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Quantitative variation as a tool for detecting human-induced impacts on genetic diversity

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Abstract

We propose quantitative genetic variation as a useful tool complementary to molecular variation in order to detect changes in biodiversity caused by different human-induced activities. We simulated a metapopulation setting under a number of realistic scenarios caused by anthropogenic activities (population isolation, reduced carrying capacity or reproductive rates, shifts in the local optima, and enhanced environmental variation or mutational rates). The effects on diversity of these scenarios were assessed for neutral variation estimated from molecular markers and for an additive quantitative trait that represents a typical morphological characteristic subject to stabilising selection promoting local adaptation to environmental conditions. The results show that monitoring quantitative genetic variation can be more informative than neutral variation to detect some human-induced environmental or genetic impacts on diversity, both at intra and interpopulation levels. We also compared the precision of diversity estimates obtained from molecular markers and quantitative traits. Under low migration rates and typical selection intensities for the quantitative trait, the precision of estimates can be substantially larger for a quantitative trait than for a single molecular marker. Thus, about 10–20 (2–4) independent markers are necessary for the precision of estimates of heterozygosity (population differentiation) from molecular markers to reach that of genetic variances (differentiation) from quantitative traits.

Keywords: Heterozygosity; Genetic variance; Population genetic differentiation; Pollution; Bottlenecks; Mutation

1. Introduction

It is clear that the majority of the recent reductions in the Earth's biodiversity can be attributed to direct human impacts on the environment (World Conservation Monitoring Centre, 1992). Such impacts are able to alter the ecosystems through population extinctions and depletions. A useful tool for monitoring the impact of human activities on natural populations is the detection of changes in genetic diversity (Belfiore and Anderson, 2001), as they can detect impacts even in the absence of population extinction or after recolonisation. Genetic diversity is overwhelmingly monitored by neutral molecular variation (Haig, 1998; Frankham et al., 2002). However, most environmental changes, even those caused by human activities, will directly affect different behavioural, anatomical, morphological, or life-history traits of the species functioning as bioindicators. Quantitative genetic variation has been suggested as an alternative or complementary tool to monitor genetic diversity in conservation contexts (Lynch, 1996; Storfer, 1996; Hedrick, 2001; Frankham et al., 2002).

The main advantage of monitoring quantitative genetic variation is that it may reveal variation more closely related to fitness and hence, it may be a better indicator of the evolutionary potential of populations (e.g. Bekessy et al., 2003). Moreover, variation for quantitative traits is polygenic, so it is more likely to be correlated with overall genetic variation than single marker

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loci. Thus, monitoring quantitative genetic variation may yield more direct information on the impact that environmental changes have on the capability of adaptation of the populations. A comparison of the degree of genetic diversity in neutral marker loci and quantitative traits can also provide insights into questions such as the importance of random genetic drift and directional natural selection as causes of population differentiation (Le Corre and Kremer, 2003; see also Toro and Caballero, 2005). In addition, the relationship between molecular variability and morphology, behaviour or life-history characteristics seems to be generally low (Ennos et al., 1997; Waldmann and Andersson, 1998; Butlin and Tregenza, 1998; Pfrender et al., 2000; Reed and Frankham, 2001; Merilä and Crnokrak, 2001; McKay and Latta, 2002), suggesting that these two types of variation can be complementary. Finally, estimates obtained from molecular markers and quantitative traits could involve different precisions. For example, a single biallelic molecular marker gives estimates of genetic distance with about the same precision as a neutral quantitative trait (Rogers and Harpending, 1983), but the precision of the marker increases with the number of alleles (Foulley and Hill, 1999; Kalinowski, 2002).

Artificial habitats, habitat fragmentation and population bottlenecks caused by human-induced activities might affect the extent and distribution of biodiversity at the genetic level. Most studies addressing the effects of environmental contaminants on genetic patterns are based on a comparison between molecular genetic variation at contaminated sites versus reference locations used as controls. In this paper, we propose quantitative genetic variation as a useful alternative or complementary tool to monitor genetic changes due to environmental contaminants. We use computer simulations to assess the potential ability of morphological versus molecular variability to detect changes in biodiversity, both at intra and interpopulation levels, caused by a variety of human-induced environmental or genetic effects. Thus, we assume a quantitative trait that represents a typical morphological characteristic subject to stabilising selection promoting adaptation to local environmental conditions in a metapopulation setting. This variation is compared to a neutral estimate from molecular markers.

Six different impacts of anthropogenic activities are simulated and compared under a number of realistic scenarios (see Hoffman and Merilä, 1999; Belfiore and Anderson, 2001; Straalen and Timmermans, 2002; Stockwell et al., 2003): (1) population isolation caused by fragmentation of habitats (artificial constructions, etc.) which will alter metapopulation structures, reducing the levels of gene flow between populations; (2) reduction in the carrying capacity of the populations (the maximum number of individuals in the population habitat as a function of the available space and resources, competitors, predators, etc.) caused by overexploitation, repeated catastrophic events, pollution, etc.; (3) reduction in reproductive rates caused by pollution; (4) shifts in the local optima to which the populations are adapted because of introduction of artificial habitats or environmental contaminants which will change habitat characteristics; and other possible effects of pollution, including (5) enhanced environmental variation; (6) increases in mutational rates. The general aim of the study is to ascertain whether monitoring quantitative variation can be more informative than molecular variation for detecting the above environmental or genetic human-induced impacts on genetic diversity, and to show that quantitative variation can be a useful tool complementary to molecular variation for conservation purposes. In addition, we compare the precision of the estimates obtained from quantitative and molecular genetic variation.

2. Models and simulation procedure

We developed a computer programme to simulate various anthropogenic impacts on the genetic diversity of natural populations. The programme allowed for a one-dimensional metapopulation with five individual populations following a stepping-stone migration model (Hartl and Clark, 1997; see Fig. 1). The number of individuals in each population (N) could vary, with the possibility of extinction and recolonisation via migration. However, the number of individuals could never achieve larger values than a maximum population size permitted (the carrying capacity), which was established at 500 per population (2500 for the whole metapopulation) to simulate sufficiently large population densities.

Migration rates (m) between adjacent populations were set at 0.001, 0.01 and 0.1 per generation. This implied that each population exchanged a proportion $m/2$ of their individuals with each neighbouring population, and those populations at the margins of the metapopulation had a rate of exchange half those of the inner ones. Note also that, because the number of individuals in each population could vary, the actual number of individuals entering or leaving the population could also vary.

The species simulated is a facultative hermaphrodite with random mating within populations and discrete

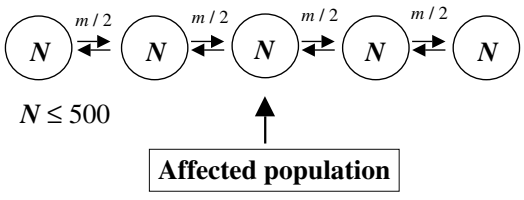

Fig. 1. Metapopulation structure. N: population size. m: per generation migration rate.

generations. Thus, new individuals were produced every generation from randomly chosen parents from each population. Each individual had a limiting reproductive rate, R, which is the maximum number of attempts it can breed. This number was set at 20 to simulate a species with moderate to high reproductive rate. Given the selection intensities investigated, this value is large enough to ensure population size recovery in most situations (see Fernández and Caballero, 2001). The sequence followed in the simulations for each generation was: migration of adults between populations, mating within populations with a maximum of R attempts per individual, and viability selection dependent on a quantitative trait (see below) until the maximum carrying capacity is produced.

2.1. Modelling neutral and quantitative trait variation

Neutral genetic variation within and between populations was monitored by a set of 100 neutral loci, initially with different alleles in all individuals, implying an initial heterozygosity of one. For the whole process, the decay in heterozygosity in neutral markers was partially compensated by a neutral mutation rate per locus and generation of 10^{-5} (Hartl and Clark, 1997, p. 163). Each mutation represented a new allele not present before in the metapopulation. As initially assigned and new mutant alleles were always different, identity-in-state equals identity-by-descent, and the average homozygosity of neutral loci was used to calculate the inbreeding coefficient of individuals.

A quantitative trait, intended to be a typical easy-to-measure morphological trait of intermediate heritability, was used as a tool to monitor genetic diversity under different anthropogenic factors. For the quantitative trait, genotypic values were controlled by an infinite number of genes of additive infinitesimal effect (the infinitesimal model; Fisher, 1918; Bulmer, 1980). This model has been shown to give robust predictions for the balance between migration and stabilising selection in structured populations (Tufto, 2000). The genotypic value of a new individual was obtained from a normal distribution with mean equal to the average genotypic value of its parents and the variance of the previous generation reduced by a factor equal to the average inbreeding coefficient of the parents. This is a model very frequently assumed to simulate quantitative trait variation for non-fitness traits (e.g. Verrier et al., 1991; Wei et al., 1996; Fernández et al., 2000; Dekkers and Chakraborty, 2001). The phenotypic value was obtained by adding an environmental effect to the genotypic value. Environmental effects were assumed to be normally distributed, with mean zero and variance $V_E = 1$. In the initial generation genotypic values were obtained from a normal distribution with mean zero and variance $V_A = 1$. Therefore, the initial genetic and phenotypic variances were one and two, respectively, and the initial heritability was 0.5. A mutational increase in genetic variance of $V_M = 10^{-3}V_E$ was assumed for the quantitative trait every generation. This is the consensus value estimated for many quantitative traits and species (Falconer and Mackay, 1996).

The quantitative trait was assumed to show some adaptive value through adaptation to optimal local environmental conditions. Fitness (w) was defined as a stabilising selection (pseudo-Gaussian) curve with an optimum θ initially set at 0 (the genotypic and phenotypic mean) and intensity V_s (basically the width of the fitness curve), so that larger values of V_s imply weaker selection. Thus, the fitness of an individual was $w = \exp\ [-(x-\theta)^2/2(V_s-1)]$ (Turelli, 1984), where x is the phenotypic value of the individual. Estimated values of V_s/V_E range from 5 to 50 (Roff, 1997), and we assumed the typical value of $V_s = 20$ (e.g. Turelli, 1984; Zhang and Hill, 2002). Inbreeding depression for this fitness value associated with the quantitative trait was modelled as a negative exponential reduction, such that the actual fitness of an individual is $w\exp(-F)$, where w is the individual fitness without inbreeding and F is its inbreeding coefficient (Keller and Waller, 2002). Because the inbreeding coefficient is calculated from the neutral markers, each individual can suffer different amounts of inbreeding depression depending on its genealogical history.

In the simulations, the fitness value of the offspring was evaluated and the new individuals might survive or die accordingly. Basically, an individual survived if a random number between 0 and 1 was smaller than its fitness value. If the individual died, new parents were randomly chosen. An individual was eligible as a parent if the number of offspring it had produced (surviving or not) had not exceeded the reproductive rate ($R = 20$). This procedure was repeated until the surviving offspring reached the population carrying capacity or all parents had spent all their R opportunities. Variation in the optima for the different populations allowed for simulating spatially heterogeneous selection. This implied different optima sampled from a normal distribution with mean zero and variance two genotypic standard deviations. For each replicate, adaptive optima were randomly allocated to the five populations of the metapopulation in the first generation, and were kept constant during all the generations simulated. We also ran some cases where the optima were placed in a clinal position in the metapopulation, with constant values of -2, -1, 0, +1 and +2 genotypic standard deviations for the consecutive populations of the metapopulation.

2.2. Effects of anthropogenic activities

In general, the simulation approach consisted of maintaining a base metapopulation until the differentiation

among populations reached an asymptotic value. The base population was then run until the change in population differentiation (F_{ST}) between consecutive generations was smaller than 0.01 (about 1000 generations). Then, a particular disturbance (simulating an environmental or genetic human-induced effect) was implemented in the central population (unless otherwise indicated) for 100 generations. Thus, we focused on the long-term impact of the following possible effects of anthropogenic activities:

Isolation (I). This was simulated by completely isolating the central population from the rest of the metapopulation by eliminating migration to and from the adjacent populations.

Reduced carrying capacity (K). The maximum population size (carrying capacity) allowed in the affected central population was reduced, producing an effective population bottleneck. We imposed reductions by factors of 10 and 100, i.e., implying carrying capacities of 50 and 5, respectively. The result is equivalent to a population bottleneck (loss of 90% and 99% of the population, respectively) after the breeding period.

Reduced reproductive rate (R). All specimens living in the affected population suffered a decrease of the maximum reproductive capability. We imposed a reduction from the usual $R = 20$ to $R = 1$ and 0.2 offspring attempts per individual and generation.

Optimum shift (O). The affected population suffered a displacement of the local optimum for the quantitative trait. We imposed a displacement of 2, 6 and 10 genotypic standard deviation units from the original optimum.

Increased environmental variance (E). Individuals from the affected population suffered an increase in environmental variance for the quantitative trait. The increases used were 25- and 100-fold, causing a rather platykurtic phenotypic distribution, and an increase in mortality in the portions of the population far apart from the local optimum.

Increased mutation rates (M). Individuals from the affected population suffered an 100 or 1000-fold increase in the per-generation mutation rate for the neutral loci and a corresponding increase in the mutational genetic variance (V_M). As the latter is directly proportional to the haploid mutation rate (Hill, 1982), the simulated changes in mutation rates are equivalent for neutral and quantitative variation.

In principle, factors (1), (2), (3) and (6) affect both the neutral and quantitative variation, whereas factors (4) and (5) affect exclusively the quantitative trait. A simulation case where no population is affected was considered as a reference for comparison with the above situations. Between 50 and 100 replicates were run for each of the treatments (different types and magnitudes of anthropogenic effects, and migration rates). Additional simulations were carried out in particular cases to assess the absence of spatially heterogeneous selection, different stabilising selection intensities, the effect of marginality (the affected population is a marginal one), and clinal local optima.

2.3. Estimates of genetic diversity

After 100 generations of each particular environmental or genetic human-induced effect, genetic diversity within and between populations was measured. Intrapopulation diversity was estimated by the heterozygosity of molecular variation (H; Nei, 1987) and by the additive genetic variance of the quantitative trait (V_A; Falconer and Mackay, 1996). These two parameters have identical expectations for purely neutral variation. Estimates were obtained only for the affected (usually central) population, and averaged over loci (H) and replicates (H, V_A).

Interpopulation diversity was measured for neutral variation by Wright's fixation index, F_{ST} (Wright, 1951; Nei, 1987), which was computed using averaged population and metapopulation mean coancestries (equation 6 of Caballero and Toro, 2002). Analogously, a similar estimate was obtained for quantitative traits using the between (V_{Ab}) and within (V_{Aw}) population genetic variances, $Q_{ST} = V_{Ab}/(2V_{Aw} + V_{Ab})$ (Spitze, 1993). A single-classification ANOVA with unequal sample sizes was used to estimate quantitative variance components (Sokal and Rohlf, 1995, p. 210) using the genotypic values of individuals. Genetic differentiation was estimated from all five populations and was averaged over loci (neutral variation) and replicates (neutral and quantitative variation). Population genetic differentiation for molecular (F_{ST}) and quantitative (Q_{ST}) traits has identical expectations for neutral variation (Whitlock, 1999), and when disequilibria among loci is of the same amount within and between populations (Le Corre and Kremer, 2003), but its relationship is under discussion for other situations (Crnokrak and Merilä, 2002; McKay and Latta, 2002; Hendry, 2002; López-Fanjul et al., 2003).

2.4. Precision of estimates

We compared the precision of the estimates of within and between population diversity by calculating the variation among replicates of the estimates of H and F_{ST} from a single molecular marker, and from V_A and Q_{ST} from a single quantitative trait. For some basic simulations we ran the metapopulation with no migration and no selection until an inbreeding coefficient 0.2 or 0.4 was reached. The marker used was allowed to have two, four or eight alleles initially at equal frequencies. The quantitative trait was controlled by one, two, five or 10 biallelic additive loci initially at equal frequencies and constant effects, so as to produce

an initial additive variance equal to the heterozygosity of a biallelic marker. An infinitesimal model as explained before was also considered. Other data from the precision of estimates were obtained from the same scenarios analysed before, allowing for migration among populations and stabilising selection for an infinitesimal quantitative trait. In the selection cases and, in order to make fair comparisons between precisions, variation of adaptive optima among populations was assigned so as to produce similar values of population differentiation from marker and quantitative variation. The variance of estimates among replicates was obtained in all cases from six sets of 50 replicates each, in order to get their standard error.

3. Results

Fig. 2 shows the average heterozygosity (H) of neutral loci and the average genetic variance (V_A) for the quantitative trait in the central population, and Fig. 3 the average differentiation for neutral loci (F_{ST}) and the quantitative trait (Q_{ST}), for the six studied effects of anthropogenic factors and different migration rates.

The continuous line in all panels represents the reference situation in which there is no anthropogenic effect on the central population, showing an increase in genetic diversity and a decrease in population differentiation with the rate of migration, as expected. The equilibrium H and V_A in these cases are below the initial values of one due to the genetic drift and selection occurred during the previous population history (heritabilities for the quantitative trait are between 0.33 and 0.44). Broken and dotted lines represent situations in which there is some anthropogenic effect of different magnitudes (see figure details).

Panels labelled I in Figs. 2 and 3 show diversity parameters after 100 generations of *isolation* of the central population. Continuous isolation produces a similar decrease, relative to the reference situation, in the amount of intrapopulation diversity both for neutral (H) and adaptive (V_A) variation (panels 2I). The effect on interpopulation differentiation is different for molecular or quantitative variation. Isolation of the central population slightly increases, relative to the reference situation, the overall population differentiation for higher migration rates, as expected, but the effect for quantitative trait differentiation (Q_{ST}, panel 3I-right) is much more pronounced than for molecular markers (F_{ST}, panel 3I-left). The reason is that an isolation of the central population causes an interruption of gene flow at the metapopulation level, impeding the homogenising effects of migration. Even though heterogeneous local optima for the quantitative trait are randomly assigned to the populations in the simulations, there can be an increase in quantitative trait differentiation between both halves of the metapopulation (right- and

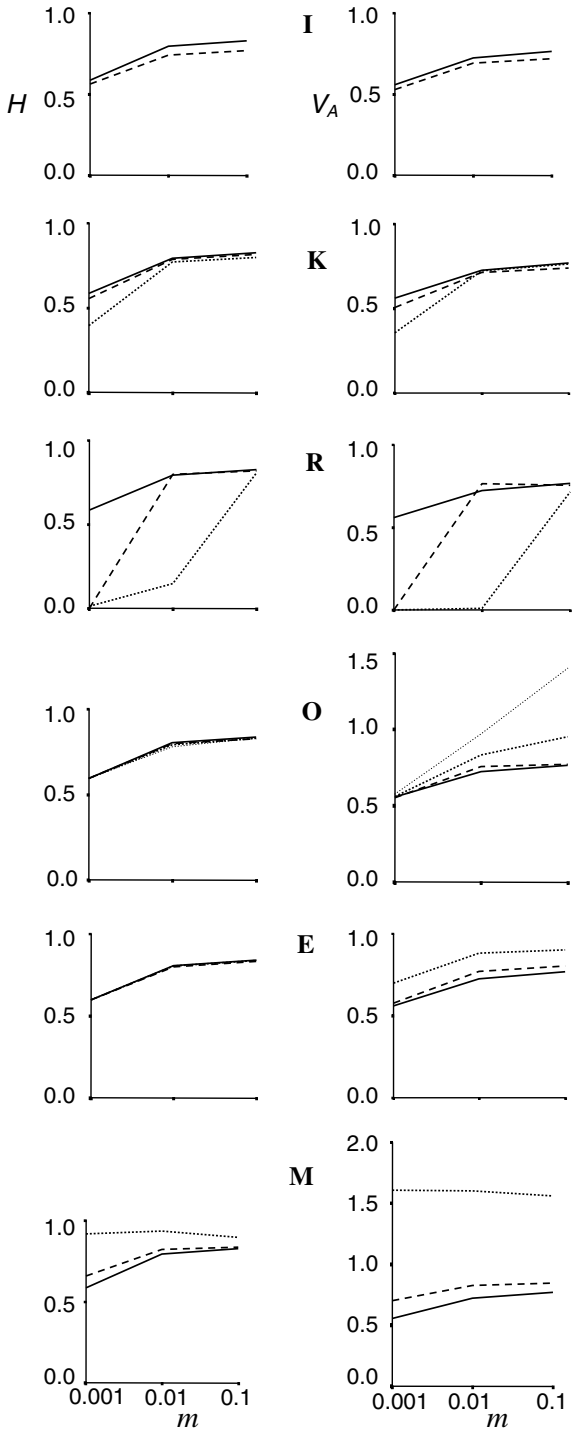

Fig. 2. Average heterozygosity (H) and genetic variance (V_A) in the central population for different migration rates (m). Continuous lines: central population not affected. Broken and dotted lines: central population affected. I: *isolation*. K: *reduced carrying capacity* (broken: $K = 50$, dotted: $K = 5$). R: *reduced reproductive rate* (broken: $R = 1$, dotted: $R = 0.2$). O: *optimum shift* (broken: 2 genotypic standard deviations, large dots: 6 genotypic standard deviations, small dots: 10 genotypic standard deviations). E: *increased environmental variance* (broken: 25-fold, dotted: 100-fold). M: *increased mutation rate* (broken: 100-fold, dotted: 1000-fold). Standard errors below 0.04 for H, and below 0.07 for V_A.

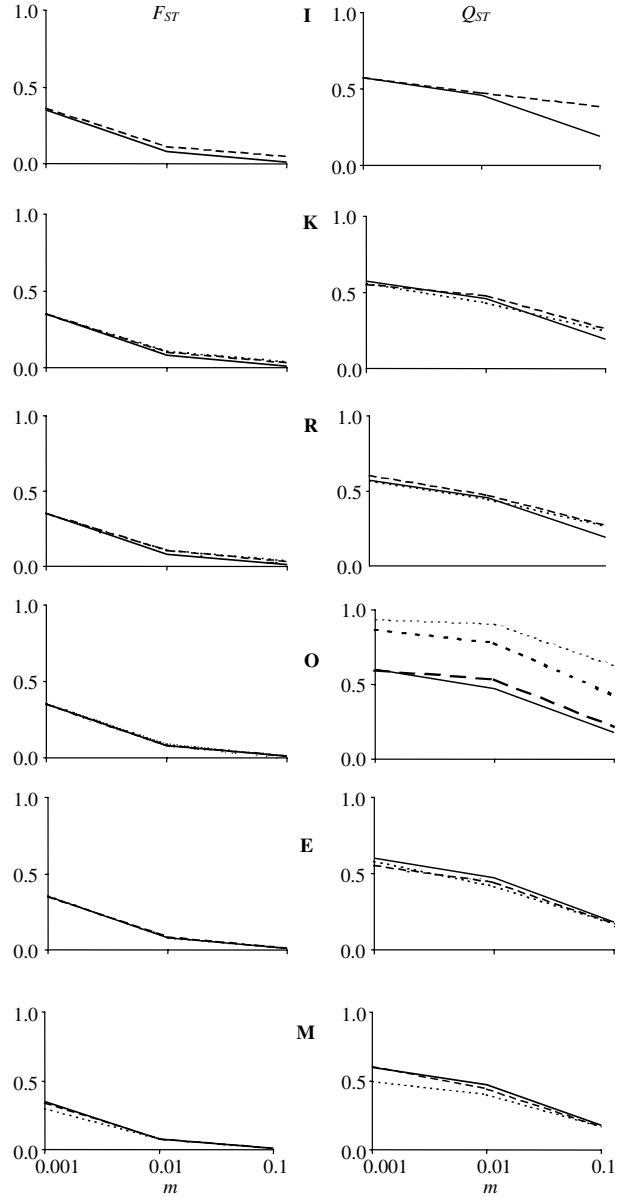

Fig. 3. Average neutral differentiation (F_{ST}) and quantitative differentiation (Q_{ST}) for different migration rates (m). Continuous lines: central population not affected. Broken and dotted lines: central population affected. See Fig. 2 for identification of panels and lines. Standard errors below 0.002 for F_{ST}, and 0.03 for Q_{ST}.

left-hand sides of the affected central population) because of different global optima in each half.

Panels labelled K in Figs. 2 and 3 show results after 100 generations of a *reduced carrying capacity* (basically a population bottleneck) in the central population, implying a constant reduced carrying capacity of $K = 50$ (broken line) and 5 (dotted line) individuals. Both neutral H and quantitative V_A are reduced, relative to the reference situation, for low migration rates and recovered at high levels of migration (panels 2K). This shows the powerful effects of migration in restoring genetic diversity despite the long-term bottleneck applied. The behaviour of neutral (F_{ST}) and quantitative (Q_{ST}) differentiation (panels 3K) is similar to those for *isolation* (panels 3I), although the effect on Q_{ST} is less marked. Because the population census size in the central population is reduced, there is less gene flow between the two halves of the metapopulation, again reducing the homogenising effects of migration. Thus, a bottleneck in the affected population produces its effective isolation, and this has an impact on the quantitative trait metapopulation differentiation when the affected population has a central position.

Panels labelled R in Figs. 2 and 3 show results after 100 generations of a *reduced reproductive rate* in the central population involving a reduced $R = 1$ (broken line) and 0.2 (dotted line) breeding attempts per individual. This effect has a major impact on the population size of the affected population, impeding the population from reaching its maximum carrying capacity. For $m = 0.001$, the central population experiences extinction in 99% of the replicates ($R = 1$ or 0.2). For $m = 0.01$, the central population becomes extinct 2% ($R = 1$) and 82% ($R = 0.2$), with an average $N = 3.3$ ($R = 1$) and $N = 1.1$ ($R = 0.2$) in the remaining cases. Finally, for $m = 0.1$ the central population never becomes extinct but the average population size becomes $N = 30.7$ ($R = 1$) and $N = 4.2$ ($R = 0.2$). It is interesting to see that, even though the average of the population size can be very low, H and V_A are completely recovered with $m \geqslant 0.01$ ($R = 1$) and $m \geqslant 0.1$ ($R = 0.2$) (see panels 2R). The larger H with respect to V_A for $m = 0.01$ is due to the fact that, in many runs, the population size has $N = 1$. In these cases V_A is assumed to be zero while H can be different from zero. With respect to population differentiation (Fig. 3R), similar conclusions are obtained as for the two previous studied effects. Again, because of the reduced population size in the affected central population, higher Q_{ST} occurs for high levels of migration (see panel 3R-right) when spatially heterogeneous selection is present.

In order to confirm the above interpretation, we simulated some extra cases with larger migration rates ($m = 0.2$ and 0.3) and different scenarios. Fig. 4a shows the difference $Q_{ST} - F_{ST}$ for the same situation as in Fig. 2. The continuous line is the reference case with no affected population. The discontinuous lines represent the cases of *isolation*, *reduced carrying capacity* ($K = 50$) and *reduced reproductive rate* ($R = 1$). For low migration rates ($m \leqslant 0.01$) the difference $Q_{ST} - F_{ST}$ is high in all cases. For larger migration rates the difference is reduced when no anthropogenic effects occur, but the difference is kept large when these occur, as gene flow is reduced between both sides of the central affected population. Fig. 4b shows a case where the affected population is placed at a marginal position. The large values of $Q_{ST} - F_{ST}$ disappear at a high migration rate for

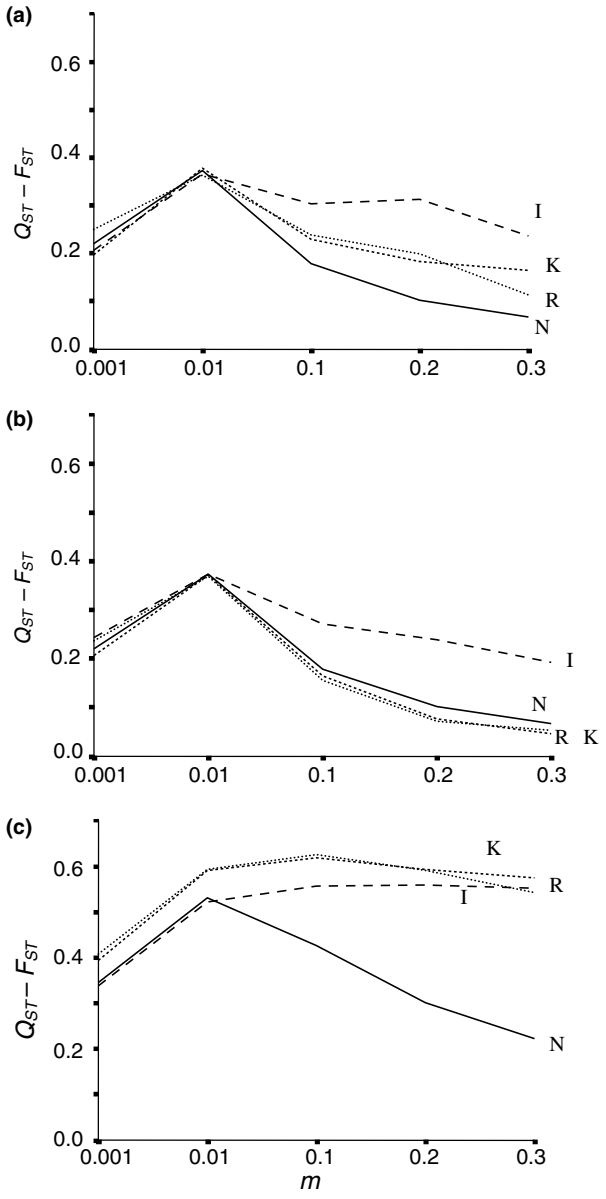

Fig. 4. $Q_{ST} - F_{ST}$ difference for a range of migration rates (m). (A) Affected central population with random spatially heterogeneous selection. (B) Affected marginal population with random spatially heterogeneous selection. (C) Affected central population with clinal adaptive local optima. N: central population not affected. I: *isolation*. K: *reduced carrying capacity* ($K = 50$). R: *reduced reproductive rate* ($R = 1$). Standard errors below 0.02.

reduced carrying capacity and *reduced reproductive rate*, whereas these are kept for the *isolation* effect, which implies a lack of gene flow in any case. Finally, Fig. 4c shows a case where the affected population is placed in a central position and the local optima are not random, but are placed in a continuous gradient (clinal local optima). In this case, a *reduced carrying capacity*, a *reduced reproductive rate* or a direct *isolation* produce a genetic bipartition of the metapopulation, causing a permanent difference between Q_{ST} and F_{ST}. This indicates that an affected population in a central position of an environmental cline, and suffering from a reduced gene flow because of environmental or genetic-induced effects, will show a substantial $Q_{ST} - F_{ST}$ difference. The immediate conclusion is that an experimental design to compare affected and non-affected populations in a lineal metapopulation setting should be made such that the putatively affected population is central to unaffected control sites.

Panels labelled O in Figs. 2 and 3 show results after 100 generations of an *optimum shift* in the central population of 2 (broken line), 6 (large dotted line) and 10 (small dotted line) genotypic standard deviations. The impact on fitness of the latter optimum shifts was quite large. The average fitness in the population before the shifts was 0.95, and this went down to 0.87, 0.40 and 0.09, respectively, immediately after applying the shifts. Despite these strong impacts on fitness, no population extinctions or reductions in the average population size were observed after 100 generations. The reason is an adaptation of the populations to the new optima. There is no effect of the shifts for neutral variation (H; panel 2O-left) but quantitative variation (V_A; panel 2O-right) is increased for a high migration rate. This is caused by the migrants coming from adjacent populations that, although adapted to different optima, still survive in the central population, increasing its variance. With respect to population differentiation (panels 3O), there is no effect on F_{ST}, whereas the optimum change causes large increases in the quantitative differentiation.

Panels labelled E in Figs. 2 and 3 show results after 100 generations of a 25-fold (broken line) and 100-fold (dotted line) *increased environmental variance* in the central population. These increases in environmental variation and hence, phenotypic variance, produce a higher mortality (the average fitness becomes 0.65 and 0.39, respectively, in the first generation after the increase in environmental variance occurs), although no extinctions occurred in the central population after 100 generations. A substantial number of extinctions occurred, however, for low migration rate ($m = 0.001$) and a 1000-fold increase in V_E (not shown). There is no effect for neutral variation (H; panel 2E-left) but quantitative variation (V_A; panel 2E-right) is increased irrespective of migration rate. The explanation is that the increased environmental variance reduces the heritability of the quantitative trait in the affected population, allowing some extreme genotypes to avoid purging selection. That is to say, the increased environmental variance reduces the selection intensity. With respect to population differentiation (panels 3E), there is no effect on F_{ST}. On the contrary, because the within population variance in the central population is increased, there is a corresponding decrease in the quantitative differentiation.

Finally, panels labelled M in Figs. 2 and 3 show results after 100 generations of a 100-fold (broken line) and 1000-fold (dotted line) *increased mutation rates* in

the central population. Although the enhanced mutation rates increase the genetic and phenotypic variance, the reduction in the average fitness is small, and no extinctions are observed. Neutral variation (H; panel 2M-left) is substantially increased for the largest change in mutation rate, though it does not reach the maximum value of one. The corresponding increase in quantitative variation (V_A; panel 2M-right) is proportionately larger. The reason for a larger proportional effect of an increase in mutation rates on quantitative versus molecular variation may be simply a question of scale, as heterozygosity can only reach a maximum value of one, whereas the genetic variance has no upper bound. With respect to population differentiation, the increase in H provokes a lower F_{ST} for low migration rates, but this is recovered for higher migration rates (panel 3M-left). The same effect, but more pronounced, is seen for quantitative differentiation (panel 3M-right).

The precision of the estimates of within and between population genetic diversity from a single molecular marker and a single quantitative trait is shown in Table 1 for a simple case involving no selection on the quantitative trait and no migration among populations. The populations are run until an inbreeding coefficient of $F = 0.2$ is reached (i.e., $F_{ST} \approx Q_{ST} \approx 0.2$, though F_{ST} is somewhat lower because there is not an infinite number of marker alleles). Results for $F = 0.4$ were also run giving similar results (not shown). As expected, the precision of a biallelic marker is about the same as that of a quantitative trait controlled by a single biallelic locus, both for within (H and V_A) and between (F_{ST} and Q_{ST}) population diversity. (The variance of heterozygosity for a neutral biallelic locus can be computed as $V(H) = 4[m_2 - m_1^2 + m_4 - 2m_3 + m_2(m_2 - m_1)]$, where m_i is the ith moment about zero of the binomial distribution, that can be obtained from Crow and Kimura, 1970, p. 335.) An increase in the number of alleles of the molecular marker increases the precision (the standard deviation is reduced) both for H and F_{ST}. This has been deduced theoretically in the case of genetic distances (Foulley and Hill, 1999). The increase in the number of loci controlling the quantitative trait substantially enhances the precision of V_A. This is due to the cancelling of random changes in gene frequency because of genetic drift occurring at different loci. (For an infinitesimal model of gene effects, the variance of V_A can be approximated using the moments about zero for a Normal (0, σ) distribution, as $V(V_A) = 2\sigma^4[(N-1)/N^2]$.) Note that the precision for a quantitative trait controlled by a few loci (say 10) is similar to that for an infinitesimal model. On the contrary, the precision of estimates of Q_{ST} does not increase with the number of loci, as deduced by Rogers and Harpending (1983).

Table 2 shows the precision of estimates under the more realistic scenario investigated previously, which includes migration among populations and stabilising selection for the quantitative trait. No human-induced effects are assumed in the central population. For each migration rate and selection regime, the estimates of diversity are very similar for the molecular marker and the quantitative trait in all cases, so that the comparisons between precisions are fair. Under no selection ($V_s = \infty$) the precision of V_A is larger than that of H for low migration rates, whereas molecular differentiation (F_{ST}) has more precision than quantitative differentiation (Q_{ST}). Under a typical intensity of selection ($V_s = 20$) and low migration rates, the precisions of both within and between-population diversity from a quantitative trait are higher than those from a single marker. We used increasing numbers of markers to ascertain how many of these are necessary to reach the precision attained by a single quantitative trait. With $V_s = 20$ and $m = 0$–0.001, between 10 and 20 independent markers are necessary for the precision of H to be the same as

Table 1
Estimates and their precisions (given as the standard deviation, SD, of estimates among replicates ± standard error, SE) from a single molecular marker with 2, 4 or 8 alleles, and a single quantitative trait controlled by 1, 2, 5, 10 biallelic loci or an infinitesimal model, for a metapopulation with no migration and no selection for the quantitative trait

Molecular marker			Quantitative trait		
Alleles	H SD(H) ± SE	F_{ST} SD(F_{ST}) ± SE	QTLs	V_A SD(V_A) ± SE	Q_{ST} SD(Q_{ST}) ± SE
2	0.40	0.16	1	0.39	0.20
	0.124 ± 0.006	0.108 ± 0.005		0.120 ± 0.003	0.112 ± 0.008
4	0.60	0.17	2	0.40	0.20
	0.108 ± 0.005	0.065 ± 0.003		0.092 ± 0.003	0.104 ± 0.008
8	0.70	0.17	5	0.40	0.19
	0.092 ± 0.003	0.043 ± 0.002		0.072 ± 0.004	0.113 ± 0.009
			10	0.39	0.19
				0.061 ± 0.002	0.099 ± 0.006
			∞	0.40	0.19
				0.049 ± 0.002	0.100 ± 0.008

The populations are run until the expected inbreeding coefficient is 0.2.

Table 2
Estimates and their precisions (given as the standard deviation, SD, of estimates among replicates ± standard error, SE) from a single molecular marker, and a single quantitative trait controlled by an infinitesimal model of gene effects, for a metapopulation with migration m and intensity of stabilising selection V_s for the quantitative trait

	Alleles	H SD(H) ± SE	V_A SD(V_A) ± SE	F_{ST} SD(F_{ST}) ± SE	Q_{ST} SD(Q_{ST}) ± SE
$V_s = \infty$					
$m = 0$	2.3 ± 0.0	0.37	0.34	0.60	0.59
		0.214 ± 0.004	0.033 ± 0.001	0.104 ± 0.002	0.182 ± 0.006
$m = 0.001$	4.9 ± 0.1	0.59	0.60	0.35	0.42
		0.142 ± 0.007	0.043 ± 0.003	0.063 ± 0.002	0.171 ± 0.004
$m = 0.01$	9.4 ± 0.2	0.80	0.80	0.08	0.11
		0.053 ± 0.002	0.057 ± 0.002	0.024 ± 0.001	0.085 ± 0.005
$V_s = 20$					
$m = 0$	2.4 ± 0.0	0.47	0.46	0.47	0.50
		0.165 ± 0.011	0.034 ± 0.001	0.080 ± 0.005	0.042 ± 0.002
$m = 0.001$	4.1 ± 0.1	0.59	0.57	0.35	0.37
		0.143 ± 0.005	0.046 ± 0.001	0.060 ± 0.002	0.038 ± 0.002
$m = 0.01$	9.3 ± 0.2	0.80	0.75	0.08	0.07
		0.053 ± 0.004	0.052 ± 0.001	0.023 ± 0.001	0.024 ± 0.001

The average number of alleles segregating for the molecular marker at the time of the analysis is indicated.

that for V_A from a single trait, and between 2 and 4 independent markers are necessary for the precision of F_{ST} to be the same as that for Q_{ST}.

4. Discussion

Although nearly all empirical studies on genetic diversity and conservation rely on molecular markers as general indicators of genetic variation (Haig, 1998; Frankham et al., 2002), quantitative variation, particularly adaptive one, may yield more interesting information on the effect of genetic and environmental changes on genetic diversity (Storfer, 1996; McKay and Latta, 2002; Bekessy et al., 2003). In this paper we have evaluated the ability of quantitative variation to detect the impact of a series of described genetic or environmental effects on genetic diversity caused by human activities under different evolutionary scenarios. The quantitative trait under screening represents a typical morphological characteristic showing some local adaptation to environmental conditions, and its variation is compared to a neutral one arising from molecular markers in a metapopulation setting.

Four of the anthropogenic effects were general ones involving all kinds of variation: population *isolation*, *reduced carrying capacity* (population bottleneck), *reduced reproductive rate* and *increased mutation rates*. Regarding the impact of these effects on intrapopulation variability (panels I, K, R and M in Fig. 2), no substantial difference was found between molecular and quantitative variation, although the impact of mutation was proportionally larger for quantitative variation than for molecular variation. This can be partially a question of scale, as heterozygosity has an upper bound of one whereas genetic variance for a quantitative trait has no upper bound, but suggests that quantitative variation can be more powerful to detect such effects than molecular variation.

Our interest was not on the impact of anthropogenic factors on the extinction of populations, as the trait under study was generally assumed to have a weak relationship with fitness. The only case where extinction appeared was that in which a reduction in the reproductive rate under low migration levels occurred (panels R in Fig. 2). In these cases, the population size of the affected population suffered from a long lasting reduction because of the low reproductive rate and reduced fitness, and new migrants were not able to recover the population size in any case. For intermediate or high migration rates, however, migration was able to restore molecular and quantitative variation, despite the low population sizes maintained. It is also remarkable that a prolonged drastic reduction in the carrying capacity (a 100-fold reduction in the population size from $N = 500$ to 5 for 100 generations) did not show an appreciable effect on diversity in the affected population (panels K in Fig. 2). This indicates that the effects of migration are really powerful in restoring genetic diversity even with very low census sizes. This may explain why the loss of heterozygotes is, sometimes, far less than expected from population bottlenecks (Amos and Balmford, 2001, and references therein; Colson and Hughes, 2004). It is also in agreement with a number of documented cases failing to show a reduced intrapopulation genetic diversity in populations exposed to environmental contaminants (Ma et al., 2000; Belfiore and Anderson, 2001 and ref-

erences therein; Ross et al., 2002 and references therein). For example, Ross et al. (2002) brought attention to the fact that a long-term exposure to metal pollution did not necessarily result in decreased genetic diversity.

The two remaining anthropogenic effects studied (*increased environmental variance* and *optimum shift*) directly affected quantitative variation. An increase in the environmental variance (panels E in Fig. 2) produces an increase in genetic variance at the quantitative trait, independent of the migration rate, which is not observed with molecular variation. The increase in environmental variance reduces the intensity of selection and more variation is maintained for the quantitative trait. The effect of a change in the local optimum of the population leads to an adaptation to the new conditions. This has been frequently observed, for example, in the cases of rapid evolution of tolerance to heavy metal pollution in plants and fungi (e.g. Taylor, 2000). Our simulations show that an adaptation to the new optima produces an increase in quantitative genetic variance, particularly for high levels of migration (see panel O-right in Fig. 2). This is explained by the immigration of individuals from neighbouring populations with different local optima, which are still able to survive in their new habitat (see, e.g. Barton, 1999, and references therein). The increase of genetic variance in the affected population is coupled with a decrease in the population fitness of about 15–30%. This is consistent with the so called migration load, the decrease in mean fitness of a population because of immigration of individuals far apart from the optimum (Lenormand, 2002). This decrease in population fitness, however, is not enough to cause an appreciable reduction in the variation at the molecular level (panel O-left in Fig. 2).

In terms of interpopulation variability (Fig. 3), two interesting results appear from quantitative variation. Under *isolation* (I), *reduced carrying capacity* (K) or *reduced reproductive rate* (R) in a central population, quantitative differentiation is maintained at high levels of migration (Figs. 3 and 4A). This occurs because all these effects, either directly (I) or indirectly (K, R), restrain the gene flow at both sides of the metapopulation, splitting it into two parts, and the effect is enhanced when the metapopulation has a clinal set of local optima (Fig. 4C). The effects produced by the population bottleneck disappear when the affected population is a marginal one (Fig. 4B) and are also likely to disappear in an island or circular stepping-stone model of subdivision. Thus, for monitoring the impacts of human-induced effects on linear metapopulation settings, the results suggest that the affected population should be better flanked by unaffected reference sites. This may be of use for the study of pollution or other anthropogenic effects in linear displays such as coastal systems or river lines.

It is also worthwhile to notice the impact of an *increased environmental variance* or an *increased mutation rate* on quantitative differentiation (panels E and M in Fig. 3). The general effect is a reduced differentiation at low migration rates that is unlikely to be observed with molecular variation. The reason is that an increase in genetic variation, produced either directly (through an increase in mutation rates) or indirectly (through reduced selection intensity because of a higher environmental variance), is responsible for a lower relative interpopulation differentiation.

The model investigated assumes presence of spatially heterogeneous selection (diversifying selection) for the quantitative trait, as this seems to be the most common form of selection in natural populations (McKay and Latta, 2002; Le Corre and Kremer, 2003). This implies that each population becomes adapted to different random (or clinal) optima, but cases were also run with no spatially heterogeneous selection (all populations have the same optima). In this situation, the values of Q_{ST} were always close to zero (except for the optimum shift effect), because stabilising selection for the quantitative trait maintained genotypes close to the common fitness optimum. Thus, absence of spatially heterogeneous selection would make quantitative differentiation of very little use in detecting metapopulation diversity changes. Situations where F_{ST} is considerably larger than Q_{ST} have been observed on some occasions (e.g. Petit et al., 2001).

In our comparison between quantitative traits and molecular marker data, we have assumed that the neutral markers are unlinked to the quantitative trait loci, as we are dealing with a single quantitative trait in a possibly multi-chromosome species. If a neutral marker were linked to a quantitative trait locus of sufficiently large effect, some of the disturbances affecting the quantitative trait would also affect the marker. However, this effect would get vanished if several unlinked markers are used in addition to the linked one. Several recent developments (see Vitalis et al., 2001 and references therein) address the issue of identifying signatures of selection among molecular markers.

Life-history traits usually exhibit large variation from environmental and nonadditive genetic sources (Falconer and Mackay, 1996; Roff, 1997), complicating the interpretation of the estimates of additive genetic components. However, many morphological traits show low levels of non-additive genetic variation, are less prone to variation from environmental sources, but still can show some adaptation to environmental conditions, making them very attractive for screening genetic diversity. We have illustrated the impact of anthropogenic activities under the basic assumption that the quantitative trait under study has an additive genetic basis. It is expected that some of the results would be differently affected under more general conditions. For example, if

the trait has a substantial dominance variance component, the enhanced inbreeding caused by an isolation or a bottleneck could produce an increase in the additive genetic variance (Robertson, 1952). Nevertheless, the possible biases in the estimation of additive genetic components are not a serious drawback in the context of detecting human induced impacts on genetic diversity, as the main objective is to compare the amount of genetic variation in affected versus non-affected populations, rather than estimating it accurately.

Genetic variation for many quantitative traits is usually difficult or impossible to assess in natural conditions, generally involving breeding and development in laboratory conditions. For some invertebrate species, however, obtaining genetic components of variation can be cheaper and faster than monitoring molecular variation, particularly when the quantitative traits of choice are easy-to-measure, such as morphological characteristics. For example, intertidal rocky shore marine species, like periwinkles (genus *Littorina*) are ovoviviparous and half/full-sib shelled embryos are available within pregnant females for estimating quantitative genetic variability of different morphological traits (Newkirk and Doyle, 1975; Carballo et al., 2001). In other instances, such as dogwhelks (genus *Nucella*), half/full-sib shelled embryos are available from laid egg masses. Our simulation conditions, although general in many aspects, refer more specifically to the above cited systems, basically species living in shore-line habitats with a patchy distribution. Thus, the system deals with a linear stepping-stone migration model in a species of a moderate to high reproductive capacity, which may be representative of many groups of marine organisms living in a littoral coast such as some algae and bivalves, as well as many gastropods (Fretter and Graham, 1980; Berger, 1983; Ward, 1990). A negative relationship between dispersal capability and degree of population differentiation in allozymes has been shown in 33 species from 27 animal groups (Bohonack, 1999), which is interpreted as that an equilibrium between genetic drift and migration is the most relevant evolutionary factor dealing with population genetic differentiation. The stepping-stone model may be more adequate than the island model for predicting the levels of population differentiation in benthic species with low dispersal capability living on the intertidal shore habitats, because in such circumstances the geographical distribution of the species occurs across one single dimension (the shore line).

A criticism to the use of quantitative traits as an alternative to molecular markers for monitoring genetic variation is that it may be easier to score several independent molecular markers than several independent quantitative traits. In this context, the precision of the estimates obtained by each approach is very relevant. Here we have shown that, under low migration rates and reasonable selection regimes, a quantitative trait is more precise than a single marker, needing about 10–20 independent markers to get a similar precision as that from a quantitative trait in the case of within-population diversity, and 2–4 markers in the case of between-population diversity. In addition, there are currently developed techniques to study multiple and independent traits simultaneously from measurements of morphological distances. The use of landmarks in morphology as well as the geometric-morphometric approach (see Bookstein, 1991; Rohlf and Marcus, 1993) permits an alternative way of getting independent variables (both geometrically and statistically) and, therefore, independent estimates of quantitative genetic variation and differentiation (see Palsson and Gibson, 2004). The number of independent variables that can be defined in this way is directly related to the number of landmarks used in the experiment (Bookstein, 1991).

In summary, we conclude that quantitative genetic variation can be a useful tool to detect some human-induced impacts on genetic diversity that cannot be detected with molecular neutral variation. At an interpopulation level, a critical issue is whether different local optima exist in the metapopulation and migration is high. In that case, quantitative information including an affected population flanked by two or more unaffected reference populations seems an appropriate setting for detecting genetic changes in the metapopulation structure. It should be remarked, however, that despite the large impacts simulated, the effects on diversity were relatively low in many cases, pointing toward the experimental difficulties in detecting genetic impacts in the presence of migration from unaffected populations.

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