Multilocus and Multitrait Measures of Differentiation for Gene Markers and Phenotypic Traits

Antoine Kremer, Anne Zanetto and Alexis Ducousso

*Institut National de la Recherche Agronomique, Laboratoire de Génétique et d'Amélioration des Arbres Forestiers,
B.P.45 Pierroton 33611 Gazinet Cedex, France*

Manuscript received September 28, 1995

Accepted for publication January 3, 1997

ABSTRACT

Multilocus measures of differentiation taking into account gametic disequilibrium are developed. Even if coupling and repulsion heterozygotes cannot be separated at the multilocus level, a method is given to calculate a composite measure of differentiation (CF_u) at the zygotic level, which accounts for allelic associations combining both gametic and nongametic effects. *Mean* and *maximum* differentiations may be relevant when multilocus measures are computed. Maximum differentiation is the highest eigenvalue of the F_{st} matrix, whereas mean differentiation corresponds to the mean value of all eigenvalues of the F_{st} matrix. Gametic disequilibrium has a stronger effect on maximum differentiation than on mean differentiation and takes into account the anisotropy that may exist between within- and between-population components of disequilibria. Multilocus mean and maximum differentiation are calculated for a set of 81 *Quercus petraea* (sessile oak) populations assessed with eight allozyme loci and two phenotypic traits (bud burst and height growth). The results indicate that *maximum* differentiation increases as more loci (traits) are considered whereas *mean* differentiation remains constant or decreases. Phenotypic traits exhibit higher population differentiation than allozymes. The applications and uses of mean and maximum differentiations are further discussed.

DIFFERENTIATION among populations for gene markers and quantitative traits has become of great interest for conservation biology and the management of natural genetic resources. In gene diversity studies, population differentiation is usually measured at the single locus level following the approaches taken by NEI (1973, 1977) or WEIR and COCKERHAM (1984). These measures do not take into account allelic associations at different loci, even when the coefficient of gene differentiation (F_{st} or G_{st}) is averaged over several loci. There are at least two reasons why multilocus approaches are seldom used to study differentiation in allogamous species: (1) in highly outcrossing species, gametic disequilibrium is expected to be extremely low (MUONA 1982) and (2) the theory for differentiation at the multilocus level has only recently been developed. When multilocus studies were undertaken, they were conducted with descriptive multivariate statistical techniques (principal component or factorial analysis) that do not provide parameters for allowing comparisons among species. In these approaches, multilocus analysis is restricted to computing various genetic distances among populations and does not provide an overall measure of differentiation taking into account specific allelic associations at different loci. Attempts to analyze multilocus differentiation have been developed in human genetics (SMOUSE and NEEL 1977; SMOUSE *et al.*

1982; LONG *et al.* 1987; EXCOFFIER *et al.* 1992) and since then adapted to conifers (YANG and YEH 1993).

Paradoxically, the multilocus methods that have been developed often assume that gametic disequilibrium is absent (LONG 1986; LONG *et al.* 1987), because multilocus gametic arrays are unknown in diploid individuals of natural populations. An exception to this is with conifers, where multilocus gametic data can be obtained by analyzing separately genes in the endosperm and the embryo. Coupling and repulsion heterozygotes cannot usually be separated in diploid tissues. As a result, estimating differentiation at the gametic level is not possible. We therefore define a composite measure of differentiation (CF_u) that accounts for allelic associations at the zygotic level, combining both gametic and nongametic effects, in much the same way as WEIR (1979, 1990) defined a composite measure of disequilibrium when coupling and repulsion heterozygotes cannot be separated. We also compare the composite measure with the original differentiation measure. Following the approach tested by LONG (1986) and LONG *et al.* (1987), the multilocus method proposed here extends the WEIR and COCKERHAM (1984) ANOVA to the multivariate case. The subdivision of the variability in the MANOVA may be differently interpreted according to the objective of the study (DAGNÉLIE 1975; TOMASSONE *et al.* 1988). The largest subdivision of variability that can be obtained by a particular combination (canonical variate) of the original variables (largest root, ROY 1953) may be one interest and the overall variability created by

Corresponding author: Antoine Kremer, INRA, B.P. 45, 33611 Gazinet Cedex, France. E-mail: antoine.kremer@pierroton.inra.fr

all canonical variates (sum of roots, HOTELLING 1951) could be another. We will refer to the *maximum* and *mean* differentiation respectfully for the two approaches and compare their response to gametic or zygotic disequilibria. Because both measures take into account disequilibria among loci, they should not be confused with single locus measures that account for population structure and are not affected by disequilibria.

More attention has recently been focused on population subdivision for quantitative traits both on a theoretical (LANDE 1992; NAGYLAKI 1994) and applied level (PROUT and BAKER 1993; SPITZE 1993; LONG and SINGH 1995; PODOLSKY and HOLTSFORD 1995). The structure of genetic variance as a function of the F -statistics has been investigated by the pioneering work of WRIGHT (1965, 1968) and FALCONER (1960) at the monolocus level and further extended to the multilocus level by ROGERS and HARPENDING (1983). Their models served as a starting point for a differentiation measure that can be compared in certain circumstances to the measures developed for gene markers and that we extended to the multitrait level.

In this work we will first present a common method of computing differentiation at different levels of investigation: monolocus, multilocus and quantitative traits. We show that ANOVA is an appropriate method for deriving measures applicable to the various levels. Second, we will provide a comprehensive view of the measures of differentiation by outlining their relationships and the way they take into account disequilibria. Finally we will further illustrate the complementary conclusions that can be drawn from different differentiation measures by giving an application to oak populations.

DIFFERENTIATION FOR GENE MARKERS

Gametic differentiation, one locus and single allele: In this study, we consider a population that is subdivided into S subpopulations, with allelic scores assessed at L loci, each locus l having A_l alleles. The total number of alleles assessed over all loci is A .

The variability for a given allele a at a given locus l can be subdivided according to a hierarchical ANOVA model developed by WEIR (1990):

$$Y_{ijk} = \mu + P_i + Z_{ij} + G_{ijk}, \quad (1)$$

where Y_{ijk} corresponds to the observation of gene k in an individual j belonging to a population i , and P_i , Z_{ij} and G_{ijk} represent the random effects due to population, individual (zygote) within population and gene within zygote. Y_{ijk} equals 1 if the gene k bears allele a and otherwise it equals 0.

From this model, F_{st} for allele a (F_{st_a}) can be calculated as the ratio of the population variance to the total variance (WEIR 1990):

$$F_{st_a} = V_P/V_Y, \quad (2)$$

where V_P is the variance due to the population source of variation and V_Y is the total variance

$$V_Y = V_P + V_Z + V_G. \quad (3)$$

V_Z and V_G are the variances due to the zygote and gene sources of variation. The different variances are estimated from the mean squares of the analysis of variance (WEIR 1990).

Gametic differentiation, one locus and multiple alleles: This model can easily be extended to the multiple allele case by using a multivariate analysis of variance instead of model (1) with $A_l - 1$ variables. In this case the original genotypic data are split into two vectors (LONG 1986) corresponding to the two alleles of a diploid individual. The total diversity is now subdivided into a set of variance covariance matrices:

$$\mathbf{Y} = \mathbf{P} + \mathbf{Z} + \mathbf{G}, \quad (4)$$

where $\mathbf{Y}$, $\mathbf{P}$, $\mathbf{Z}$, $\mathbf{G}$ are the (co)variance matrices corresponding to the total diversity ($\mathbf{Y}$), and respectively to the population ($\mathbf{P}$), zygote ($\mathbf{Z}$) and gene ($\mathbf{G}$) effects. A $\mathbf{F}_{st}$ matrix can now be defined from the matrix of (co)variances similarly to the definition of F_{st} at a single allele.

$$\mathbf{F}_{st} = [\mathbf{Y}]^{-1/2} \mathbf{P} [\mathbf{Y}]^{-1/2}. \quad (5)$$

There are $(A_l - 1)$ measures of F_{st} corresponding to the $(A_l - 1)$ eigenvalues of the matrix $\mathbf{F}_{st}$. LONG (1986) and LONG *et al.* (1987) have taken the mean value of all eigenvalues as measure of differentiation, which can be related to the trace of $\mathbf{F}_{st}$:

$$F_{st_l} = (1/(A_l - 1)) TR\{\mathbf{F}_{st}\}, \quad (6)$$

where TR denotes the trace of a matrix.

Because the sum of squares can also be expressed as the sum of all pairwise differences between vectors (LI 1976; EXCOFFIER *et al.* 1992), LONG *et al.* (1987) have shown that in the multiple allele case, F_{st} for locus l (F_{st_l}) can also be calculated as the mean of the standardized distances (in the $\mathbf{Y}$ metric) between all populations.

$$F_{st_l} = (1/S^2) \sum_{i < i'}^S D_{i,i'}^2, \quad (7)$$

where $D_{i,i'}^2$ is the standardized distance between population i and i' .

$$D_{i,i'}^2 = (1/(A_l - 1)) (\mathbf{p}_i - \mathbf{p}_{i'})^T [\mathbf{Y}]^{-1} (\mathbf{p}_i - \mathbf{p}_{i'}), \quad (8)$$

where $\mathbf{p}_i$ is the vector of allelic frequencies of populations i containing $A_l - 1$ elements. Note that the distances in (7) are not summed over all pairwise combinations of populations (including reciprocal or populations with themselves) as in the definition of G_{st} by NEI (NEI 1987, p. 163).

Gametic differentiation, multiple loci: The extension to the multiple locus case is straightforward: the

TABLE 1
Multilocus measures of differentiation at the gametic level

	Gametic equilibrium ^a	Gametic disequilibrium
Mean differentiation	$\overline{F_{st_l}}$	$(1/(A - L)) TR[\mathbf{Y}]^{-1/2} \mathbf{P} [\mathbf{Y}]^{-1/2}$
Maximum differentiation	$F_{st_{\max}}$	$\lambda_{\max} \{[\mathbf{Y}]^{-1/2} \mathbf{P} [\mathbf{Y}]^{-1/2}\}$

^a $\overline{F_{st_l}}$ is the mean value of F_{st_l} over all loci and $F_{st_{\max}}$ is the maximum value of differentiation at the single locus level.

multivariate ANOVA is now calculated on the basis of $(A - L)$ alleles over all loci. The original vectors and matrices of (4) have $(A - L)$ terms and F_{st_l} at the multilocus level (F_{st_m}) can be calculated from the matrix $\mathbf{F}_{st_m}$. At this point, two measures of differentiation may be of interest for analysing the genetic structure of populations:

1. The mean differentiation ($F_{st_{mo}}$) over all loci. The mean differentiation is given by the mean value of all the diagonal terms of $\mathbf{F}_{st_m}$, which is also equal to the mean values of all eigenvalues (λ_i).

$$F_{st_{mo}} = (1/(A - L)) TR\{\mathbf{F}_{st_m}\}. \quad (9)$$

2. The maximum differentiation ($F_{st_{ma}}$) that can be obtained with particular combinations of loci. The eigenvalues of $\mathbf{F}_{st_m}$ are expected to be different, and the highest value ($\lambda_{\max}$) represents the largest differentiation that can be obtained with a specific combination of allelic frequencies corresponding to the canonical variates.

$$F_{st_{ma}} = \lambda_{\max}\{\mathbf{F}_{st_m}\}. \quad (10)$$

The expression of mean and maximum differentiation are compared in Table 1. When there is no gametic disequilibrium, $\mathbf{Y}$ and $\mathbf{P}$ are diagonal matrices, and the mean differentiation is the mean of all F_{st_l} values over all loci. However, if disequilibrium is absent the maximum differentiation is given by the largest single locus F_{st_l} value ($F_{st_{\max}}$).

The construction of the vectors corresponding to (4) requires that the multilocus gametic arrays be known, if gametic disequilibrium has to be taken into account in the differentiation (Table 3). However, in most organisms, multilocus gametic arrays are not known. LONG (1986, p. 634) decided to equal all terms of matrix $\mathbf{Y}$ to 0 that corresponded to combinations of alleles belonging to different loci, implicitly assuming that there was no gametic disequilibrium. In this case the F_{st_m} is the mean of all F_{st_l} (as in Equation 9). Strictly speaking this is not a differentiation measure that includes specific allelic combinations at different loci. By using haploid data in conifers YANG and YEH (1993) were able to obtain gametic arrays and calculate F_{st_m} taking gametic disequilibrium into account.

Zygotic differentiation: We developed a method that

calculates multilocus differentiation at the diploid level and takes into account allelic associations belonging to different loci. The method is similar to that described at the gametic level.

Since gametic arrays cannot usually be obtained from diploid data, the proposed ANOVA model limits the subdivision of Y_{ijk} to the individual level unlike model (1). The ANOVA model corresponding to the zygotic value (X_{ij}) is now

$$X_{ij} = \mu + P_i + \overline{Z}_{ij}. \quad (11)$$

$\overline{Z}_{ij}$ is the mean individual effect, within which the effects of each allele cannot be separated. In comparison to model (1), $\overline{Z}_{ij}$ for a diploid individual can also be written as

$$\overline{Z}_{ij} = Z_{ij} + (G_{ij1} + G_{ij2})/2. \quad (12)$$

In contrast to model (1), X_{ij} is now a zygotic value. For a given allele a if ij is a homozygote aa , then X_{ij} equals 1; if ij is a heterozygote aa' , then X_{ij} equals 0.5. This system of scoring is similar to the one used by SMOUSE *et al.* (1982). For each individual there is now only one vector of data corresponding to the given zygote in comparison to the two vectors used by LONG (1986) corresponding to the two alleles at a diploid locus (Table 3).

Similarly to (2), the differentiation at the zygotic level is defined as CF_{st} , standing for composite measure of differentiation.

$$CF_{st_n} = V_P/V_X, \quad (13)$$

where

$$V_X = V_P + V_{\overline{Z}}. \quad (14)$$

As with the gametic arrays, the extension to multiple alleles and multilocus can now be made, replacing $\mathbf{Y}$ by $\mathbf{X}$ in (5) or (8) and $(A_l - 1)$ by $(A - L)$. The matrix of the multilocus composite differentiation can now be defined as

$$\mathbf{CF}_{st_m} = [\mathbf{X}]^{-1/2} \mathbf{P} [\mathbf{X}]^{-1/2}. \quad (15)$$

As with gametic differentiation, we suggest as differentiation measure mean differentiation ($CF_{st_{mo}}$) and maximum differentiation ($CF_{st_{ma}}$).

$$CF_{st_{mo}} = (1/(A - L)) TR\{\mathbf{CF}_{st_m}\}, \quad (16)$$

TABLE 2
Multilocus measures of differentiation at the zygotic level

	Mean differentiation		Maximum differentiation	
	Gametic equilibrium ^a (F_{st_m})	Gametic disequilibrium (CF_{st_m})	Gametic equilibrium ^a ($F_{st_{ma}}$)	Gametic disequilibrium ($CF_{st_{ma}}$)
Hardy-Weinberg equilibrium	$\overline{F_{st_l}}$	$(1/(A - L)) \text{TR}[\mathbf{U}]^{-1/2} \mathbf{P} [\mathbf{U}]^{-1/2}$	$F_{st_{max}}$	$\lambda_{\max} \{[\mathbf{U}]^{-1/2} \mathbf{P} [\mathbf{U}]^{-1/2}\}$
Hardy-Weinberg disequilibrium	$\overline{CF_{st_l}}$	$(1/(A - L)) \text{TR}[\mathbf{X}]^{-1/2} \mathbf{P} [\mathbf{X}]^{-1/2}$	$CF_{st_{max}}$	$\lambda_{\max} \{[\mathbf{X}]^{-1/2} \mathbf{P} [\mathbf{X}]^{-1/2}\}$

$\mathbf{U} = \mathbf{P} + 2\mathbf{\bar{Z}}$ and $\mathbf{X} = \mathbf{P} + \mathbf{\bar{Z}}$, where $\mathbf{P}$ and $\mathbf{\bar{Z}}$ are the variance covariance matrices corresponding to model (9). TR indicates the trace of a matrix, and $\lambda_{\max}$ is the highest eigenvalue of a matrix.

^a F_{st_l} and CF_{st_l} are the mean values of all single locus differentiation and $F_{st_{max}}$ and $CF_{st_{max}}$ are the maximum values of differentiation at the single locus level.

$$CF_{st_{ma}} = \lambda_{\max}\{\mathbf{CF}_{st_m}\}. \quad (17)$$

Like the composite measure of genotypic disequilibrium introduced by WEIR (1990), CF_{st_m} is a composite measure of differentiation taking into account allelic associations at the gametic and at the nongametic level. More specifically it accounts for differentiation due to allelic combinations in a given zygote, whether these combinations are due to gametic associations or not.

Comparison between gametic differentiation and zygotic differentiation: The comparison is restricted to the single allelic case. In addition to F_{st} the two other F -statistics (F_{is} and F_{it}) can be calculated from model (1) (WEIR 1990):

$$F_{is_n} = V_Z / (V_Z + V_G), \quad (18)$$

$$F_{it_n} = (V_P + V_Z) / V_Y. \quad (19)$$

From formula (12) it can be shown that

$$V_{\bar{Z}} = V_Z + V_G/2. \quad (20)$$

As a result,

$$CF_{st_n} = V_P / (V_P + V_Z + V_G/2). \quad (21)$$

By comparing (2) and (21), it can be seen that zygotic composite differentiation (CF_{st_n}) is always greater than gametic differentiation (F_{st_n}). This difference can be more clearly determined by using the F -statistics.

From (2), (19) and (21) it can be shown that

$$CF_{st_n} = 2F_{st_n} / (1 + F_{it_n}). \quad (22)$$

If the populations are in random mating equilibrium ($F_{is_n} = 0$; $F_{it_n} = F_{st_n}$), then a direct relationship exists between the gametic and zygotic differentiation measures.

$$CF_{st_n} = 2F_{st_n} / (1 + F_{st_n}). \quad (23)$$

Conversely, if populations are in random mating, than the gametic differentiation F_{st_n} can be derived from the ANOVA computed at the zygotic level (model 11). From (20) and (13), F_{st_n} becomes

$$F_{st_n} = V_P / (V_P + 2V_{\bar{Z}}). \quad (24)$$

Table 2 summarizes the different differentiation mea-

asures that can be obtained from multilocus data at the diploid level. If Hardy Weinberg equilibrium is assumed, F_{st} values can be derived from the model developed at the zygotic level (11). However, when Hardy Weinberg equilibrium is absent, only composite measures can be calculated at the zygotic level, and these will always be larger than the F_{st} values. For computation purposes, Table 3 presents the coding of data and construction of vectors for the multivariate ANOVA.

DIFFERENTIATION FOR QUANTITATIVE TRAITS

Single locus: We consider a total population that is subdivided into S subpopulations, for which a given phenotypic trait is assessed. We further assume that the trait is controlled by one locus, and that there are no dominance effects. The total variation of the trait can be subdivided according to an ANOVA model similar to (11) in which

$$W_{ij} = \mu + P_i + A_{ij}, \quad (25)$$

where P_i and A_{ij} correspond to the random effect of population i , and the random effect of the additive value of individual j in population i . By further assuming that the additive variance is the same in each subpopulation, then

$$V_W = V_P + V_A, \quad (26)$$

where V_W is the total variance, V_P is the variance due to the population subdivision, and V_A is the mean additive variance within the populations.

As with zygotic differentiation we define the differentiation measure at locus l for a quantitative character as being $CF_{st_{ql}}$,

$$CF_{st_{ql}} = V_P / (V_P + V_A). \quad (27)$$

If $F_{st_{ql}}$ and $F_{it_{ql}}$ are the two F -statistics at the locus controlling the quantitative character, then WRIGHT (1965) and COCKERHAM (1969) have shown that

$$V_P = 2F_{st_{ql}}V_A, \quad (28)$$

TABLE 3
Scoring procedure for calculation of differentiation measures at the multilocus level

Gametic differentiation				Zygotic differentiation		
Gametes		Elements of vector	<i>y</i>		Genotype	Elements of vector
(1)	(2)		(1)	(2)		
A1:A1		A1	1	1	A1 A1	A1
B1:B2		A2	0	0	B1 B2	A2
		B1	1	0		B1
A1:A3		A1	1	0	A1 A3	A1
B2:B2		A2	0	0	B2 B2	A2
		B1	0	0		B1
A1:A2		A1	1	0	A1 A2	A1
B2:B1		A2	0	1	B2 B1	A2
		B1	0	1		B1

Gametic and zygotic arrays are given for two loci (*A* and *B*; *A* being a triallelic locus, and *B* a diallelic locus). For locus *A* they are two elements in the vector (*A1* and *A2*), and there is only one for locus *B* (*B1*). Phase is supposed to be known in the case of gametic differentiation (gametes originating from the male and female parent are separated by a colon). Phase is unknown for the zygotic differentiation. *y* and *x* are the multilocus vectors corresponding to the gametic [model (1)] and zygotic arrays [model (11)]. For gametic arrays there are two vectors for a given genotype corresponding to the two gametes, and only one vector for zygotic arrays.

$$V_A = (1 + F_{it_{qt}} - 2F_{st_{qt}}) V_a, \quad (29)$$

where V_a is the additive variance of the total population. The differentiation for a quantitative character is therefore

$$CF_{st_{qt}} = 2F_{st_{qt}} / (1 + F_{it_{qt}}). \quad (30)$$

The differentiation for a quantitative character is the same as for the composite measure at the zygotic level for a gene marker (22 and 30).

Multiple loci: The extension to the multiple locus case has been made by ROGERS and HARPENDING (1983) assuming that there are no epistatic effects using a similar approach to the ANOVA model (25). If we assume that there is no gametic disequilibrium among the loci contributing to the quantitative character, then

$$V_P = 2R_o V_a, \quad (31)$$

where R_o (in Roger and Harpending's notation) is the mean of all the F_{st_i} values for the loci contributing to the quantitative character. If the populations are furthermore in random mating, then

$$V_A = (1 - R_o) V_a. \quad (32)$$

The resulting multilocus differentiation measure for a quantitative trait $F_{st_{qm}}$ is now (KREMER 1994) as follows:

$$F_{st_{qm}} = 2R_o / (1 + R_o) \quad (33)$$

or (FALCONER 1960)

$$F_{st_{qm}} = V_P / (V_P + 2V_A). \quad (34)$$

Once again the differentiation measure for a quantitative trait has a form similar to the composite measure

at the zygotic level, when populations are in random mating equilibrium. However differentiation measures for a quantitative trait can only be compared with the mean differentiation for gene markers, due to the assumptions used for calculating R_o .

Multiple traits: The multitrait case can be analyzed similarly to the multilocus case, with a multivariate ANOVA model adapted from (25).

$$\mathbf{W} = \mathbf{P} + \mathbf{A}, \quad (35)$$

where $\mathbf{W}$, $\mathbf{P}$ and $\mathbf{A}$ are the (co)variance matrices corresponding to the different effects given in (25). The concept of mean and maximum differentiation may also be used for quantitative traits, based on the $\mathbf{F}_{st_q}$ and the $\mathbf{CF}_{st_q}$ matrix given by

$$\mathbf{CF}_{st_q} = [\mathbf{W}]^{-1/2} \mathbf{P} [\mathbf{W}]^{-1/2}, \quad (36)$$

$$\mathbf{F}_{st_q} = [\mathbf{P} + 2\mathbf{A}]^{-1/2} \mathbf{P} [\mathbf{P} + 2\mathbf{A}]^{-1/2}. \quad (37)$$

There has been a somewhat different approach proposed by ZHIVOTOVSKY (1985), who estimates and subdivides the standardized generalized variance (SGV). SGV is the scalar multivariate analogue to the variance in the univariate case. When applied to (35), SGV can be calculated for each of the three matrices as their determinant. However, as shown by ZHIVOTOVSKY (1985), the additive relationship between the different SGVs is lost in the multivariate case:

$$SGV(\mathbf{W}) \geq SGV(\mathbf{P}) + SGV(\mathbf{A}). \quad (38)$$

Complete additivity can only be obtained if the pattern of between-population variation is similar to the pattern of within-population additive variation. If so, the level of differentiation can be given by $SGV(\mathbf{P}) /$

SGV(W). Due to the lack of additivity we used the $\mathbf{CF}_{stq}$ approach rather than the SGV approach.

STATISTICAL TESTS FOR MULTIVARIATE DIFFERENTIATION

The null hypothesis of no multivariate differentiation can be stated by (ITO 1962)

$$H_0: \lambda_1 = \lambda_2 = \lambda_3 = \dots = 0,$$

where the λ_i are the different eigenvalues of the $\mathbf{F}_{st}$ (or $\mathbf{CF}_{st}$) matrices. Unlike in univariate analysis of variance there are three different criteria that can be used for testing the null hypothesis (TOMASSONE *et al.* 1988):

- (C1) WILKS's criterion (WILKS 1932) $\Pi(1 - \lambda_i)$,
- (C2) PILLAI's criterion (PILLAI 1955) $\Sigma \lambda_i$,
- (C3) ROY's largest root (ROY 1953) $\lambda_{\max}$.

Any of the three criteria can be used to reject the null hypothesis H_0 at a preassigned level α . The exact distribution, under the null hypothesis, of the three criteria are known (TOMASSONE *et al.* 1988). However there are differences in the powers of the tests depending on the alternative hypothesis that is tested (ITO 1962; PILLAI and JAYACHANDRAN 1968; TOMASSONE *et al.* 1988). If H_1 is $\lambda_1 \neq 0$ and all other λ_i are 0, the power of the largest root (criteria C3) is higher than the others. If H_1 is all λ_i different from zero, then criteria C1 and C2 show higher powers. We therefore would recommend using criteria C3 if maximum differentiation is of concern and C1 (or C2) if mean differentiation is to be tested. These testing procedures could be used for quantitative traits (multitrait differentiation). However for marker data (multilocus differentiation), an empirical method based on permutational analysis can also be used as an alternative to overcome the deviations to the basic assumptions of parametric tests (EXCOFFIER *et al.* 1992). A null hypothesis is constructed where individuals are randomly sampled from the global population and assigned to each subpopulations by keeping sample sizes equal to the original data set. Any observed value of multilocus F_{st} can then be compared to the null distribution for testing for significance of F_{st} .

MAXIMUM VS. MEAN DIFFERENTIATION

It must be emphasized that both measures as defined in (9) and (10) take into account disequilibria among loci or traits. Therefore these measures should not be considered as measures of population structure (as are monolocus measures of F_{st} for gene markers) from which inferences on gene flow will be made, but only as measures of differentiation taking into account disequilibria. Their comparison with single locus differentiation should be made only if the objective is to estimate the proportion of differentiation due to disequilibria. However the two measures may differ in the way they account for disequilibria. Furthermore dis-

equilibria *per se* can be subdivided in two different components (the between- or within-population) and these components may differentially affect the two measures of differentiation. The contribution of disequilibria and its components to the two multilocus differentiation measures will be addressed in a case study where both gene markers and quantitative traits were assessed. It must also be noted that, because MANOVA uses covariances for estimating multivariate differentiation, only disequilibria of the second order are taking into account in both mean and maximum differentiation. If higher orders are of interest, then log linear methods should be preferred (PEREZ DE LA VEGA *et al.* 1991).

Although the definitions of maximum and mean differentiation are comparable in terms of eigenvalues, their values should be compared with caution when comparisons are based on different number of loci.

Maximum differentiation will account for variations due to the variables (locus or traits) exhibiting the highest single variable differentiation and disequilibrium among them. When more variables are added in the MANOVA models, there will therefore be a tendency of increase of $\lambda_{\max}$ that can be attributed to two different causes: (1) a mechanical one due to the increase of the number of variables and (2) a genetic one due to the disequilibrium between the new variables and the previous ones. For example, in the case of multilocus analysis, if more loci are added, chances of introducing a locus exhibiting higher single locus differentiation value than the previous ones increase with the number of loci. Therefore the mechanical effect due to the number of loci limits comparisons of $\lambda_{\max}$ values between studies conducted with different number of loci. Even if the number of loci is the same, but the loci are different, comparisons may be affected by the mechanical effect. However this restriction does not impede comparisons between $\lambda_{\max}$ and $F_{st\max}$ (maximum value at the single locus level, Table 1), made on the same loci, to separate the effects of the two causes on the level of differentiation (see Figure 1 and 2 in the case study). Therefore for identifying the effects to disequilibria on multilocus differentiation it is recommended to compare $\lambda_{\max}$ and $F_{st\max}$. Finally the mechanical effect of the number of loci on $\lambda_{\max}$ values will be more pronounced when the number of loci is low.

Mean differentiation as maximum differentiation accounts for single variable differentiation and disequilibria. But, because it is a mean value over all eigenvalues it will be less affected by the number of variables considered than maximum differentiation. As more variables are added, the mechanical cause will be less pronounced and comparisons across studies may be more reliable.

A CASE STUDY: POPULATION DIFFERENTIATION IN OAKS

Multilocus measures of gene differentiation, materials and methods: A set of 81 populations of *Quercus petraea*

FIGURE 1.—Variation of multilocus differentiation (F_{st_m}), as a function of the number of loci associated, assuming Hardy Weinberg equilibrium in the populations. —■—, maximum differentiation ($F_{st_m} = \lambda_{\max}\{F_{st}\}$) taking into account gametic disequilibrium; —△—, maximum differentiation ($F_{st_m} = F_{st_{\max}}$) assuming no gametic disequilibrium; —●—, mean differentiation ($F_{st_m} = (1/(A - L))\{F_{st}\}$) taking into account gametic disequilibrium; —○—, mean differentiation ($F_{st_m} = F_{st}$) assuming no gametic disequilibrium. The arrow separating the two curves ($\lambda_{\max}\{F_{st}\}$ and $F_{st_{\max}}$) indicates the effect of gametic disequilibria on gametic differentiation.

sampled throughout the natural range of the species was used to assess gene diversity at eight isozyme loci. Enzymes were extracted from 120 seeds per population. The seeds were randomly chosen from a seedlot harvested on an area varying from 15 to 20 hectares. Exact data of the origin of the populations and electrophoretic procedures are given in ZANETTO and KREMER (1995), and Mendelian inheritance of markers was verified in MÜLLER-STARCK *et al.* (1996). The enzyme systems were as follows: acid phosphatase (ACP, EC 3.1.3.2), alanine aminopeptidase (AAP, EC 3.4.11.1), diaphorase (DIA, EC 1.6.4.3), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), leucine aminopeptidase (LAP, EC 3.4.11.1), menadione reductase (MR, EC 1.6.99.2), phosphoglucosomerase (PGI, EC 5.3.1.9), phosphoglucosomutase (PGM, EC 2.7.5.1).

Monolocus and multilocus differentiation measures were calculated from univariate and multivariate ANOVA models (1) and (11). The (co)variances used to estimate the differentiation measures were obtained by equating the mean squares and crossproducts to their expectations. Multilocus differentiation measures were computed for all associations of loci (from two to eight). For each association, all combinations of loci were considered (28 for two and six loci; 56 for three and five; 70 for four; eight for seven and one; one for eight). Using Pillai's criterion (PILLAI 1955) all tests for multilocus differentiation were significant.

Multilocus measures of gene differentiation, results: The multilocus measures of mean differentiation on the whole were constant (2.5% for $F_{st_{mo}}$, 4.9% for $CF_{st_{mo}}$) whatever the combination of loci considered indicating that the single locus measures F_{st_i} were of

FIGURE 2.—Variation of composite multilocus differentiation (CF_{st_m}), as a function of the number of loci associated. —■—, maximum differentiation ($CF_{st_m} = \lambda_{\max}\{CF_{st}\}$) taking into account zygotic disequilibrium; —△—, maximum differentiation ($CF_{st_m} = CF_{st_{\max}}$) assuming no zygotic disequilibrium; —●—, mean differentiation ($CF_{st_m} = (1/(A - L))\{CF_{st}\}$) taking into account zygotic disequilibrium; —○—, mean differentiation ($CF_{st_m} = CF_{st}$) assuming no zygotic disequilibrium. The arrow separating the two curves ($\lambda_{\max}\{CF_{st}\}$ and $CF_{st_{\max}}$) indicates the effect of disequilibria on zygotic differentiation.

similar magnitude (Figures 1 and 2). However maximum differentiation steadily increased from 2.9 to 4.6% for F_{st_m} and from 5.7 to 8.9% for CF_{st_m} when gametic disequilibrium was considered to be absent. The increase in differentiation was even larger when gametic disequilibrium was considered present: from 2.9 to 6.5% for F_{st_m} , and from 5.7 to 12.2% for CF_{st_m} . When the multilocus maximum differentiation measures were computed with the combination of eight loci, differentiation taking disequilibrium into account were 40% higher than the values obtained when disequilibrium was not considered. The steadily increase of maximum differentiation is partly due to the mechanical effect related to the number of loci but also to disequilibria among loci illustrated by the difference between $\lambda_{\max}$ and $F_{st_{\max}}$ on Figures 1 and 2. The increase of maximum differentiation associated to the maintenance of constant values for mean differentiation indicates that disequilibria are mostly absorbed by the first canonical variate: as shown by Figures 1 and 2, disequilibria have stronger effects on maximum differentiation than on mean differentiation.

It is worth noting that the association of only two loci (AAP, LAP) accounts for a major portion of differentiation (9.8% for CF_{st_m} for these two loci and 12.2% for all eight loci) (Table 4). Interestingly these two loci are closely linked as shown by the cosegregation analysis (MÜLLER STARCK *et al.* 1996) and exhibit significant gametic disequilibrium in the populations (KREMER and ZANETTO 1996).

Genetic differentiation of quantitative traits, materials and methods: A subset of 21 populations from the sample of 81 used for the isozyme survey was used for

TABLE 4
Multilocus differentiation for specific associations of loci

Locus associations	Mean differentiation				Maximum differentiation			
	Gametic equilibrium ²		Gametic disequilibrium		Gametic equilibrium		Gametic disequilibrium	
	$F_{st_{mo}}$	$CF_{st_{mo}}$	$F_{st_{ma}}$	$CF_{st_{ma}}$	$F_{st_{ma}}$	$CF_{st_{ma}}$	$F_{st_{ma}}$	$CF_{st_{ma}}$
AAP-LAP	0.0238	0.0464	0.0275	0.0531	0.0464	0.0886	0.0514	0.0978
AAP-LAP-MR	0.0229	0.0449	0.0270	0.0521	0.0464	0.0886	0.0591	0.1114
AAP-LAP-MR-PGI	0.0213	0.0416	0.0259	0.0500	0.0464	0.0886	0.0618	0.1164
AAP-LAP-MR-PGI-PGM	0.0221	0.0433	0.0258	0.0496	0.0464	0.0886	0.0633	0.1190
AAP-LAP-MR-PGI-PGM-GOT	0.0216	0.0422	0.0252	0.0487	0.0464	0.0886	0.0644	0.1212
AAP-LAP-MR-PGI-PGM-GOT-ACP	0.0221	0.0432	0.0256	0.0502	0.0464	0.0886	0.0653	0.1221
AAP-LAP-MR-PGI-PGM-GOT-ACP-DIA	0.0217	0.0424	0.0248	0.0480	0.0464	0.0886	0.0653	0.1224

field plantation. These populations originated mostly from the Western part of the natural distribution (DUCOUSSO *et al.* 1996). The material was collected in the fall 1987 according to the procedure outlined earlier. The seeds were sown in four replicates in a nursery located in Brittany (North West France). When the seedlings were 3 years old, they were outplanted in the forest in four different sites. The results presented here correspond to a site located near the nursery, in Brittany (Fôret de la Petite Charnie). The field experimental design comprised five complete blocks. Within each block, each population was represented by two discontinuous plots of 24 trees. Overall each population was represented by $24 \times 2 \times 5 = 240$ trees. In the spring of the sixth season (3 years after plantation) bud burst was recorded with a scoring procedure. Scores varied between 0 (dormant bud) to 5 (fully developed bud). At the end of the 6th season the total height of the trees was recorded.

The data were analyzed with the following ANOVA model:

$$W_{ijk} = \mu + P_i + b_j + (Pb)_{ij} + \epsilon_{ijk}. \quad (39)$$

Where W_{ijk} is the phenotypic value of a tree, P_i is the population effect, b_j is the block effect, $(Pb)_{ij}$ is the effect due to the population*block interaction and ϵ_{ijk} is the individual effect (phenotypic value of a tree within a population). P_i and ϵ_{ijk} are considered as random effects and b_j as a fixed effect. The total variance can now be subdivided according to model (39):

$$V_W = V_P + V_{Pb} + V_\epsilon. \quad (40)$$

The differentiation measures were calculated as

$$CF_{st_{qm}} = V_P / (V_P + h^2(V_{Pb} + V_\epsilon)), \quad (41)$$

and in case of Hardy Weinberg equilibrium:

$$F_{st_{qm}} = V_P / (V_P + 2h^2(V_{Pb} + V_\epsilon)), \quad (42)$$

where h^2 is the narrow sense heritability of the trait. V_Z , V_P and V_ϵ were estimated by equating mean squares of

ANOVA to their expectations. Because progenies were not available for each population, the additive variance could not be estimated for each population. We therefore assumed that the heritability of the trait (h^2) was the same for each population. For the multitrait analysis, a multivariate ANOVA was used following model (39).

Genetic differentiation of quantitative traits, results: We used heritability values for bud burst and height growth (Table 5) that were within the range of experimental results obtained in forest trees (CORNELIUS 1994). In general the differentiation coefficients were higher than 20% for both traits regardless of the heritability values. Furthermore, the differentiation levels were comparable for the two traits, even though they are highly dependent on the heritability values. If comparisons are to be made with differentiation levels obtained with gene markers, precautions should be taken so that conclusions can be drawn for comparable parameters. Differentiation for quantitative traits should be compared with *mean* multilocus zygotic differentiation for gene markers ($CF_{st_{qm}}$ with $CF_{st_{ma}}$, or $F_{st_{qm}}$ with $F_{st_{ma}}$). Comparison of data from Tables 4 and 5 clearly indicate that population differentiation is much higher for morphological and phenological traits than for allozymes. The differences in differentiation should be even much larger, considering that the sample of populations for allozymes covers a larger geographic range than for quantitative traits.

TABLE 5
Population differentiation for bud burst and height growth in *Q. petraea*

Bud burst			Height growth		
h^2 ^a	$F_{st_{qm}}$	$CF_{st_{qm}}$	h^2 ^a	$F_{st_{qm}}$	$CF_{st_{qm}}$
0.20	0.431	0.602	0.05	0.482	0.651
0.30	0.336	0.503	0.10	0.318	0.483
0.40	0.274	0.431	0.15	0.237	0.383
0.50	0.233	0.378	0.20	0.189	0.318

^a h^2 , Narrow sense heritability of the trait.

TABLE 6
Multitrait differentiation measures in *Q. petraea* populations

h^2 ^a		Zhivotovsky's measures of differentiation ^b		Mean differentiation				Maximum differentiation			
				Noncorrelated traits ^c		Correlated traits ^d		Noncorrelated traits ^c		Correlated traits ^d	
Bud burst	Height	$W = P + 2A$	$W = P + A$	$F_{st_{qmo}}$	$CF_{st_{qmo}}$	$F_{st_{qma}}$	$CF_{st_{qma}}$	$F_{st_{qma}}$	$CF_{st_{qma}}$	$F_{st_{qma}}$	$CF_{st_{qma}}$
0.20	0.05	0.437	0.606	0.457	0.623	0.446	0.612	0.482	0.651	0.532	0.695
0.50	0.20	0.200	0.331	0.211	0.348	0.208	0.341	0.233	0.378	0.267	0.421
0.20	0.20	0.272	0.420	0.310	0.460	0.305	0.450	0.431	0.602	0.443	0.614
0.50	0.05	0.320	0.476	0.409	0.515	0.351	0.502	0.482	0.651	0.496	0.663
0.30	0.10	0.312	0.473	0.358	0.493	0.321	0.481	0.336	0.503	0.394	0.565

^a h^2 , narrow sense heritability of the trait.

^b Zhivotovsky's measure of differentiation = $\{|P|/|W|\}^{1/2}$.

^c The within (r_w) and between (r_b) correlation are assumed to be equal to zero.

^d Corresponding to the real data ($r_w = 0.017$) and ($r_b = -0.310$).

The multitrait differentiation measures were calculated according to Table 2 by replacing matrices **X** and **U** by **W** and (**P** + **2A**), respectively, according (35) (Table 6). Using Pillai's (PILLAI 1955) criterion, multitrait differentiation was significant at $P = 0.05$. For comparative purposes, Zhivotovsky's differentiation measures were also added on Table 6, that are close to values of mean differentiation (correlated characters). Due to the lack of additivity between the different components in Zhivotovsky's subdivision of **W**, these measures were later discarded.

There is a clear discrepancy between the patterns followed by the maximum and the mean differentiation (Table 6). The multitrait maximum differentiation, in the case of correlated traits, is always higher than that observed when the traits are assumed to be independent, (differentiation for the trait showing the largest differentiation). The multitrait mean differentiation for correlated traits is lower than the mean differentiation of single traits. This discrepancy is due to the anisotropy between the within- and between-population (co)variances matrices. Height growth and bud burst were negatively correlated at the population level ($r_b = -0.310$) whereas the within population correlation was close to zero ($r_w = 0.017$). As a result, the multitrait between-population variance decreases when compared to the single trait variability, whereas the multitrait total variation remains stable. We further investigated the influence of the anisotropy, or isotropy between r_b and r_w on the level of maximum and mean differentiation. We used the data when the heritability of height growth was assumed to be 0.10, and the heritability for bud burst 0.30. Calculations were done for the composite measures of differentiation. When the traits were considered to be noncorrelated at the between- and within-population level, the composite differentiation was 0.483 for height growth and 0.503 for bud burst, the multitrait mean differentiation was 0.493 and the multitrait maximum differentiation was 0.503. The

within- and between-population variances were those of the original data, and the covariances were calculated according to the imposed r_b and r_w values. Four different cases were considered:

1. The two correlations are of the same magnitude and of the same sign (Figure 3a). The multitrait mean differentiation varies from 0.493 to 0.500 and the multitrait maximum differentiation varies from 0.503 to 0.563 as r_b and r_w approach 1. The overall effect of the isotropy on the level of differentiation is low, and is of similar magnitude on the mean and maximum differentiation. Identical results are obtained regardless of whether r_b and r_w are negative or positive.
2. The two correlations are of the same magnitude but of the opposite sign (Figure 3b). The multitrait mean differentiation steadily increases from 0.493 to 0.500, but at a lower rate as in the previous case. However the maximum differentiation increases linearly from 0.503 to 1. There is a strong effect of the opposite sign of the correlations on the level of maximum differentiation. Identical results are obtained whether r_b is positive and r_w negative, or r_b negative and r_w positive.
3. There is no correlation at the within population level ($r_w = 0$), and r_b either increases from 0 to 1 or decreases from 0 to -1 (Figure 3c). The multitrait mean differentiation steadily decreases from 0.493 to 0.330, and is lower than the mean differentiation when the two traits are independent. This case corresponds to our data as shown in Table 6. The multitrait maximum differentiation increases from 0.503 to 0.660.
4. There is no correlation at the between population level ($r_b = 0$), and r_w either increases from 0 to 1 or decreases from 0 to -1 (Figure 3d). The multitrait mean differentiation increases from 0.493 to 0.663, and the maximum differentiation from 0.503 to 1.

FIGURE 3.—Variation of composite multitrait differentiations ($CF_{st_{gm}}$) as a function of the correlations between height growth and bud burst. Heritability was considered to be 0.30 for bud burst, and 0.10 for height growth. The variances of both traits were those of the data set. Covariances were calculated according to the imposed r_b (between population correlation) and r_w (within population correlation) values. —■—, maximum composite differentiation ($CF_{st_{gm}}$); —□—, mean composite differentiation ($CF_{st_{gm}}$).

These four cases illustrate how the differentiation measures are sensitive to the differences of the (co)variance matrices at the within and between population levels.

- The multitrait mean differentiation is hardly affected at all when both correlations r_b and r_w are of the same magnitude (Figure 3a), and even if they are of the opposite sign (Figure 3b). It is lower than the mean of the single trait differentiation, when there is no within population correlation, regardless of the magnitude of r_b (Figure 3c). It is higher than the mean of the single trait differentiation, when there is no between population correlation, regardless of the magnitude of r_w (Figure 3d).
- The multitrait maximum differentiation is always larger than the largest single trait differentiation (Figure 3, a–d). It is particularly sensitive to the opposite sign of correlations of r_b and r_w (Figure 3b), whereas mean differentiation is not affected by the anisotropy between the two correlations.

DISCUSSION

Variance components as a method for measuring differentiation: The proportion of diversity attributed to

population subdivision has been extensively used in population genetics for analyzing the structure of diversity in natural populations (HAMRICK *et al.* 1992). Its popularity is mainly due to (1) its multiple meanings, ranging from Wright's F -statistics (WRIGHT 1968) to Nei's coefficient of differentiation (NEI 1973) and (2) its *a posteriori* estimate of gene flow in the frame of the island model in spite of the controversial issue of its estimation. Simulation studies, as well as experimental data, have however shown that when the number of populations is large, the two estimation procedures [Nei's gene diversity approach, and the variance components methods of WEIR and COCKERHAM (1984)] give similar results (CHAKRABORTY and LEIMAR 1987). We extended the variance components approach of Weir and Cockerham to the multilocus case as did LONG (1986) and LONG *et al.* (1987), but we also took into account the covariances among allele frequencies to account for specific associations among alleles at the same or at different loci. In this case, the evolutionary significance in terms of coancestry (WEIR and COCKERHAM 1984) had to be discarded. We used the differentiation measure as a descriptive measure of the among population variation, regardless of the evolutionary

causes that created differentiation. The variance components approach can easily be extended to the multilocus level as part of the general linear model or with multivariate ANOVA (LONG 1986). The advantage of using analysis of variance is related to the generality of the estimation procedures. The ANOVA approach is particularly appropriate also in the multilocus case. When a large number of loci are analyzed, the number of existing genotypes in a population survey can be extremely high, limiting the application of Nei's. Finally the ANOVA approach also has the advantage of allowing comparisons with data obtained with quantitative characters by population biologists, because similar models are used for the data analysis. Keeping in mind that multilocus mean differentiation values for gene markers are to be compared with differentiation values of quantitative traits, our example shows that phenotypic traits such as bud burst or height growth show higher differentiation among populations than allozyme loci. This discrepancy is primarily due to the different evolutionary factors affecting allozymes and phenology in oaks. In a previous study, we showed that the geographic structure of allozyme diversity in *Q. petraea* follows a longitudinal gradient and results primarily from the postglacial recolonization routes (ZANETTO and KREMER 1995). In contrast to allozymes, the geographic pattern of variation of bud burst follows a latitudinal pattern (DUCOUSSO *et al.* 1996), as a consequence of selection pressures most probably due to latitudinal variation of defoliating insects.

Multilocus measures of differentiation: mean vs. maximum differentiation: Associations among alleles at different loci originate from several potential causes such as drift (OHTA and KIMURA 1969), Wahlund effects (NEI and LI 1973; SINNOCK 1975), population subdivision when migration is limited (OHTA 1982a,b), epistatic selection (PEREZ DE LA VEGA *et al.* 1991) and hitchhiking effects (LAURIE-AHLBERG and WEIR 1979). These associations have usually been studied by calculating gametic disequilibria for two locus associations, and log linear models or multivariate descriptive statistics for multiple loci (SPIELMAN and SMOUSE 1976; SMOUSE and NEEL 1977). However only a few attempts have been made to quantify the amount of differentiation created by such allelic associations. YANG and YEH (1993) have applied the ANOVA model to haploid data to estimate F_{st_m} in a similar way to us, whereas LONG (1986) and LONG *et al.* (1987) did not take into account the correlation between allelic frequencies in the multivariate ANOVA. However by showing that Euclidean distances between allele frequencies of different populations can be related to Wright's F_{st} , LONG *et al.* (1987) established a link between the univariate and multivariate approach. Both methods were already associated by CHAKRABORTY (1980) who established a relationship between the single measure of differentiation (Nei's G_{st}) and a multilocus measure of SPIELMAN and SMOUSE (1976) that is derived from the Mahalanobis distance.

If differentiation due to specific allelic associations needs to be measured, a number of different alternatives can be chosen according to the specific combinations that are considered. We therefore found it useful to separate the mean differentiation from the maximum differentiation, even if both measures take into account gametic disequilibria. There are at least three reasons why we focused on maximum differentiation. First, maximum differentiation may provide an indication on evolutionary causes of differentiation, as indicated by empirical results of population differences obtained with multivariate techniques (WHEELER and GURIES 1982; MERKLE *et al.* 1988; LAGERCRANTZ and RYMAN 1990; KREMER and ZANETTO 1996). In these examples the population values of the first canonical variate (the one having the largest eigenvalue) were correlated with geographical or historical data; maximum differentiation could therefore be related to systematic evolutionary factors. If, for example, gametic disequilibrium is due to epistatic selection, differentiation may be increased for those loci affected by disequilibria, in contrast to other combinations of loci. As a result, discrepancies of differentiation values are expected with different loci combinations. This is the case in our example (Table 4), where differentiation at the two locus level for AAP-LAP accounts for a major part of the total differentiation that can be observed at the eight locus level. These loci may in fact be identified by the correlation between their allele frequencies and the eigenvector corresponding to the largest eigenvalue in the eight loci analysis. On the other hand, if disequilibrium is due to stochastic causes, it would have affected all loci and resulted in similar levels of differentiation. Second, in comparison to mean differentiation, maximum differentiation takes into account the discrepancy that may exist between the (co)variance matrices at the between- and within-population level (Figure 3b). Contrasting patterns of these matrices may be observed in natural populations due to different levels of gametic disequilibria and should be a component of differentiation. For example, in allogamous plants, within population disequilibrium is generally low due to random mating (MUONA 1982), whereas between population disequilibrium can be high due to historical causes (different origins of populations). The contrasting pattern of within and between population disequilibria for multilocus allozymic data (KREMER and ZANETTO 1996) and for quantitative traits (Figure 3c) has clearly shown that the anisotropy affects more maximum differentiation than mean differentiation. Third, maximum differentiation appears to be better adapted for measuring differentiation due to disequilibrium than mean differentiation according to the experimental results that were obtained in our examples (Figure 1 and 2). Although maximum differentiation is inflated by a mechanical effect due to the number of loci, as compared to mean differentiation, there are some indi-

cations that maximum differentiation absorbs more of the disequilibria among loci than mean differentiation. This is shown by the steadily increase of maximum differentiation associated to the constant value of mean differentiation (on Figures 1 and 2) as more loci are added in the analysis. As a result disequilibria effects appear to be unevenly distributed among the different canonical variates.

Gametic and zygotic differentiation: At a multilocus level for a diploid organism, allelic differentiation can only be estimated if coupling and repulsion heterozygotes can be identified, unless one has access to gametic tissues like in conifers. Based on the ANOVA model, we developed an alternative method that accounts for differentiation when phase is unknown, which we called composite differentiation. However the method overestimates differentiation as defined by WEIR and COCKERHAM (1984). The bias may be important especially at low values of F_{st} and is due to the fact that the total variation comprises only half of the within individual variance (Equation 21). As shown in our example, CF_{st} is almost twice as high as F_{st} . When populations are in HW equilibrium, one may calculate F_{st} values from CF_{st} data. However, when there is evidence of Hardy-Weinberg disequilibrium, it is recommended to calculate composite measures of differentiation.

The authors acknowledge the thoughtful comments of C. DILLMANN, M. LASCoux, R. J. PETIT, O. PONS on earlier versions of this manuscript, and two anonymous reviewers for their suggestions.

LITERATURE CITED

- CHAKRABORTY, R., 1980 Relationship between single- and multi-locus measures of gene diversity in a subdivided population. *Ann. Hum. Genet. Lond.* **43**: 423–428.
- CHAKRABORTY, R., and O. LEIMAR, 1987 Genetic variation within a subdivided population, pp. 89–120 in *Population Genetics and Fishery Management*, edited by N. RYMAN and F. UTTER. Sea Grant Program, University of Washington Press, Seattle.
- COCKERHAM, C. C., 1969 Variances of gene frequencies. *Evolution* **23**: 72–84.
- CORNELIUS, J., 1994 Heritabilities and additive genetic coefficients of variation in forest trees. *Can. J. For. Res.* **24**: 372–379.
- DAGNÉLIE, P., 1975 *Analyse statistique à plusieurs variables*. Les Presses Agronomiques de Gembloux, Gembloux, Belgium.
- DUCCOUSO, A., J. P. GUYON and A. KREMER, 1996 Latitudinal and altitudinal variation of bud burst in western populations of Sessile oak (*Quercus petraea* (Matl.) Liebl.). *Ann. Sci. Forest.* **53**: 775–782.
- EXCOFFIER, L., P. E. SMOUSE and J. M. QUATTRO, 1992 Analysis of molecular variance inferred from metric distance among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.
- FALCONER, D. S., 1960 *Introduction to Quantitative Genetics*. Oliver and Boyd, Edinburgh and London.
- HAMRICK, J. L., M. J. GODT and S. L. SHERMAN-BROYLES, 1992 Factors influencing levels of genetic diversity in woody plant species. *New Forest* **6**: 95–124.
- HOTELLING, H., 1951 A generalized *T* test and measure of multivariate dispersion. *Proceedings of the Second Berkeley Symposium Mathematical Statistics and Probability*, Berkeley, pp. 23–41.
- ITO, K., 1962 A comparison of the powers of two multivariate analysis of variance tests. *Biometrika* **49**: 455–462.
- KREMER, A., 1994 Diversité génétique et variabilité des caractères phénotypiques chez les arbres forestiers. *Genet. Sel. Evol.* **26**: 105s–123s.
- KREMER, A., and A. ZANETTO, 1996 Geographic structure of gene diversity in *Quercus petraea* (Matl.) Liebl. II: multilocus patterns of variation. *Heredity* (in press).
- LAGERCRANTZ, U., and N. RYMAN, 1990 Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozymic variation. *Evolution* **44**: 38–53.
- LANDE, R., 1992 Neutral theory of quantitative genetic variance in an island model with local extinction and colonization. *Evolution* **42**: 455–466.
- LAURIE-AHLBERG, C. C., and B. S. WEIR, 1979 Allozymic variation and linkage disequilibrium in some laboratory populations of *Drosophila melanogaster*. *Genetics* **92**: 1295–1314.
- LI, C. C., 1976 *Population Genetics*. Boxwood, Pacific Grove, CA.
- LONG, A. D., and R. S. SINGH, 1995 Molecules versus morphology: the detection of selection acting on morphological characters along a cline in *Drosophila melanogaster*. *Heredity* **74**: 569–581.
- LONG, J. C., 1986 The allelic correlation structure of Gainj and Kalam-speaking people. 1. The estimation and interpretation of Wright's *F* statistics. *Genetics* **112**: 629–647.
- LONG, J. C., P. E. SMOUSE and J. W. WOOD, 1987 The allelic correlation structure of Gainj and Kalam-speaking people. 2. The genetic distance between population subdivisions. *Genetics* **117**: 273–283.
- MERKLE, S. A., W. T. ADAMS and R. K. CAMPBELL, 1988 Multivariate analysis of allozyme variation patterns in coastal Douglas-fir from the southwest Oregon. *Can. J. For. Res.* **18**: 181–187.
- MÜLLER-STARCK, G., A. ZANETTO, A. KREMER and S. HERZOG, 1996 Inheritance of isoenzymes in sessile oak (*Quercus petraea* (Matl.) Liebl.) and offspring from interspecific crosses. *For. Genet.* **3** (1): 3–14.
- MUONA, O., 1982 Potential causes for multilocus structure in predominantly outcrossing populations. *Silva Fennica* **16**: 107–114.
- NAGYLAKE, T., 1994 Geographical variation in a quantitative character. *Genetics* **136**: 361–381.
- NEI, M., 1973 Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA* **12**: 3321–3323.
- NEI, M., 1977 *F* statistics and analysis of gene diversity in subdivided populations. *Ann. Hum. Genet.* **41**: 225–233.
- NEI, M., and W. H. LI, 1973 Linkage disequilibrium in subdivided populations. *Genetics* **75**: 213–219.
- OHTA, T., 1982a Linkage disequilibrium with the island model. *Genetics* **101**: 139–155.
- OHTA, T., 1982b Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proc. Natl. Acad. Sci. USA* **79**: 1940–1944.
- OHTA, T., and M. KIMURA, 1969 Linkage disequilibrium due to random genetic drift. *Genet. Res.* **13**: 47–55.
- PEREZ DE LA VEGA, M., P. GARCIA and R. W. ALLARD, 1991 Multilocus genetic structure of ancestral Spanish and colonial Californian populations of *Avena Barbata*. *Proc. Natl. Acad. Sci. USA* **88**: 1202–1206.
- PILLAI, K. C. S., 1955 Some new test criteria in multivariate analysis. *Ann. Math. Stat.* **26**: 177–121.
- PILLAI, K. C. S., and K. JAYACHANDRAN, 1968 Power comparisons of tests of equality of two covariance matrices based on four criteria. *Biometrika* **55**: 335–342.
- PODOLSKY, R. H., and T. P. HOLTSFORD, 1995 Population structure of morphological traits in *Clarkia dudleyana*. I. Comparison of *F*_{st} between allozymes and morphological traits. *Genetics* **140**: 733–744.
- PROUT, T., and J. S. F. BARKER, 1993 *F* statistics in *Drosophila buzzatii*: selection, population size and inbreeding. *Genetics* **134**: 369–375.
- ROGERS, A. R., and H. C. HARPENDING, 1983 Population structure and quantitative characters. *Genetics* **105**: 985–1002.
- ROY, S. N., 1953 On a heuristic method of test construction and its use in multivariate analysis. *Ann. Math. Stat.* **24**: 220–238.
- SINNOCK, P., 1975 The Wahlund effect for the two locus model. *Am. Nat.* **109**: 565–570.
- SMOUSE, P. E., and J. V. NEEL, 1977 Multivariate analysis of gametic disequilibrium in the Yanomana. *Genetics* **85**: 733–752.
- SMOUSE, P. E., R. S. SPIELMAN and M. H. PARK, 1982 Multiple-locus allocation of individuals to groups as a function of the genetic variation within and differences among human populations. *Am. Nat.* **119**: 445–463.

- SPIELMAN, R. S., and P. E. SMOUSE, 1976 Multivariate classification of human populations. I. Allocation of Yanomama Indians to villages. *Am. J. Hum. Genet.* **28**: 317–331.
- SPITZE, K., 1993 Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics* **135**: 367–374.
- TOMASSONE, R., M. DANZART, J. J. DAUDIN and J. P. MASSON, 1988 *Discrimination et classement*. Masson, Paris.
- WHEELER, N. C., and R. P. GURIES, 1982 Population structure, genic diversity, and morphological variation in *Pinus contorta* Dougl. *Can J. For. Res.* **12**: 595–606.
- WEIR, B. S., 1979 Inferences about linkage disequilibrium. *Biometrics* **35**: 235–254.
- WEIR, B. S., 1990 *Genetic Data Analysis*. Sinauer Associates, Sunderland, MA.
- WEIR, B. S., and C. C. COCKERHAM, 1984 Estimating F -statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.
- WILKS, S. S., 1932 Certain generalizations in the analysis of variance. *Biometrika* **24**: 471–494.
- WRIGHT, S., 1968 *Evolution and the Genetics of Populations. The Theory of Gene Frequencies*. Vol 2. University of Chicago Press, Chicago.
- WRIGHT, S., 1965 The interpretation of population structure by F -statistics with special regard of mating. *Evolution* **19**: 395–420.
- YANG, R. C., and F. C. YEH, 1993 Multilocus structure in *Pinus contorta* Dougl. *Theor. Appl. Genet.* **87**: 568–576.
- ZANETTO, A., and A. KREMER, 1995 Geographic structure of gene diversity in *Quercus petraea* (Matt.) Liebl. 1. Monolocus patterns of variation. *Heredity* **75**: 506–517.
- ZHIVOTOVSKY, L. A., 1985 Some methods of analysis of correlated characters, pp 423–432 in *Proceedings of the Second International Conference on Quantitative Genetics*, edited by B. S. WEIR, E. J. EUGENE, M. M. GOODMAN and G. NAMKOONG. Sinauer Associates, Sunderland, MA.

Communicating editor: B. S. WEIR