

NEWS AND VIEWS

COMMENT

 G_{ST} is still a useful measure of genetic differentiation — a comment on Jost's D

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In a recent issue of *Molecular Ecology*, Jost (2008) suggests that the widely used measure of genetic differentiation G_{ST} and its relatives do not measure differentiation. His conjecture is based on the now well-known dependence of G_{ST} on average within population heterozygosity (H_S) which prevents G_{ST} from taking values larger than average homozygosity ($1 - H_S$; Jin & Chakraborty 1995; Charlesworth 1998; Nagylaki 1998; Hedrick 1999, 2005). To eliminate the influence of heterozygosity on G_{ST} , Jost (2008) suggests that genetic diversity is quantified in terms of effective number of alleles rather than heterozygosity, and he derives an alternative measure, D , claimed to be independent of heterozygosity. He proposes that D should replace G_{ST} when differentiation is the quantity of interest.

We are not convinced that G_{ST} has served its time as a measure of genetic differentiation. Whereas G_{ST} is mathematically constrained by H_S , there is no such constraint on D , implying that D can take any value in the range 0–1 regardless of H_S . The lack of such a restriction does not imply that D is expected to change independently of heterozygosity, however. We argue that D shares the same problems of dependence on H_S and mutation rate as G_{ST} and its relatives, and that these problems are sometimes even more pronounced for D than for G_{ST} .

There is current and renewed interest in the assessment of diversity in ecology and population genetics (e.g. Pavoine *et al.* 2005; Ricotta & Szeidl 2006; Jost 2007). The D measure suggested by Jost quantifies diversity at a gene locus as the inverse of gene identity ($1/J$), equivalent to the effective number of alleles (Crow & Kimura 1970), rather than as $1 - J$ which is a basic quantity for G_{ST} . An attractive characteristic of an approach using $1/J$ is that it is based on purely mathematical requirements for measures of diversity, and that it lends itself to partitioning diversity in a hierarchical way that is intuitively appealing.

However, despite the utility of measures based on $1/J$ for apportioning the allelic diversity at a locus, there are other

circumstances where G_{ST} is more appropriate. This measure has to a large extent been used to describe population structure with special focus on the effects of genetic drift and migration, which are the evolutionary forces reflecting demographic population characteristics that under selective neutrality are expected to affect all loci in the same way. As pointed out by Slatkin (1991), for assessment of population structure it would be desirable to have a measure of differentiation that does not confound the purely demographic processes of genetic drift and migration with purely genetic processes such as mutation. Unfortunately, as has become increasingly obvious during the past decade (e.g. Hedrick 1999), G_{ST} is affected by mutation and heterozygosity in a way that sometimes makes it difficult to compare results obtained from loci with markedly different mutation rates.

The point we make here is that Jost's D is also affected by mutation and heterozygosity in a way that can be even more pronounced than for G_{ST} . Thus, although based on an approach for quantifying variation that is appealing for apportioning allelic diversity, the D measure shares the same limitations as G_{ST} when it comes to comparing divergence at loci with different mutation rates. In addition, in some cases D approaches mutation–migration–drift equilibrium at a markedly slower rate than G_{ST} , which has implications for its usefulness when estimating migration rates assuming equilibrium.

G_{ST} is defined as

$$G_{ST} = \frac{H_T - H_S}{H_T}, \quad (\text{eqn 1})$$

where H_S and H_T are the expected heterozygosities within subpopulations and for the total population, respectively (Nei 1975). H_T and H_S are both smaller than unity and $H_T \geq H_S$, which implies that G_{ST} goes towards zero when H_S approaches unity, and that G_{ST} cannot exceed $1 - H_S$. Thus, with an H_S of, say, 0.80, which is commonly observed at, e.g. microsatellite loci, G_{ST} cannot exceed 0.20 even in situations where all the populations are segregating for completely non-overlapping sets of alleles. This constraint can create problems when analyzing divergence patterns at genetic markers displaying different levels of variation, for example, when comparing G_{ST} estimates obtained by allozymes or single nucleotide polymorphisms (SNP) vs. microsatellites (Ryman & Leimar 2008).

Nei's G_{ST} is typically used for describing the average amount of differentiation observed over multiple loci, and the quantities H_S and H_T then represent averages over all the loci examined. In contrast, as implied by the derivations in Jost (2008), the D measure is intended to describe the variation at a single locus. Keeping this distinction in mind, and using the same quantities as for G_{ST} , Jost's D for a locus can be written as

$$D = \frac{H_T - H_S}{1 - H_S} \frac{s}{s - 1}, \quad (\text{eqn 2})$$

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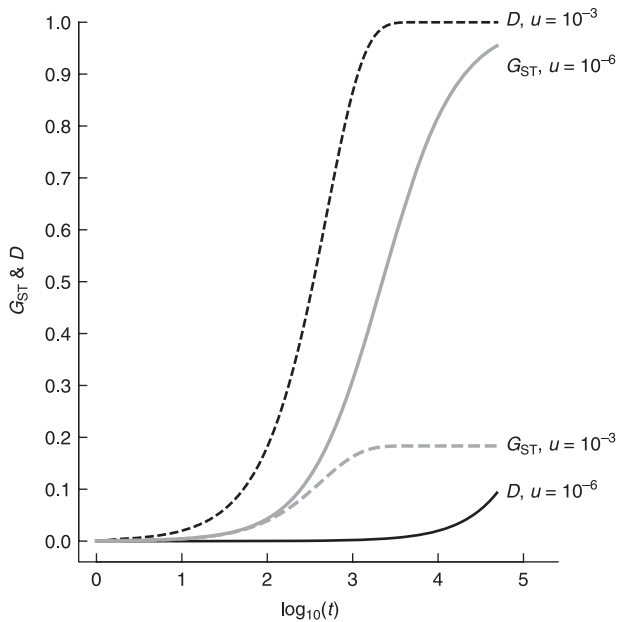


Fig. 1 Change of differentiation (D and G_{ST}) over time (t ; 50 000 generations, base-10 log scale) at two mutation rates ($u = 10^{-6}$ and $u = 10^{-3}$) among $s = 10$ completely isolated ($m = 0$) populations of effective size $N = 1000$. At $t = 0$ the 10 populations are assumed to represent copies of a single population in mutation–drift equilibrium.

where s is the number of subpopulations (Jost 2008, equation 11). Unlike G_{ST} , there is no constraint on D to be smaller than $1 - H_s$, and D can take values in the range 0–1 regardless of the magnitude of H_s . D does not change independently of H_s during the differentiation process, however, and as exemplified below it is affected by heterozygosity and mutation in similar ways as G_{ST} .

Numerical examples

To illustrate the effect of heterozygosity and mutation on D , we describe the change of G_{ST} and D during the differentiation process, assuming an island model of migration among s subpopulations, and an infinite allele model for mutation. Under those assumptions, the expected change of H_s and H_T (and thereby of G_{ST} and D) from one generation (t) to the next ($t + 1$) can be obtained from the recurrence equations for gene identity between and within subpopulations (Nei 1975; Li 1976; see Ryman & Leimar 2008 for details of application).

Figure 1 depicts the change of G_{ST} and D over the first $t = 50\,000$ generations after separation for $s = 10$ completely isolated (migration rate $m = 0$) populations of effective size $N = 1000$ at two different mutation rates ($u = 10^{-6}$ and $u = 10^{-3}$). In the first generation ($t = 0$) the 10 populations are assumed to represent copies of a single population in mutation–drift equilibrium, implying that equilibrium conditions within populations will persist throughout the differentiation process. Specifically for Fig. 1, this means that H_s remains at 0.004 and 0.800 for the mutation rates $u = 10^{-6}$ and $u = 10^{-3}$, respectively (e.g. Crow & Kimura 1970, equation 7.2.2).

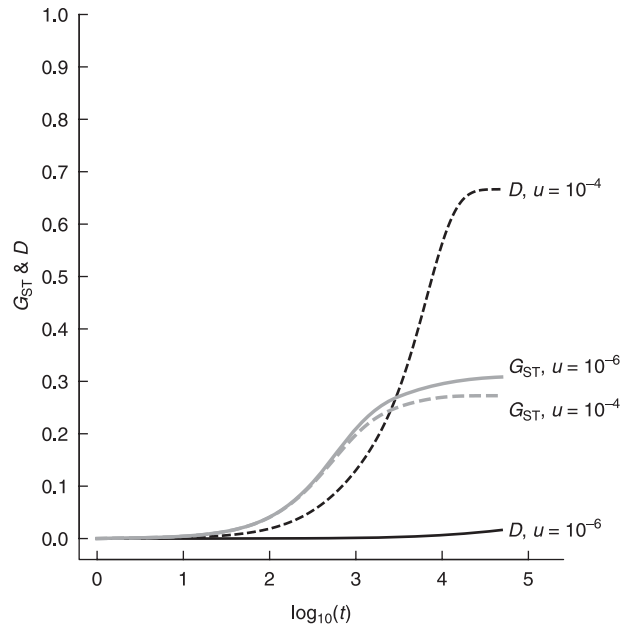


Fig. 2 As Fig. 1 except that the mutation rates are $u = 10^{-6}$ and $u = 10^{-4}$, and $m = 0.0005$.

There is an obvious effect of mutation on G_{ST} in Fig. 1. The two mutation rates result in steady-state values of $G_{ST} = 0.996$ and $G_{ST} = 0.183$ for $u = 10^{-6}$ and $u = 10^{-3}$, respectively, and the approach to equilibrium is much faster at the higher rate than at the lower one (cf. Ryman & Leimar 2008). Under complete isolation, as in the present case, the equilibrium value of D is unity (1) regardless of mutation rate, but before this state has been reached (i.e. during the transition phase) D is strongly affected by mutation, even more strongly than G_{ST} . Thus, comparing divergence estimates obtained from genetic markers with different mutation rates, such as microsatellites and SNPs, is expected to be associated with problems regardless of which of the two measures that is applied when measuring divergence.

Both measures are affected by mutation also when isolation is incomplete ($m > 0$), as exemplified in Fig. 2. Here the divergence scenario is similar to the previous one (Fig. 1), except that the mutation rates are 10^{-6} and 10^{-4} and the migration rate is $m = 0.0005$. Under those conditions, the effect of mutation is clearly more pronounced on D than on G_{ST} . Thus, in this case the signal from the demographic processes of migration and drift is more confounded by mutation when measuring differentiation by Jost's D rather than G_{ST} (Fig. 2).

The effect of heterozygosity on D is obvious also when considering loci with identical mutation rates. Ryman & Leimar (2008) showed that during the transition phase, the change of G_{ST} is not only dependent on mutation, but also on the heterozygosity in the base population from which the populations diverged. The same phenomenon is true for the D measure. As an example, Fig. 3 depicts the first 1000 generations after divergence for two scenarios where $s = 10$ completely isolated subpopulations ($m = 0$) of effective size $N = 1000$ diverge from ancestral populations with different heterozygosities. The mutation rate is $u = 10^{-4}$, and the base population is assumed to be either in mutation–

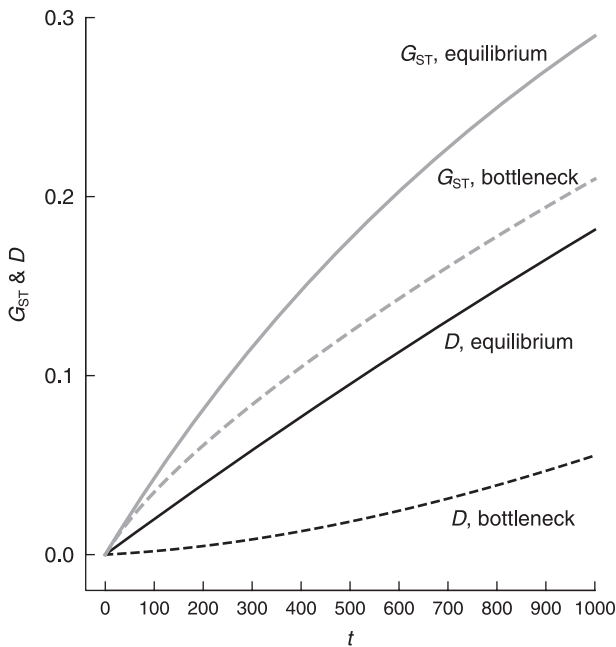


Fig. 3 Change of differentiation (D and G_{ST}) over time (t ; 1000 generations) at different initial heterozygosities representing mutation–drift equilibrium and a bottleneck event among $s = 10$ completely isolated ($m = 0$) populations of effective size $N = 1000$ when $u = 10^{-4}$. At $t = 0$, the 10 populations are assumed to represent copies of a single population with heterozygosity 0.286 or 0.0286 for the equilibrium and bottleneck scenarios, respectively.

drift equilibrium ($H_S = 0.286$), or to have passed through a bottleneck resulting in a heterozygosity of only 10% of the equilibrium value ($H_S = 0.0286$).

The lower initial heterozygosity of the bottlenecked population results in a slower change of G_{ST} than when starting out from equilibrium conditions, and the behaviour of D is similar (Fig. 3). Clearly, there is an effect of heterozygosity on the change of D during the transition phase, and in the present example, it is obviously more pronounced than that on G_{ST} . In Fig. 3, the distinct responses of D and G_{ST} to different initial heterozygosities during the very first part of the divergence process also stress a fundamental difference between the two measures. While the two curves for G_{ST} change in a similar way for the first *c.* 50 generations, because of identical population sizes and migration rates in the two scenarios, those for D take off in different directions from the very beginning. The explanation for the difference is that when mutation rates are low, the expected value of G_{ST} varies in the same way for all selectively neutral loci in a subdivided population, and this holds approximately also for higher mutation rates (cf. Ryman & Leimar 2008). Jost's measure D , on the other hand, lacks this property.

As an illustration of this difference between D and G_{ST} , we may consider the very basic situation of an island model with no migration and no mutation ($m = u = 0$) and an infinite number of subpopulations ($s = \infty$). Here, the change of G_{ST} over time (t) is determined completely by the effective size (N) through the relation $G_{ST} = 1 - [1 - 1/(2N)]^t$ (Wright 1965). In contrast, the cor-

responding expression for D also contains the initial heterozygosity (e.g. for large N the slope of D at $t = 0$ is $H_S(0)/\{[1 - H_S(0)]2N\}$), which can be shown through analysis of the above-mentioned recursion equations of gene identity between and within populations as in Ryman & Leimar (2008).

Approach to equilibrium

Another characteristic of D seems to be that it approaches equilibrium considerably slower than G_{ST} unless mutation rate is high. As seen in Fig. 1, for example, D and G_{ST} are both close to their respective equilibrium values after about 1000 generations at the higher mutation rate $u = 10^{-3}$. In contrast, at $u = 10^{-6}$ the rate of approach to equilibrium is strikingly slower for D than for G_{ST} . Similar differences are seen in Fig. 3, where $u = 10^{-4}$ for both initial heterozygosities. With the parameter values used for Fig. 3, the equilibrium values are 0.69 and 1.0 for G_{ST} and D , respectively, regardless of initial heterozygosity. Assuming that both measures are reasonably close to their respective equilibrium when they have reached 90% of the limiting value, the time to this state is *c.* $t = 6600$ generations for G_{ST} , whereas it takes almost twice that time (*c.* 11 500 generations) for D when starting out from the higher initial heterozygosity ($H_S = 0.286$ at $t = 0$). For the bottleneck scenario, where initial heterozygosity is $H_S = 0.0286$, the corresponding times are *c.* 8100 and 13 000 generations for G_{ST} and D , respectively.

The slower approach to equilibrium of D may make it less useful than G_{ST} for estimating migration rates from observed levels of differentiation. As stressed by Jost (2008), the accuracy of such estimates depends strongly on whether the populations examined are in migration–mutation–drift equilibrium. It is one of the attractive characteristics of G_{ST} that it approaches equilibrium relatively quickly and may reach a state close to equilibrium long before other components of gene diversity (Crow & Aoki 1984; Chakraborty & Leimar 1987; Ryman & Leimar 2008). Our present numerical evaluations suggest that in practical applications assumptions of equilibrium are likely to be violated more frequently for D than for G_{ST} .

Conclusions

We see a use for Jost's D in situations where interest is focused on a single locus of particular relevance for some question, for example the highly polymorphic major histocompatibility complex (MHC) locus in the context of disease resistance and genetic conservation. Here, the D measure provides a means for ranking groups of populations with respect to their degree of differentiation, and for identifying hierarchical levels corresponding to major shifts in genetic composition.

Nevertheless, as is clear from our results, the D measure cannot be interpreted exclusively in terms of basic population genetics quantities such as population size and gene flow. This means that D is not a useful measure when interest is focused on demographic processes such as genetic drift and migration, rather than on the particular mutational characteristics of the sampled loci.

Assessing the effects of the demographic processes of migration and drift should ideally be carried out using a measure that is not obscured by genetic processes such as mutation. Slatkin (1995) showed that the R_{ST} measure meets this

requirement under a perfect stepwise-mutation model (SMM), but the practical applicability of this observation may be limited because no group of loci seems to mutate entirely according to this model (Balloux & Lugon-Moulin 2002). Likewise, G_{ST} is not perfect in reflecting demographic processes because of its dependence on mutation and heterozygosity. As we have shown, Jost's D suffers from similar problems, and the problems are sometimes even more pronounced for D than for G_{ST} . The same is true for Hedrick's (2005) standardized measure (Ryman & Leimar 2008), and there is currently no correction available that accounts for all effects of mutation on G_{ST} .

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The authors share an interest in evolutionary biology, O.L. focusing on the evolution of phenotypic and genetic variation in heterogeneous environments, and N.R. on the genetic effects of harvesting and supplementing natural populations. Their concern about the interpretation of G_{ST} and other measures of genetic differentiation was triggered when comparing divergence estimates in Atlantic herring obtained using different sets of markers.

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