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Original article

Genetic and phenological differentiation between introduced and natural populations of *Quercus rubra* L

JB Daubree, A Kremer

INRA, laboratoire de génétique et d'amélioration des arbres forestiers, BP 45, 33611 Gazinet, France

Summary — Gene diversity within populations of *Q rubra* was compared between 23 introduced stands and 9 geographic regions within the natural range for 4 enzymes encoded by 4 polymorphic loci. Gene diversity within populations was, in general, higher in introduced stands than in geographic regions, due to differences in allelic frequency profiles. For 2 loci, there were directional increases of frequencies of rare alleles in introduced stands as compared to geographic regions, whereas the mean number of alleles was lower in the former populations. Similarly, intraspecific variation among 15 introduced stands was compared to geographic variation among 18 origins in the natural range for bud flush and leaf coloration in experimental plantations established in France. There was a clinal latitudinal variation for both phenological traits in the natural range. The introduced populations occupied an intermediate position in the rankings for both phenological traits. A hypothesis of genetic differentiation between introduced and natural populations is proposed in light of the results obtained.

allozymes / bud flush / leaf coloration / genetic differentiation / *Quercus rubra* L

Résumé — Différenciation génétique entre les populations introduites et celles de l'aire naturelle du chêne rouge d'Amérique (*Quercus rubra* L). *La diversité génétique intrapopulation chez* Q rubra *L a été étudiée dans 23 peuplements introduits et 9 régions géographiques de l'aire naturelle avec l'aide de 4 isozymes contrôlés par 4 locus polymorphes. Cette diversité est plus élevée dans les peuplements introduits, à cause des différences de profils des fréquences alléliques, alors que le nombre moyen d'allèles par population est plus faible en Europe que dans l'aire naturelle. Pour 2 loci, les fréquences d'allèles rares sont systématiquement plus élevées dans les peuplements introduits. De la même manière, la variabilité intraspécifique a été étudiée sur un échantillon de 15 populations introduites et 18 populations de l'aire naturelle pour le débourrement et la coloration automnale des feuilles. Les populations de l'aire naturelle manifestent une variabilité suivant un gradient latitudinal. Les populations de l'aire introduite se singularisent par leur position intermédiaire dans le classement des provenances pour les 2 critères phénologiques. L'hypothèse d'une différenciation génétique entre les populations américaines et européennes est émise à la lumière de ces résultats.*

allozyme / débourrement / coloration des feuilles / différenciation génétique /* Q rubra *L

INTRODUCTION

Northern red oak (*Q rubra* L) was introduced in Europe during the 17th century (Bauer, 1953; Timbal *et al,* 1993). It was first planted in botanical collections before being planted in forests at the end of the last century. Plantations were established all over Europe except in Mediterranean regions and in Scandinavia. It is currently widely used for afforestation in France where a nationwide tree improvement program is planned. Stands established in Europe are usually of unknown origin, but have certainly resulted from successive generations of the original introductions rather than from direct importation of seeds from the natural range.

The objective of the present contribution was to compare genetic variation between introduced and natural populations by means of allozymes and phenological traits; it was not to study genetic variation *per se* by means of a large number of loci and on various quantitative traits, but rather to put emphasis on those traits that show evidence of genetic differentiation between both origins. As a result, in the case of allozymes, the analysis has been restricted to components of genetic variation that would mostly reveal genetic differentiation (frequency of rare alleles). Since most introduced populations are of unknown origins, there is some suspicion that they resulted from founder effects, which could easily be detected by comparing rare allele frequencies between European and North American populations. Phenological traits exhibit, in general, latitudinal trends of variation in forest trees due to either photoperiodic or heat-sum responses (Wright, 1976). The important differences of latitudinal distribution and climatic conditions between the natural and introduced range of distribution of *Quercus rubra* should therefore contribute to genetic differentiation for phenological traits.

Regional genetic variation studies were conducted on allozymes (Schwarzmann and Gerhold, 1991) and range-wide studies on growth and adaptive traits (Kriebel *et al,* 1976, 1988). Fragmentary data exist on intraspecific variation of introduced populations (Krahl-Urban, 1966), but no attempt has been made so far to compare genetic variation among populations between both continents.

MATERIALS AND METHODS

Genetic variation was assessed by means of allozymes and phenological traits in populations from the natural range and populations introduced into Europe.

Allozyme variation

A total of 23 French stands were sampled (fig 1a). Introduced stands are usually of small size (between 1 and 10 ha), over 40 years of age and of unknown origin. Stands are located in the geographic regions where northern red oak is used for afforestation (northeast, southwest and central parts of France). Bulked collections of seeds were made for the establishment of provenance tests in France. A random sample of 60 seeds was taken from each seed lot for electrophoretic studies.

The material from the natural range originated from existing combined provenance and progeny tests planted during the past 10 years in France. Nine geographic regions were delineated and, from each, 20 open-pollinated progenies coming from different stands within the region (depending upon the collection available) were selected to represent a sample of the region (fig 2a). Number of stands per region varied between 1 and 5; within a given region, stands were separated by less than 2° in latitude or longitude. For electrophoretic studies, 5 seedlings were sampled in each progeny (100 seedlings/geographic region).

Four enzymes (phosphoglucose isomerase EC 5.3.1.9, phosphoglucomutase EC 2.7.5.1, malate dehydrogenase EC 1.1.1.37, shikimate

dehydrogenase EC 1.1.1.25) were separated from crude homogenates of root radicles (extraction buffer, see Tobolski, 1978) or buds (extraction buffer, see Müller-Starck and Ziehe, 1991). Enzymes were separated by standard starch–gel electrophoresis. Gel compositions and electrophoretic procedures are detailed elsewhere (Zanetto *et al*, this volume). Zymograms of buds and roots of identical genotypes exhibited the same banding pattern (Daubree, 1990). The enzymes corresponded to 4 coding loci (PGI, PGM, MDH, SKDH, respectively).

Estimation of genetic parameters

Allelic frequencies (p_i) were calculated for each population (stand or geographic region) and within population gene diversities (or expected heterozygosity) were computed ($H = 1 - \Sigma\ p_i^2$) and averaged over all loci. Rare allele frequencies were compared between introduced and artificial populations. Rare alleles ($p_i < 0.05$) were regrouped in a single class within each population and for each locus.

Due to experimental constraints, collections could not be made with the same sampling strategy in the natural and introduced range. However, the different sampling schemes used were choosen so that they do not affect the precision of within-population expected heterozygosity and of rare allele frequencies. The variance of these parameters, when progenies are sampled, can be calculated using the method of Brown and Weir (1983) and compared to the variance in bulk collections. These calculations were made by postulation that there is no selfing in *Q rubra* (Schwarzmann and Gerhold, 1991). For a given locus with 2 alleles ($p_1 = 0.95$ and $p_2 = 0.05$) and with the sampling procedures used in this study, the standard errors of expected heterozygosity are 0.036 for bulk collections (assuming that all 60 seeds originated from different parents) and 0.039 for progeny collections. Similarly the standard errors for rare allele frequency ($p = 0.05$) are 0.019 for bulk collections and 0.022 for progeny collections.

Variation of phenological traits

Fifteen introduced stands were sampled in France, Germany, and the Netherlands (fig 1b) and 18 populations in the natural range (fig 2b). Collections in each stand were made as bulked seed lots (provenances) or single tree progenies (4–13/stand). A combined provenance and progeny test was established with 2-year-old seedlings in Ibos on the Pyrénées foothills. Entries of the test were either provenances or progenies.

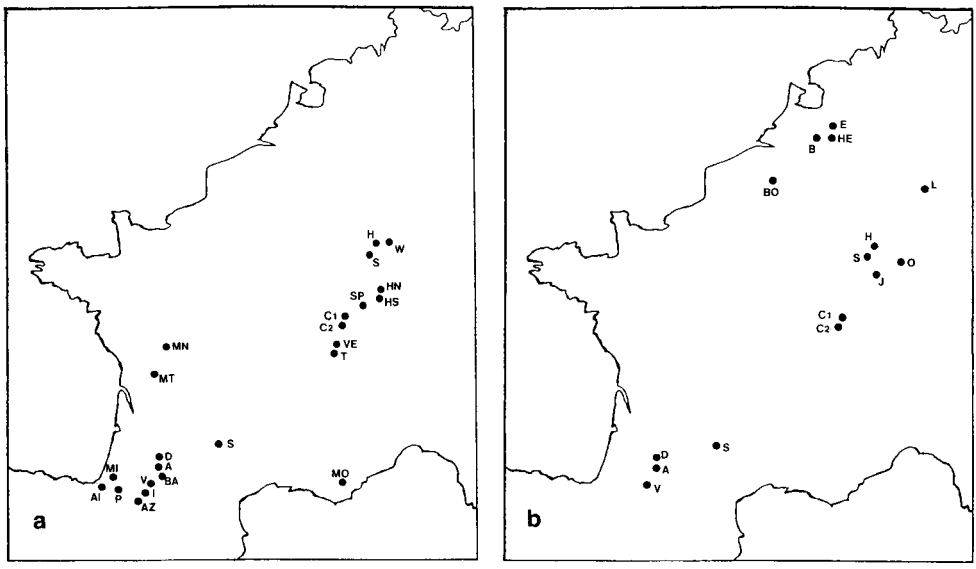

Fig 1. Location of introduced populations: **a)** for the allozyme study; **b)** for phenological traits.

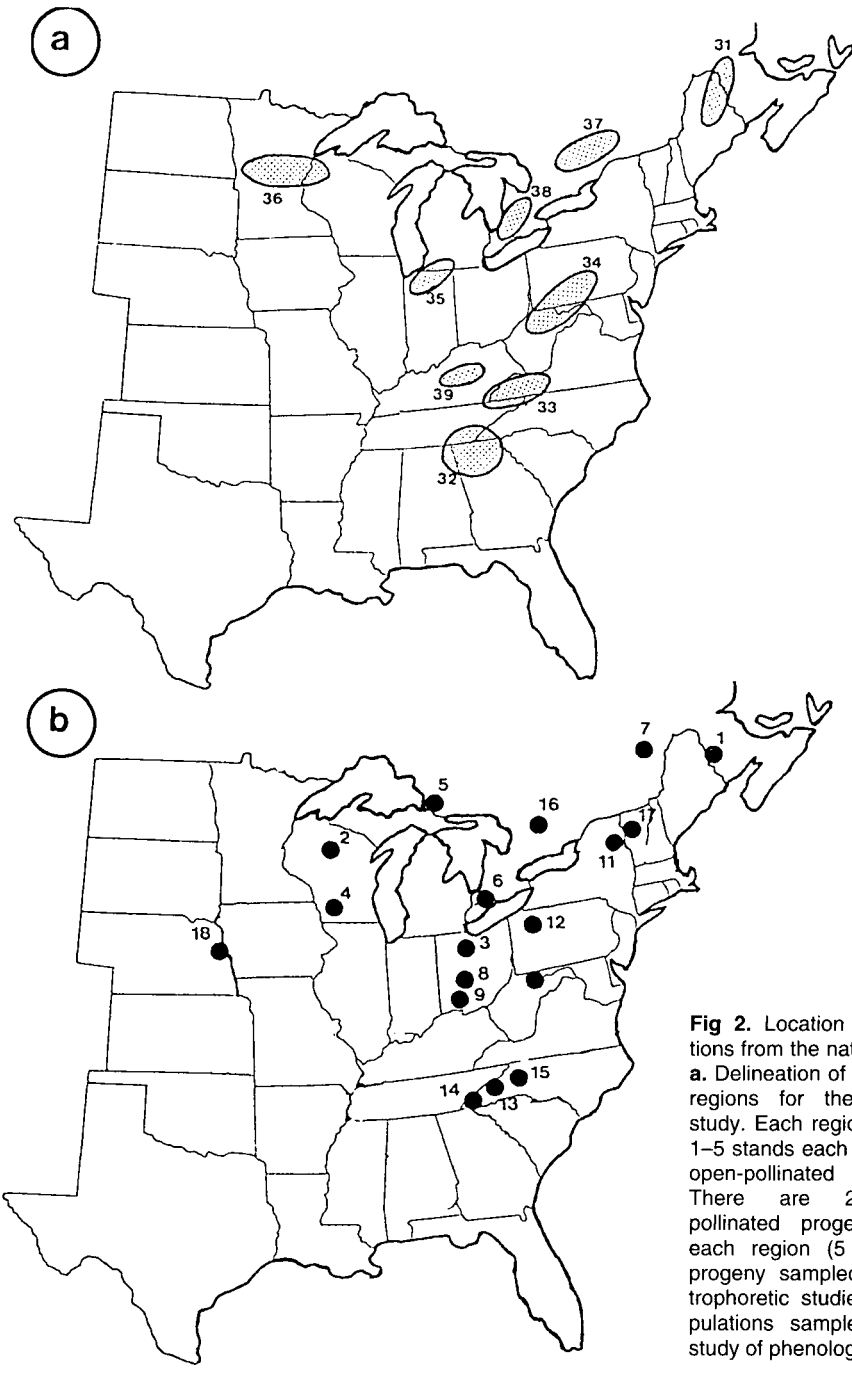

Fig 2. Location of populations from the natural range. **a.** Delineation of geographic regions for the allozyme study. Each region contains 1–5 stands each comprising open-pollinated progenies. There are 20 open-pollinated progenies from each region (5 seedlings/progeny sampled for electrophoretic studies). **b.** Populations sampled for the study of phenological traits.

The experimental design in the nursery was a complete block design (4 blocks, 102 entries, and a variable number of seedlings/plot). The experimental design in the field was an incomplete block design (81 blocks, 102 entries, 16 entries/block, 6 trees/plot).

Due to experimental constraints and availability of material, it was not possible to make the isozyme survey and the phenological assessments on the same populations. However, there is some overlap in the sampling between both studies (fig 1a, b).

At the end of the first growing season (November 1980), leaf coloration was assessed in the nursery using a scoring system (1 (green)–5(brown). In the spring of 1984, when trees were 4 years old, flushing was recorded in the field experiment with a grading system (1 (dormant bud) – 5 (beginning of stem elongation). Only the population level was used in calculations, *eg,* means were calculated over several progenies when the population was composed of progenies.

RESULTS

Allozyme variation

Twenty-one alleles were identified in the natural range over the 4 loci and 21 in the introduced stands; 20 were common to both continents and 1 specific to each continent (frequency 0.002 in each continent).

Introduced stands showed higher gene diversity than regions in the natural range at the 4 loci studied (table I). The difference was not due to variation in the number of alleles: there were rather fewer alleles in a given introduced stand than present over a geographic region in the natural range. The difference was mainly due to variation in frequency profiles between the 2 origins.

Over the whole survey, locus PGI had 2 common alleles (overall mean frequency 0.60 and 0.31) and 3 rare alleles. An allele was defined as rare when its mean frequency over all populations was < 0.05. The frequencies of the rare alleles were summed in one single class (table I). Although a few introduced stands (C1, MO) exhibited unusually high or low frequencies of rare alleles, there was a general trend towards increased rare allele frequencies in the introduced stands.

Locus PGM showed a similar pattern. There was only 1 common allele (overall mean frequency 0.92) and 5 rare alleles. Again, extremely variable frequencies could be observed in a few introduced stands (AZ, HN, MO); the pattern of a systematic increase in the frequency of rare alleles in introduced stands was also seen. The *t*-test was not significant between artificial and introduced populations ($P = 0.11$) mainly because of the important variation of the rare allele frequencies in introduced stands (MO, AZ).

Locus MDH had 1 common allele (overall mean frequency 0.97) and 3 rare alleles. No differences in frequency of rare alleles was noted between the 2 origins.

Locus SKDH had 3 common alleles (mean frequency 0.33, 0.11, 0.55) and 2 extremely rare alleles.

The trend towards a systematic increase in the frequencies of rare alleles for loci PGI and PGM was responsible for the higher gene diversity in European stands. The unusual variation of the frequencies of rare alleles in a few introduced stands accounted for the higher genetic differentiation among introduced stands as compared to differentiation among geographic regions in the natural range (G_{st} values are respectively 3.3 and 1.8%).

Geographic variation of phenological traits

Analysis of variance indicated significant differences between natural and introduced origins for leaf coloration and bud flushing.

Table I. Gene diversity, number of alleles and frequency of rare alleles in geographic regions of the natural range and introduced populations.

Region [a] *Natural range*	*H* [b]	*N* [c]	*Frequency of rare alleles* [d] *PGI*	*PGM*	*MDH*
31 Québec	0.317	13	0.150	0.066	0.040
32 Georgia	0.280	13	0.017	0.024	0.065
33 Smoky Mnts	0.285	16	0.050	0.046	0.026
34 Pennsylvania	0.289	19	0.045	0.045	0.029
35 Michigan	0.222	14	0.036	0.026	0.011
36 Minnesota	0.311	16	0.027	0.055	0.052
37 North Ontario	0.276	16	0.017	0.055	0.021
38 South Ontario	0.291	17	0.052	0.033	0.035
39 Kentucky	0.165	12	0.011	0.022	0.006
Means	0.271	15.1	0.045	0.041	0.032
Stand [a] *Introduced populations*					
BA Bassoues	0.341	16	0.089	0.072	0.030
A Averon Berg	0.363	13	0.083	0.092	0.076
D Doat	0.357	14	0.151	0.066	0.066
SE Serenac	0.294	14	0.147	0.028	0.015
C2 Chaux Royale	0.287	13	0.242	0.025	0.000
C1 Chaux 1re C	0.336	11	0.118	0.076	0.035
MN Montmorillon	0.305	13	0.121	0.033	0.017
MT La Mothe	0.284	14	0.100	0.045	0.008
I Ibos	0.329	13	0.102	0.100	0.017
AZ Azereix	0.375	12	0.070	0.275	0.090
V Vic Bigorre	0.301	15	0.050	0.056	0.017
AI Ainhoa	0.336	13	0.088	0.110	0.028
HS Hardt Sud	0.336	14	0.109	0.042	0.029
HN Hardt Nord	0.320	12	0.076	0.150	0.000
S Schopperten	0.346	13	0.083	0.083	0.042
P Prechacq	0.378	12	0.167	0.108	0.067
MI Mixe	0.352	13	0.066	0.122	0.052
H La Houve	0.289	11	0.144	0.008	0.042
T La Tranclière	0.272	13	0.064	0.050	0.042
VE La Vernée	0.334	16	0.144	0.092	0.033
W Wissembourg	0.314	12	0.129	0.033	0.050
SP Seppois-Pfet	0.332	11	0.078	0.074	0.008
MO La Môle	0.426	11	0.000	0.469	0.021
Means	0.330	13	0.105	0.096	0.034
t-test [e]	3.85	3.16	3.26	1.66	0.27
Probability of a larger value	< 0.001	0.005	0.003	0.110	> 0.50

[a] See figure 2a for location of regions and figure 1a for introduced populations. [b] H: within region or stand diversity. [c] N: total number of alleles (4 loci). [d] Frequency of rare alleles not reported for locus SKDH having only extremely rare alleles. Frequencies were summed over all rare alleles for each locus. [e] Comparison of mean values between regions of the natural range and introduced stands.

A clear clinal pattern of variation appeared in the natural range as shown in figure 3a and b. Northern origins flushed earlier and leaf coloration changed earlier. No latitudinal or longitudinal trend of variation was noticeable in the introduced distribution range. Overall the range of variation of scores for bud-flushing and leaf coloration were less in the European than in the North American populations. For bud-flushing most of the introduced origins were located in the mid part of the ranking, origins from the natural range occupied the bottom and top of the rankings. These observations are illustrated in figure 4 where the position of the introduced stands is strikingly clustered separately from the natural range populations.

DISCUSSION AND CONCLUSION

Comparison of variations of allozymes and phenological traits indicate clearly that European populations of northern red oak may already have differentiated from the natural range populations.

A few introduced stands have resulted certainly from founder effects as shown by the unusually high or low frequencies of rare alleles. However, for loci PGI and PGM, there is a directional increase of their frequencies. Had genetic drift been the only acting force, rare alleles would either have disappeared or increased. But there is a general increase in frequencies of rare alleles that can only be attributed to a systematic force acting directly or indirectly on these loci. Natural selection pressures are different on the 2 continents. Natural regeneration of northern red oak is extremely difficult in its natural range (Crowe, 1988) but, in Europe, *Q rubra* is an invasive species. The causes of the differences in regeneration success are unknown but are being investigated (Steiner, personal communication). However, in general, seed collected from introduced stands is of better quality than seed from the natural range, probably because their major parasites are absent in Europe. As a result, one might expect a release of selection pressures in introduced stands. For example, it has been shown that European stands are more sensitive to *Phythoptora cinnamomi* than stands from the natural range (Robin, 1991). Similar directional change of rare allele frequencies (locus LAP) has been found in beech between populations sensitive and tolerant to forest decline in Germany (Müller-Starck and Ziehe, 1991).

Variations in bud-flushing and leaf coloration in natural populations showed continuous latitudinal variation in experimental plantations established in France. For bud-flushing, these results were different from range-wide studies conducted in provenance tests planted in the natural range which indicated a northwest-to-southeast trend of variation (Kriebel *et al,* 1976, Schlarbaum and Bagley, 1981). For leaf coloration, similar patterns of variation were observed in both plantations (Deneke, 1974; Schlarbaum and Bagley, 1981).

Comparison of the rankings of the introduced populations with those from the natural range indicates that the former originated from the central part of the natural distribution (fig 4) and/or were established from a mixture of several origins. However, the latter hypothesis is not supported by the allozyme data. The total number of alleles identified in European and North American stands was the same, except for 1. They included rare alleles, some of which were confined to specific geographic origins. As a result, one can infer that introduced populations originate from various regions of the natural range. Their intermediate ranking for phenological traits (fig 4) can therefore be interpreted as the consequence of directional selective pressures in Europe since their introduc-

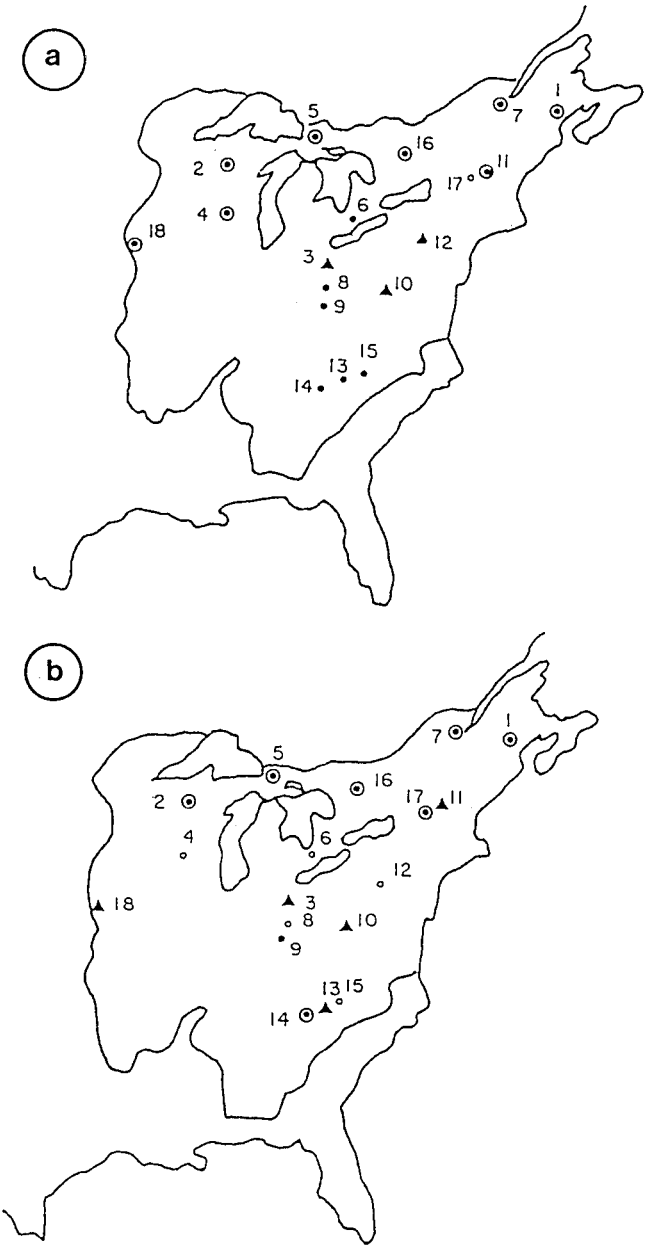

Fig 3. Geographical variation of phenological traits in the natural range. **a.** Mean scores for bud flush. Scores vary from 5 (expanding leaves) to 1 (dormant bud). To illustrate population mean values, these were separated into 4 classes as indicated on the figure. ⊙ > 4; o = 3.4–4; ▲ = 2.7–3.4; • = < 2.7. **b.** Mean scores for leaf coloration. Scores vary between 5 (brown leaves) to 1 (green leaves). ⊙ ≥ 2.8; o = 2.5–2.8; ▲ = 2.3–2–5; • = ≤ 2.3.

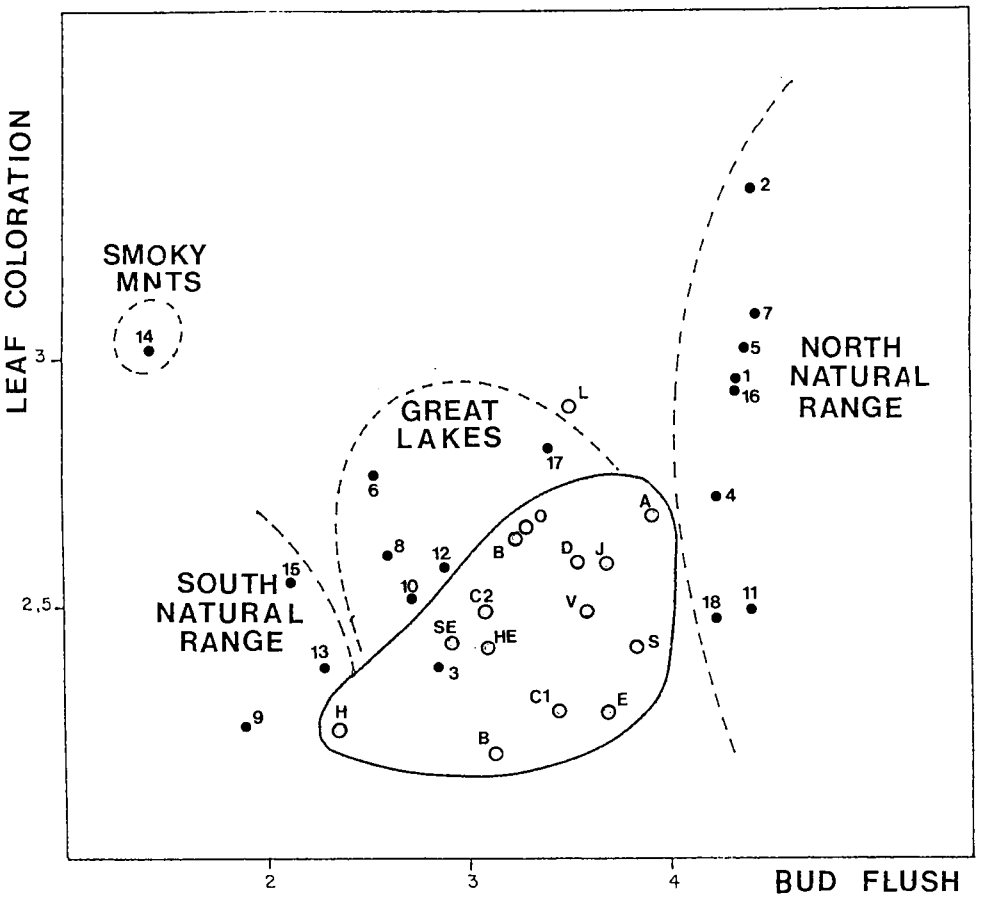

Fig 4. Comparison of variation between introduced and natural origins for phenological traits. Mean population values for leaf coloration are shown on the y axis and bud flush on the x axis. Populations from the natural range are identified by numbers and introduced stands are identified by letters. These letters are the same as these cited in figure 1b. Numbers refering to populations of the natural range correspond to populations shown on figure 2b.

tion. The distribution of northern red oak in Europe covers a narrower latitudinal range than in North America. Introduced early-flushing and late-growing trees may have been progressively eliminated in natural regeneration in Europe due to their sensitivity to late or early frosts.

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