Sampling within the genome for measuring within-population diversity: trade-offs between markers

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Abstract

Experimental results of diversity estimates in a set of populations often exhibit contradictory patterns when different marker systems are used. Using simulations we identified potential causes for these discrepancies. These investigations aimed also to detect whether different sampling strategies of markers within the genome resulted in different estimates of the diversity at the whole genome level. The simulations consisted in generating a set of populations undergoing various evolutionary scenarios which differed by population size, migration rate and heterogeneity of gene flow. Population diversity was then computed for the whole genome and for subsets of loci corresponding to different marker techniques. Rank correlation between the two measures of diversity were investigated under different scenarios. We showed that the heterogeneity of genetic diversity either between loci (genomic heterogeneity, GH) or among populations (population heterogeneity, PH) varied greatly according to the evolutionary scenario considered. Furthermore, GH and PH were major determinants of the level of rank correlation between estimates of genetic diversities obtained using different kinds of markers. We found a strong positive relationship between the level of the correlation and PH, whatever the marker system. It was also shown that, when GH values were constantly low during generations, a reduced number of microsatellites was enough to predict the diversity of the whole genome, whereas when GH increased, more loci were needed to predict the diversity and amplified fragment length polymorphism markers would be more recommended in this case. Finally the results are discussed to recommend strategies for gene diversity surveys.

Keywords: AFLP markers, correlation, genetic diversity, genome heterogeneity, microsatellites, simulation

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Introduction

Measuring gene diversity within natural populations with the help of molecular markers follows a three-step sampling procedure: sampling of populations, sampling of individuals within populations and sampling of gene loci within the genome of individuals. Up until now, most of the attention has been directed to the sampling of populations and individuals. Sampling variance and confidence intervals were developed through analytical calculations and were applied for defining optimal sampling strategies (Brown & Weir 1983; Zhang & Allard 1986). However, sampling within the genome has received much less attention, mostly because the distribution of genetic polymorphism within the genome is often unknown.

Recent advances in molecular technology have greatly increased the number of genetic markers available for assessing genetic diversity within populations. The random amplified polymorphic DNA (RAPD; Williams et al. 1990) and the amplified fragment length polymorphism (AFLP; Vos et al. 1995) methods are techniques for which individuals can be scored at a large number of loci that are randomly distributed within the genome. Conversely, for microsatellites, only a limited number of loci can be scored with the same amount of resources available.
In comparison with RAPD and AFLP markers, the use of microsatellites requires preliminary molecular analysis to design the primers for their amplification. On the other hand, these two marker systems do not share the same genetic properties: microsatellites are highly informative (multiallelic and co-dominant) at a single locus, whereas AFLP markers (AFLPs) are usually dominant and biallelic. As a consequence, we considered that two extreme sampling strategies could be adopted when molecular markers are chosen for a genetic diversity study: (i) sampling of numerous poorly informative markers randomly distributed within the genome (AFLPs) or (ii) selection of a few highly informative markers (microsatellites).

Using simulations, we investigate here the trade-offs between markers for measuring diversity in natural populations. Do the number of AFLP loci compensate for their low information at single loci? Or do the multiallelism and co-dominance of microsatellites compensate for their low number of loci? The question of the trade-offs between markers was also raised by results in the literature that showed that levels of within-population diversity were only seldom correlated when their estimates were performed with different markers (for a review see Kremer 1998; Mariette 2001). Why do populations not rank in a comparable order when diversity is estimated with different markers? If a set of populations went through different evolutionary histories involving drift and migration, do the multiallelism and co-dominance of microsatellites compensate for their low number of loci? The question of the trade-offs between markers was also raised by results in the literature that showed that levels of within-population diversity were only seldom correlated when their estimates were performed with different markers (for a review see Kremer 1998; Mariette 2001). Why do populations not rank in a comparable order when diversity is estimated with different markers?

Using simulations, we investigate here the trade-offs between markers for measuring diversity in natural populations. Do the number of AFLP loci compensate for their low information at single loci? Or do the multiallelism and co-dominance of microsatellites compensate for their low number of loci? The question of the trade-offs between markers was also raised by results in the literature that showed that levels of within-population diversity were only seldom correlated when their estimates were performed with different markers (for a review see Kremer 1998; Mariette 2001). Why do populations not rank in a comparable order when diversity is estimated with different markers? If a set of populations went through different evolutionary histories involving drift and migration, two different types of markers should rank them similarly, even if the markers corresponded to genomic regions with different mutation rates. However, this is only rarely the case in experimental results (Dubreuil & Charcosset 1998; Lannér-Herrera et al. 1997b; Le Corre et al. 1997a; Mariette et al. 2001).

We attempt here to identify the reasons for these unexpected results by monitoring the evolution of diversity in a set of populations under various evolutionary scenarios. The metaPop software (Le Corre et al. 1997b) was modified to model the genome of individuals and the evolution of diversity within populations along generations. We compute the correlation of diversity assessed with different markers and the whole genome, and we compare advantages and drawbacks of different markers with different properties and different distributions within the genome, as AFLPs and microsatellites. Finally, we provide arguments for reasoning strategies to assess diversity with different marker techniques in natural populations.

Material and methods

Simulations

For monitoring the evolution of diversity measured with different types of markers in a set of populations, we used a modified version of the software metaPop (Le Corre et al. 1997b). This software is designed to model the evolution of structured populations connected by gene flow. The individuals in the populations are plants represented by their nuclear genotypes. The software simulates the action of migration, drift, selection, mutation and demography on a set of populations. In this study, generations were discrete and nonoverlapping, and selection was assumed to be absent.

Construction of a genome for each individual. In metaPop, the genome is defined by the total number of loci, the number of linkage groups and the recombination rates between successive loci along the linkage groups. Here the genome consisted of 1000 loci equally distributed in 10 linkage groups, with a recombination rate of 0.005 between two successive loci. Each linkage group consisted therefore of 100 loci evenly distributed with recombination rates between successive loci being 0.005. Of course loci on different groups were independent. Thus, linkage groups were of equal size: 49.5 cm using the formula of Kosambi (1944), and the total genome length was 495 cm. Mutation rates were sampled from a nonuniform distribution and assigned to loci independently along the linkage groups. In this study, the distribution of mutation rates consisted of nine classes: from $10^{-3}$ to $10^{-7}$ with a sampling probability of 2.5% for $10^{-7}$, 5% for $5 \times 10^{-7}$, 10% for $10^{-6}$, 20% for $5 \times 10^{-6}$, 25% for $10^{-5}$, 20% for $5 \times 10^{-5}$, 10% for $10^{-4}$, 5% for $5 \times 10^{-4}$ and 2.5% for $10^{-3}$. For all loci, the model of mutation rate was the Infinite Allele Model and the maximum number of alleles was 250. The diversity calculated over the total number of loci was considered as the diversity at the whole genome level.

Selection of loci within the genome for assessing diversity. Genetic diversity was monitored at several subsets of loci. They correspond to different marker systems. In this study, we aimed to contrast a high number of biallelic dominant loci with a few co-dominant multiallelic loci, which we will, respectively, denote ‘AFLPs’ and ‘microsatellites’. Once the numbers of AFLP and microsatellite loci were defined, they were sampled randomly among the existing 1000 loci of the whole genome, with the constraint that they should have the same number of marker loci per linkage group. If, for example, 50 AFLP loci were scored, five loci were randomly selected among the 100 existing within each linkage group. Some loci may be linked on the same linkage group just by chance. We did not record the recombination rates among the marker loci. However since recombination is included as a genetic process in the simulations, the diversity computed for the marker loci accounts for recombination. Mutation rates of AFLP loci were sampled within the distribution of mutation rates indicated for the whole genome. Mutation rates of microsatellites were randomly sampled in a distribution comprising five classes: 5% for $10^{-4}$, 20% for $5 \times 10^{-4}$, 50%
for $10^{-3}$, 20% for $5 \times 10^{-3}$, 5% for $10^{-2}$. For the biallelic dominant loci, $M$ and $m$ denote the dominant and null alleles at a locus. In order to simulate dominant biallelic loci, we merged the existing alleles (obtained via the mutation process at each locus) into two alleles $M$ and $m$. Half of the alleles were assigned to $M$, and the second half to $m$.

**Simulation of a metapopulation.** A set of plant populations was created, which were interconnected by pollen and seed flow once the number of populations, population sizes and migration rates among populations were defined. Migration was modelled by defining symmetric gene flow matrices (one for pollen flow and one for seed dispersal), where migration rates were introduced for all pairwise associations of populations. The choice of the parent plants, the sampling of gametes and their combination to form zygotes were all performed stochastically to reproduce meiosis and fertilization in panmictic populations.

**Running simulations.** The simulations proceeded as follows:

(i) The starting point was a unique base population consisting of a set of $Ni$ individuals that were homozygous at each locus. $Ni$ is the population size at the metapopulation level.

(ii) This initial population underwent random mating during 100,000 generations. New alleles were created by mutation, an equilibrium between mutation and drift was progressively reached and gene diversity approached an asymptotic value.

(iii) At this equilibrium, allele frequencies were computed at each locus in the base population. Then 25 subpopulations comprising each $N$ individuals ($N = Ni/25$) were created with allele frequencies sampled according to the allele frequencies in the base population.

(iv) Finally, these subpopulations evolved during an additional 3000 successive generations following a given evolutionary scenario as will be indicated in the following text.

**Input parameters**

The input parameters that we used are indicated in Table 1. In all simulations the number of populations was 25. These populations were spatially organized on a square grid system. Our main objective was to compare diversity estimates with different marker systems and different sampling intensities within the genome. The number of loci was set to three values for each marker system covering the range reported in experimental studies and beyond: 500, 200 and 50 loci for AFLPs and 50, 20 and five loci for microsatellites. These loci were always evenly distributed among linkage groups, and randomly within linkage groups.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Input parameters for the simulations of the genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome</td>
<td></td>
</tr>
<tr>
<td>Total genome</td>
<td>1000 loci, 10 linkage groups</td>
</tr>
<tr>
<td>Mutation rates</td>
<td>11 classes from $10^{-7}$ to $10^{-2}$</td>
</tr>
<tr>
<td>Recombination rates</td>
<td>0.005 between successive loci</td>
</tr>
<tr>
<td>Markers</td>
<td></td>
</tr>
<tr>
<td>AFLPs</td>
<td>500 loci, 50 on each linkage group</td>
</tr>
<tr>
<td>Sample size</td>
<td>500, 200, 50</td>
</tr>
<tr>
<td>Mutation rate</td>
<td>nine classes from $10^{-7}$ to $10^{-3}$</td>
</tr>
<tr>
<td>Microsatellites</td>
<td>50 loci, five on each linkage group</td>
</tr>
<tr>
<td>Sample size</td>
<td>50, 20, 5</td>
</tr>
<tr>
<td>Mutation rate</td>
<td>five classes from $10^{-4}$ to $10^{-2}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Evolutionary scenarios tested with the island model and with the two-dimensional stepping-stone model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen (m_p) and seed (m_s) migration rates</td>
<td>Population size</td>
</tr>
<tr>
<td></td>
<td>N = 1000</td>
</tr>
<tr>
<td>m_p = 0.01</td>
<td>Case 1</td>
</tr>
<tr>
<td>m_s = 0.0001</td>
<td></td>
</tr>
<tr>
<td>m = 0.0051</td>
<td></td>
</tr>
<tr>
<td>m_p = 0.0005</td>
<td>Case 3</td>
</tr>
<tr>
<td>m_s = 0.0001</td>
<td></td>
</tr>
<tr>
<td>m = 0.000035</td>
<td></td>
</tr>
</tbody>
</table>

As for migration and population size, we compared the results given by the different marker systems in four contrasting situations for which pollen migration rates and population size took different values as shown in Table 2. Seed migration rates were constant across all simulations ($10^{-4}$). Within each case of Table 2, we furthermore considered two seed and pollen migration models, an island model and a two-dimensional stepping-stone model. In total, eight different combinations of input parameters corresponding to eight different evolutionary scenarios were considered. For each scenario 10 different simulations were run to account for stochastic variations.

The two contrasting migration models we used correspond to two different mechanisms generating some differentiation among populations for their diversity. Since pollen flow was equally distributed among all populations in the island model, difference among populations in the level of diversity may only be produced by stochasticity. However, in the two-dimensional stepping-stone model, populations located at the edges of the grid system received less migrating genes than the more central populations. These uneven migration rates acted as a systematic force to create heterogeneity in within-population diversity in addition to stochastic effects. The stepping-stone model was therefore used here as a deterministic mechanism to
generate heterogeneity in the level of diversity between populations due to uneven migration rates among populations. Therefore in the text, the two migration models used should be understood as follows: the island model as homogeneity of gene flow, the stepping-stone model as heterogeneity of gene flow.

Outputs of simulations

At each generation, Nei’s genetic diversity \( H \) (Nei 1987) was calculated for the different sets of markers in each population and at the whole genome level. For dominant markers, two types of analyses were performed. First, Nei’s genetic diversity was directly calculated at each locus \( i \) on the \( P_i \) and \( Q_i \) frequencies, \( P_i \) being the frequency of the \( MM \) and \( Mm \) genotypes pooled together and \( Q_i \) being the frequency of the \( mm \) genotypes. \( P_i \) would correspond to the frequency of the band that an experimenter would observe. Phenotypic diversity is calculated as:

\[
H_p = 1 - P_i^2 - Q_i^2.
\]

This analysis will be denoted ‘P analysis’. Second, a genotypic analysis of dominant markers was performed in the software: \( p_i \) and \( q_i \) being the frequencies of the alleles responsible for the presence and absence of bands. Assuming that populations were under Hardy–Weinberg equilibrium,

\[
P_{MM} = p_i^2
\]

\[
P_{Mm} = 2p_iq_i
\]

\[
P_{mm} = q_i^2
\]

Then \( p_i \) and \( q_i \) were simply deduced from \( P_i \) and \( Q_i \):

\[
p_i = 1 - \sqrt{Q_i}
\]

\[
q_i = \sqrt{Q_i}
\]

The genotypic diversity, assuming Hardy–Weinberg equilibrium, was calculated as:

\[
H_G = 1 - p_i^2 - q_i^2
\]

This analysis will be denoted ‘G analysis’.

The interlocus variance of \( H_{Si} \) (the mean within-population diversity at a given locus \( i \)) over all 1000 loci was calculated to measure the heterogeneity of diversity within the genome (\( GH \), genome heterogeneity) for each evolutionary scenario. The interpopulation variance of \( H_i \) (the mean diversity over all loci within population \( i \)) among populations was also calculated to measure the heterogeneity of diversity among populations (\( PH \), population heterogeneity). Both \( GH \) and \( PH \) were expressed as coefficients of variation and were always computed at the whole genome level.

\[
G_H = \left( \frac{1}{1000} \sum (H_{Si} - H_S)^2 \right)^{1/2} / H_S
\]

\[
P_H = \left( \frac{1}{25} \sum (H_i - H_S)^2 \right)^{1/2} / H_S
\]

where \( H_S \) is the mean within population diversity over all 1000 loci and all populations, \( H_i \) is the mean diversity over all 1000 loci within population \( i \), and \( H_{Si} \) is the mean within population diversity at locus \( i \).

From generation 0 to generation 3000, we computed Spearman’s rank correlation coefficient (Sokal and Rohlf 1995) between \( H_{G, \text{genome}} \), measured on the whole genome, and \( H_{P, \text{marker}} \), measured with each category of marker. The rank correlation was therefore computed on 25 pairs of data.

The equilibrium values of correlation estimates, obtained at the end of the simulations, were compared between markers and the whole genome. The equilibrium value of correlations was calculated as the mean value over the last 100 generations. As we were mainly interested in the comparison of diversity among marker systems, we did not consider different mutation models for different loci or markers in the genome (stepwise-mutation model vs. infinite allele model). Different mutation mechanisms are expected to affect diversity in its absolute value. But the effect would be of similar magnitude in different populations, hence its effect on the rank correlation is expected to be meaningless. As a consequence, microsatellites should be understood here in a restricted way as hypervariable loci following an infinite allele mutation model.

By monitoring the rank correlation in the eight different scenarios, we intended to identify the effect of the following three sources of variation: population size (1000 vs. 100), migration rates (\( 5 \times 10^{-3} \) vs. \( 3.5 \times 10^{-4} \)) and gene flow heterogeneity (island vs. stepping-stone model).

Results

Impact of the different scenarios on the within-population diversity

As expected, the eight different scenarios resulted in contrasted levels of within-population diversity (Fig. 1). When measured over the whole genome (1000 loci) after 3000 generations, the mean within-population diversity
varied between 0.060 and 0.509. There were differences for the rate at which the within-population diversity reached equilibrium, this rate was much slower when the migration rate was lower (Fig. 1e–h vs. Fig. 1a–d).

These scenarios also induced heterogeneity for the within-population diversity (population heterogeneity, \(PH\)), and among loci within the genome (genomic heterogeneity, \(GH\)). For example, the two migration models differed markedly for \(PH\). As expected, populations were much more heterogeneous for their level of diversity in the stepping-stone model than in the island model (Fig. 1a vs. Fig. 1b, Fig. 1c vs. Fig. 1d, Fig. 1e vs. Fig. 1f, and Fig. 1g).
vs. Fig. 1b). PH was in general three times higher for the stepping-stone than for the island model. It was also lower for high population size or high migration rates.

GH, as measured by the coefficient of variation of diversity among all 1000 loci, varied markedly in response to migration rates. At high migration rates (Fig. 1a–d), there was only a slight increase during the process, whereas at low migration rates (Fig. 1e–h), the heterogeneity within the genome increased more. There were also important differences of GH according to population size. Scenarios with low population sizes (Fig. 1c,d,g,h) were characterized by extremely high GH values (from 139% to 256%), in comparison to scenarios with high population sizes (Fig. 1a,b,e,f). The impact of the migration model on GH was however, limited.

Evolution of the correlations along the generations
Whatever the evolutionary scenario and whatever the marker system, the correlations of diversity between a set of marker loci and the total genome were low during the first generations and then increased constantly towards an equilibrium. Figure 2 illustrates the general pattern that was observed. There were important differences among the scenarios for (i) the length of the period taken by the correlation to reach an asymptotic value and (ii) the variation of the correlation between successive generations.

For high migration rates (Fig. 2a–d), correlations reached an equilibrium value within less than 500 generations. An exception is case 1 (island model), which is characterized by the lack of any variation of diversity among populations (see Fig. 1b).

For low migration rates (Fig. 2e–h), the correlations increased more progressively, but did not reach asymptotic values within 3000 generations.

Homogeneity of gene flow among populations (island model) was characterized by a larger stochasticity of the correlation over successive generations (Fig. 2b vs. Fig. 2a, Fig. 2d vs. Fig. 2c, and Fig. 2f vs. Fig. 2e).

Impact of different evolutionary factors on the final values of the correlations

Effect of population size. In general the correlation of diversity between marker loci and the whole genome increased when population size decreased from 1000 to 100 individuals. This trend was quite pronounced in the island model (Fig. 2b vs. Fig. 2d, and Fig. 2f vs. Fig. 2h) and much less in the stepping-stone model. For example, in the island model and for a pollen migration rate of 0.01, the correlation between 50 microsatellites and the whole genome was 0.278 for a population size of 1000 (Fig. 2b), and increased to 0.717 when the population size was 100 (Fig. 2d). The reduction of population size contributed to the inflation of the heterogeneity in the level of diversity among populations (Fig. 1b,f). Within the island model this general trend was, however, much more pronounced for microsatellites than for AFLPs. The difference of correlations for different population sizes was smaller for AFLPs. For 500 loci, the correlation increased from 0.367 (Fig. 2b) to 0.637 (Fig. 2d) for N decreasing from 1000 to 100.

Effect of migration rates. The effect of migration was quite complex. The major difference observed was related to the migration model. For the stepping-stone model, there was usually a reduction in the correlations as migration rate decreased. For m_p varying between 1% and 0.05%, the correlation between microsatellites and the whole genome decreased from 0.904 (Fig. 2a) to 0.521 (Fig. 2e) for 50 microsatellites. This trend did not occur for AFLPs. In most cases the value of the correlation did not change for AFLPs when migration rates decreased. In the island model, a decrease of pollen flow induced a slight increase of the correlations between the whole genome and the marker (Fig. 2b vs. Fig. 2f, Fig. 2d vs. Fig. 2h), whatever the population size and whatever the marker.

Effect of the heterogeneity of gene flow. The correlations measured in the island model were in most cases lower than for the stepping-stone model for a given set of parameters, except in case 4 (Fig. 2b vs. Fig. 2a, Fig. 2d vs. Fig. 2c, Fig. 2f vs. Fig. 2e). The difference could be quite important between the two migration models: from 0.904 (Fig. 2a) to 0.278 (Fig. 2b) for 50 microsatellites. Because gene flow was evenly distributed among all populations in the case of the island model, the populations tended to reach the same level of diversity as indicated by the low PH values (Fig. 1b). However, in the case of the stepping-stone model, populations located at the edges of the 5 × 5 grid received fewer migrating genes from the other populations than populations located in the central part of the grid. A gradient of diversity has therefore been formed from the internal to the external part of the grid. The level of correlation is therefore likely to be related to the level of population differences for their diversity. We extended the simulations of the stepping-stone model to the case were the populations at the edges of the grid system exchanged genes in a torus-like situation. The results obtained (data not shown here) were strictly comparable to the simulations conducted under the island model. The results support the conclusion that the stepping-stone model induces an important heterogeneity of diversity among populations located at the edges than the more internal populations, as was already revealed by the higher PH values obtained under the stepping-stone model.

Overall, the impact of the different evolutionary factors on the correlation were clearly illustrated by PH and GH.
At constant GH, the increase of PH always resulted in a corresponding increase of the correlation, as shown by the comparison of the stepping-stone models with the island models (for example Fig. 1a,b as compared to Fig. 2a,b). Similarly at constant PH, the increase of GH most generally resulted in a decrease of the correlation (for example Fig. 1e,d compared with Fig. 2e,d, or Fig. 1c,h compared with Fig. 2c,h). Hence PH and GH had opposite effects on the correlation. Furthermore, as some evolutionary factors had similar effects on GH and PH, their effect on the correlation between markers is unpredictable. For example, the reduction of population sizes or the reduction of migration rates increased both PH and GH (Fig. 1), which in turn had contrasting impacts on the correlation. The highest correlation
was obtained when a high value of PH was associated with a reduced value of GH (Fig. 2c), and the lowest when PH was low or a low PH value was associated with a higher GH value (Fig. 2b,f). We further tested other mechanisms that would generate higher PH values to verify its effect on the rank correlation. The additional simulations involved different population sizes among the 25 populations of a given scenario. For example, we monitored the evolution of PH and the rank correlation when the population size varied among the 25 populations between 400 and 800, instead of being constant as on Figs 1 and 2 (data not shown), all other parameters being constant. Variation in population size resulted in the increase of asymptotic PH values from 0.025 (for a population size of 1000, stepping-stone model) to 0.045 (population size varying from 400 to 800, stepping-stone model). The increase of PH had a stronger effect on the increase of the rank correlation, in comparison to the case where populations were of constant size 1000 (data not shown).

Trade-offs between markers for measuring diversity

First of all, as expected, the final value of the correlation increased as a function of the number of loci for a given marker (Fig. 3). The increase can be quite substantial as shown in Fig. 3(e), where the correlation varied from 0.146 to 0.521 when the number of microsatellite loci varied from 5 to 50 (case 3, stepping-stone model). Besides the expected effect of the number of loci on the level of the correlation, there were important differences according to the markers. Microsatellites exhibited more variable results across the different evolutionary scenarios. For example, the final values of correlation varied between 0.262 and 0.904 among the eight evolutionary scenarios when diversity was assessed with 50 microsatellite loci. Microsatellites proved to be the best predictors of the whole genome when the migration rate was high (Fig. 3a–d) but they were only poorly predictive at low migration rates (Fig. 3e–h). On the other hand, AFLPs were more robust, as they did not exhibit such a large range of variation. The final value of the correlation varied between 0.349 and 0.906 among the eight scenarios when diversity was assessed with 500 AFLP loci. The predictive value of a given marker system in comparison to another marker system is therefore variable according to the evolutionary scenario. In order to illustrate the trade-offs between markers, we compared their predictive value (correlation) for different sampling strategies (Fig. 2 and Fig. 3). In general, cases 3 and 4 benefited more from the increase of AFLP loci for predicting the diversity (Table 3). The number of loci in these cases compensated more efficiently the poor information available at each locus. On the other hand, in cases 1 and 2, the increase of AFLP loci, by a magnitude of 4–10 times the number of microsatellite loci, did not improve the prediction of the whole diversity in comparison to microsatellites.

<table>
<thead>
<tr>
<th>( N_{AFLP} / N_{microsatellites} )</th>
<th>Migration model</th>
<th>Scenarios where ( r_{AFLP} ) is:</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Stepping-stone</td>
<td>&gt; ( r_{SSR} ) = ( r_{SSR} ) &lt; ( r_{SSR} )</td>
</tr>
<tr>
<td>10</td>
<td>Island</td>
<td>3 and 4</td>
</tr>
<tr>
<td>4</td>
<td>Stepping-stone</td>
<td>1, 3 and 4</td>
</tr>
<tr>
<td>4</td>
<td>Island</td>
<td>1 and 3</td>
</tr>
</tbody>
</table>

*Ratio of the number of loci between AFLP and microsatellites.

Comparison between the correlations measured with the phenotypic analysis and with the genotypic analysis on AFLPs

For dominant markers as AFLP, diversity was computed either at the phenotypic level, by considering the frequencies of genotypes having a band, and at the genetic level by considering allelic frequencies for alleles responsible for the presence or absence of a band. Correlations between diversity estimates at the phenotypic and genetic level were of similar magnitude when population size was low (Fig. 2c,d,g,f), as expected, due to the higher fixation index values in those scenarios. When fixation rates are high, genotypic frequencies are closer to allelic frequencies (\( P_{mm} = q_i^2 + F p_i q_i \), where F is the fixation index) and a better congruence between diversity at the phenotypic and genetic level is expected. Hence differences between the correlations calculated at the two levels were higher when population sizes were higher. An interesting result was that the correlations computed for AFLP at the phenotypic level were still higher than those computed with microsatellites in cases 3 and 4 (Fig. 2e,f).

Discussion

This study was inspired by results often observed in the literature showing that diversity estimates were only rarely correlated when assessed with different marker systems. By using simulations under different evolutionary scenarios we intended to identify hypotheses to interpret these results. Some of the scenarios tested were selected to fit for temperate forest trees, which are known to exhibit large population sizes, important pollen flow (Hamrick et al. 1992) and a strong asymmetry between pollen and seed flow, and which are our model species. We aimed to address diversity at the whole genome level, but limited our computations to fully neutral markers by omitting the consideration of selection in the evolutionary scenarios.
As many marker systems used today are still anonymous and most probably located in noncoding regions, our conclusions would still be relevant. Diversity in nonneutral markers and in quantitative trait loci (QTLs) controlling adaptive traits are considered elsewhere (papers in preparation).

**Why do gene diversity surveys provide contrasting results among markers?**

Comparative analysis of gene diversity in natural populations with different markers (Zhang et al. 1993; Wolff...
et al. 1994; Isabel et al. 1995; Dubreuil & Charcosset 1998; Lannér-Herrera et al. 1996; Le Corre et al. 1997a) indicate generally that estimates of diversity made with different markers are not correlated (Kremer 1998; Mariette, 2001). Our simulation studies indicated that low correlations may be expected in three different situations: (i) low heterogeneity of populations for their diversity, (ii) high heterogeneity within the genome associated to low sampling efforts of markers within the genome, and (iii) recently created populations.

Low differentiation of populations for their diversity. For large populations and for high gene flow (case 1 of our simulations), populations tended to be poorly differentiated for their allelic frequencies and to show similar levels of genetic diversity. This was particularly the case in the island model. In this case, the differences in levels of diversity may be so small among populations that the ranking of populations that was observed was only generated by the random variation of diversity. An extreme case of such a scenario is illustrated by case 1 in the island model (Fig. 2b). According to these results, temperate forest tree species are the more likely among plant species to show low correlation of diversity among markers. First, they have colonized temperate regions for only a few hundred generations. Second, they tend to have large population sizes due to their life history traits. Populations of tree species such as Quercus are characterized by large population sizes and high gene flow (see for example Streiff et al. 1999). From our results, one can expect low correlations between markers for measuring diversity in these populations.

High heterogeneity within the genome. In the different simulations tested in this work, the increase of GH (at constant PH) was moderately associated with a decrease of the correlation of genetic diversity between a marker system and the total genome. The impact of GH is furthermore higher for more limited sampling of loci within the genome. This may be a trivial statistical interpretation of the low correlations usually observed, which was confirmed in all the scenarios tested in our simulations (Fig. 3). Whatever the evolutionary scenario, we showed that correlations between a type of marker and the whole genome increased with the number of markers sampled. This was also the case when two types of markers were compared (data not shown). For example the correlation between diversity estimated with five microsatellite loci and the diversity estimated with 50 AFLPs was always lower than 0.300.

Nonequilibrium situations. Our results indicated that, before within-population diversity reached an equilibrium between drift, migration and mutation (first generations of simulations), a substantial discrepancy between diversity assessed with markers and diversity over the whole genome was observed. During the first generations, correlations between markers were also very low, whatever the sample size of markers. The period of nonequilibrium situation varied according to the different scenarios. They lasted longer when the migration rate was low. This could explain the low correlations observed for example by Le Corre et al. (1997a) on Quercus petraea or by Isabel et al. (1995) on Picea mariana. Fewer than 300 generations of tree populations have elapsed since the last glaciations and the genetic equilibrium may not have been reached (Kremer 1994). The absence of correlation between markers which was observed on such species may be explained by the departure of the studied populations from genetic equilibrium.

Which strategy should be adopted to assess genetic diversity within populations?

Our simulations indicated that the different scenarios that were tested resulted in different levels of genomic and population heterogeneities (GH and PH). When summarizing the results across the different scenarios (Figs 1, 2 and 3), it appeared that GH and PH are to be considered as the key points for deciding upon a strategy for measuring within-population diversity. The comparison among the eight scenarios clearly showed that the final value of the correlation of diversity measured with a marker system and the whole genome is dependent on the level of heterogeneity of populations for their diversities (PH) and to a lesser extent on GH. As soon as an evolutionary factor increased PH (for example, in our case, the heterogeneity of gene flow), the correlation increased as well, whatever the marker system (Fig. 4). As a check, when we used different population sizes in the same set of
populations, we found that \(PH\) and the correlations between the genome and each marker were higher than when all population sizes were the same (data not shown). However, in most cases, \(PH\) is unknown when a gene diversity study is undertaken. Our results showed how \(PH\) may vary according to other different criteria (Table 4). For example, large population size and high migration rates tend to decrease the final \(PH\) values. If populations are at genetic equilibrium and differentiated for their diversity, any marker system with a sufficiently large number of loci will sort them correctly according to their level of diversity.

Genomic heterogeneity \((GH)\) showed important differences among the different scenarios and increased when population size or migration rates decreased (Fig. 1e–h). As \(GH\) increases, higher numbers of loci are needed to assess population diversity. Interestingly the comparisons of the correlation among the different marker systems indicated that cases 3 and 4 corresponded to situations where the increase of the number of loci improved the correlation, and where the trade-offs between markers were the most demonstrative. Larger \(GH\) values are to be expected when migration rate and population size decrease. As drift effects are more pronounced, the variations of allele frequencies among loci are expected to increase, and consequently \(GH\) as well. In those cases, it is recommended that the number of loci for assessing diversity be increased, even if the markers are poorly informative at each locus. Because AFLPs allow the sampling within the genome to be increased at low cost, they would be recommended for use in those cases.

Although dominant markers are subject to biases in the estimation of population genetics parameters when diploid material is used (Lynch & Milligan 1994; Krutovskii et al. 1999; Zhivotovsky 1999), our simulations (phenotypic analysis of AFLP loci, Figs 2 and 3) indicated that they could be efficient in estimating the diversity at the whole genome level. However, at least four times more dominant markers are to be used in order to attain the same efficiency as with co-dominant markers. When migration rates are higher and populations exhibit lower \(GH\), at least 10 times more dominant AFLPs markers would be required to supplement the prediction of co-dominant hypervariable markers.

This simulation study showed the interest of performing comparative genetic diversity analyses between different kinds of markers, both at theoretical and experimental levels. We chose to describe the evolution of populations of constant sizes, without selection. Our work should be extended to populations undergoing extinction and colonization. Moreover, the comparison between markers should be completed by the introduction of selected genes within the genome.

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References


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Table 4 Effects of different evolutionary forces on population heterogeneity and genomic heterogeneity (trends inferred from Fig. 1)

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<thead>
<tr>
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<th>Genomic heterogeneity (GH)</th>
<th>Population heterogeneity (PH)</th>
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<tbody>
<tr>
<td></td>
<td>Duration to equilibrium*</td>
<td>Delta†</td>
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<tr>
<td>Population size</td>
<td>++</td>
<td>–</td>
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<tr>
<td>Migration rates</td>
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<tr>
<td>Heterogeneity of gene flow</td>
<td>0</td>
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*Duration of the period taken by GH or PH to reach an equilibrium value.
†Difference of GH or PH between initial value (generation 0) and final value (generation 3000).
‡Final value of GH and PH.


This study was performed at the Population Genetics and Genetic Improvement of Forest Trees Laboratory of INRA Bordeaux/Pierroton in France as part of Stéphanie Mariette’s doctoral research. During her PhD, Stéphanie has compared genetic markers to assess diversity within natural populations. Valérie Le Corre develops simulation models in the field of plant population genetics, with a special interest in metapopulation dynamics and response to natural selection. Frédéric Austerlitz develops mathematical models in population genetics. Antoine Kremer has long-standing interests in the organization and dynamics of genetic variability in forest trees, particularly oaks.