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A COMPARISON OF TWO METHODS OF CALCULATING G_{ST}, A GENETIC MEASURE OF POPULATION DIFFERENTIATION[1]

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G_{ST} is a genetic statistic describing differentiation of populations and has frequently been compared with Hamrick and Godt's (1989) review of the plant literature. We show here that some comparisons may be inappropriate if G_{ST} was calculated in a different way than that used by Hamrick and Godt (HG). An alternative method advocated by Nei is mathematically different from the HG technique, occasionally resulting in different G_{ST} values. We reviewed 695 studies that appeared between 1990 and September 1999 that cited Hamrick and Godt (1989) and found that many of these calculated G_{ST} according to Nei's method (46%), with the majority of these papers (61%) including comparisons to Hamrick and Godt's review. We suggest that if G_{ST} estimates are compared across studies, it is most appropriate to calculate them the same way. However, we found that in most cases, the magnitude of difference in G_{ST} values was small, suggesting that qualitative comparisons of G_{ST} estimates between most studies are probably valid. Nevertheless, we have identified theoretical and empirical situations in which large differences in G_{ST} values are likely to arise. Thus, we advise future investigators to carefully consider which method to use in calculating G_{ST} for a given data set.

Key words: genetic variation; G_{ST}; Hamrick and Godt; population differentiation.

One of the most frequently cited papers on genetic variation in plants is Hamrick and Godt's (1989; often referred to as 1990) review entitled ''Allozyme diversity in plant species.'' This paper examined the literature to study patterns of genetic variation (e.g., number of alleles per locus, percentage of polymorphic loci, etc.) according to a variety of different traits, including life form, geographic range, and breeding system. Since the review was first published 11 yr ago, it has been cited in increasing numbers as researchers use the data set as a point of reference for their own studies (Fig. 1).

A genetic statistic that has frequently been compared with the Hamrick and Godt (1989) review is G_{ST}, a measure of population differentiation. Values of G_{ST} range from zero to one, with low values indicating that little variation is proportioned among populations (high values denote that a large amount of variation is found among populations). The purpose of this paper is to point out that there are two common but different methods of calculating G_{ST}, one used by Hamrick and Godt (1989) and the original method advocated by Nei (1973). For clarity, these will be referred to as the HG and Nei methods throughout this paper. We show here that these two methods can give different results in certain cases. Thus, conclusions drawn from a comparison of G_{ST} with the Hamrick and Godt review may be inappropriate if G_{ST} is calculated using the Nei method. Furthermore, we determine how frequently this miscalculation has occurred in the literature by reviewing 695 studies published in the 11 yr since the review appeared.

As one of Nei's (1973) genetic diversity statistics, G_{ST} is defined as the proportion of genetic diversity that resides among populations. It is equivalent to Wright's (1951) F_{ST} when there are only two alleles at a locus, and, in the case of multiple alleles, G_{ST} is equivalent to the weighted average of F_{ST} for all alleles (Nei, 1973). G_{ST} is also similar to Weir and Cockerham's (1984) θ, except that the latter accounts for effects of uneven sample sizes and number of sampled populations. Although rare, θ may take on negative values (Weir, 1996). G_{ST} is calculated from the total genetic diversity in the pooled populations (H_T) and mean diversity within each population (H_S) as:

$$G_{ST} = \frac{H_T - H_S}{H_T} = \frac{D_{ST}}{H_T} \quad (1)$$

or alternatively as:

$$G_{ST} = 1 - \frac{H_S}{H_T} \quad (2)$$

where D_{ST} is the total genetic diversity distributed among populations. H_S is calculated is the mean of H_e values over all populations, where H_e is the expected proportion of heterozygous loci per individual ($H_e = 1 - \Sigma\, p_i^2$, where p_i is the frequency of a given allele; Nei, 1973). Unbiased estimates of H_S can also be obtained for small sample sizes (Nei, 1978; Nei and Chesser, 1983; but see Cockerham and Weir, 1986) and few populations (Nei, 1986). Because H_S represents a subset of the total diversity found in H_T, it can never be more than H_T. Using allele frequencies, Nei's (1973) genetic statistics (H_T, H_S, D_{ST}, G_{ST}) are calculated for each locus (Table 1).

The two methods of calculating G_{ST} differ in when the mean

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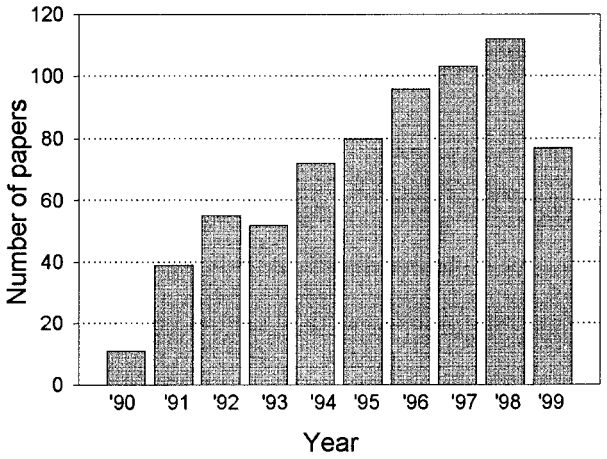

Fig. 1. The number of papers published each year that have cited Hamrick and Godt (1989 or 1990), as given in the Science Citation Index (see text). Results for the last year are as of 19 September 1999.

is calculated. In the HG method, G_{ST} values are first calculated for each locus and then averaged over only polymorphic loci (see Berg and Hamrick, 1997). This can be written as the following, where N_p is the number of polymorphic loci and i represents the ith locus:

$$\frac{\sum_{i=1}^{N_p}\left(\frac{H_{T_i}-H_{S_i}}{H_{T_i}}\right)}{N_p}=\bar{G}_{ST} \tag{3}$$

$$\frac{\sum_{i=1}^{N_p}\left(\frac{H_{T_i}}{H_{T_i}}-\frac{H_{S_i}}{H_{T_i}}\right)}{N_p}= \tag{4}$$

$$\frac{\sum_{i=1}^{N_p}\left(1-\frac{H_{S_i}}{H_{T_i}}\right)}{N_p}= \tag{5}$$

$$\frac{N_p-\sum_{i=1}^{N_p}\left(\frac{H_{S_i}}{H_{T_i}}\right)}{N_p}= \tag{6}$$

$$\frac{N_p}{N_p}-\frac{\sum_{i=1}^{N_p}\left(\frac{H_{S_i}}{H_{T_i}}\right)}{N_p}= \tag{7}$$

$$1-\frac{\sum_{i=1}^{N_p}\left(\frac{H_{S_i}}{H_{T_i}}\right)}{N_p} \tag{8}$$

In the Nei method, H_T and H_S values are first averaged across all loci, and G_{ST} is calculated from these mean values according to Eq. 1. Although both monomorphic and polymorphic loci are usually used in calculations of H_T and H_S (Nei, 1986), Nei's G_{ST} is unaltered by the inclusion of monomorphic loci because they contribute to both the numerator and denominator; in effect, they cancel each other out (i.e., N is absent in Eq. 14 below). Nei's G_{ST} can be rewritten as the following, where N is the number of all loci:

TABLE 1. Sample G_{ST} loci table in which the following statistics are calculated for each of three loci: mean diversity within each population (H_S), total genetic diversity in the pooled populations (H_T), the total genetic diversity distributed among populations (D_{ST}), and the measure of population differentiation (G_{ST}). In the HG method, G_{ST} values are averaged over polymorphic loci to obtain the mean G_{ST} value. In the Nei method, H_T and H_S values are first averaged across all loci, and G_{ST} is calculated from these mean values according to Eq. 1.

Locus	H_S	H_T	D_{ST}	G_{ST}
1	H_{S_1}	H_{T_1}	D_{ST_1}	G_{ST_1}
2	H_{S_2}	H_{T_2}	D_{ST_2}	G_{ST_2}
3	H_{S_3}	H_{T_3}	D_{ST_3}	G_{ST_3}
Mean	$\bar{H}_S$	$\bar{H}_T$	$\bar{D}_{ST}$	$\bar{G}_{ST}$

$$\frac{\bar{H}_T-\bar{H}_S}{\bar{H}_T}=\bar{G}_{ST} \tag{9}$$

$$\frac{\left(\frac{\sum_{i=1}^{N}H_{T_i}}{N}\right)-\left(\frac{\sum_{i=1}^{N}H_{S_i}}{N}\right)}{\left(\frac{\sum_{i=1}^{N}H_{T_i}}{N}\right)}= \tag{10}$$

$$\frac{\frac{1}{N}\left(\sum_{i=1}^{N}H_{T_i}-\sum_{i=1}^{N}H_{S_i}\right)}{\frac{1}{N}\left(\sum_{i=1}^{N}H_{T_i}\right)}= \tag{11}$$

$$\frac{\sum_{i=1}^{N}H_{T_i}-\sum_{i=1}^{N}H_{S_i}}{\sum_{i=1}^{N}H_{T_i}}= \tag{12}$$

$$\frac{\sum_{i=1}^{N}H_{T_i}}{\sum_{i=1}^{N}H_{T_i}}-\frac{\sum_{i=1}^{N}H_{S_i}}{\sum_{i=1}^{N}H_{T_i}}= \tag{13}$$

$$1-\frac{\sum_{i=1}^{N}H_{S_i}}{\sum_{i=1}^{N}H_{T_i}} \tag{14}$$

As evident in Eqs. 8 and 14, the HG and Nei methods of calculating G_{ST} are not mathematically identical. Both methods will yield the same value in only a few rare cases. First, if all populations are completely differentiated from one another, all of the diversity will lie among populations, rather than within them ($H_S = 0$). If this is true for all loci, both methods yield G_{ST} values of one. This can also occur when values of H_T are identical over all loci. Second, if the total diversity is contained within each population ($H_S = H_T$) for all loci, a G_{ST} value of

TABLE 2. Percentages of the 203 published studies in which G_{ST} could be assigned to either the Nei or Hamrick and Godt (HG) method. In these cases, the method could be confidently determined ($Nei_{confident}$, $HG_{confident}$) or was inferred (Nei_{infer}, HG_{infer}) (see text). Numbers in parentheses are the actual number of studies within each category.

Method	Percentage of all studies (no. of studies)	Percentage of studies comparing to Hamrick and Godt (no. of studies)
$Nei_{confident}$	32.0[a] (65)	61.5 (40)
Nei_{infer}	13.8 (28)	60.7 (17)
Nei total	45.8 (93)	61.3 (57)
$HG_{confident}$	50.7[a] (103)	73.8 (76)
HG_{infer}	3.9 (8)	75.0 (6)
HG total	54.7 (111)	73.9 (82)

[a] Includes one study in which G_{ST} was calculated using HG and Nei methods on different data sets.

zero will result using both methods. Finally, HG's and Nei's G_{ST} will be equivalent if values of (H_S/H_T) are identical for all loci. If any of the above cases is not true for at least one locus (e.g., $0 < H_S < H_T$), G_{ST} values calculated using the Nei and HG methods will differ to some extent from one another.

MATERIALS AND METHODS

We examined the circumstances under which the HG and Nei methods would yield different G_{ST} values and determined how frequently Nei's G_{ST} had been compared to Hamrick and Godt (1989) by reviewing studies appearing between 1990 and 19 September 1999 that cited the paper. Using the Science Citation Index (Institute for Scientific Information [ISI], Philadelphia, Pennsylvania, USA), 695 studies were retrieved that fit this criterion. For each study, the following were recorded: (1) what, if any, genetic statistic was calculated (e.g., G_{ST}, F_{ST}, or θ) and (2) whether the statistic was compared to G_{ST} values presented in Hamrick and Godt (1989). For papers that calculated G_{ST}, the reported information was then used to determine which method (Nei or HG) was employed. This was accomplished by recalculating G_{ST} both ways, using a reported G_{ST} loci table and then determining which value matched the reported number. In a few cases, reported G_{ST} values had been calculated by the Nei method, but were unbiased for sample size (Nei and Chesser, 1983) and population number (Nei, 1986). If a loci table was not given, it was generated using published allele frequencies. In some cases, not enough information was given to accurately determine how G_{ST} was calculated (see below).

Of the 695 studies that cited Hamrick and Godt (1989), a large number (45%) did not calculate any statistics. Several other papers included various measures of population differentiation, such as F_{ST} (15%), θ (3%), ϕ_{ST} (<1%), and δ (<1%). Over a third of the total papers (36% or 252 studies) reported a G_{ST} value. Of these, 49 studies contained insufficient information so that (1) neither G_{ST} value could be recomputed, (2) only one value could be recalculated and this did not match the reported G_{ST}, or (3) both values could be recalculated but neither matched the reported number. These studies were not considered further.

Of the 203 remaining studies in which G_{ST} could be recalculated, the method (HG or Nei) was confidently determined in 167 papers (82%) because enough data were given to calculate G_{ST} using both methods. This set of papers will hereafter be referred to as $HG_{confident}$ or $Nei_{confident}$, depending upon which method could be confidently assigned. In the remaining 36 papers (18%), the method could not be established with certainty because insufficient data made it impossible to calculate G_{ST} according to both methods. For example, mean H_T and H_S values were sometimes reported (allowing computation of Nei's G_{ST}), but tables of allele frequencies or G_{ST} values across loci were not given (i.e., HG's G_{ST} could not be calculated). In these papers, the G_{ST} value that could be recalculated matched the reported G_{ST} within the scope of rounding error (approximately ±0.01). These studies will henceforth be referred to as HG_{infer} or Nei_{infer}, depending upon which method could be inferred.

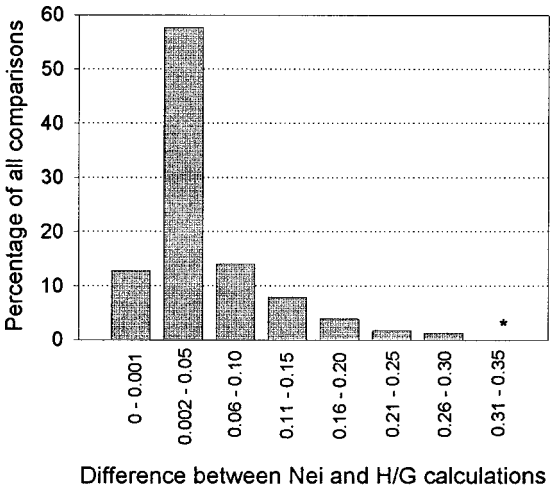

Fig. 2. Range of differences between HG and Nei G_{ST} values shown as a percentage of all 227 comparisons. Only one study could be assigned to the last category.

RESULTS

Nearly half (46%) of the papers in which the method could be confidently determined or inferred used Nei's method to calculate G_{ST} (Table 2). Regardless of whether the method was inferred or known, ~61% of these papers contained comparisons to Hamrick and Godt's (1989) review. These comparisons would be incorrect if empirical HG and Nei G_{ST} values were substantially different from one another, as previously indicated by theory. To determine how often these values actually diverged and the magnitude of such a difference, we compared the recalculated HG and Nei G_{ST} values in 167 studies in which the method could be confidently determined. Several of these studies contained multiple G_{ST} values for different species (considered different data sets), resulting in 227 different pairwise comparisons.

Differences between actual HG and Nei G_{ST} values ranged from 0 to 0.31 (Fig. 2), with the Nei method usually resulting in higher values (Fig. 3). Of the 227 comparisons, 30 gave similar values (±0.001) for both methods and over half of the comparisons (69%) yielded a difference of 0.05 or lower (Fig. 2). However, there were 35 comparisons (15% of all studies) in which the two G_{ST} values differed by >0.10. Overall, HG and Nei G_{ST} values were significantly different from one another in the 227 comparisons (Wilcoxon signed rank test, $T_s = 7721$, $P = 0.0001$), although the assumptions of this test may be partially violated because taxa were treated as independent and some may be phylogenetically related (Gitzendanner and Soltis, 2000).

DISCUSSION

Several factors may contribute to some of the observed differences between HG and Nei G_{ST} values. An examination of

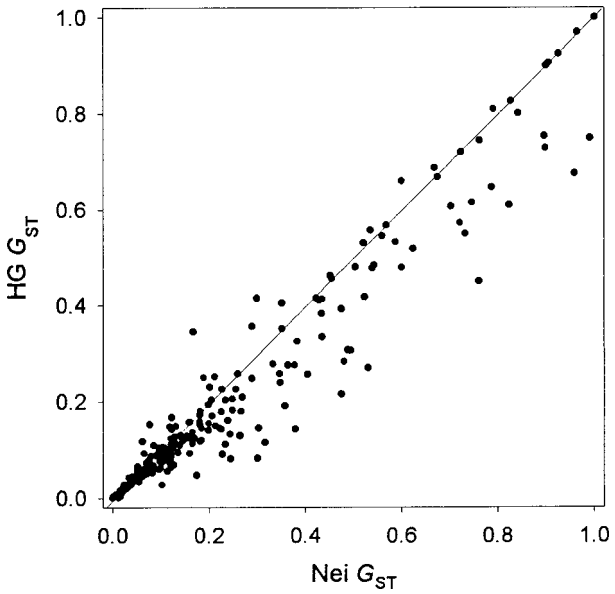

Fig. 3. Comparison of G_{ST} values calculated with the HG and Nei methods for each of 227 populations or species in published papers. ● represents a single study. Identical G_{ST} values fall on the diagonal line.

studies with large differences revealed a wide range of G_{ST} values across individual loci (~0.01–0.80). In contrast, papers in which there were no substantial differences between the two values (≤0.002) generally had a lower range of G_{ST} values across loci (0.0–0.30), although there were some exceptions. In addition, similar G_{ST} values were obtained in rare cases in which populations were either completely fixed for different alleles ($H_S = 0$) or had identical allele frequencies ($H_S = H_T$) for all loci (two cases noted earlier). A wide range of G_{ST} values usually resulted from a mixture of uneven allele frequencies across populations at some loci (resulting in high G_{ST} values) and similar allele frequencies across populations at other loci (low G_{ST}). Uneven frequencies at individual loci were largely due to fixation of different alleles and/or the loss of a common allele within a few populations, which could occur as a result of a reduction in effective population size and subsequent genetic drift. A low range of G_{ST} values typically reflected a similarity of allele frequencies across all populations for all loci.

The HG and Nei G_{ST} values may also diverge because of a difference in their underlying mathematical properties. As apparent in Fig. 3, the Nei method gave relatively higher G_{ST} values than the HG technique in a number of papers. This is an example of Jensen's inequality, a mathematical property of nonlinear functions (Hansen, 2000). Essentially, the difference between the HG and Nei methods is how a ratio (H_S/H_T) is averaged before it is subtracted from one (Eq. 2). In this particular case, Jensen's inequality states that the mean of a ratio (the HG method) will always be less than the ratio of the means (the Nei method), assuming that both the numerator (H_S) and denominator (H_T) are independent of one another. If true, the HG estimate of G_{ST} should be relatively lower than the Nei estimate. In an analysis of ten studies with large G_{ST} differences, H_T and H_S values were less correlated (more independent) with one another ($r = 0.61$, $P = 0.58$) than in ten other studies in which there was no difference between Nei and HG values ($r = 0.98$, $P = 0.0001$). Thus, there are certain cases in which the Nei method will give relatively higher G_{ST} values than the HG method simply because of the way means are calculated.

TABLE 3. Example demonstrating that if H_T increases relative to H_S for a locus, Nei's G_{ST} will increase while HG's value remains the same. Two populations are initially fixed for two different alleles at the first locus, such that Nei's G_{ST} of 0.87 is greater than the HG value of 0.56. A third population fixed for a third allele is then added at the first locus (locus 2 remains unchanged), resulting in an increase in Nei's G_{ST} (0.89) while the HG value remains 0.56.

	Two populations				Three populations			
Locus	H_S	H_T	D_{ST}	G_{ST}	H_S	H_T	D_{ST}	G_{ST}
1	0.00	0.50	0.50	1.00	0.00	0.67	0.67	1.00
2	0.08	0.09	0.01	0.11	0.08	0.09	0.01	0.11
Mean	0.04	0.30	0.26	0.56	0.04	0.38	0.34	0.56

A closer examination also revealed that the Nei method appears to be more sensitive to interlocus variation in allele frequencies than the HG technique. If H_T increases relative to H_S for a locus, Nei's G_{ST} will increase while HG's value remains the same. For example, the addition of a third population fixed for a third allele to a system results in an increase of Nei's G_{ST}, while the HG value remains unchanged (Table 3). As more populations and fixed alleles are added to the first locus, Nei's G_{ST} increases, while HG's value remains the same.

Frequently, estimates of G_{ST} are used to predict other genetic phenomena, including gene flow (i.e., $N_em = (1 - G_{ST})/4G_{ST}$; Wright, 1951) and the number of populations needed to sample a certain level of genetic variation (i.e., proportion of variation $= 1 - G_{ST}^n$; Ceska, Affolter, and Hamrick, 1997). For example, the second equation is used for sampling and preserving germplasm of rare, threatened, and endangered species. Although the applicability of these approaches has been debated (Bossart and Prowell, 1998; Whitlock and McCauley, 1999), they represent important and relatively common applications of G_{ST}. To determine whether different G_{ST} values would yield differing predictions, we inserted the HG and Nei G_{ST} values from the 227 paired comparisons into each equation. Typically, the estimate of gene flow, N_em, was relatively higher when the HG G_{ST} was used than when the Nei G_{ST} was employed. Although estimates of N_em were significantly different overall using the two methods (Wilcoxon signed rank test, $T_s = -6772$, $P = 0.0001$), >75% of the differences were <1.0, and the majority was <0.50 (Fig. 4). Consequently, it is only when the estimates of G_{ST} are strongly different that conclusions may be erroneous. The effect on the number of populations to sample was less dramatic. We compared the proportion of variation that would be captured with $N = 2$, 5, 10, and 12 populations sampled, using either the HG or Nei G_{ST} value. Differences only occurred at $N = 5$ populations with the HG estimate predicting that slightly more of the variation would be captured for any given G_{ST} value (Fig. 5).

Implications—Ultimately, the researcher must decide which method (HG or Nei) to use, based on the objectives of the study. When making comparisons to other studies, it is most appropriate to use similar methods. For example, if a G_{ST} value is to be compared to Hamrick and Godt's (1989) review, G_{ST} should be calculated using the HG method. If the comparison involves another study that used the Nei method, G_{ST} should be calculated according to Nei. However, as we have demonstrated here, in some cases, qualitative comparisons of es-

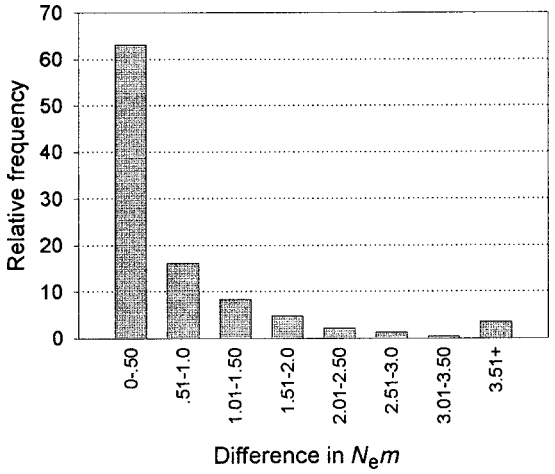

Fig. 4. Relative frequency of differences in estimates of gene flow (N_em) calculated using the HG or Nei G_{ST} value. $N = 227$ paired estimates.

timates calculated in different ways are unlikely to lead to erroneous conclusions. Still, major differences in G_{ST} values are possible, especially in situations in which conservation issues apply, such as populations that are fragmented or have experienced a bottleneck (when G_{ST} is likely to be used). We suggest that researchers should fully explore their data rather than relying solely on mean statistics to draw conclusions and make predictions. In certain cases, investigators should be particularly concerned about comparisons using the two methods. Data sets where we found the greatest difference in G_{ST} values also showed higher mean D_{ST} values, more loci with a D_{ST} >0.05, and a higher proportion of fixed or nearly fixed loci relative to loci with alleles evenly distributed across populations. Consequently, the variance in diversity statistics across loci may be more informative than means of these statistics.

If a comparison to a published study is not intended, an additional factor to consider in choosing an appropriate method is whether one technique is mathematically or biologically more meaningful than the other. Unfortunately, there is no clear answer about which method is best to use. From a mathematical viewpoint, there is no inherent reason to prefer taking the mean of ratios over the ratio of means, but the HG method is more likely to generate lower results than Nei's method (i.e., Jensen's inequality). At present, there is no way of knowing which value best represents population genetic structure in a given situation. The HG method gives equal weight to loci with high and low H_T values, which may result in a range of different G_{ST} values across loci; the Nei method would be less affected by such loci (J. Hamrick, University of Georgia, personal communication). The Nei method may be more biologically meaningful, as it is more sensitive to variation in allele frequencies across populations (the very factor promoting population differentiation). However, investigators should consider using other genetic measures instead of G_{ST} (see below) if a comparison to Hamrick and Godt's (1989) review is not intended. For example, θ is advantageous because it has a real biological definition (correlation of uniting games) and is unbiased with respect to sample size and the number of sampled populations (Weir and Cockerham, 1984).

Based on our review, we have several suggestions for future studies in which G_{ST} values are presented. First, the method of calculation should be clearly explained; many studies have cited Nei (1973) without any further explanation. At the very least, the HG method can be described as "G_{ST} values were averaged over polymorphic loci," while the Nei method can be expressed as "the mean values of H_T and H_S over all loci were used to calculate G_{ST} according to Nei (1973)." Second, G_{ST} values should be reported for individual loci (along with H_T, H_S, and D_{ST}) to facilitate calculations of both HG and Nei G_{ST} values and to allow an examination of variation in these statistics. Several studies only reported mean values of H_T, H_S, and G_{ST} for the species or population(s) in question. Third, sample sizes should be given in allele frequency tables, as the absence of this information makes it impossible to recalculate G_{ST} values. Fourth, scientists should carefully consider whether biased or unbiased estimates of H_T and H_S are appropriate for the data, especially when studying rare species (see Nei, 1978, 1986; Nei and Chesser, 1983; Chakraborty and Danker-Hopfe, 1991). Finally, there was some confusion in the literature over the use of H_T, H_S, H_{es}, and H_{ep} when comparing

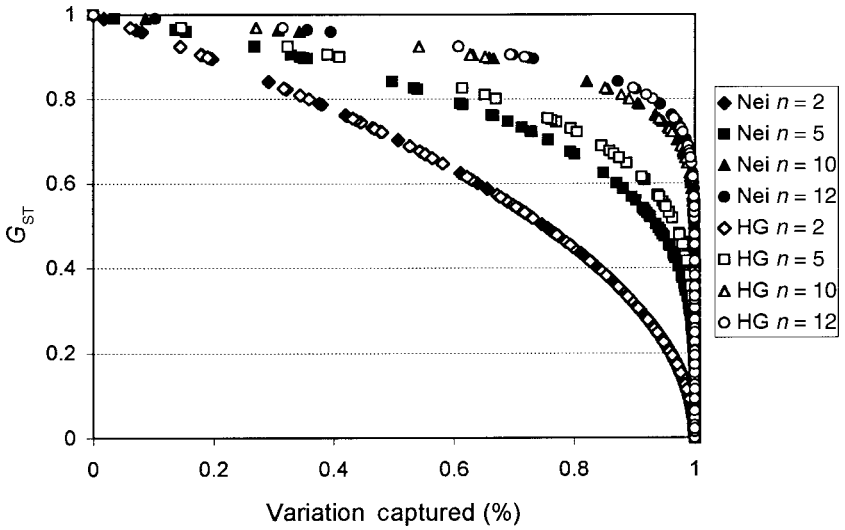

Fig. 5. Differences in the percentage of genetic variation that could be captured given differences in the estimate of G_{ST} and number of populations sampled. $N = 227$ paired estimates.

results to Hamrick and Godt (1989). In that review, genetic diversity was calculated over all loci (monomorphic and polymorphic) as H_{es} at the species level by pooling across all populations, and as H_{ep} at the population level (mean of population values). These statistics are analogous to Nei's (1973) H_T and H_S, respectively. However, the H_T and H_S values reported in Hamrick and Godt (1989) are calculated over polymorphic loci only, and as such, are not directly comparable with Nei's H_T and H_S. Thus, it is important that the researcher state whether all loci or only polymorphic loci were used to calculate H_T and H_S.

In this paper, we have been concerned with G_{ST} as a measure of population differentiation because it is commonly used and there was an urgent need to clarify how it is calculated. Whether or not G_{ST} is most appropriate as a measure of population structure is yet another issue. Several other statistics do exist (e.g., F_{ST}, θ, R_{ST}, ϕ_{ST}) and may be more suitable than G_{ST} in many situations. For example, G_{ST} is dependent on sample sizes and number of populations, in addition to its reliance on Hardy-Weinberg genotype proportions (P. Lewis, University of Connecticut, personal communication), conditions that may be violated when analyzing small isolated populations. Although Nei (1986) and Nei and Chesser (1983) suggested that unbiased estimates of G_{ST} can be obtained through modifications of the original formula (but see Cockerham and Weir, 1986), others have argued that θ is a better estimator (Weir and Cockerham, 1984; but see Chakraborty and Danke-Hopfe, 1991). In view of this debate, investigators should include raw genotype counts in published studies so that θ can be recomputed if necessary for comparison with other studies. Although a discussion of these genetic statistics is beyond the scope of the current paper, the researcher should carefully consider which statistic is best for the system under investigation.

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