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Original article

Estimation of *Fagus sylvatica* L mating system parameters in natural populations

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Summary — The mating system of beech (*Fagus sylvatica* L) was investigated using polymorphism at 4 allozyme loci and the multilocus model of Ritland and Jain (1981). Beech appears to be a highly outcrossing species: the outcrossing rate ranges from 0.94 to 1. No significant differences were found in outcrossing rates according to environmental factors or among or within trees. Comparison of single-locus and multilocus estimates indicated that little or no inbreeding occurred. Outcross pollen pool was not homogeneous and heterogeneity in pollen allelic frequencies was observed even among nearby trees. A possible explanation may be the temporal variability of the pollen pool due to variation in flowering time and to matings between phenologically synchronous trees.

mating system / outcrossing rate / pollen heterogeneity / beech

Résumé — Estimation des paramètres du mode de reproduction de *Fagus sylvatica* L. *Le mode de reproduction du hêtre* (Fagus sylvatica) *a été étudié à l'aide de 4 marqueurs alloenzymatiques (GOT1, MDH1, SOD1 et IDH1) et du modèle multilocus de Ritland et Jain (1981) dans 4 populations françaises : l'une en forêt d'Issaux dans les Pyrénées-Atlantiques, les trois autres dans le massif de l'Aigoual (La Serreyrèdes, Plo du Four et Sommet) (tableau I). Dans la forêt d'Issaux, 3 parcelles présentant des physionomies différentes ont été étudiées : une parcelle à forte densité (forêt), une autre située en lisière de forêt et la troisième formée d'arbres isolés. Les questions abordées dans cette étude sont les suivantes : i) quel est le taux d'autofécondation du hêtre en conditions naturelles ? ii) existe-t-il des variations de ce taux dans l'espace et dans le temps ? iii) existe-t-il une hétérogénéité du pollen à l'intérieur des populations ? Le hêtre est une espèce hautement allogame : le taux d'allofécondation est compris entre 0,94 (Aigoual) et 1 (Issaux) (tableau II). Ces estimations correspondent à des taux d'autofécondation inférieurs à la valeur moyenne (13%) calculée à partir des observations de Nielsen et Schaffalitzky-de-Muckadell (1954). Aucune différence significative n'a été mise en évidence selon les variations des facteurs de l'environnement entre les taux d'allofécondation observés. Ce taux ne varie pas non plus significativement d'un arbre à l'autre ou entre les secteurs d'un même arbre. Les taux très élevés d'allofécondation chez cette espèce autocompatible pourraient s'expliquer par certaines caractéristiques de sa biologie florale. La comparaison des estimations uni- et multilocus du taux d'allofécondation montre un niveau nul ou très faible de consanguinité. Une analyse de variance à 2 facteurs montre qu'il n'y a pas de variation de fréquence allopollinique d'un secteur à l'autre* *de la couronne d'un arbre : les secteurs d'un même arbre ont donc pu être considérés comme des répétitions aléatoires. En revanche, le nuage allopollinique est hétérogène : i) d'un arbre à l'autre et les fréquences alléliques du pollen peuvent être différentes même entre individus voisins (IDH1, tableau III), ii) entre les peuplements (GOT1 et MDH1). Dans la forêt d'Issaux cette hétérogénéité est maximale pour les arbres isolés (tableau V). À l'Aigoual, il n'y a pas d'hétérogénéité interpeuplements mais une forte hétérogénéité à l'intérieur de 2 des peuplements (tableau VI). Ces phénomènes peuvent s'expliquer par la variabilité du nuage pollinique dans le temps en raison de décalages à déterminisme génétique de la période de floraison (jusqu'à 20 j) et de la reproduction entre arbres synchrones d'un point de vue phénologique. Ce modèle pourrait expliquer, en particulier, l'hétérogénéité de l'allopollen entre arbres voisins non synchrones. Cependant, il devrait conduire, au cours du temps, à une structuration des populations en groupes d'arbres précoces et d'arbres tardifs, ce qui n'a pas été observé. En fait, il existe entre les individus les plus précoces et les plus tardifs toutes les classes intermédiaires : la distribution des arbres en fonction de leur période de floraison est à peu près normale, ce qui induit des classes chevauchantes d'individus.*

mode de reproduction / allofécondation / hétérogénéité du pollen / hêtre

INTRODUCTION

The estimation of mating system parameters is necessary to understand population genetic structures and species evolution. Mating systems affect the distribution, maintenance and evolution of population genetic variability (Allard, 1975; Brown, 1979). In plants many mating systems can be found, from autogamy to allogamy through different degrees of self-fertilization. Most mating system mathematical estimation methods are based on the mixed mating model which involves self-fertilization and panmictic outcrossing without selection (Fyfe and Bailey, 1951; Brown and Allard, 1970). Maternal self-fertilization *(s)* and outcrossing *(t)* rates are the quantitative parameters generally used to describe the mating system.

In long-lived trees, most *s* and *t* estimations have been carried out on temperate wind-pollinated conifers in natural populations (for review see Mitton, 1992). Few studies have been carried out on angiosperm trees: *Eucalyptus* (Brown *et al*, 1975; Phillips and Brown, 1977; Moran and Brown, 1980), tropical trees (O'Malley and Bawa, 1987; O'Malley *et al*, 1988) and anemophilous species like *Quercus ilex* (Yacine, 1987), *Alnus crispa* (Bousquet *et al*, 1987) and *Juglans regia* (Rink *et al*, 1989).

Mating system parameters vary both between and within species. Intraspecific variation can occur with altitude (Neale and Adams, 1985a), stand density (Farris and Mitton, 1984; Knowles *et al*, 1987), flowering period (El Kassaby *et al*, 1988) and between and within individual maternal parents (El Kassaby *et al*, 1986, 1987).

Fagus sylvatica L (European beech) is a monoecious, anemophilous, and self-fertile but mainly outcrossing species (Nielsen and Schaffalitzky-de-Muckadell, 1954; Thiébaut and Vernet, 1981). The self-fertilization mean rate was estimated at 13% (Nielsen and Schaffalitzky-de-Muckadell, 1954) under controlled conditions. Beech genetic structure is rather similar (Cuguen, 1986) to the isolation-by-distance model of Wright (1943, 1946). This model assumes limited gene flow and associated self-fertilization and outcrossing within neighbourhoods. Thus it assumes an increase of relatedness which contributes to total inbreeding with self-fertilization. Two arguments support this hypothesis: (i) self-fertilization alone cannot explain the high heterozygote deficit observed in European beech stands (Cuguen *et al*, 1988; Comps *et al*, 1990); and (ii) Cuguen (1986) observed genotypic sub-population differentiation due to limited gene flow, mainly pollen flow.

In this study we will try and answer 3 questions. (i) What is the self-fertilization rate of beech in natural conditions? (ii) Is there spatial, temporal, inter- or intra-individual variation in this self-fertilization rate? (iii) Does pollen–pool heterogeneity exist within the population?

MATERIALS AND METHODS

Sampling

Material was sampled according to several hierarchized organization levels from a wide level between populations located in 2 distant regions to the lowest level between several crown sectors within each tree. This sampling may allow us to detect possible variations of mating system parameters and the influence of the environmental factors: wind, beechwood physiognomy and stand density on outcrossing rate (table I).

Estimation of *t* was carried out from maternal families in 2 mountain regions (table I): (i) the northern slope of the Aigoual mountain (Cevennes) where 3 stands (Serreyredes, Plo du Four, Sommet) were chosen within 3 distinct populations; and (ii) the Atlantic Pyrenees where 3 physiognomically different stands (Isolated trees, Edge of forest, Forest) were chosen in the Issaux forest. In the Issaux forest, the crown of each mother-tree was stratified into 4 sectors according to a horizontal plane (detection of position influence) and to a vertical plane chosen to detect the prevailing wind influence in the case of isolated trees and of that of the 2 closest neighbours in the other stands.

Sampled material and biochemical methods

Alloenzymatic analysis were carried out: (i) on cortical tissue and dormant buds to determine

Table I. Environmental characteristics of the stands studied.

	Region					
	Pyrenees			*Cevennes : Aigoual mountain*		
	Population					
	Issaux			*Serreyredes*	*Plo du Four*	*Sommet*
	Isolated	*Edge of forest*	*Forest*			
Altitude	1 000	1000	1 000	1 200	1 400	1 505
Stand type	Pasture land	Edge of dense forest	Dense forest	Dense forest	Open forest	Edge of dense forest
Wind influence	Very strong	Strong	Weak	Weak	Very strong	Strong
1	Isolated trees	8	8	10	15	10
2	70–375	Near neighbours	Near neighbours	20	25–90	25
Number of sampled trees	5	5	5	9	17	20

1, mean distance between close neighbours; 2, distance between sampled trees.

each maternal tree genotype; and (ii) on dormant beech-nuts (40 from each sampling unit, trees or sectors in the Pyrenees, 30 in Cevennes) collected from maternal parents. Electrophoretic conditions were as previously described (Thiébaut *et al*, 1982; Merzeau *et al*, 1989). Four unlinked polymorphic loci (Merzeau, 1991), GOT1, MDH1, SOD1 and IDH1 were assayed.

Data analysis

Multilocus (t_m) and single-locus (t_s) outcrossing rates were estimated jointly with outcrossing pollen gene frequencies (p) using the maximum likelihood approach of Ritland and Jain (1981) and Ritland and El Kassaby (1985). The assumptions used were those of the mixed mating model (Fyfe and Bailey, 1951): (i) each mating event is a result of either a random outcross (with probability t) or a self-fertilization (with the probability s); (ii) the probability of an outcross is independent of the maternal genotype; (iii) all embryos have equal fitness regardless of mating event; and (iv) outcross pollen pool gene frequencies are homogeneous over the array of the sampled maternal parents. Estimates were calculated for each stand (t_m and p) and for each sampled unit, sector or tree (t_{mi} and p_i). Variances were calculated from the inverted information matrix (Ritland and El Kassaby, 1985).

Variability was estimated either from variance analysis in case of hierarchical sampling (Issaux) after arc-sinus square-root transformation (OPEP program, Baradat, 1985) or using the G-test in the other case (Aigoual). When G tests showed a significant heterogeneity ($P < 0.05$), they were completed by multiple comparison tests (Sherrer, 1984).

RESULTS

Outcrossing rate

No influence of height or crown sector was found when comparisons were made using global estimates of the outcrossing rate or using 2-way anova carried out on individual estimates. Thus, sectors of 1 tree can be pooled to obtain better estimates based on a higher number of observations.

In Issaux, multilocus estimates (t_m) ranged from 0.986 to 1.022; outcrossing was complete in isolated trees, lower than but not significantly different from 1 in the other 2 stands (table II). In the Aigoual forest t_m was close to 0.940 within the 3 stands and was significantly lower than 1 in 2 cases. Single-locus estimates (t_s) ranged from 0.826 to 1.123 in Issaux and from 0.658 to 1.260 in Aigoual (table II). Heterogeneity over loci was significant within 1 stand in Issaux (isolated trees) and within the 3 Aigoual stands. Outcrossing rate estimates differed at each locus from one stand to another. Mean single locus estimates (t_s) (weighted by $1/V$) were similar to that from their corresponding multilocus population estimates (t_m).

In the Issaux stands, tree multilocus estimates were close to 1 and no intra-stand individual heterogeneity was found using Ritland and El Kassaby's method (1985) (table II). In spite of a rather high heterogeneity of t_{mi} within Aigoual stands, the values were not significant; most of values indicated complete outcrossing.

Pollen pool (Issaux)

The 2-way anova revealed no significant variation of allopollen frequencies between crown sectors. In edge-of-forest and forest stands no relation was found between one allele frequency in the pollen pool received by any tree sector and the genotype of the facing tree. The sectors of each tree can be considered as random repetitions (*ie* replications).Thus it became possible to carry out a nested anova through p_i estimates. This revealed significant heterogeneity between stands for 2 loci (GOT1 and MDH1) and within stands for 1 locus (IDH1) (table III).

Table II. Estimates of the outcrossing rate in Issaux and Aigoual forest stands.

Outcrossing rate	*Pyrenees*			*Aigoual*		
	Issaux			*Plo du Four*	*Serreyredes*	*Sommet*
	Isolated	*Edge of forest*	*Forest*			
$\hat{t}_m$	1.022 (0.017)	0.986 (0.020)	0.988 (0.023)	0.938* (0.028)	0.941 (0.042)	0.935* (0.028)
$\hat{t}_s$						
GOT1	0.859* (0.048)	0.977 (0.051)	1.051 (0.059)	0.902 (0.063)	0.658*** (0.088)	1.260 (0.071)
MDH1	1.060 (0.054)	0.911 (0.062)	0.857* (0.057)	0.767*** (0.063)	0.873 (0.100)	0.836* (0.064)
SOD1	0.826* (0.049)	1.123 (0.067)	1.001 (0.058)	1.007 (0.072)	1.012 (0.107)	0.919 (0.072)
IDH1	1.065 (0.042)	1.043 (0.045)	1.006 (0.053)	1.053 (0.056)	1.079 (0.084)	0.931 (0.061)
$\hat{t}_s$	0.957 (0.024)	1.012 (0.027)	0.978 (0.022)	0.936 (0.031)	0.903 (0.047)	0.975 (0.033)
χ^2_{Het}	21.57***	6.34 NS	6.47 NS	12.82**	13.27**	21.95***
$\Delta\hat{t}_{mi}$ V_o/V_e	0.005/0.020	0.005/0.016	0.020/0.041	0.129/0.094	0.126/0.082	0.111/0.075
	NS	NS	NS	NS	NS	NS

* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$; NS non-significant; $\hat{t}_s$, minimum variance mean; χ^2_{Het}, heterogeneity chi-square; $\Delta\hat{t}_{mi}$, individual estimate variations of the outcrossing rate between trees; V_o, observed variance of t_{mi} between trees; V_e, expected variance computed according to Ritland and El Kassay (1985). Standard errors are given in parentheses.

Table III. Issaux forest: nested anova on individual outcrossing pollen gene frequencies.

		Test F *values*			
		GOT1	*MDH1*	*SOD1*	*IDH1*
Sources of variation	df				
Among stands	[2.12]	7.79**	6.58*	0.73	1.67
Within stands	[12.45]	1.57	1.63	1.52	2.35*
Between-stand variance / Between + intra-stand variance	(%)	78.83	74.36	0.00	18.82

* 0.01 < P < 0.05; ** 0.001 < P < 0.01.

Analysis of variance carried out for each stand revealed differences in within-stand variability organization. The pollen pool heterogenity between trees appears at distinct loci from one stand to another (table IV). A discriminant analysis using all loci shows that this heterogeneity is highest between isolated trees (mean equality Bartlett's test) (table V). Mahalanobis's distance matrices show different organizations according to stands: (i) edge-of-forest, no significant distance; (ii) forest, one tree (118) does not receive the same outcross pollen as its

Table IV. Issaux forest: within-stand anova on outcrossing pollen gene frequencies.

Stands	*Test* F *values (4 and 13 df)*			
	GOT1	*MDH1*	*SOD1*	*IDH1*
Forest	1.05	3.21*	0.71	2.49*
Edge	3.10*	0.88	1.73	2.12
Isolated	1.83	1.29	2.85+	2.36+

* 0.01 < P < 0.05; + 0.05 < P < 0.10.

Table V. Issaux forest: matrices of Mahalonobis distances between trees, computed from the AFD on the fourth outcrossing pollen gene frequencies (GOT1, MDH1, SOD1 and IDH1).

Stands	*Trees*	*110*	*111*	*112*	*118*
Forest	111	NS			
	112	NS	NS		
	118	*	**	***	
	122	NS	NS	NS	**
	Trees	*L1*	*L2*	*L3*	*L4*
Edge	L2	NS			
	L3	NS	NS		
	L4	NS	NS	NS	
	L5	NS	NS	NS	NS
	Trees	*176*	*177*	*180*	*181*
Isolated	177	NS			
	180	NS	*		
	181	*	*	NS	
	182	**	*	***	***

* 0.01 < P < 0.05; ** 0.001 < P < 0.01; *** P < 0.001; NS : non-significant.

Table VI. Aigoual: among and within-stand variations of outcrossing pollen gene frequencies.

	GOT1	*MDH1*	*SOD1*	*IDH1*
Between stands				
Plo du Four	0.641 (0.032)	0.724 (0.032)	0.770 (0.029)	0.870 (0.021)
Serreyredes	0.628 (0.037)	0.753 (0.038)	0.742 (0.043)	0.877 (0.029)
Sommet	0.623 (0.023)	0.742 (0.031)	0.735 (0.027)	0.853 (0.023)
G test	NS	NS	NS	NS
*Within stands (*G *test)*				
Plo du Four	**	**	**	**
Serreyredes	NS	NS	NS	NS
Sommet	**	**	*	**

* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; NS non-significant. Standard errors are given in parentheses.

neighbours; and (iii) isolated trees, outcross pollen heterogeneity is the highest (7 significant distances). In Aigoual populations, there is no inter-stand heterogeneity but only a high within-stand heterogeneity in the Sommet (edge of dense forest) and in Plo du Four (open forest) (table VI).

DISCUSSION

Outcrossing rate

In this study beech is shown to be a highly outcrossing species with low (Aigoual) or zero (Issaux) self-fertilization rates: estimates are lower than the mean value (13%) calculated from the observations of Nielsen and Shaffalitzky-de-Muckadell (1954). Few wind-pollinated species show outcrossing rate estimates as high as in Issaux forest: *Pseudotsuga menziesii* (Neale and Adams, 1985b), *Pinus contorta* ssp *latifolia* (Epperson and Allard, 1984), *Quercus ilex* (Yacine, 1987). For most conifers, the estimates are quite similar to Aigoual estimates with values ranging from 0.90 to 0.97. The forest angiosperms that have been studied up to now show higher selfing rates than the beech but most of them are entomophilous: *Eucalyptus pauciflora* (Phillips and Brown, 1977), *Bertholletia excelsa* (O'Malley *et al*, 1988), *Robinia pseudoacacia* (Surles *et al*, 1990).

The only significant variation of the outcrossing rate found in beech occurs between 2 stands each of them located in 1 of the 2 studied regions. No altitude influence was detected as opposed to other observations (Phillips and Brown, 1977; Neale and Adams, 1985a). Estimations are the same in dense stands and in isolated trees. This confirms the results of Neale and Adams (1985b) and Furnier and Adams (1986). However, different results were obtained in other species: the relation between density and outcrossing rate is either positive (Farris and Mitton, 1984; Knowles *et al*, 1987) or negative (Ellstrand *et al*, 1978; Ritland and El Kassaby, 1985). Wind does not have any influence either, even in open stands.

Now we have to answer the following questions. Are the high estimates obtained for beech realistic? Does bias occur to induce an overestimation of the actual outcrossing-rate? Pollen heterogeneity is the most frequent violation of the mixed-mating model. However, it was shown (Shaw *et al,* 1981; Ennos and Clegg, 1982; Brown *et al,* 1985) that this heterogeneity leads to an underestimation of the outcrossing rate (Wahlund effect). When few loci are used, even multilocus outcrossing rates may be underestimated. In the studied stands, both factors should have induced a low apparent outcrossing rate. This was not observed. Thus our estimation using only 4 loci seems valid.

A second bias may be due to selection between mating and analysis periods. Inbreeding depression was shown to be low in beech (Nielsen and Shaffalitzky-de-Muckadell, 1954). In our study, outcrossing rate estimations were carried out from dormant seeds so that only early post-zygotic selection could occur. Nilsson and Wästljung (1987) used rate of production of empty seeds to evaluate the selfing amount in beech. However, their selfing estimates might have overestimated the actual selfing rate due to parthenocarpy phenomena (Nielsen and Shaffalitzky-de-Muckadell, 1954; Oswald, 1984).

Thus outcrossing rate seems to be very high. For this self-compatible species, this rate may be explained by some characteristics of its floral biology (Nielsen and Schaffalitsky-de-Muckadell, 1954). First, male flowers are often located at the basis of annual boughs and female flowers often at their upper part (hercogamy). Secondly, female flower stigmas are receptive about 5 d before pollen release (protogyny) and because leafing-out and flowering are simultaneous, the male flowers do not shed their pollen until the leaves have expanded, which hinders pollen circulation through the crown. Finally, leafing-out and flowering occur from the bottom towards the top of the tree, so that synchronism only exists between pollen shedding male flowers of the lower crown and receptive female flowers of the upper crown. However, the probability of upwards pollen movement is low and selfing possibilities are limited.

Pollen pool

The results show an heterogeneity of pollen gene frequencies both between stands within a forest and between trees within a stand whatever their distance. The study of homozygote mother descendants in other species often revealed an heterogeneity of genotype frequencies (Brown *et al,* 1975; Knowles *et al,* 1987). However, this only concerns the total pollen and it becomes difficult to know which of the 2 pollen components is responsible for this heterogeneity. In beech, the low selfing rate and the lack of individual outcrossing rate variability are 2 arguments in favour of an outcross pollen heterogeneity. Thus outcrossings are not panmictic, contrary to one hypothesis of the mixed-mating model, which implies: (i) that male gene flows are limited, and (ii) that the population studied is subdivided into genetically distinct subpopulations.

Gene flow may be limited in space and outcross pollen-pool frequencies are heterogeneous as a result of clustering of related individuals (family substructuring) in the population. Thus gene flows limited to closed neighbours over time leads to an increase in relatedness. Differences between multilocus (t_m) and single-locus (t_s) outcrossing rates are interpreted as a sign of consanguineous (non-self) matings (Shaw *et al,* 1981; Shaw and Allard, 1982), even if these differences cannot be tested. The lowest t_s estimates would occur for loci showing a pollen heterogeneity. This is not always the case in the studied stands. Moreover, t_s is lower than t_m in only 2 stands: in Serreyredes (Aigoual) and, paradoxically, in isolated trees (Issaux). The research of a homogamic mating excess due to a relation between maternal and pollinisator genotypes may allow the detection of matings between relatives. According to Ritland (1985), the amount of the effective selfing caused by consanguineous matings is directly estimated from the slope of the regression line of outcrossing-pollen gene frequencies on the additive value of the maternal genotype. Only 2 regression coefficients are significant (Plo du Four: MDH1, 0.263* and SOD1: 0.306*).

Thus, whatever the method, proofs of mating between related neighbours are weak; and these matings paradoxically occur within a stand where pollen heterogeneity was not detected (Serreyredes) or in open stands (Issaux, isolated trees; Plo du Four). Moreover, differences in outcross pollen frequencies would have to occur preferentially between distant trees if neighbours mate amongst themselves.

Phenological heritable differences (up to 20 d in beech) could also explain pollen heterogeneity. Thus, at any time, only some individuals participate in reproduction, and variations in pollen gene frequencies during flowering period could induce a temporal structuration. This model can explain an outcross pollen heterogeneity between no synchronous closed neighbours, like in tree 118 (Issaux, Forest) which blossoms much later than its neighbours. However, if synchronous trees have similar alleles, intraclass phenological matings would over time lead to an excess of homogametic matings, and to a spatial structuring of reproductive phenology classes and, consequently, of genotypes and alleles. Such patches of early or late trees were not observed within the studied stands.

In fact, due to protogyny, one tree may be fertilizated by slightly earlier individuals. This could favour negative assortative matings. Moreover, the tree distribution according to their flowering period is approximately normal, which induces overlapping classes. At last, the beginning and the length of the flowering period vary according to annual climatic conditions. This can induce interannual variations of phenological gaps and class overlaps. These variations may delay the occurrence of a gametic structuration and may induce an inbreeding increase. This is all the more important as the generation number is perhaps too small, so that the consequences of gene-flow limitation are perceptible.

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