Intraclass Correlation of Polychotomous Responses of Lodgepole Pine to Infection of Western Gall Rust: A Simulation Study

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(Received 14th November 1997)

Abstract

Monte Carlo simulations were conducted to (i) examine the theory that permits the intraclass correlation (t_o) for a dichotomous or polychotomous trait to be adjusted to a continuous scale (t) and (ii) investigate sampling properties of estimated t_o. Simulations consist of nine parametric values of t = 0.025 to 0.225 representing heritability from 0.1 to 0.9 for half-sib families, four sets of family numbers and sizes, 10 dichotomous and 10 polychotomous traits and 1,000 replicates per 'treatment' combination. Simulation results substantiate and complement the well-known simulations for dichotomous traits. The theory was less adequate with extreme incidences, few infection classes and high levels of t. Smaller family numbers and/or sizes caused higher frequencies of negative estimates of t_o particularly with extreme incidences or few infection classes. We also analyzed the polychotomous responses of 291 lodgepole pine open-pollinated families in three geographic regions in west central Alberta to infection of western gall rust. Estimated t_o for geographic regions had much larger sampling variability. Estimated t_o for families within regions was considerably inflated, likely due to the presence of non-additive genetic variance and/or confounding stand effect. Inadequacy of the theory particularly with extreme incidences and few infection classes suggests a need to explore direct estimation procedures based on generalized linear models.

Key words: Intraclass correlation, threshold trait, liability model, Monte Carlo simulation, estimation of genetic parameters.

FDC: 165.3; 165.53; 453; 172.8 *Endocronartium harknessii;* 174.7 *Pinus contorta;* (712.3).

Introduction

Many traits in forest genetics and tree breeding, such as tree mortality, tree vigor and pest resistance, have discontinuous distributions but their variation cannot be adequately explained according to the Mendelian inheritance. Estimation of genetic parameters for such traits may be complicated because the true distribution cannot be observed. A simple solution to this difficulty proposed for dichotomous traits is to assign arbitrary values, 0 and 1, to the two phenotypic classes and to calculate the intraclass correlation or heritability from these values using the analysis of variance (ROBERTSON and LERNER, 1949; ELSTON, 1977; FOULLEY et al., 1990). Similar analysis has also been suggested for polychotomous traits (COCHRAN, 1954) and has been carried out for polychotomous responses to pest infection in forest trees (e.g., YANCHUK et al., 1988; HOFF and SUN, 1994; WU et al., 1996). However, an obvious caveat of this approach is the dependence of estimated genetic parameters on the frequencies of phenotypic classes. While the arc-sine square-root transformation of pest incidence is often recommended (e.g., COCHRAN, 1940; BECKER and MARSDEN, 1972), it remains to be seen the impact of such transformations on estimation of genetic parameters.

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The alternative remedy to remove this dependence on frequency is to use a model of a continuous underlying distribution of liability in which individuals are "affected" if they exceed a certain threshold value of liability (WRIGHT, 1934; ROBERTSON and LERNER, 1949; DEMPSTER and LERNER, 1950; HILL and SMITH, 1977). The intraclass correlation or heritability estimated on the (0, 1) scale can be adjusted to a frequency independent correlation or heritability on the continuous scale by making use of the properties of the normal curve (ROBERTSON and LERNER, 1949; HILL and SMITH, 1977). Adequacy of such adjustment has been examined in several simulation studies (e.g., VAN VLECK, 1972; OLAUSSON and RÖNNINGEN, 1975; MERCER and HILL, 1984; MCGUIRK, 1989). However, these studies have focused their examinations on dichotomous traits and on a small number of replications. GIANOLA (1979) generalized the adjustment to continuous scale by allowing for the analysis of polychotomous traits. Furthermore, there is little information about effects of sampling options (family number and size), infection frequencies and number of infection classes on the distributional behaviors of second order statistics such as intraclass correlation and heritability for threshold traits. The objectives of our simulation study are to examine the adequacy of adjustment to continuous scale for intraclass correlation of threshold traits and to provide an empirical assessment of statistical properties of estimated intraclass correlation from simulated experiments. We also analyzed polychotomous responses of lodgepole pine (*Pinus contorta* DOUGL. ssp. *latifolia* ENGELM.) to infection of western gall rust (WGR) [*Endocronartium harknessii* (J.P. MOORE) Y. HIRATSUKA].

Materials and Methods

Simulations

Computer generated observations, y, from a normal distribution with mean 0 and variance 1, i.e., $y \sim N(0, 1)$. There were a total of ns observations in s groups each of size n generated for each simulated sample. Following WEIR (1996, p. 55), we employed 1,000 replicated samples to adequately study the distributional behavior of estimated intraclass correlations for threshold traits. We used four sets of values of s and n to examine the effects of group number and size on estimation of intraclass correlation: (i) s = 300 and n = 30; (ii) s = 100 and n = 90; (iii) s = 30 and n = 100; and (iv) s = 30 and n = 30. Thus, observations y with the group structure could be expressed as the sum, $y = b + w$, of group effects, $b \sim N(0, t)$, and within-group deviations, $w \sim N(0, 1 - t)$, where t is the intraclass correlation. Nine parametric values of t = 0.025 to 0.225 were used in simulations to represent heritabilities of h^2 = 0.1 to 0.9 for half-sib families. Random effects b and w are independently distributed with $\sigma^2_b + \sigma^2_w = \sigma^2_y = 1$.

The generated observations, y, were grouped into "infected" and "normal" classes by truncating on phenotypic values corresponding to 10 incidences, p_1 = 0.05, p_2 = 0.10, …, p_{10} = 0.50. Because of symmetry, the results expected for incidences > 0.5 are the same as for those < 0.5. Thus, ten dichotomous traits

each with a binomial distribution were produced and the ith dichotomous trait (x_i) was defined as

$$x_i = \begin{cases} 1, & \text{if } y > \Phi^{-1}(p_i) \\ 0, & \text{otherwise} \end{cases} \qquad (1)$$

where $\Phi^{-1}(p_i)$ is the threshold corresponding to p_i with $\Phi(.)$ being the cumulative distribution function of underlying normal variable (y). We also defined nine polychotomous traits (i.e., number of infection classes ≥ 3) based on the defined dichotomous variables. Thus, a polychotomous trait with k infection classes (w_k) was simply the sum of the first k-1 dichotomous variables, i.e.,

$$w_k = \sum_{i=1}^{k-1} x_i , \qquad k = 3, 4, ..., 11 \qquad (2)$$

Equation (2) is equivalent to a direct definition of polychotomous traits (e.g., GIANOLA, 1979) where $w_k = i$ if $\Phi^{-1}(p_{i-1}) < y \leq \Phi^{-1}(p_i)$ for $i = 0, 1, 2, ..., k$-1 with special values of $p_0 = 0$ and $p_k = 1$. However, our definition (2) shows the relationships between individual dichotomous traits (x_i) and polychotomous traits (w_k). For each of 1,000 replicates of generated data, one-way analysis of variance (ANOVA) was computed and intraclass correlation was estimated for y and for each and every dichotomous and polychotomous traits defined. Averages of estimated intraclass correlations ($\hat{t}_o$) across 1,000 replicates were multiplied by four to obtain corresponding estimates of heritability. The theoretical values of intraclass correlation for polychotomous traits is given in GIANOLA (1979),

$$t_o = \frac{\left[\sum_{i=1}^{m} a_i (z_{i-1} - z_i)\right]^2}{\sum_{i=1}^{m} a_i^2 p_i - \left(\sum_{i=1}^{m} a_i p_i\right)^2} t \qquad (3)$$

where z_i is the height of the ordinate of the normal curve at the ith threshold point with two trivial values, $z_0 = z_m = 0$ and a_i is the score for the ith category. The scores can be the raw scores ($a_i = i$) or a set of "optimal" scores [$a_i = (z_{i-1} - z_i) / p_i$] leading to maximum intraclass correlation or heritability (GIANOLA and NORTON, 1981). For dichotomous traits (m = 2), equation (3) reduces to

$$t_o = \frac{z^2}{p(1-p)} t \qquad (4)$$

as shown in ROBERTSON and LERNER (1949) and HILL and SMITH (1977).

Experimental Data

The data used in this study was derived from a greenhouse inoculation experiment carried out during 1992 to 1994 to evaluate responses of lodgepole pine to WGR infection (YANG et al., 1997). A total of 291 open-pollinated families in the inoculation experiment were phenotypically superior trees in natural stands of three geographic regions in west central Alberta: 100 families from region B1, 97 from region B2 and 94 from region C [see DHIR and BARNHARDT (1993) for detailed description of the geographic regions]. Thirty seedlings per family were grown for inoculation. Seedling growth conditions and inoculation procedures were detailed in YANG et al. (1997). Assessment of WGR incidence was made for each seedling at 6 months after inoculation based on the 0 to 5 rating system by KLEIN (1991): 0 = no symptoms; 1= visible discolouration or a definite indication of infection, such as acute bending of stem; 2 = a definite canker but no swelling; 3 = some swelling with rough bark and open necrotic canker; 4 = partial gall, often with rough bark and necrotic canker; and 5 = complete gall formation. Following HOFF and SUN (1994), we also developed a (0 to 1) rating system, where 0 = absence of symptoms and 1 = presence of symptoms (i.e., combining scores 1 to 5 in the (0 to 5) rating system).

ANOVA was carried out for WGR infection scores using the following linear model:

$$Y_{ijl} = \mu + R_i + S_{ij} + e_{ijl}$$

where Y_{ijl} is the infection score of the lth seedling of the jth family in the ith region, μ is the overall mean, R_i is the effect of ith region, S_{ij} is the effect of the jth family in the ith region and e_{ijl} is the residual representing variability within families. All effects except for μ were considered random and unrelated, with zero means and variances σ^2_R, σ^2_S, and σ^2_e, respectively. We carried out the F-test of significance for our categorical data. However, the assumption of normality required for the F-test was obviously not met. For this reason, we also partitioned the likelihood-ratio statistics (G^2) (AGRESTI, 1990) for the 291 x 6 contingency table for six-class infection data and the 291 x 2 contingency table for two-class infection data into components due to regions and families within regions. Both F-test and G^2-test showed significant differences among regions and families within regions.

Following KEMPTHORNE (1969, Ch. 13) and FALCONER and MACKAY (1996, Ch. 9), we defined two intraclass correlations, t_1 and t_2, as proportions of the total variance due to among-region and among-family components, respectively. The formulae for computing these and related intraclass correlations are given in *table 1*. Estimates of t_1 and t_2 are given by (cf. *Table 1*),

$$\hat{t}_1 = \frac{\hat{\sigma}^2_R}{\hat{\sigma}^2_R + \hat{\sigma}^2_F + \hat{\sigma}^2_e} \qquad (5a)$$

$$= \frac{MSR - K_1 MSF - (1 - K_1) MSE}{MSR + (K_2 - K_1) MSF + [K_1 - K_2 - (1 - k_2)] MSE},$$

and

$$\hat{t}_2 = \frac{\hat{\sigma}^2_R + \hat{\sigma}^2_F}{\hat{\sigma}^2_R + \hat{\sigma}^2_F + \hat{\sigma}^2_e} \qquad (5b)$$

$$= \frac{MSR + (K_2 - K_1) MSF + (K_1 - K_2 - 1) MSE}{MSR + (K_2 - K_1) MSF + [K_1 - K_2 - (1 - k_2)] MSE},$$

where $K_1 = k_1/k_3$ and $K_2 = k_2/k_3$. The approximate standard errors of $\hat{t}_1$ and $\hat{t}_2$ were computed using the general formula of KEMPTHORNE (1969, p. 246–247).

Results and Discussion

Simulations

Figure 1 presents observed ($\hat{h}^2_o = 4\hat{t}_o$) and expected heritabilities ($h^2_o = 4t_o$) for the 19 dichotomous traits corresponding to levels of incidence from 0.05 to 0.95 at equal interval of 0.05 for the sampling setting of s = 300 and n = 30. The other three

Table 1. – Layout of analysis of variance, variance components and their relations to intraclass correlations in analyzing polychotomous responses of lodgepole pine to western gall rust.

Source	df	Mean Squares	Expected Mean Squares[a]	Variance Component
Regions	2	*MSR*	$\sigma_e^2 + k_1\sigma_F^2 + k_2\sigma_R^2$	$\sigma_R^2 = t_1\sigma_T^2$
Families (regions)	288	*MSF*	$\sigma_e^2 + k_3\sigma_F^2$	$\sigma_F^2 = (t_2 - t_1)\,\sigma_T^2$
Error	8,470	*MSE*	σ_e^2	$\sigma_e^2 = (1 - t_2)\,\sigma_T^2$

where $\sigma_T^2 = \sigma_R^2 + \sigma_F^2 + \sigma_e^2$

$$t_2 - t_1 = \frac{\sigma_F^2}{\sigma_R^2 + \sigma_F^2 + \sigma_e^2}$$

$$t_1 = \frac{\sigma_R^2}{\sigma_R^2 + \sigma_F^2 + \sigma_e^2}$$

$$1 - t_1 = \frac{\sigma_F^2 + \sigma_e^2}{\sigma_R^2 + \sigma_F^2 + \sigma_e^2}$$

$$t_2 = \frac{\sigma_R^2 + \sigma_F^2}{\sigma_R^2 + \sigma_F^2 + \sigma_e^2}$$

$$1 - t_2 = \frac{\sigma_e^2}{\sigma_R^2 + \sigma_F^2 + \sigma_e^2}$$

[a]) σ^2_R is variance due to regions; σ^2_F is variance due to families within regions; σ^2_e is error variance; k_1 = 30.37; k_2 = 2918.80; and k_3 = 30.10.

sampling options produced the nearly identical trajectory though the four sampling options differed markedly in the sampling variability of estimated heritabilities (see below for detailed discussion). Each point on the solid line was the average of 1,000 estimated heritabilities for an incidence and true heritability (i.e. heritability on the continuous scale). The expected heritabilities (dash line) were calculated using equation (4). For example, when p = 0.05, h^2_o = [(0.1031)^2/(0.05)(0.95)] h^2 = 0.2239 h^2. For each of nine true heritabilties, $\hat{h}^2_o$ and h^2_o were symmetrically distributed with a maximum value at an incidence of p = 0.5. Good agreements between $\hat{h}^2_o$ and h^2_o were evident, particularly when the true heritability was low ($h^2 \leq 0.3$). When the true heritability was high, observed heritabilities tended to be slightly higher than their expected values. Such deviations from the expected heritabilities were intensified when incidences were extreme. Similar results were found in other simulation studies (VAN VLECK, 1972; OLAUSSON and RÖNNINGEN, 1975; MERCER and HILL, 1984; MCGUIRK, 1989) though these studies except for MCGUIRK (1989) had a limited number of replicated simulations.

Both $\hat{h}^2_o$ and h^2_o were smaller than the true values (h^2) across the whole range of incidences (*Fig. 1*). The more extreme incidences, the greater the differences between $\hat{h}^2_o$ or h^2_o and h^2. For example, for h^2 = 0.1, the observed heritabilities across ten incidences (0.05 to 0.5) estimated with s = 300 and n = 30 were, respectively, $\hat{h}^2_o$ = 0.023, 0.036, 0.044, 0.050, 0.055, 0.058, 0.061, 0.063, 0.064 and 0.064. The corresponding expected values were h^2_o = 0.024, 0.034, 0.043, 0.049, 0.054, 0.058, 0.060, 0.062, 0.063, and 0.064. Similarly, for h^2 = 0.9, $\hat{h}^2_o$ = 0.265, 0.365, 0.429, 0.476, 0.510, 0.536, 0.554, 0.567, 0.574, and 0.577; and h^2_o = 0.202, 0.308, 0.383, 0.441, 0.484, 0.518, 0.543, 0.560, 0.570 and 0.572. Similar observed heritabilities were found for other three sampling settings. The reason for reduced $\hat{h}^2_o$ or h^2_o as compared to h^2 is the change in both phenotypic and genetic variances when the scale is shifted from continuous to discrete. It is evident that while the phenotypic variance [i.e., the denominator in equation (4)] has a maximum of 0.25 at incidence p = 0.5, the phenotypic variance of the continuous variable y was unity. Correspondingly, since $z \leq (2\pi)^{-1/2} < 0.4$, the factor z^2 represented the approximate decrease in the additive genetic variance in shifting from the continuous to binomial scale. Thus, the observed heritabilities for dichotomous traits need to be adjusted to the continuous scale as $\hat{h}^2 = p(1\text{-}p)/z^2\,\hat{h}^2_o$ [cf. equation (4)].

The four different sampling options (varying group numbers and sizes) had little effect on averages of 1,000 replications of estimated heritabilities across all incidences as they all produced the nearly same trajectory as shown in *figure 1* for s = 300 and n = 30. However, the sampling variability as measured in terms of standard deviation of 1,000 replications (results not shown) varied greatly among the four sampling options. Ranking of the magnitude of standard deviations for the four sampling options was consistent for almost all combinations of simulated incidences and heritabilities: (s = 100 and n = 90) < (s = 300 and n = 30) < (s = 30 and n = 100) < (s = 30 and n = 30). To further examine the sampling effect, we counted the number of negative among-family variance components from 1,000 estimates (h^2 = 0.1) or 9,000 estimates (all nine levels of heritabiltiy) for the four sampling options (*Table 2*). Most negative estimates occurred at h^2 = 0.1, but the frequency of occurrence varied substantially among the sampling options. For example, the range of frequencies at incidence of 0.5 was from 4 for (s = 100 and n = 90) to 320 for (s = 30 and n = 30). Judging from the frequencies of the negative variance

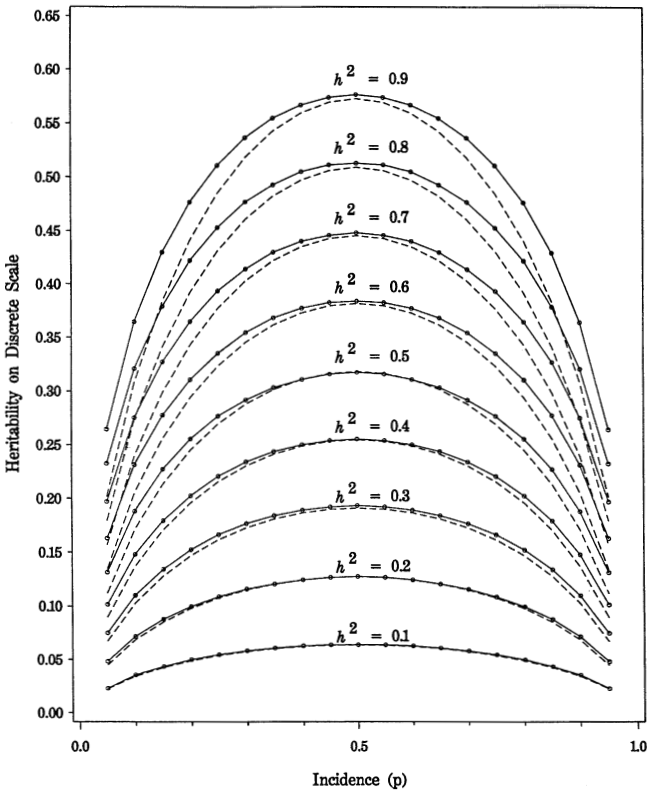

Figure 1. – The observed (solid line) and expected (dash line) relationships between heritability on binomial scale and incidence level (p) for given true heritability (h^2) on underlying continuous scale. Each of the observed values was an average of 1,000 estimated intraclass correlations based on 300 half-sib families and 30 individuals per family. The expected values for heritability on binomial scale were computed using equation (4).

components, ranking of the four sampling options was again: (s = 100 and n = 90) < (s = 300 and n = 30) < (s = 30 and n = 100) < (s = 30 and n = 30). It is also evident that the frequencies increased with decreasing incidences.

Underestimation of heritabilities for polychotomous traits was reduced as the number of infection classes increased (*Fig. 2*). Again, there was little effect of the different sampling options on the averaged heritabilities across 1,000 replications, so, only the results from the sampling setting of s = 300 and n = 30 were shown. When there was an infinite number of classes (i.e., underlying normal distribution itself), the observed heritabilities were almost identical to their respective parametric values. Good agreement between the observed and expected [using both optimal and raw scores] heritabilities was found when there were many classes and/or when parametric values were small. The adjustment for discontinuity using equation (3) was necessary, particularly when the number of classes was less than six and when h^2 was greater than 0.4. Thus, when there are many infection classes, categorical data, for all practical purposes, may be analyzed as if they are continuously varying data. For example, in our previous study of responses of lodgepole pine to infection of western gall rust (six infection classes), we found similar results from the analysis of variance based on transformed data (for normality) and raw (categorical) data (YANG et al., 1997).

As in dichotomous traits, the effect of sampling on heritabilities of polychotomous traits was judged by standard errors (results not presented) and frequency of negative among-family variance components (*Table 3*). They revealed the same ranking of the four sampling options as that for the dichotomous traits: (s = 100 and n = 90) < (s = 300 and n = 30) < (s = 30 and n = 100) < (s = 300 and n = 30). In fact, when s = 100 and n = 90, there was no negative among-family variance

Table 2. – Number of negative among-group variance components found from 1,000 estimates for h^2=0.1 and from a total of 9,000 estimates for h^2=0.1 to h^2=0.9 for 10 levels of incidences [0.05 (0.05) 0.5] with four sets of group number (s) and group size (n).

		Incidence									
s	n	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
h^2 = 0.1 (1,000 estimates)											
300	30	40	0	1	0	0	0	0	0	0	0
100	90	4	0	0	0	0	0	0	0	0	0
30	100	73	20	17	6	8	5	3	2	0	0
30	30	320	225	175	139	134	121	118	110	105	95
Total (9,000 estimates)											
300	30	41	0	1	0	0	0	0	0	0	0
100	90	4	0	0	0	0	0	0	0	0	0
30	100	82	22	17	6	8	5	3	2	0	0
30	30	725	350	248	183	171	140	131	127	121	114

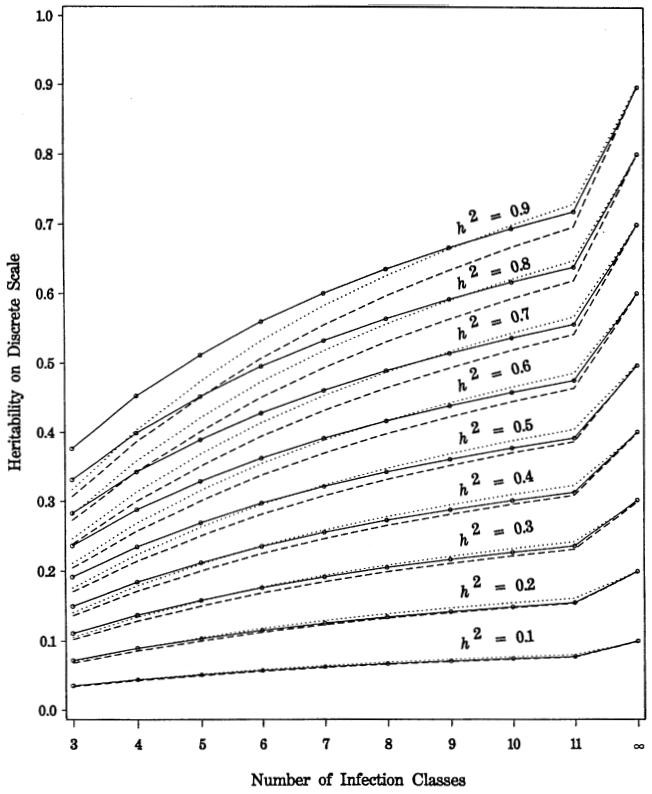

Figure 2. – The observed (solid line) and expected relationships between heritability on multinomial scale and number of infection classes for given true heritability (h^2) on underlying continuous scale. Each of the observed values was an average of 1,000 estimated intraclass correlations based on 300 half-sib families and 30 individuals per family. The dot and dash lines represent the expected values for heritability on multinomial scale using equation (3) when the scores are the raw scores and the 'optimal' scores, respectively.

component at each of 10 infection classes. Again, most negative estimates occurred at $h^2 = 0.1$, but the frequency of occurrence varied substantially among the sampling options. In general, the number of negative estimates decreased with increasing number of infection classes. As expected, there were fewest negative estimates from the normal data ($k = \infty$).

For the normal data, the expected probability of negative estimates of the among-family variance component was computed by making use of the *F*-distribution (cf. SEARLE et al., 1992, p. 66–69),

$$P\{\hat{\sigma}_b^2 < 0\} = P\left\{F_{(s-1)}^{s(n-1)} > 1 + \frac{nt}{1-t}\right\}$$

For example, when $s = 30$, $n = 30$ and $h^2 = 0.1$, the expected probability for $F = 1.769$ with 870 and 29 degrees of freedom is 0.03. Thus, of 1,000 estimates of the among-family variance component, the expected number of negative estimates is 30, which is close to the observed value of 28 for $k = \infty$. While such a calculation of the expected probability is not yet available for categorical data, it is evident from *tables 2* and *3* that extreme incidences (dichotomous traits) and few infection classes (polychotomous traits) lead to a marked increase in the probability of the negative estimates of the among-family variance component.

From a different standpoint, some negative estimates are expected. Since the among-family variance component (σ^2_b) is the same as the intraclass correlation (t) in our simulations, the expected range for σ^2_b is $-(n-1)^{-1}$ to 1 (SNEDECOR and COCHRAN, 1980, p. 243-244).

Furthermore, the estimation of intraclass correlations for a quantitative trait has an inherent downward bias as shown in PONZONI and JAMES (1978):

$$E(\hat{t} - t) \approx \frac{-2(1-t)[t + (1-t)/n][t + (1-t)/sn]}{s-1}$$

Clearly, this downward bias is inversely related to family numbers and sizes for a given true intraclass correlation. For categorical data, further downward bias would occur, particularly with the extreme incidences (dichotomous traits) and few infection classes (polychotomous traits) as clearly indicated by the high frequencies of negative estimates of intraclass correlations under these cases (*Tables 2* and *3*).

The adjustment in equation (3) or (4) would apply only when the distribution of the underlying liability is normal or unimodal (ROBERTSON and LERNER, 1949; FALCONER and MACKAY, 1996). If the liability is distributed bimodally or multimodally due to major gene and/or environmental effects, there could be no scale transformation to make its distribution normal. In other words, quantitative genetic analysis of the liability is valid only if it has a polygenic basis (i.e., it is controlled by many genes each with relatively small effects). Nevertheless, heritability on the binomial or multinomial scale are often adjusted to the underlying continuous scale, despite unknown genetic mechanisms underlying threshold traits. There are several advantages in working on the underlying scale. For example, when comparing heritability estimates obtained in different breeding populations, it is of interest to know whether observed differences truly reflect different scope for genetic improvement or they are simply a consequence of different incidences or infection classes in the populations.

Consistent higher observed than expected heritabilities (i.e., $\hat{h}^2_o > h^2_o$), particularly with extreme incidences and few infection classes, is probably due to the presence of non-additive genetic variance during the change in scale from normal to the binomial or multinomial distribution. This is because theoretical calculations based on equations (3) and (4) have assumed a linear relationship between additive genetic effects on the two scales (DEMPSTER and LERNER, 1950; GIANOLA, 1979). Numerical results by DEMPSTER and LERNER (1950) showed that the amount of non-additive genetic variance for dichotomous traits increased with increasing departure from incidence of 0.5 and with increasing true heritability (h^2). Given the inadequacy of the theory [i.e., equations (3) and (4)] particularly with extreme infection frequencies and few infection classes, it is desirable to explore direct estimation procedures based on generalized linear models (e.g., GILMOUR et al., 1985).

While selection of a threshold trait is similar to that of a continuously varying trait, genetic gain may be quite different. For mass selection, the genetic gain for the threshold trait is the product of $i\, h^2_o\, \sigma_o$, where i is the selection differential and σ^2_o is the phenotypic variance for the threshold trait. Since $h^2_o < h^2$ as shown in our simulations, genetic gain for the threshold trait is always less than that for a continuously varying trait. In light of our simulation results, it is also clear that genetic gain increases as the number of infection classes increases, reaching to the upper limit when the number of classes is infinite (i.e., continuously varying trait itself). Thus, the estimated heritability and appropriate variance components for threshold traits needs to be adjusted to continuous scale in order to compare with the genetic gain from selection for the quantitative trait. In addition, selection differential for

Table 3. – Number of negative among-group variance components found from 1,000 estimates for $h^2=0.1$ and from a total of 9,000 estimates for $h^2=0.1$ to $h^2=0.9$ for 10 different classes [3, 4, ..., 11, ∞(normal)] with four sets of family number (s) and family size (n).

		Number of infection classes									
s	n	3	4	5	6	7	8	9	10	11	∞
h^2 = 0.1 (1,000 estimates)											
300	30	2	0	0	0	0	0	0	0	0	0
100	90	0	0	0	0	0	0	0	0	0	0
30	100	21	8	5	5	3	3	2	1	0	0
30	30	222	172	123	102	89	75	66	65	60	28
Total (9,000 estimates)											
300	30	2	0	0	0	0	0	0	0	0	0
100	90	0	0	0	0	0	0	0	0	0	0
30	100	22	8	5	5	3	3	2	1	0	0
30	30	372	242	168	131	108	87	74	72	66	30

Table 4. – Estimates of variance components and intraclass correlations.

Source	df	Six Classes	Two Classes
Regions	2	0.2535	0.0134
Families (regions)	288	0.6909	0.0222
Error	8,470	3.0775	0.1210
Estimates on discontinuous scales:			
$\hat{t}_1$		0.0630±0.0431	0.0854±0.0563
$\hat{t}_2$		0.2348±0.0374	0.2270±0.0489
Adjustment to continuous scale:			
$\hat{t}_1$		0.1177±0.0805	0.1798±0.1185
$\hat{t}_2$		0.4386±0.0699	0.4778±0.1029

threshold traits does not depend primarily on the proportion selected as parents (f), but on the incidence of desired class (p). It is well known (e.g., VAN VLECK, 1972; FALCONER and MACKAY, 1996) that the maximum selection differential is obtained when $p = f$. The greater the difference between p and f, the less effective is the selection.

Experimental results

Estimates of components of variance due to differences among regions and among families within regions are presented in *table 4*. Total variance for the six infection classes was apportioned into 6.30% for among-region variance, 17.18% for among-family variance and 76.52% for within-family variance. Apportionments of the total variance for the two infection classes were 8.54% for among-region variance, 14.16% for among-family variance and 77.30% $^1(\Sigma^i_{j=1} p_j)$, where p_j = 0.1870, 0.0300, 0.0212, 0.0555, 0.0788 and 0.6276 for six infection classes or p_j = 0.187 and 0.8130 for two infection classes. Averages of 1,000 $\hat{t}_1$ values were 0.1482 ± 0.1525 (normal data), 0.0917 ± 0.0836 (six classes), 0.0677 ± 0.0662 (two classes) and averages of 1,000 $\hat{t}_2$ values were 0.4582 ± 0.0083 (normal data), 0.3222 ± 0.0672 (six classes), 0.2520 ± 0.0636 (two classes).

Thus, the rankings of both $\hat{t}_1$ and $\hat{t}_2$ were the same: normal data > six classes > two classes, confirming the conclusion established from earlier simulations (*Fig. 2*). Judging from the large size of standard errors for $\hat{t}_1$ in the three cases, it is possible to observe the unexpected ranking of $\hat{t}_1$ (two classes) > $\hat{t}_1$ (six classes) as shown in *table 4*. On the other hand, because of much smaller standard errors for $\hat{t}_2$ in the three cases, the observed ranking of $\hat{t}_2$ (two classes) < $\hat{t}_2$ (six classes) was consistent with the expectation.

While geographic regions and families within regions were significant sources of variation in WGR infection, the largest percentage of the variation (> 75%) was due to within-family effect. Apart from possible micro-environmental effects (e.g., temperature and light intensity), the large seedling-to-seedling variation in open-pollinated families is likely indicative of the large number of effective pollen parents and storage of considerable additive genetic variation within such families (depending on sibships) for outcrossing lodgepole pine.

Genetic interpretation of the estimated intraclass correlations requires clear definitions of the reference populations and assumptions about genetic composition of variance components. Individual heritability (h_i^2) estimated from a random sample of half-sib families taken from a random mating population is given by,

$$h_i^2 = \frac{4(\hat{t}_2 - \hat{t}_1)}{1 - \hat{t}_1}. \qquad (6)$$

Conceptually, our data consisted of two-level nested sampling: a random sample of three geographic regions and random samples of 94 to 100 half-sib families within regions. However, family effect was confounded with stand (provenance) effect because only one family was selected in target stands (DHIR and BARNHARDT, 1993). Furthermore, non-additive genetic variance may exist in among-provenance variance (LAND et al., 1987). Therefore, unless the stand effect can be separated from the family effect and/or the assumption of complete additivity of genetic variance of stand effect is warranted, equation (6) will always lead to an upward biased estimate of individual heritability. YANCHUK et al. (1988) estimated that the among-family variance accounted for 3.13% of the total variance in WGR resistance. Using this number, the estimates of individual heritability were: $\hat{h}^2_{io} = 0.156$ for the two classes and $\hat{h}^2_{io} = 0.157$ for the six classes. These estimates were within the range of estimated heritability given in other studies for WGR resistance [e.g., 0.14 in YANCHUK et al. (1988) and 0.17 in HOFF and SUN (1994)]. When being adjusted to the continuous scale based on equation (3) or (4), the adjusted estimates were $\hat{h}^2_i = 0.328$ for the two classes, $\hat{h}^2_i = 0.230$ ("optimal" scores) and $\hat{h}^2_i = 0.243$ (raw scores) for the six classes.

Conclusions

This simulation study is among the first to examine the effects of sampling options (family number and size), infection frequencies and number of infection classes on the distributional behaviors of estimated among-family variance components for threshold traits. Our results substantiate and complement the previous simulation studies for a dichotomous trait in that the estimated intraclass correlations for the dichotomous or polychotomous trait were always less than those for the underlying quantitative trait ("liability"). Results also showed that the theory (i.e., adjustment to continuous scale) is less satisfactory when (i) the incidence is far from 0.5; (ii) the number of infection classes is limited and (iii) the true intraclass correlation is moderate to high. The expected downward bias of estimated intraclass correlations was relatively small for quantitative traits but sizeable for dichotomous or polychotomous traits as indicated by substantially higher frequencies of negative estimates particularly with extreme incidences and few infection classes, and with small family numbers and sizes (*Tables 2* and *3*).

The analysis of polychotomous responses of lodgepole pine to WGR showed that estimates of intraclass correlations for geographic regions ($\hat{t}_1$) had large sampling variability. This is probably due to sampling of only three regions and consequently obscured the pattern of intraclass correlations based on different infection classes as revealed in our simulation study. On the other hand, the ranking of estimates of intraclass correlation for families within regions ($\hat{t}_2$) was consistent with the pattern of intraclass correlations based on different infection classes. However, the estimates were considerably inflated, likely due to confunding provenance/family effects and the presence of non-additive genetic variance.

It is desirable to adjust the estimated intraclass correlations for dichotomous or polychotomous traits to the underlying continuous scale for comparing the estimates from different populations. Such comparison is able to discern if any differences observed among populations truly reflect unequal scope for genetic improvement or if they are simply a consequence of different incidences in the populations. However, the adjustment is less satisfactory with extreme incidences and few infection classes. This appears to be a consequence of lack of perfect linear (additive) relationship between genetic values on the two scales. It is perhaps desirable to explore direct estimation procedures based on generalized linear models.

Acknowledgements

We thank Dr. PH. BARADAT and an anonymous reviewer for their helpful comments. This research was funded jointly by the Natural Sciences and Engineering Research Council of Canada grants OGP3983 (R.-C.Y.), OGP2282 (F.C.Y.), and Alberta Land and Forest Service (N.K.D.).

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Genetic Variation of *Pinus brutia* from Islands of the Northeastern Aegean Sea

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(Received 24th November 1997)

Abstract

The present study concentrated on *Pinus brutia* TEN. one of the main forest species of the Aegean islands and one of the most important low-elevation Mediterranean conifers. In this report the amount and structure of genetic diversity of four *Pinus brutia* populations which forms an integral prerequisite for breeding efforts and for the protection of the species genetic resources, is presented. One population per island was sampled from four islands of the north-eastern Aegean namely Lesvos, Chios, Samos and Thasos. Seven isoenzymic loci (*Dia-1*, *Idh-1*, *Lap-1*, *Mdh-1*, *Mdh-4*, *Pgd-1* and *Pgi-2*) were identified and used for the evaluation of genetic variability in the above populations. Five of the loci studied were polymorphic, while a total of 17 alleles were detected. All populations presented significant amounts of genetic diversity and heterozygosity. The levels of genetic diversity parameters were higher than those of earlier reports regarding this species, but in agreement with values reported for conifer trees in general. Genotypic frequencies of the population samples were in agreement with those expected from HARDY-WEINBERG expectations. Results also point towards the absence of inbreeding and random genetic drift. Most of the genetic diversity of *Pinus brutia* (97.9%) was found within populations and only 2.1% among populations. Most of the alleles studied were common for all populations. Some differences among populations were detected for rare alleles. Populations present low values of NEI's genetic distance and CAVALLI-SFORZA and EDWARDS' chord distance. Two groups were revealed in the respective dendrograms: the first group formed by the populations of Lesvos, Chios and Samos, and the second by the Thasos population. The significance of these results in breeding and forest management practice is briefly discussed.

Key words: *Pinus brutia*, isoenzymes, Aegean, population genetics.

FDC: 165.3; 174.7 *Pinus brutia*; (495).

1. Introduction

Pinus brutia TENORE subsp. *brutia* is one of the main forest tree species of the Aegean islands, Greece. The species attains a tree form with a height of 15 m to 20 m, sometimes even 30 m with a usually straight main stem (ATHANASIADIS, 1986; PANETSOS, 1981). *P. brutia* populations present considerable phenotypic variation and plasticity (ISIK, 1986; PANETSOS, 1981). The distribution of *P. brutia* in the eastern Mediterranean basin and its ability to grow in adverse climatic and soil conditions make this species very important for multiple purpose forestry. Moreover in favorable sites *P. brutia* exhibits a significant growth potential, while the presence of mechanisms for regeneration after fire makes the species irreplaceable in the delicate Mediterranean ecosystem (PANETSOS, 1986).

Factors such as island geographic isolation, long term negative selection due to needs in wood and resin, soil mosaic, climatic variability due to differences in altitude, as well as forest fires, are expected to have contributed to the species present genetic structure. The destruction of *Pinus brutia* forests in the Aegean islands calls for the frequent employment of artificial reforestations. Protection of the local genetic resources and selection of the suitable planting material aquires a high importance in the frame of possible genetic divergence due to the island population subdivision and differentiation. One potential means to face the increasing demands for quality wood production (construction wood, ship-yard wood etc.) in the Aegean islands is the use of genetically improved material. The basic prerequisite for genetic improvement and for the protection of genetic resources is the study of genetic variability. In this paper an analysis of the isoenzymic genetic variability in natural populations of *Pinus brutia* from the islands of north-eastern Aegean is presented.