On Estimating Relatedness Using Genetic Markers

Gerald S. Wilkinson; Gary F. McCracken

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ON ESTIMATING RELATEDNESS USING GENETIC MARKERS

GERALD S. WILKINSON[1] AND GARY F. MCCRACKEN
Department of Zoology, University of Tennessee, Knoxville, TN 37916

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Recently, two methods for estimating relatedness (sensu Michod and Hamilton, 1980) using genotypic data have been proposed, but opposite conclusions have been reached regarding their efficacy. Pamilo and Crozier (1982) and Pamilo (1984) have shown that regression can return estimates of the average degree of relatedness among individuals within a group that closely agree with expected or specified levels of kinship, and their methods have been used in a number of studies on social insects (Pamilo and Varvio-Aho, 1979; Pamilo, 1981, 1982, 1983; Crozier et al., 1984; Ward and Taylor, 1981; Ward, 1983; McCauley and O'Donnell, 1984). In contrast, Schwartz and Armitage (1983) calculated Rogers' (1972) mean index of genetic similarity, S, between pairs of yellow-bellied marmots within colonies and then examined the relationship between S and relatedness, independent estimates of which were obtained for each colony by pedigree analysis. Although the relationship between S and

[1] Present address: Institute for Behavioral Genetics, Campus Box 447, University of Colorado, Boulder, CO 80309.

relatedness was positive, Schwartz and Armitage found that levels of relatedness could not be estimated precisely using genetic similarity. They concluded that allozyme data may be used only qualitatively to imply relatedness among members of social groups.

Our object is to compare the utility of these methods for assessing kinship in groups given sampling regimes and data acquisition that are reasonable for studies of vertebrate populations. Our simulations differ from those of Pamilo and Crozier (1982) and Crozier et al. (1984) in that we simulate a greater range of average levels of relatedness and sample fewer numbers of groups. We expand on the analyses of Schwartz and Armitage (1983) by simulating loci with common alleles at lower frequencies than were available in their marmot study. Thus, we examine the possibility that limitations of the data they had available may have biased their evaluation of this statistic.

Methods

Populations of at least 2,000 individuals were generated using Monte Carlo simulations. For each population, the mating system, number of loci (m), and number (n) and frequency (p) of alleles segregating at each locus were specified. Loci were unlinked, and offspring sex ratios were unbiased. Genotypes for the first generation were randomly sampled from a population with Hardy-Weinberg genotype frequencies. Parents of the second generation were drawn at random from offspring of the first. By adjusting the number of females mating with each male and the number of offspring per female, we created, in three simulated generations, populations which allowed sampling groups of full-sibs, half-sibs, cousins, or randomly chosen individuals. Actual relatedness within each group (r) was calculated using path analysis by searching pedigrees to find all common ancestors.

To limit the number of permutations to compare we simulated individuals containing four biallelic loci; however, the methodology can be applied to any number of alleles per locus. The frequency of the common allele at all loci in a population was set in the parental generation at 0.5, 0.75, or 0.9. Samples from each simulated population consisted of 3, 6, or 10 groups, each with 5, 10, or 20 individuals per group. We calculated the two statistics described below for every combination of allele frequency, group number (c), and group size (N) for sampled groups of full-sibs, half-sibs, cousins, and randomly drawn individuals.

To provide m independent estimates of relatedness, we calculated the genotypic correlation (Stanton, 1960; Crozier et al., 1984; Pamilo, 1984), among groups (Equation 1). Here, $p_{h,j}$ is the mean frequency of allele h in group j, P_h is the mean frequency of allele h in the population, $g_{hh,j}$ is the mean frequency of genotype hh in group j, and G_{hh} is the mean frequency of genotype hh in the population. This correlation coefficient can be interpreted as the average degree of relatedness within a group (Pamilo and Crozier, 1982; Pamilo, 1984). In this paper, we present the mean and range of $\hat{r}$ obtained over the four loci examined in each set of groups to illustrate the variation that can be expected from a typical multi-locus data set. Pamilo and Crozier (1982) provide jackknifed estimates of confidence limits for the related regression coefficient, b.

$$\hat{r} = \frac{1 - \sum_{h=1}^{n} P_h^2 - \frac{1}{c}\sum_{j=1}^{c}\left(1 - \sum_{h=1}^{n} p_{h,j}^2\right) - \frac{1}{c}\sum_{j=1}^{c}\frac{1}{N_j - 1}\left[\left(1 - \sum_{h=1}^{n} p_{h,j}^2\right) - \frac{1}{2}\left(1 - \sum_{h=1}^{n} g_{hh,j}\right)\right]}{1 - \sum_{h=1}^{n} P_h^2 - \frac{1}{2}\left(1 - \sum_{h=1}^{n} G_{hh}\right)} \quad (1)$$

To examine how each varied factor (r, c, N, and p) in our simulations influenced the accuracy of $\hat{r}$, we performed one-way ANOVAs between each factor and the absolute value of the difference between $\hat{r}$ and r obtained over the four loci. All negative values of $\hat{r}$ were truncated to zero for this analysis.

Following Schwartz and Armitage (1983), we calculated the mean genetic similarity among groups over loci (Equation 2). In this equation, N_j is the number of individuals in group j, and $p_{h,i,j,k}$ is the frequency of allele h at locus i in individual k (or l) from group j (i.e., 0, 0.5, or 1). The 95% confidence limits around each estimate were derived by jackknifing the z-transformation (Sokal and Rohlf, 1981 p. 798) of the similarity index among groups.

$$S = \frac{1}{cm}\sum_{i=1}^{m}\sum_{j=1}^{c}\frac{2}{N(N-1)}\sum_{k=1}^{N_j-1}\sum_{l=k+1}^{N_j} 1 - \left[\frac{1}{2}\sum_{h=1}^{n}(p_{h,i,j,k} - p_{h,i,j,l})^2\right]^{1/2} \quad (2)$$

Copies of the simulation and statistical programs for use on an Apple II series machine with 64K of memory can be obtained by sending a blank 5.25 inch diskette and sufficient return postage to the first author.

Results

The results show that $\hat{r}$ obtained from four loci is often a good predictor of r (Fig. 1). However, the magnitude of the ranges of values indicate that $\hat{r}$ obtained from a single locus can be misleading.

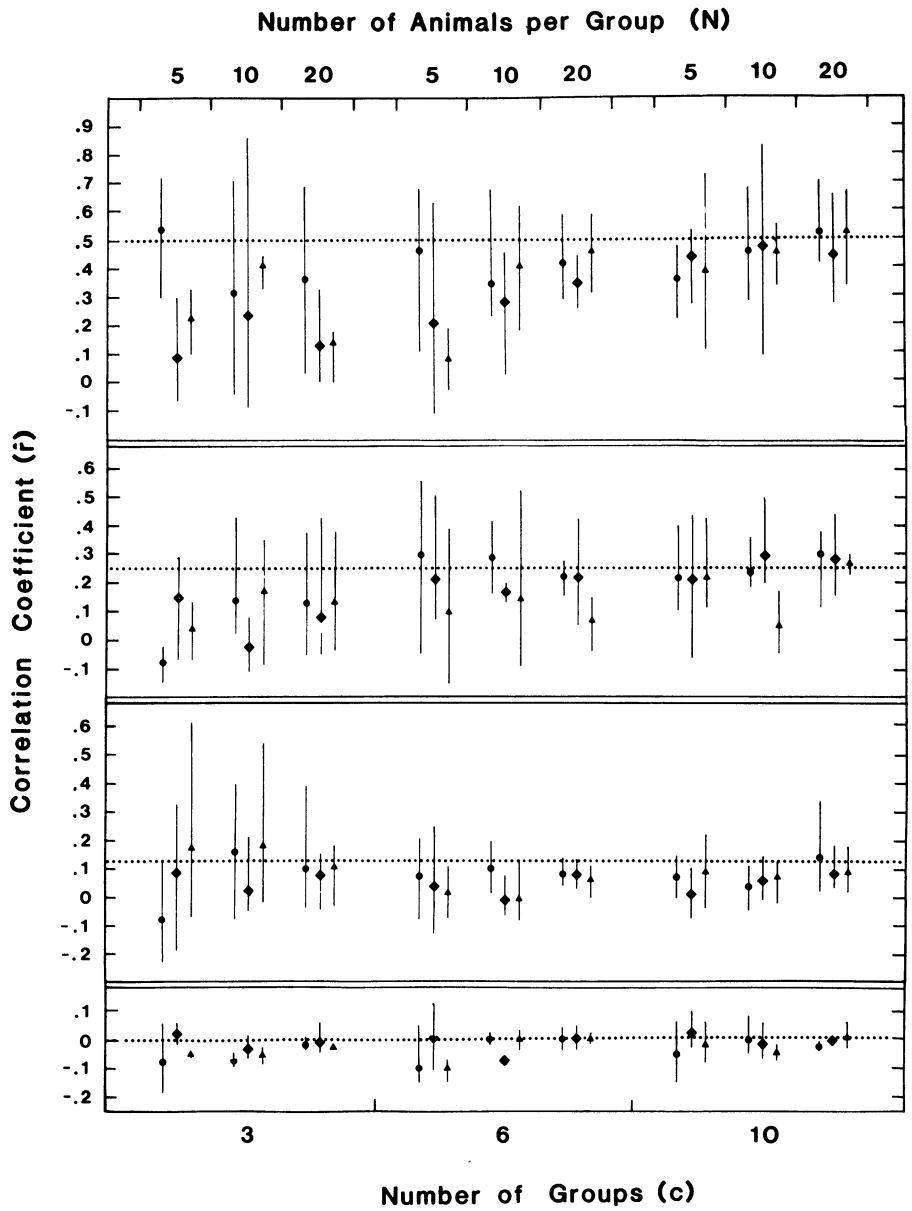

FIG. 1. Mean and range for the genotypic correlation coefficient ($\hat{r}$) calculated from four biallelic loci as a function of the number of groups sampled (c) and the size of each group (N). The degree of relatedness (r) within each group is indicated in each panel by a dotted line. The frequency of the common allele is designated by symbols (●, $p = 0.5$; ◆, $p = 0.75$; ▲, $p = 0.9$).

Without detailed discussion, results from additional simulations suggest that the accuracy of $\hat{r}$ in estimating r increases asymptotically with the number of variable loci.

The factor with the greatest effect on the accuracy of $\hat{r}$ was relatedness itself (Table 1). The mean absolute deviation around the full-sib estimates (0.145) was an order of magnitude greater than the mean deviation around the groups assembled at random (0.014). The mean deviations for the other two sets

TABLE 1. Summary of one-way ANOVAs between the level of relatedness (r), number of groups (c), group size (N), and allele frequency (p) and the absolute difference between the expected value of r and the mean r obtained by the correlation method.

Factor	Sum of squares		Degrees of freedom		F
	Factor	Error	Factor	Error	
r	0.2500	0.5556	3	104	15.58
c	0.0797	0.7256	2	105	5.77
p	0.0221	0.7833	2	105	1.48
N	0.0070	0.7983	2	105	0.46

of groups decreased in magnitude with r. This pattern is to be expected, since the maximum variance of a binomial distribution occurs when $r = 0.5$. Consequently, estimates of $\hat{r}$ among distantly or very closely related ($r \gg 0.5$) individuals will necessarily be more accurate than those for full sib or mother-offspring groups.

Of the remaining factors, the next most influential was the number of groups (Table 1). The mean deviations for simulations containing 3, 6, and 10 groups were 0.109, 0.072, and 0.043, respectively. The distribution of alleles appeared to be important only when the frequency of the common allele was not near 0.5. The mean deviation for all simulations using $p = 0.5$ was 0.055, while for $p = 0.75$ it was 0.086, and for $p = 0.9$ it was 0.09. Our simulations and those of Pamilo and Crozier (1982) show a trend to underestimate relatedness when $p < 0.5$, particularly when there are few groups (Fig. 1). This bias is partially ameliorated by estimating $\hat{r}$ by the jackknife procedure. For the range of group sizes examined, the number of individuals in a group had relatively little effect on the size of the deviation between the mean estimate and true value of r. The mean deviations for groups of 5, 10, and 20 were 0.086, 0.073, and 0.066.

Scrutiny of S and its confidence limits for each variable (Fig. 2) indicates that 1) at all levels of relatedness, S increases with the frequency of the common allele and 2) these increases in S are proportionately greater at lower levels of relatedness. This frequency dependence of S leads to two conclusions. First, use of this index requires calibration of each data set using either pairs of individuals with independently determined relatedness, or (as is done here) simulated groups with matching allele frequencies and known levels of relatedness. Second, loci with alleles at intermediate frequencies provide more information because the regression of r on S has a steeper slope than when loci are less polymorphic.

With one exception ($c = 3$, $N = 5$, $p = 0.9$, Fig. 2) the correlation between r and S was positive for all sets of groups; however, in every set of groups the 95% confidence limits around S overlapped broadly across two or more relatedness categories. As expected, the highest predictability of r from S occurs when $p = 0.5$ and the lowest when $p = 0.9$. At this allele frequency, the confidence limits of the similarity estimates overlapped among all levels of relatedness in every set of groups. The confidence limits about S decrease with the number of individual pairwise comparisons, $cN(N-1)/2$, but even for the expected best case ($c = 10$, $N = 20$, $p = 0.5$), the confidence limits of S for randomly related individuals overlap with those of both cousins and half-sibs. To examine the effect that increasing the number of variable loci has on these confidence limits, we simulated ten independent biallelic loci and calculated mean similarity within groups of full-sibs where $c = 10$, $N = 20$, and $p = 0.5$. When compared to the equivalent four locus simulation, the increase to ten loci resulted in an over four-fold reduction in the confidence limits ($S = 0.783$, limits = 0.823–0.735 for four loci; $S = 0.785$, limits = 0.795–0.774 for ten loci).

DISCUSSION

From these results and those of Pamilo and Crozier (1982) we offer the following cautions and guidelines regarding estimation of relatedness by correlation. First, $\hat{r}$ obtained from single locus data should not be considered reliable unless there are many groups (a minimum of ten, preferably more). If the biology of the organism necessitates a smaller sampling regime, then this statistic should be estimated from mean values taken over as many independent loci (i.e., no linkage disequilibria) as possible. Second, $\hat{r}$ obtained from loci in which the common allele occurs at frequencies greater than 0.5 are less reliable and frequently underestimate r more than $\hat{r}$ calculated from loci with a more equal distribution of allele frequencies. This problem is exacerbated if sample sizes are small. One solution is to omit such loci from an estimate; however, these often include the majority of loci resolved by electrophoresis. A better alternative is to design sampling regimes that allow scoring sufficient numbers of individuals and groups. Third, if the expectation for r is near 0.5, then the magnitude of error around $\hat{r}$ will be greater than if the expectation is either greater or smaller than 0.5. In this situation, increasing the number of loci used is the most effective way of reducing the sampling variance in $\hat{r}$. Finally, this method assumes that all individuals in every group are from one panmictic population. If there is local genetic subdivision between groups due to drift, then $\hat{r}$ will not accurately estimate r within groups. Pamilo (1984) suggests a hierarchical analysis to detect such situations.

Our results indicate that, given sufficient information, the multilocus similarity index could provide a useful estimate of r. However, the number of robustly polymorphic loci and/or number of large groups necessary to permit precise estimation of r is likely to prohibit its practical application in most

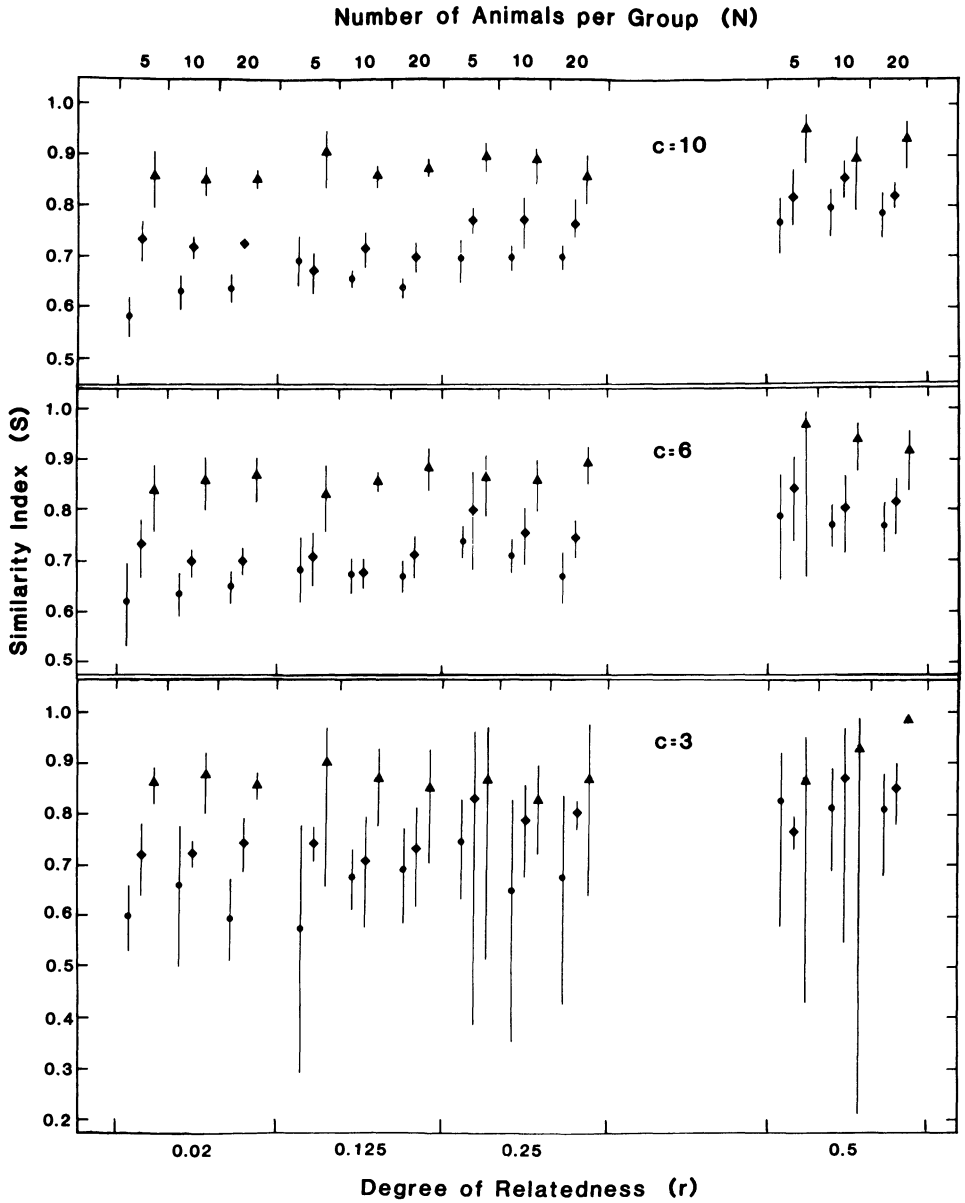

FIG. 2. Mean and 95% confidence limits calculated by jackknifing the multilocus genetic similarity index (S) as a function of c, r, N, and p (variable definitions given in Figure 1).

cases. Therefore, we believe that the correlation technique is preferred for estimating mean relatedness within groups. However, it must be recognized that the correlation method does not provide information on the variance of r among groups. If this latter issue is of interest, then a calibrated genetic similarity index could be used in cases where sufficient genetic information is available. Alternatively, if a limited amount of pedigree information is available, relatedness may be estimated from a reconstructed genealogy obtained by maximum likelihood methods (Cannings and Thompson, 1981 p. 110). Thompson (1976) provides details of an algorithm which has been used to reconstruct ac-

curate genealogies of up to 100 individuals on the basis of 10 loci and additional information such as age, mating system, and sibship size.

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FAUNAL TURNOVER IN SOUTH AMERICAN FOSSIL AVIFAUNAS: THE INSUFFICIENCIES OF THE FOSSIL RECORD

STORRS L. OLSON
Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560

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Vuilleumier (1984 p. 1391) has presented what he has termed "the first quantitative analysis of faunal dynamics in fossil South American birds." I believe, however, that the data used therein to attempt to document "faunal dynamics" and "turnover" are in considerable part illusory. Even if all of the genera and families listed by Vuilleumier were valid and correctly identified, which many are not, the fossil record of birds in South America is simply too scattered in time and space, and specimens too few, to derive the sorts of generalizations regarding faunal "turnovers" attempted by Vuilleumier.

Apart from some seabirds and a single fossil from the Miocene of Colombia, the published fossil record of birds in South America for the entire Tertiary is restricted to Argentina, Uruguay, and southern Brazil. Although, as Vuilleumier (1984 p. 1386) notes, similar biases have not prevented paleomammalogists from drawing generalizations that are assumed to be valid for the rest of the continent, there is simply no comparison between the much richer and much more intensively studied mammalian fossil record and that currently available for birds.

Except for the fossil record from the Argentinian Cenozoic, discussed below, most of Vuilleumier's data are derived from two large late Quaternary avifaunas from opposite sides of the continent in Ecuador and Peru (Campbell, 1976, 1979) and in