybridization in the same stand. Seeds were collected on trees from both species that differed in their pollination environment (defined as the proportion of each oak species surrounding the seed tree), and seedlings were grown in a glasshouse for 3 yr before being subjected to morphological measurements. Only when considered together in multivariate analyses were morphological markers informative with regard to hybridization. The distribution of the individual values on the first axis of the discriminant analysis of the morphological traits was bimodal for the offspring of *Q. robur* (with one peak shifted in the direction of *Q. petraea*), especially for those trees

surrounded by allospecifics, whereas the offspring of *Q. petraea* had a unimodal distribution. This supports the previous interpretation of unidirectional interspecific gene flow based on isozymes. Molecular studies based on molecular markers (RAPD) further confirmed these results (Bacilieri *et al.*, 1996b).

Controlled pollination studies carried out in several countries in Europe (Fig. 6) indicate that this asymmetric gene flow has a genetic basis. In the study of Steinhoff (1998), 40 485 flowers of *Q. robur* and *Q. petraea* were artificially pollinated between 1989 and 1996. A total of 5261 acorns and 1933 plants developed from these crosses, including 568 hybrids. Most hybrids were from the combination *Q. robur* (female) \times *Q. petraea* (male). In fact, *Q. petraea* females appear to be more 'choosy' in both inter- and intraspecific crosses (Table 5). As a consequence, the success rate of the interspecific crosses *Q. robur* (female) \times *Q. petraea* (male) was as high as that of crosses between *Q. petraea* individuals. In other oak species complexes, asymmetric introgression are caused by differences in flowering phenology (Belahbib *et al.*,

Table 4 A selection of studies comparing the reaction of *Quercus robur* and *Q. petraea* to abiotic and biotic traits

Study	Trait	Main findings
Cochard <i>et al.</i> (1992)	Drought	Studies on excised branches showed that <i>Q. robur</i> was more vulnerable than <i>Q. petraea</i> to water-stress induced cavitation.
Epron & Dreyer (1993)	Drought	Effect of drought was assessed experimentally under natural conditions. Slight differences were observed, <i>Q. robur</i> displaying smaller leaves, lower chlorophyll contents, and a larger stomatal conductance at equivalent net assimilation rates than <i>Q. petraea</i> .
Ponton <i>et al.</i> (2001)	Drought	The carbon isotope discrimination approach was used to test whether the two species present distinct water use efficiencies. Leaves and dated ring sequences were sampled in 10 pairs of adult trees growing side by side. <i>Q. robur</i> had larger isotopic discrimination than <i>Q. petraea</i> and therefore lower intrinsic water use efficiency (−13%).
Schmull & Thomas (2000)	Waterlogging	Seedlings of <i>Q. robur</i> and <i>Q. petraea</i> were cultivated in lysimeters and subjected to waterlogging for several weeks. The growth of <i>Q. robur</i> was less affected by waterlogging than that of <i>Q. petraea</i> . The decrease in the hydraulic conductance was smallest in <i>Q. robur</i> .
Wagner and Dreyer (1997)	Waterlogging	Potted seedlings were submitted to contrasted irradiance regimes in a greenhouse and shortly after to waterlogging. <i>Q. petraea</i> experienced more photosynthesis dysfunctions, suggesting increased sensitivity to waterlogging. After the stress, <i>Q. robur</i> behaved best, recovering high levels of predawn water potential, slowly reopening stomata and reincreasing net assimilation rates.
Marcais & Cael (2000)	Fungi	Young saplings were inoculated in the glasshouse with <i>Collybia fusipes</i> . Susceptibility of <i>Q. robur</i> saplings was higher than that of <i>Q. petraea</i> saplings.
Liesebach & Stephan (2000)	Fungi	Seedlings of <i>Q. robur</i> were more severely attacked by powdery mildew (<i>Microsphaera alphitoides</i>) than those of <i>Q. petraea</i> .
Thomas <i>et al.</i> (2002)	Factor interaction (biotic and abiotic)	Using evidence ranging from experiments with young trees to monitoring on a supraregional scale, it was shown that hydromorphic site conditions render <i>Q. robur</i> more susceptible to drought stress than <i>Q. petraea</i> as a result of an impairment of root growth in the subsoil. Furthermore, excess nitrogen, in combination with drought stress, results in decreased foliar concentrations of allelochemicals in <i>Q. robur</i> , thereby making that species more susceptible to insect defoliation.

2001) or by decreased male fitness of one of the two species – the environmental emasculation hypothesis – (Williams *et al.*, 2001), but none of these two mechanisms seems to be operating in the case of *Q. petraea* and *Q. robur* (Bacilieri *et al.*, 1995).

The 'resurrection' process: emergence of distinct entities following introgression

Although asymmetric gene flow may indeed favour the 'resurrection' of *Q. petraea* through preferential backcross of the initial F1 hybrids with other *Q. petraea* trees, the completion of this process would seem to necessitate additional conditions such as a large number of generations; very high selection pressures in favour of *Q. petraea*; and a low number of species-related Quantitative Trait Loci (QTLs) clustered on one or at most very few linkage groups. The latter prediction seems necessary because the introgression of many independent traits simultaneously is very difficult, requiring very high sample sizes and a considerable number of generations (Hospital, 2001).

Some but not all of these criteria appear to be fulfilled in oaks. Population sizes are often very large and individual oak trees are very prolific, especially if one considers their life-long productivity. This should allow very high selection pressures, especially on seedlings and young saplings. Furthermore, early studies on oaks seemed to point out the existence of a limited number of genes involved in species differentiation. Stebbins (1950, pp. 64–65) noted that 'the progeny of both artificial and natural oak hybrids [...] usually segregate so sharply that even among a relatively small number of individuals the parental types can be recovered'. This led him to infer that 'the number of genes by which species of oaks differ from each other is considerably smaller than in [...] most other plant groups'. In support of this observation, morphologically intermediate individuals are rare in most mixed stands (Kremer *et al.*, 2002), which suggests that the number of generations needed for 'resurrection' of a morphologically typical *Q. petraea* must in fact be quite low. However, recent work has shown that the number of QTLs involved in the differentiation between *Q. robur* and *Q. petraea* is high, involving at least nine of the 12 pairs of chromosomes (Saintagne *et al.*, 2003),



Fig. 6 Controlled crosses in oaks at the INRA research centre in Cestas.

Table 5 Results of the controlled crosses of *Quercus robur* and *Quercus petraea* carried out in Germany

		Female parent <i>Q. robur</i>	<i>Q. petraea</i>
Male parent	<i>Q. robur</i>	11.3% (7000)	0.8% (12 000)
	<i>Q. petraea</i>	5.3% (9000)	5.8% (9000)
	Selfing	1.3% (2000)	0.0% (2000)

Percentages of flowers yielding acorns in each type of cross and approximate number of inflorescences used for each cross (in brackets), after Steinhoff (1998).

so a low number of loci does not seem at this point to account for the observation of rapid segregation. To resolve this puzzle, one ought to consider that fixed genetic differences between species have not been detected so far; rather, only frequency differences are known between species, in contrast to other hybridizing oak species (Craft *et al.*, 2002). In fact, it is only

when several traits or markers are considered together in multivariate analyses that well-distinct clusters of individuals corresponding to the two species emerge. This may be caused by allelic associations at different QTLs (including epistasis) or by genetic redundancy in morphogenesis. Clearly, further work is needed to describe the underlying mechanisms of species morphological integration.

The resurrection phase may be accompanied by the introgression of other adaptive traits. In principle, populations emerging after an episode of hybridization and introgression should be better adapted than those arising from regular seed dispersal if QTLs involved in local adaptation (but unrelated to species differentiation) are transferred between species during that process. Preliminary data suggest that geographic trends in the timing of bud burst are similar across the two oak species (A. Ducousso, unpublished); although convergence could also account for this observation, it fits well with the hypothesis that local adaptations are transferred from the more pioneer to the later successional species during invasion.

Research perspectives

Additional research could test critical aspects of the model presented here. Although unusually detailed phylogeographic studies have been carried out in this oak complex (Petit *et al.*, 2002b,a), location of colonization routes and timing of expansion have not been attributed to particular species. Methods to identify subfossil oak remains such as wood to the species level combined with the development of macrofossil databases may help reconstruct the timing of colonization by each species. Newly developed techniques to uncover DNA from wood (Dumolin-Lapègue *et al.*, 1999; Deguilloux *et al.*, 2002, 2003) and new markers displaying large frequency differences between the two species (Bodénès *et al.*, 1997a; Muir *et al.*, 2000) could be combined for that purpose. Such markers, if applied to seeds collected on *Q. robur* trees, could be used to determine the species that contributed the male gamete. In particular, the existence of long-distance inter-specific gene flow (the first stage of our model) may be tested by applying this method to isolated *Q. robur* stands.

Full parentage analyses of seedlings growing in mixed stands or subsequently evaluated in progeny tests could permit us to classify them into pure parental or hybrid origins. This would allow evaluating the behaviours of hybrids and of pure parental taxa, by relying on the morphology of the parents of each plant rather than on that of the plants themselves (not always a good indicator of hybrid status).

Controlled-crosses have been used to produce hybrids whose morphology can be investigated, but interpretations are complicated by differences between leaf morphology of mature and young trees (Kleinschmit *et al.*, 1995). The oldest hybrid plants were seven years old and grew as well as their intraspecific sibs, but with clear indication of maternal effects (Steinhoff, 1998). Reanalysis of such data at a later stage could help evaluate hybrids' appearance and fitness. Backcrosses or F2 may further allow investigating the segregation of the traits involved in species distinction and should help understand the mechanisms of species morphological integration.

Conclusions

Succession can be defined as the directional change in time of the species composition and vegetation physiognomy of a single site (Finegan, 1984). The facilitation hypothesis of succession, wherein changes caused by a species or group of species make the environment suitable for the next species or group of species, has been much disputed because of its apparent teleological character. One way in which species may interact, but one that has rarely been considered in this context, is through exchange of genetic material. In fact, if species involved in succession are closely related and have the potential to hybridize, the study of the control of gene flow between them becomes of particular interest.

Organization into a species complex (*sensu* Pernès, 1984)

could slow down speciation rates by dynamically maintaining species distinction through disruptive selection, in a kind of steady state very different from incipient speciation. This could increase species longevity by boosting their dispersal potential, as the range colonized by related species will be more accessible to invasion if hybridization and introgression are possible; not only seeds but also pollen can then be used as vehicles for invasion.

Studies involving other oak species (Belahbib *et al.*, 2001) or other tree species, both wind- and insect-pollinated (Potts *et al.*, 2003), indicate that pollen-mediated invasion could be a widespread phenomenon in species where pollen flow predominates over seed dispersal (i.e. a large fraction of plant taxa; Petit & Vendramin, in press). The transfer of local adaptations from the early colonist into the later successional species is not a prerequisite in this model but could operate in synergy. Recently, displacement of native by invasive species has attracted much interest and it has been shown that when closely related native and invasive species are involved, genetic introgression often plays an important role (Huxel, 1999). Pollen-mediated dispersal into the range of another species could therefore play a significant role in both natural and artificial invasions (Petit *et al.*, in press).

If hybridization and introgression were associated with speciation events, they should be rarely detected in trees, which are long-lived not only as individuals but also as species (Stebbins, 1950; Levin & Wilson, 1976), and hence experience proportionally fewer episodes of speciation than other plants. (This may strike as odd for genera such as *Quercus* that are so species-rich, but the genus dates back from at least the Oligocene, some 30 million years ago, and many oak species seem to have remained virtually unchanged since the Miocene, especially in temperate areas (Stebbins, 1950; Nixon, 1993).) Although we are not aware of surveys of the frequency of hybridization as a function of the life-history traits of plants, Stebbins (1950) and Raven (1976) have suggested that woody groups experience rather more interspecific gene flow than other plant groups. If true, this would provide support for 'modest' roles for hybridization in trees that are essentially disconnected from speciation events.

According to Schluter (2001), persistence of ecologically differentiated populations in the face of gene flow could point to ecological speciation, but alternative mechanisms can produce such patterns. In fact, the current role of hybridization may or may not be related to the mechanism of speciation, that is, it is not necessarily an 'adaptation' but it could represent an 'exaptation' at the species level (Gould, 2002). We stress this point because suggestions for evolutionary roles for (relatively) rare events such as hybridization regularly lead to accusations of Panglossian attitudes (Gould & Lewontin, 1979). For instance, when Potts & Reid (1988) first suggested that hybridization could play a role in the dispersal of eucalypts, Schemske & Morgan (1990) objected that adaptation for this function seemed unlikely.

Remarkably, in the *Q. robur*–*Q. petraea* model, the preferred direction of interspecific gene flow appears to match the ecological dynamics, but the conditions under which unilateral reinforcement of reproductive barriers may evolve remain unknown. This asymmetric barrier may have evolved for reasons unrelated to pollen-mediated dispersal (e.g. as a by-product of more stringent mate choice in *Q. petraea*, also observed in intraspecific mating events) and have then become co-opted by selection at higher levels. Note that in any case the interpretation critically depends on the level of organization considered. For instance, the ‘suicidal’ behaviour of the pioneer *Q. robur* whose flowers are receptive to the pollen of *Q. petraea* seems hard to understand, whereas considering the prospects of gametes involved in interspecific encounters (which may in fact be quite good) could lead to a different conclusion. Bruno *et al.* (2003) recently advocated a better integration of facilitation into ecological theory, but warned that it will challenge some of our most cherished paradigms. Evolutionary theory as well may be hard pressed by the necessity to incorporate genetic facilitation.

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