Neighbour joining trees, dominant markers and population genetic structure

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Population genetic theory for 'traditional' codominant loci showing low levels of allelic diversity (eg allozymes) has been well characterised and evaluated. In contrast, appropriate methods for the analysis of data from more recently developed marker systems are still being refined. For multilocus dominant markers such as amplified fragment length polymorphisms (AFLPs) and randomly amplified polymorphic DNA (RAPDs), the methods of data analysis can be split into two main categories. In population-based approaches, population allele frequencies are compared to obtain some measure of the partitioning of genetic diversity into within- and between-population components. In contrast, individual-based approaches use individual multilocus genotypes as the unit of analysis. Inferences on population processes such as gene flow are based on inter-relationships among individual samples as visualised on phenetic diagrams such as neighbour joining trees. Using a simulation approach coupled with neighbour joining analyses, we show

that while the underlying population genetic structure is an important determinant of tree shape in the analysis of dominant data, the number of loci examined also affects the topology. At low levels of population differentiation (eg $F_{ST} = 0.07$), mutually exclusive clustering of individuals into their respective populations can occur when sufficiently large numbers of loci are scored (eg 250 loci, typical of many AFLP studies). In contrast, unresolved star-shaped topologies can be recovered at higher levels of population differentiation ($F_{ST} > 0.15$) when lower numbers of loci are employed (eg 50 loci, typical of many RAPD studies). Thus, the relationship between tree topology and the extent of genetic structuring of populations is contingent upon the number of dominant loci scored. The consequences of these findings for the biological interpretation of individual-based analysis of dominant data sets are discussed.

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Introduction

Traditional descriptors of population genetic structure (eg F_{ST}) were developed for the analysis of population samples scored for genetic markers showing codominant inheritance and low levels of allelic diversity (typically two to four alleles per locus). A substantial body of population genetic theory appropriate for such markers has provided us with an understanding of how estimates of F_{ST} are influenced by parameters such as migration rate, population size and mutation rate (Wright, 1978; Nei, 1987). Using this knowledge, it is now possible (with caution, and making a number of assumptions) to infer biological processes such as dispersal patterns from estimates of F_{ST} calculated from traditional codominant markers (eg allozymes) in natural populations.

In recent years, a much wider range of genetic markers with significantly different properties have become available (Karp *et al.*, 1998). These can be divided into two main classes: (1) locus-specific hypervariable microsatellite markers and (2) multilocus arbitrary fingerprinting techniques (eg randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs)).

The fact that both these classes of markers have different properties from their predecessors means that new methods of analysis are required if they are to be used to make inferences about population processes.

Microsatellite markers differ from allozyme markers in their stepwise pattern of mutation, their high mutation rates and their high levels of allelic diversity. Several recent reviews have highlighted the problems with using traditional estimators of F_{ST} derived from microsatellite data to infer such things as levels of gene flow among populations (Hedrick, 1999; Balloux *et al.*, 2000; Balloux and Lugon-Moulin, 2002). This has stimulated the development of alternative methods that are based either on models of microsatellite evolution (eg R_{ST} ; Slatkin, 1995) or population assignment tests (Waser and Strobeck, 1998).

In the current paper, we focus on the analysis and interpretation of population data derived from arbitrary fingerprinting techniques such as RAPDs, ISSRs (inter-simple sequence repeats) and AFLPs. These generate large numbers of polymorphic markers by the amplification or nonamplification of arbitrary DNA sequences scattered throughout the genome (Williams *et al.*, 1990; Zietkiewicz *et al.*, 1994; Vos *et al.*, 1995). One clear advantage of these approaches is the large number of loci that can be scored as biallelic characters. RAPD and ISSR studies are typically based on data from 30 to 100 loci, whereas it is not unusual to score up to 250 loci in AFLP studies. The major disadvantage of using arbitrary

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fingerprinting techniques is the dominant nature of the data obtained (Lynch and Milligan, 1994; Harris, 1999). Of the three potential genotypic classes in a diploid individual (X_1X_1 , X_1X_2 , X_2X_2), only two character states are recorded: band presence (scored as $1 = X_1X_1$ and X_1X_2) and band absence (scored as $0 = X_2X_2$).

Two strategies have been adopted for utilising this dominant suite of markers to study population structure. The first involves population level analysis. It requires scoring of substantial numbers of individuals across a range of populations. Where the breeding system of the populations is known, population allele frequencies can be estimated using appropriate unbiased estimators and F_{ST} is then determined (Lynch and Milligan, 1994). Alternatively, especially where the breeding system is not known, either a Bayesian approach to estimating F_{ST} can be performed (Holsinger *et al*, 2002) or a genetic distance matrix based on phenotypic data can be calculated, and the variation partitioned into its among- and between-population components to calculate an analogue of F_{ST} (ϕ_{ST} ; Excoffier *et al*, 1992). Inferences about levels of migration among populations, or population history, are then made in the normal way based on population genetic theory that relates F_{ST} to population processes.

The second strategy for utilising dominant marker data typically involves sampling a smaller number of individuals per population, but each is scored at a large number of loci. The multilocus dominant marker phenotypes of individuals are then used to generate a genetic distance matrix. This information is subjected to phenetic analysis to produce a visual representation (a tree-based diagram or principal coordinates plot (PCO)) of the genetic relationships among individuals in the sample (eg Winfield *et al*, 1998; Clausen *et al*, 2000; Drummond *et al*, 2000; van der Merwe *et al*, 2000; Sawkins *et al*, 2001; Barth *et al*, 2002; Nan *et al*, 2003).

The shape of the tree or PCO plot then forms the basis for making population genetic inferences about the populations from which the individuals were derived. For instance, grouping of individuals from different populations in the same cluster of a tree has been interpreted as evidence for gene flow between these populations; location of individuals from different populations in mutually exclusive clusters has been taken as evidence for genetic isolation of the populations.

The representation of genetic relationships among individuals using these techniques is commonly undertaken. However, the interpretation of the resulting trees in terms of the genetic behaviour of the populations involved is not straightforward, and is not based on any formal quantitative population genetic model or theory. Despite this, the use of phenetic diagrams using dominant data to infer population genetic processes appears to be gaining widespread and uncritical acceptance.

The objective of this paper is to emphasise some potential pitfalls inherent in this practice. To do this, we present a simple illustrative example. We explore how the commonly used neighbour joining tree-based approach for analysing arbitrary fingerprinting data relates to known information on population structure. We show how variation in the extent of genetic differentiation among populations, the number of markers scored and the interaction of these factors affect tree topology. Our

purpose is not to provide guidelines for population genetic interpretation of phenetic trees, but rather to emphasise some factors that make this process problematic when practised in isolation.

Materials and methods

Overview

The procedure adopted can be divided into four steps:

- (1) An individual-based computer model simulates the genetic behaviour of some populations isolated to different degrees. The populations are polymorphic at a large number of selectively neutral, unlinked loci (250). This number is chosen because it is close to the upper limit of the number of loci routinely included in population genetic studies using dominant markers. The simulation generates multilocus codominant genotypes of all individuals in the populations.
- (2) Traditional population genetic methods are then used to quantify the partitioning of genetic variation between populations by calculating F_{ST} using all of the codominant data.
- (3) The same codominant data are then converted to multilocus dominant phenotypes. A genetic distance matrix among individuals is then calculated and used to generate a neighbour joining tree. To illustrate the effect of using different numbers of dominant genetic markers, data from either all 250 or subsamples of 125 and 50 loci are used to construct neighbour joining trees.
- (4) The extent to which individuals from different populations are clustered on distinct tree branches is then assessed, and compared with the quantitative measure of the partitioning of genetic information between populations (F_{ST}). F_{ST} estimates from the full codominant data set represent a well-understood standard, against which the tree-based analyses of the dominant data are compared.

Simulation of genetic drift

Simulations were performed using the individual-based, discrete generation model Easypop1.7.1 (Balloux, 1999). All populations simulated contained 100 diploid, random mating individuals. At the beginning of each simulation, populations were polymorphic at 250 unlinked, codominant, selectively neutral, diallelic loci (X) in which the frequency of both alleles (X_1 , X_2) was 0.5. Genetic divergence between populations was then generated in two ways:

- (i) Divergence in isolation: Genetic drift was simulated in two populations (designated A and B) genetically isolated for up to 75 generations. Multilocus, codominant genotypes of all individuals in the two populations were stored from independent simulations at five-generation intervals from five to 75 generations.
- (ii) Divergence with migration: Each simulation involved 10 populations. Drift and migration between populations occurred under an island model with zygotic migration at a rate m per generation.

Simulated values of m varied from 0.0025 ($Nm = 0.25$ migrants per generation) to 0.08 ($Nm = 8$ migrants per generation). The migration rates represent the probability of any one individual being a migrant (ie the rates are not deterministic). Simulations were run for 100 generations. Two populations (designated A and B) were then selected at random from the 10 in the simulation. The complete multilocus codominant genotypes from all 200 individuals in these two populations were used in subsequent analyses.

All simulations were replicated five times.

Calculation of F_{ST}

F_{ST} , measuring the partitioning of genetic variation between the two populations was calculated from the complete codominant multilocus data set using GENEPOP 3.3. (Raymond and Rousset, 1995). F_{ST} values derived from our simulations of populations diverging in isolation were compared with the expected F_{ST} values:

$$F_{ST} = 1 - (1 - 1/2N)^t$$

where t is the number of generations of isolation (Weir, 1996).

Individual tree-based analysis

Codominant multilocus data from all 200 individuals were converted to dominant data using the convention $X_1X_1=1$, $X_1X_2=1$, $X_2X_2=0$, where 1 represents the presence and 0 represents the absence of a band in a typical RAPD, ISSR or AFLP analysis. This approach is designed to mimic sampling of diploid individuals using dominant markers.

Two approaches were used to construct distance matrices among individuals. In the first, we used a simple Euclidean distance measure:

$$E = n[1 - (n_{xy}/n)]$$

where n is the total number of polymorphic bands and n_{xy} is the number of characters shared by individuals x and y (shared presences and shared absences). This approach has been used by many authors for arbitrary fingerprinting data in both tree- and AMOVA-based analyses (eg Ayres and Ryan, 1997; Arens *et al*, 1998; Carino and Daehler, 1999; Greef and Triest, 1999; Amsellem *et al*, 2000; Comes and Abbott, 2000; Auge *et al*, 2001).

The use of Euclidean distances has been criticised on the grounds that the shared absence of a band may be attributable to multiple different factors and is not necessarily a homologous character state (Harris, 1999). However, this is not an issue in our simulations where sharing of the character states 1 or 0 represent true homologies (the simulations start with a biallelic system at panmixia with no subsequent mutation; there is no homoplasy).

Nevertheless, we also calculated distance among individuals using Jaccard's (1908) similarity coefficient (calculated in R version 4.0 (Casgrain and Legendre, 2001) and imported into PAUP* as a user-specified distance). This is an asymmetrical distance that excludes

shared band absence (Harris, 1999). Jaccard's distance = $1 - S$, where

$$S = a/(a+b+c)$$

and a is the number of bands shared between accessions x and y , b is the number of bands in accession x and c is the number of bands in accession y .

Neighbour joining trees (Saitou and Nei, 1987) were constructed for each simulation from matrices of Euclidean distances and corresponding Jaccard's coefficients and visualised as unrooted phylograms using PAUP* (Swofford, 2000). Not surprisingly, given the 'clean' nature of the simulated data, virtually identical results were obtained. We report here only the results using the Euclidean metric. Separate trees were calculated using information from 250, 125 or 50 loci. The population identity of each individual (A or B) was indicated on the tree.

Results

Classification of tree types

The trees generated by the neighbour joining analysis could be classified into three classes, represented in Figure 1. In the first (Star, Figure 1a), individuals from the two populations A and B were intermingled. In the second, significant structure was apparent, but up to 5% of individuals were included within the cluster occupied by the alternate population (misclassified (Mc), Figure 1b). In the third type of tree, individuals from populations A and B were clearly separated in different clusters (mutually exclusive (ME), Figure 1c). These categories were subsequently used as a convenient shorthand for describing the outcome of neighbour joining tree analysis under different population and sampling scenarios.

Figure 1 Stylised trees to illustrate different topologies among individuals from each of 2 populations (A and B). (a) Shows an unresolved 'star'-shaped topology. (b) Shows the two populations falling into two groups, but with one Mc individual. (c) Shows ME clustering of the individuals from the two populations.

F_{ST} and tree shape after genetic divergence in isolation
Figure 2 summarises the comparison of F_{ST} values and tree shape when two populations diverge in isolation (the raw data is presented in Appendix A). As anticipated, the F_{ST} value rises as the number of generations in isolation between the two populations increases. The F_{ST} values derived from our simulations match closely the expected values (Appendix A).

For 250 loci, when the populations diverged for five generations ($F_{ST}=0.02\text{--}0.025$), a star-shaped topology was recovered (Figure 2). Greater structure was observed, however, after only 10 generations in isolation ($F_{ST}=0.046\text{--}0.052$) with less than 10 individuals being Mc in each simulation. After 15 generations, and at low values of F_{ST} (eg $F_{ST}=0.072\text{--}0.078$), the individuals fell into ME clusters.

For 125 loci the star-shaped topologies still occur for up to 10 generations in isolation ($F_{ST}=0.046\text{--}0.052$), and Mc individuals occurred for up to 45 generations ($F_{ST}=0.187$) (Figure 2). ME clustering occurred in some simulations after 20 generations ($F_{ST}=0.090$), but only after approximately 30 generations ($F_{ST}=0.124\text{--}0.132$) did this pattern predominate.

For 50 loci, the star-shaped topology was the predominant topology for up to 25 generations approxi-

mately in isolation ($F_{ST}=\text{ca. } 0.1$), and occurred for up to 40 generations in isolation ($F_{ST}=0.171$) (Figure 2). Mc individuals occurred at high F_{ST} values (eg $F_{ST}=0.297$; up to 70 generations in isolation).

As the number of loci scored decreases, the transition to the ME tree type occurs after a greater number of generations, and at a higher F_{ST} value (Figure 2). The tree shape obtained for a given level of genetic divergence thus depends on the number of loci scored. In addition, as the number of loci used for constructing the trees decreases, the variance increases, such that the transition zones between the different tree topologies (star–Mc–ME) become more diffuse with fewer loci (Figure 2).

F_{ST} and tree shape under divergence with migration

Table 1 shows the results of individual simulations with migration rates ranging from 0.0025 to 0.08. It is important to stress that for these simulations there are two sources of Mc individuals. These correspond to (a) individuals with multilocus genotypes that are by chance more similar to those from the foreign population than their own population and (b) immigrant individuals.

For 250 loci for high migration rates ($Nm>6$ per generation) a star topology is found. For intermediate migration rates ($6>Nm>1$) the tree topologies tend to be structured, with variable numbers of individuals Mc (Mc type). For low migration rates and high F_{ST} values ($F_{ST}>0.25$; $Nm<0.05$) individuals from the two populations tend to fall into ME clusters (ME type). Table 2 shows the expected numbers of migrants for the different migration rates. It can be seen that the number of Mc individuals at intermediate migration rates broadly corresponds to the expected number of immigrants (Table 2).

As was the case for divergence in isolation, as the number of loci decreases, a greater level of population differentiation (in this case lower migration rates) is required before the transition from a star-shaped topology to ME clustering occurs. For 125 loci, star-shaped topologies are recovered until a migration rate of 0.02 and an $F_{ST}=0.081$ are reached (Table 1). For 50 loci, star-shaped topologies were recovered up to a migration rate of 0.015 and an $F_{ST}=0.124$ (Table 1).

Discussion

The simulations described here indicate that it is inadvisable to draw population genetic inferences solely from the topology of neighbour joining trees generated from distance matrices based on arbitrary fingerprinting data. As the simulations illustrate, there is no straightforward relationship between genetic isolation and the partitioning of genetic variation among populations on the one hand, and clustering of individuals on the neighbour joining tree on the other. The results are contingent on the number of markers scored.

When a very large number of markers are available, as in AFLP analyses, clustering of groups can occur at very low F_{ST} values. In the simulation of two populations diverging in isolation with 250 loci, very strong structuring (ME type) is shown between populations when the F_{ST} value is only 0.07 (Figure 2). As the number of loci used decreases, ME clustering occurs at increasingly higher levels of population differentiation. For 250 loci, the highest F_{ST} value at which there is a Mc

Figure 2 Relationship between F_{ST} and tree shape for two populations diverging in isolation for five to 75 generations, analysed for 250, 125 and 50 loci, respectively.

Table 1 Relationship between migration rate, F_{ST} and tree topology from simulated data under divergence with migration

Migration rate	F_{ST}	Tree shape		
		250 loci	125 loci	50 loci
0.0025	0.3231	ME	ME	ME
0.0025	0.3334	ME	ME	ME
0.0025	0.2746	1 Mc	2 Mc	2 Mc
0.0025	0.2920	ME	ME	1 Mc
0.0025	0.3499	ME	ME	ME
0.005	0.2506	ME	ME	1 Mc
0.005	0.2319	1 Mc	1 Mc	3 Mc
0.005	0.2511	ME	ME	7 Mc
0.005	0.2437	1 Mc	1 Mc	2 Mc
0.005	0.2353	1 Mc	1 Mc	1 Mc
0.01	0.1943	ME	ME	3 Mc
0.01	0.1770	ME	2 Mc	6 Mc
0.01	0.1528	ME	2 Mc	4 Mc
0.01	0.1690	ME	ME	2 Mc
0.01	0.1721	1 Mc	ME	2 Mc
0.015	0.1238	1 Mc	8 Mc	Star
0.015	0.1527	1 Mc	1 Mc	4 Mc
0.015	0.1264	2 Mc	2 Mc	Star
0.015	0.1256	1 Mc	4 Mc	9 Mc
0.015	0.1141	2 Mc	3 Mc	6 Mc
0.02	0.1078	5 Mc	5 Mc	Star
0.02	0.1173	2 Mc	3 Mc	10 Mc
0.02	0.1059	5 Mc	3 Mc	Star
0.02	0.1132	1 Mc	2 Mc	6 Mc
0.02	0.0808	6 Mc	Star	Star
0.03	0.0614	6 Mc	8 Mc	Star
0.03	0.0704	8 Mc	Star	Star
0.03	0.0579	7 Mc	Star	Star
0.03	0.0656	5 Mc	Star	Star
0.03	0.0579	7 Mc	Star	Star
0.04	0.0497	Star	Star	Star
0.04	0.0531	8 Mc	Star	Star
0.04	0.0614	9 Mc	Star	Star
0.04	0.0533	9 Mc	Star	Star
0.04	0.0481	Star	Star	Star
0.06	0.0360	10 Mc	Star	Star
0.06	0.0337	Star	Star	Star
0.06	0.0317	Star	Star	Star
0.06	0.0335	Star	Star	Star
0.06	0.0250	Star	Star	Star
0.08	0.0307	Star	Star	Star
0.08	0.0303	Star	Star	Star
0.08	0.0294	Star	Star	Star
0.08	0.0202	Star	Star	Star
0.08	0.0267	Star	Star	Star

Star = star-shaped topologies; Mc = between 1 and 10 misclassified individuals (with the number of Mc individuals specified); ME = mutually exclusive clusters.

individual for divergence in isolation is $F_{ST}=0.0715$, for 125 loci it is $F_{ST}=0.1873$ and for 50 loci it is $F_{ST}=0.297$ (Figure 2, Appendix A). There is also an increasing amount of noise, and the transitions between the different tree topologies becomes more protracted with fewer loci.

In the absence of any knowledge of F_{ST} an intuitive interpretation of strong structure in a neighbour joining tree may be that the populations involved are genetically isolated. Yet, this is not the case. Where large numbers of loci are scored structured trees are produced after a small number of generations in isolation (eg 10, Figure 2), or with up to one migrant per generation (Table 1). Indeed, the chances of finding structured trees may be even higher than suggested by our simulations. The reason is

Table 2 Expected and observed number of misclassified individuals for differing migration rates

Migrants per population per generation	Expected Mc	Observed			Mean F_{ST}
		250 loci	125 loci	50 loci	
0.25	0.5	0.2	0.4	0.6	0.3146
0.5	1	0.6	0.6	2.8	0.2425
1	2	0.2	0.8	3.4	0.1730
1.5	3	1.4	3.6	Star	0.1285
2	4	3.8	5.4	Star	0.1050
3	6	6.6	Star	Star	0.0626
4	8	Star	Star	Star	0.0531
6	12	Star	Star	Star	0.0320
8	16	Star	Star	Star	0.0275

Expected number of Mc individuals is the expected number of immigrants in each population. The observed number of misclassified individuals and the mean F_{ST} estimates are based on five simulations at each migration rate.

that in the results reported here, all individuals in the population were included in the neighbour joining trees. In a typical field-based study, only a proportion of individuals would be sampled. Under these circumstances, the probability of sampling recent migrants would be low, and the distinctions between groups might appear even more pronounced on the neighbour joining tree at low levels of F_{ST} .

This problem with overestimating the genetic distinctiveness of groups is likely to increase in the future. Our simulations used between 50 and 250 loci. However, as the automation of fragment analyses improves and the costs of techniques such as AFLPs come down, the number of markers used in empirical population level studies is likely to increase (eg Cresswell *et al*, 2001 used 765 polymorphic loci in their AFLP analysis of *Lolium* diversity). In these circumstances neighbour joining trees may be able to distinguish groups between which genetic divergence is so small as to be biologically insignificant.

This must be borne in mind when data such as these are used in any applied context to guide genetic management of populations. Just because individuals from two separate populations form ME clusters in a neighbour joining tree, this does not necessarily imply that there is no gene exchange, or that, in a conservation scenario, the populations should be treated as different units for management.

Although the simulations have highlighted problems with simple genetic interpretations of tree topology, they nevertheless suggest that when large numbers of markers are available it may be possible to recognise recent migrants within populations from their multilocus genotypes and assign them to their source population (Waser and Strobeck, 1998). Thus in the drift-migration simulations, the number of 'Mc' individuals within populations corresponds well with the number of migrants anticipated in the previous generation. And while it is known that simple phenetic-based methods of assignment such as this are weak, it supports the notion that the use of more formalised assignment tests for the study of population genetic processes represents a potentially useful application of the resolving power of large numbers of polymorphic molecular markers (Waser and Strobeck, 1998; Pritchard *et al*, 2000).

We stress that the results presented here (tree shape in relation to F_{ST} for a given number of polymorphic markers) do not form the basis for direct extrapolation based on 'real' data (eg obtaining an estimate of F_{ST} from the shape of a tree and a known number of polymorphic markers). (1) Firstly, the simulated data are clean; all 1's and all 0's are homologous. Real data will contain varying levels of noise attributable to comigrating nonhomologous bands and, if Euclidean distances are used, nonhomologous shared absences. The level of this noise in the data will be an additional contributing factor in the determination of tree topology. (2) The results presented here are for panmictic random mating sub-populations. Localised subpopulation differentiation and/or selfing may lead to marked multilocus structure among individuals that will affect their relationships in an individual-based clustering diagram for a given value of F_{ST} . (3) Our starting frequency of alleles for each locus was 0.5. In real data sets, low-frequency variants at individual loci are likely to alter the power of discrimination between populations for a given number of polymorphic loci. (4) The number of simulations we have carried out have been sufficient to illustrate clearly the differences between tree topologies when the same individuals are scored for different numbers of loci. The number of simulations undertaken was not designed to estimate formally the mean and variance of F_{ST} values under which these topology transitions occur (and given the previously mentioned caveats in the interpretation of our simulation results, any such estimates would be of doubtful biological relevance).

Instead, our take home message is that simple population genetic interpretation of phenetic cluster diagrams is problematical. In addition to the underlying population genetic structure, a key determinant of tree shape is the number of loci analysed. The same biological situation can result in different tree topologies if studied with different numbers of markers. While this fact may come as no surprise to many population geneticists, the prevalence of this approach, especially in the plant molecular ecology literature, suggests that the limitations involved deserve wider appreciation.

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Appendix A

Results from individual simulations (as summarised in Figure 2) on the relationship between F_{ST} and tree topology from simulated data for two populations diverging in isolation. The observed F_{ST} values are from our simulations, the expected F_{ST} values are derived from $F_{ST} = 1 - (1 - 1/2N)^t$, where t is the number of generations of isolation (Weir, 1996), as seen in Table A1.

Table A1 Results from individual simulations (as summarised in Figure 2) on the relationship between F_{ST} and tree topology from simulated data for two populations

Generations in isolation	Observed F_{ST}	Expected F_{ST}	Number of loci		
			250 loci	125 loci	50 loci
5	0.0229		Star	Star	Star
5	0.0255		Star	Star	Star
5	0.0254		Star	Star	Star
5	0.0249		Star	Star	Star
	Mean 0.0247	0.0248			
10	0.052		4 Mc	Star	Star
10	0.0461		8 Mc	Star	Star
10	0.0509		1 Mc	Star	Star
10	0.0483		4 Mc	Star	Star
10	0.0519		1 Mc	8 Mc	Star
	Mean 0.0498	0.0489			
15	0.0715		1 Mc	3 Mc	Star
15	0.0746		ME	1 Mc	Star
15	0.0746		ME	7 Mc	Star
15	0.0721		ME	5 Mc	Star
15	0.0780		ME	4 Mc	Star
	Mean 0.0742	0.0724			
20	0.0944		ME	1 Mc	Star
20	0.1059		ME	2 Mc	Star
20	0.1037		ME	1 Mc	Star
20	0.0972		ME	1 Mc	Star
20	0.0896		ME	ME	6 Mc
	Mean 0.0982	0.0954			
25	0.1061		ME	1 Mc	Star
25	0.1192		ME	2 Mc	Star
25	0.0987		ME	1 Mc	Star
25	0.1139		ME	1 Mc	5 Mc
25	0.1358		ME	ME	5 Mc

Table A1 Continued...

Table A1 (continued)

Generations in isolation	Observed F_{ST}	Expected F_{ST}	Number of loci		
			250 loci	125 loci	50 loci
	Mean 0.1147	0.1178			
30	0.1248		ME	ME	Star
30	0.1312		ME	ME	1 Mc
30	0.1267		ME	ME	4 Mc
30	0.1262		ME	1 Mc	Star
30	0.1323		ME	ME	6 Mc
	Mean 0.1282	0.1396			
35	0.1654		ME	ME	2 Mc
35	0.1431		ME	ME	7 Mc
35	0.1481		ME	1 Mc	4 Mc
35	0.1553		ME	ME	2 Mc
35	0.1626		ME	ME	2 Mc
	Mean 0.1549	0.1609			
40	0.1725		ME	ME	ME
40	0.1890		ME	ME	1 Mc
40	0.1720		ME	ME	3 Mc
40	0.1980		ME	ME	9 Mc
40	0.1711		ME	ME	Star
	Mean 0.1805	0.1817			
45	0.2021		ME	ME	8 Mc
45	0.1873		ME	1 Mc	2 Mc
45	0.1691		ME	ME	1 Mc
45	0.2446		ME	ME	ME
45	0.2097		ME	ME	2 Mc
	Mean 0.2026	0.2019			
50	0.2261		ME	ME	1 Mc
50	0.2216		ME	ME	1 Mc
50	0.2021		ME	ME	3 Mc
50	0.2133		ME	ME	ME
50	0.2139		ME	ME	ME
	Mean 0.2154	0.2217			
55	0.2317		ME	ME	ME
55	0.2391		ME	ME	ME
55	0.2270		ME	ME	ME
55	0.2210		ME	ME	ME
55	0.2274		ME	ME	2 Mc
	Mean 0.2292	0.2410			
60	0.2484		ME	ME	ME
60	0.2500		ME	ME	ME
60	0.2391		ME	ME	1 Mc
60	0.2379		ME	ME	ME
60	0.2693		ME	ME	ME
	Mean 0.2489	0.2597			
65	0.2820		ME	ME	ME
65	0.2892		ME	ME	2 Mc
65	0.2748		ME	ME	ME
65	0.2464		ME	ME	ME
65	0.2347		ME	ME	5 Mc
	Mean 0.2654	0.2781			
70	0.3098		ME	ME	ME
70	0.2970		ME	ME	1 Mc

Table A1 Continued...

Table A1 (*continued*)

<i>Generations in isolation</i>	<i>Observed F_{ST}</i>	<i>Expected F_{ST}</i>	<i>Number of loci</i>		
			<i>250 loci</i>	<i>125 loci</i>	<i>50 loci</i>
70	0.2782	0.2959	ME	ME	ME
70	0.2952		ME	ME	ME
70	0.3087		ME	ME	ME
	Mean 0.2978				
75	0.3068	0.3134	ME	ME	ME
75	0.3424		ME	ME	ME
75	0.3413		ME	ME	ME
75	0.2772		ME	ME	ME
75	0.3442		ME	ME	ME
	Mean 0.3224				