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Genetic differentiation of neutral markers and quantitative traits in predominantly selfing metapopulations: confronting theory and experiments with *Arabidopsis thaliana*

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Summary

The comparison of the genetic differentiation of quantitative traits (Q_{ST}) and molecular markers (F_{ST}) can inform on the strength and spatial heterogeneity of selection in natural populations, provided that markers behave neutrally. However, selection may influence the behaviour of markers in selfing species with strong linkage disequilibria among loci, therefore invalidating this test of detection of selection. We address this issue by monitoring the genetic differentiation of five microsatellite loci (F_{ST}) and nine quantitative traits (Q_{ST}) in experimental metapopulations of the predominantly selfing species *Arabidopsis thaliana*, that evolved during eight generations. Metapopulations differed with respect to population size and selection heterogeneity. In large populations, the genetic differentiation of neutral microsatellites was much larger under heterogeneous selection than under uniform selection. Using simulations, we show that this influence of selection heterogeneity on F_{ST} can be attributable to initial linkage disequilibria among loci, creating stronger genetic differentiation of QTL than expected under a simple additive model with no initial linkage. We found no significant differences between F_{ST} and Q_{ST} regardless of selection heterogeneity, despite a demonstrated effect of selection on Q_{ST} values. Additional data are required to validate the role of mating system and linkage disequilibria in the joint evolution of neutral and selected genetic differentiation, but our results suggest that F_{ST}/Q_{ST} comparisons can be conservative tests to detect selection in selfing species.

1. Introduction

In subdivided populations, the genetic differentiation of quantitative traits is governed mainly by migration, genetic drift and selection. The issue of the respective contribution of selection versus other forces to population structure is a central theme of evolutionary biology (Merilä & Crnokrak, 2001; McKay & Latta, 2002), because local adaptation, stemming from a spatial heterogeneity of selection, is involved in several important evolutionary processes, such as speciation (Schluter, 2001; Via, 2001) and maintenance of genetic diversity (e.g. Levene, 1953). The influence of selection on population structure can be inferred by comparing the genetic differentiation of presumably selected traits with that of presumably neutral molecular markers. The theory was developed a few decades ago (Wright, 1951; Rogers, 1986; Lande, 1992) and generalized recently to any population structure (Whitlock, 1999); it predicts that the genetic differentiation of a neutral polygenic trait is identical to the genetic differentiation of a single, neutral locus. Hence, the genetic differentiation of neutral markers (F_{ST}; Wright, 1951) represents a null hypothesis for the expected amount of differentiation of quantitative traits (Q_{ST}; defined by Spitze, 1993) due to migration and drift only. If the observed differentiation of a quantitative trait is significantly higher than the differentiation of molecular markers, the trait most likely undergoes diversifying selection. In contrast, significantly smaller values of Q_{ST} than F_{ST} suggest the action of uniform selection.

The test of the relative importance of selection versus drift based on a comparison of F_{ST} and Q_{ST}

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has been applied recently to natural populations (for reviews see Merilä & Crnokrak, 2001; McKay & Latta, 2002). These studies show that the differentiation of quantitative traits is generally higher than that of molecular markers, suggesting a predominant role of diversifying selection in natural populations. This conclusion is consistent with available estimations of selection in natural populations, revealing ubiquitous local selection in the wild (Kingsolver *et al*., 2001; Hereford *et al*., 2004). In addition, the genetic differentiation of traits seems to be weakly but significantly correlated with that of neutral markers (Merilä & Crnokrak, 2001). Whether this correlation is an artifact or is biologically meaningful is still debated (Crnokrak & Merilä, 2002; Hendry, 2002; Latta & McKay, 2002) and the mechanisms involved remain to be elucidated.

The conclusion of ubiquitous diversifying selection in nature and the significance of a correlation between F_{ST} and Q_{ST} depend on assumptions underlying the measurement of genetic differentiation. Merilä & Crnokrak (2001) pointed out that differences between Q_{ST} and F_{ST} can arise due to biased estimations of differentiation, which may occur for both quantitative traits and molecular markers. Using the present experimental evolution study, we previously demonstrated that Q_{ST} values were primarily influenced by selection heterogeneity, despite biasing factors such as genotype by environment interactions (Porcher *et al*., 2004*a*). Here, we focus on the genetic differentiation of molecular markers. The use of F_{ST} as a null hypothesis for the differentiation of quantitative traits requires a neutral behaviour of molecular makers. This assumption may be violated in numerous circumstances, either because some markers undergo natural selection (e.g. enzymes; Tracey *et al*., 1975; Farris & Mitton, 1984; Watt *et al*., 1996), or because the behaviour of effectively neutral markers is influenced by selection at physically or statistically linked loci. Indirect selection, due to positive selection (hitchhiking; Maynard-Smith & Haigh, 1974) or purging of deleterious mutations (background selection; Charlesworth *et al*., 1993), may become genome-wide in highly inbreeding species (Charlesworth *et al*., 1993).

In this paper, we study the influence of positive selection and its spatial heterogeneity on the genetic differentiation of molecular markers in a predominantly selfing species, using a combination of experimental and theoretical approaches. We also compare the genetic differentiation of molecular markers with that of quantitative traits. Experimental data are obtained from initially unstructured experimental metapopulations of *Arabidopsis thaliana* that evolved during eight generations under controlled conditions of drift and selection heterogeneity. Using simulation studies, we specifically explore the effects of initial associations among loci on the differentiation of presumably neutral molecular markers. We conclude by addressing the achievability of detecting selection from a comparison between Q_{ST} and F_{ST} in predominantly selfing plant species with strong linkage disequilibria among loci.

2. Materials and methods

(i) *Experimental setup*

(a) *General description*

Twelve metapopulations of *A. thaliana*, each consisting of 20 populations, were grown in the greenhouse for eight generations (Fig. 1). Metapopulations differed with respect to heterogeneity of selection and population size. Six metapopulations were submitted to uniform directional selection for a short life cycle, i.e. selection was identical in all populations (see below). Six metapopulations were under heterogeneous selection for the duration of life cycle, with 10 of 20 populations under selection for a short life cycle and no artificial selection on the other 10 populations. Within each selection regime, population size was 10, 25 or 100 plants per population, resulting in total metapopulation sizes of 200, 500 and 2000 plants. Plant density was constant across population size. For each population size and selection treatment, two replicate metapopulations were established. Migration among local populations within a metapopulation occurred through seeds only and followed an island-like model, where a given population received migrants from a single, randomly chosen population, with rate $m = 0{\cdot}02$.

The initial populations were all sampled at random from the same pool of seeds. This pool of seeds was a mixture of 10 different F_2 lines obtained from crosses involving 14 parental lines from natural populations from France and the UK, together with a male-sterile mutant determined by a nuclear recessive mutation (for details regarding crosses see Lavigne *et al*., 2001). We expect the average initial genetic differentiation to be close to zero for both quantitative traits and molecular markers, because all populations were sampled from the same pool of seeds. The sampling procedure is also likely to create strong initial associations among loci, despite one-generation selfing of heterozygous F_1 individuals (hence recombination) to obtain the F_2 generation (see Section 3 for expected linkage disequilibria among microsatellite loci, estimated from data on parental genotypes and table of crosses).

Note that a metapopulation is defined here as a set of local populations connected by migration (Hanski & Gilpin, 1991), so that extinction and colonization events, which are often associated with the metapopulation concept, are not considered. Our

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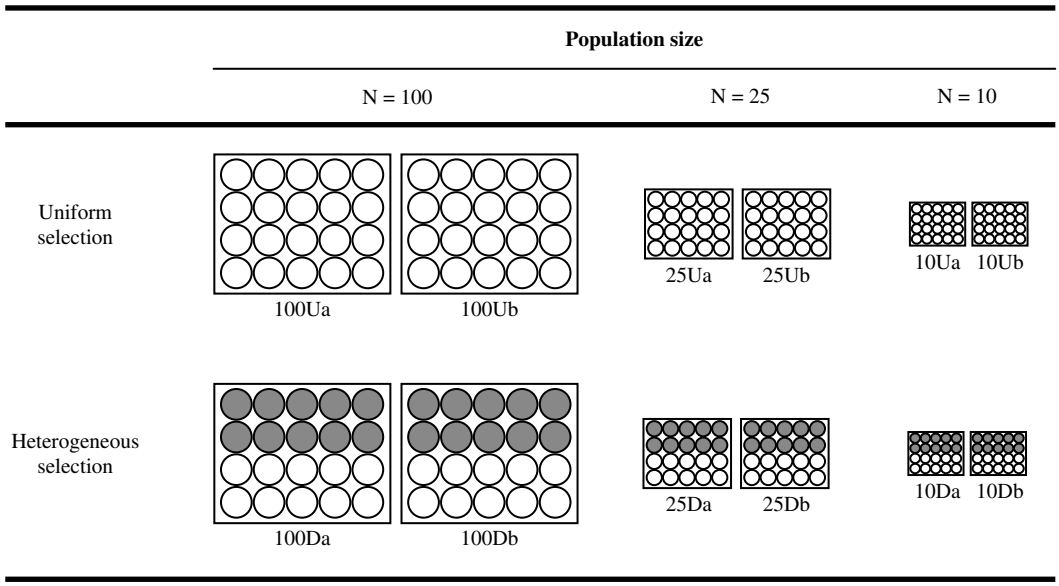

Fig. 1. The 12 experimental metapopulations. A metapopulation (square) consists of 20 populations (circles) connected by migration following an island-like model (the spatial distribution of population does not affect the patterns of gene flow). Metapopulations differ with respect to population size (100, 25 and 10 plants per population) and selection regime: under uniform selection (U), all populations within a metapopulation are selected for a short life cycle (white circles); under heterogeneous selection (D), 10 populations are selected for a short life cycle (white circles) and 10 populations are not submitted to artificial selection (grey circles). Two replicate metapopulations (a and b) are available for each combination of population size and selection regime.

experimental setup hence models a scenario of connected populations recently derived from a single, large population and evolving under selection, drift and migration.

(b) *Experimental conditions of each generation*

Each generation, seeds were sown following a regular grid, watered with a solution containing 0·15% fungicide (Dericlor, Ciba Geigy) and left for 1 week in the dark at 4 °C to break dormancy. Density was constant across population sizes using different pot sizes: 26·4 cm² (10 plants), 86·25 cm² (25 plants) and 350 cm² (100 plants), one population per pot. After germination, the plants were grown in a climate-controlled compartment of a greenhouse under a 16 h light/8 h dark photoperiod, 15 °C night and 20 °C day, and watered twice a week. The newly sown populations consisted of 88% seeds harvested from hermaphrodites within the population, 2% migrant seeds from another randomly chosen population within the metapopulation and 10% seeds harvested from male-sterile individuals within the population. The contribution of outcrossed male-sterile plants increases the low outcrossing rate of *A. thaliana* up to 10% (Porcher *et al.*, 2004*b*). Selection for precocity was applied by stopping any watering when first fruits ripened in either of the two metapopulations within a selection and population size treatment. Once watering of the plants was stopped, they were left to dry. In metapopulations under heterogeneous selection, the 10 populations that were not under artificial selection for precocity were watered until all plants matured. These populations were the same from generation to generation.

By selecting plants via hydric stress, we selected not only for plant precocity but also for other traits such as ability to produce seeds rapidly under severe drought. Our aim was not to apply a formal directional selection on one specific trait, but to generate uniform or heterogeneous selection across populations on a variety of traits, hence mimicking selection in natural conditions. Previous measurements performed on this experimental setup suggest that we successfully achieved this. The average selection differential over all measured traits was $s = 0{\cdot}28$ (Porcher *et al.*, 2004*a*), a value comparable to selection measured in natural populations (average selection gradient = 0·22; Kingsolver *et al.*, 2001). In addition, under the heterogeneous selection regime, we observed significant differences in selection differentials between the populations under artificial selection for precocity and the populations under no artificial selection (Porcher *et al.*, 2004*a*), indicating spatial heterogeneity in the strength of directional selection.

(c) *Measurements and data analyses*

Microsatellite diversity: At generation 8, 10 plants were sampled in five populations from metapopulations with 25 and 100 plants per population, and

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seven plants were sampled in seven populations from metapopulations with 10 plants per population. We also included the 15 parent plants in this analysis. DNA was extracted from leaves using the Chelex (Biorad) protocol from Bucheli *et al.* (2001), and analysed at five microsatellite loci, each located on a different chromosome: Nga162, Nga138, Nga132, Nga106 and Nga128 (Bell & Ecker, 1994). PCR amplifications were performed using a PTC 100 thermal cycler (MJ Research), with 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. Each reaction (10 μl) contained 1 μl of 10× reaction buffer (50 mM KCl, 0·1% Triton X-100, 10 mM Tris-HCl, pH 9·0), 10 μM of dNTP, 0·2 μg/μl bovine serum albumin (BSA), 1·5 mM $MgCl_2$, 2·5 pmol of each primer (the forward primer labelled with fluorescence), 0·25 U of *Taq* DNA polymerase (Promega), and approximately 10 ng of sample DNA. PCR products were separated in 6% polyacrylamide gels and visualized by scanning the gel with a FMBIO II scanner (Hitachi Genetic Systems).

In each metapopulation, we estimated genetic differentiation (F_{ST}) and average heterozygote deficiency (F_{IS}) via the Weir & Cockerham's estimators θ and f respectively (Weir & Cockerham, 1984), using the Fstat software (Goudet, 1995). Standard deviations and confidence intervals of F_{ST}s were calculated using jackknives and bootstraps over loci respectively. The significance of F_{ST}s was tested by permuting individuals among populations (Goudet, 1995). The influence of population size (genetic drift) and selection heterogeneity on F_{ST} values was tested using an analysis of variance (GLM, SAS software, SAS Institute, 2000), with the model:

$$Y_{ijk} = siz_i + reg_j + (siz \times reg)_{ij} + R_{ijk}$$

where Y_{ijk} is a F_{ST} value, *siz* stands for population size ($i = 1, 2, 3$), *reg* for selection regime ($j = 1, 2$) and R_{ijk} is a residual error term within population size and selection regime. Population size and selection regime effects were treated as fixed.

Quantitative traits. Details regarding the measurements of quantitative traits and estimation of their genetic diversity can be found in Porcher *et al.* (2004*a*). Here, we only provide information relevant to this study.

At generations 2 and 8, we analysed nine quantitative traits in six populations per metapopulation using families obtained by selfing. The six populations were those used for microsatellite analyses, plus or minus one population. In metapopulations under heterogeneous selection, the six populations consisted of three populations under selection for a short life cycle and three populations with no selection. Nine traits describing plant morphology and phenology were measured, either during plant growth (duration of the rosette stage (DRS) = number of days between sowing and bolting, time to flowering (FT) = number of days between bolting and flowering, and number of rosette leaves at bolting (NRL)) or at harvest (plant height (PH), height of the first fruit (HFF), height of the first branch (HFB), number of primary branches (NPB), total number of flowering branches (NFB) and total number of fruits (NF)).

In each metapopulation and for each trait, we estimated the within-population (V_{WP}) and between-population (V_{BP}) components of genetic variance using a full-sib design and an analysis of variance. The genetic differentiation was estimated as $Q_{ST} = (1 + F_{IS})\ V_{BP}/[(1 + F_{IS})\ V_{BP} + 2\ V_{WP}]$ (Wright, 1969). To estimate confidence intervals for Q_{ST}, we performed bootstraps over populations ($N = 1000$). In metapopulations under heterogeneous selection, bootstrapping was performed within each selection treatment (selection or no selection), over three populations only.

(ii) *Model for the joint evolution of the genetic differentiation of quantitative traits and the differentiation of neutral markers*

In a predominantly selfing species, the evolution of neutral markers is likely influenced by selection at other loci because a highly inbreeding mating system reduces the effective recombination rate (Liu *et al.*, 1998). This effect of the mating system is probably increased with strong initial associations among loci. We analysed the role of initial linkage disequilibria using a modified version of the Metapop program (Le Corre *et al.*, 1997) to model the evolution of a polygenic trait in metapopulations experiencing different levels of selection heterogeneity. This program simulates the genetics of subdivided populations; each individual is characterized by its genotype (n loci). Each generation, each population within a metapopulation undergoes selection, mating via selfing or outcrossing, and migration. We do not consider mutation here because we focus on short-term evolution (20 generations in the simulations); mutation is unlikely to have a major influence on the genetic differentiation on such short time scales. Parameter values are chosen according to the experimental setup. A metapopulation consists of 20 populations connected by 2% migration, following the island-like model and containing $N = 100$, 25 and 10 individuals. All individuals have identical selfing rate (=0·9), which correctly mimics outcrossing in the experimental metapopulations (not shown), although male-sterile individuals are not explicitly considered.

A genotype consists of five diallelic neutral markers and n_s diallelic loci controlling a quantitative trait via identical additive effects $\alpha = +1$ or -1 ($n_s = 10$ or 50). The loci encoding the trait are hereafter named

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quantitative trait loci (QTL). All loci are physically unlinked. Selection heterogeneity is modelled according to the patterns of selection for precocity in the experimental populations: the trait is submitted to directional selection with standardized selection differential s_1 in 10 populations among 20, and is neutral ($s_2=0$) in the other 10 populations. The fitness w of phenotype x is $w=1+sx/\sigma_x$, where σ_x is the standard deviation of the phenotype, calculated over all individuals in a population. We investigate the effects of selection heterogeneity on genetic differentiation by varying selection differentials in populations under selection, from $s_1=0$ to 1, which encompasses the range of selection differentials measured in the experimental metapopulations (Porcher *et al.*, 2004*a*). Identical selection differentials across populations therefore corresponds to neutrality ($s_1=s_2=0$). Moderate uniform selection, comparable to that in the experimental populations ($s_1=s_2=0{\cdot}3$; average selection differential measured in the experimental populations $s=0{\cdot}28$, Porcher *et al.*, 2004*a*), is also considered for comparative purposes but is not incorporated in the analysis of the influence of selection heterogeneity on genetic differentiation.

To study the influence of initial linkage disequilibria on the genetic differentiation of markers, we vary the strength of initial associations as follows. In the initial populations, individual genotypes are generated by randomly sampling a diploid genotype at one locus (marker or QTL) among two alleles with identical frequency ($f=0{\cdot}5$). For each copy of the genome, the alleles of subsequent loci are then identical to the allele of the first locus with probability P; they differ with probability $(1-P)$. All individuals are generated independently in each population, so that the initial genetic differentiation is close to zero, except for sampling variability. We vary the strength of associations from $P=0{\cdot}5$ (all loci sampled at random) to 1 (all loci identical within a genome copy), which generates Lewontin's (1964) measure of linkage disequilibrium from $D'=0{\cdot}06$ ($N=100$, $P=0{\cdot}5$) to 1 ($P=1$). The initial linkage disequilibrium is always larger than zero, due to finite population size and sampling effects creating associations across loci.

Simulations are run for 20 generations, and we monitor the genetic differentiation of the quantitative trait (Q_{ST}), of molecular markers, and of QTL (F_{ST}). For each set of parameter values (population size, selection heterogeneity, and initial linkage disequilibrium), 50 simulations are run.

3. Results

(i) *Initial linkage disequilibrium across markers loci*

In the experimental metapopulations, the initial linkage disequilibria among microsatellite loci were strong, as expected from the limited number of crosses used to create the initial pool of seeds. Normalized expectations of linkage disequilibrium (Lewontin, 1964; Hedrick, 1987), calculated using the parental genotypes, the table of crosses and the contribution of the different crosses to the initial pool of seeds (Lavigne *et al.*, 2001), ranged from $D'=0{\cdot}32$ (N128 × N168) to 0·58 (N139 × N162) (Table 1).

Table 1. *Initial linkage disequilibria among microsatellite loci. The expected value of D′, a normalized measure of linkage disequilibrium (Lewontin, 1964), was calculated from the parental genotypes and the crossing scheme*

	Microsatellite markers			
	N128	N162	N168	N106
N139	0·52	0·58	0·43	0·54
N128	–	0·43	0·32	0·45
N162		–	0·33	0·49
N168			–	0·35

(ii) *Genetic differentiation of molecular markers and quantitative traits in the experimental metapopulations*

All metapopulations exhibited significant differentiation at microsatellites loci after eight generations of evolution from an initially unstructured situation, as indicated by F_{ST}s significantly exceeding zero (Table 2). This genetic differentiation is attributable to migration and drift, and, in some cases, to selection heterogeneity. Population structure was primarily influenced by drift and migration, as indicated by the highly significant effect of population size on F_{ST}s ($P<0{\cdot}0001$, Table 3), with higher F_{ST}s in smaller populations (Table 2). Note that the effects of drift and migration cannot be distinguished in this experimental setup: they vary jointly due to constant frequency (not number) of migrants. Genetic differentiation of markers was also driven by selection and selection heterogeneity, as indicated by a significant effect of selection regime on F_{ST}s (Table 3), with higher values under heterogeneous than under uniform selection (Table 2). The effect of selection depended on population size (significant regime × size interaction, Table 3) and was appreciable in large populations only (ANOVA within population size, selection regime effect: $P=0{\cdot}008$, $N=100$ and $P=0{\cdot}022$, $N=25$). In large populations ($N=100$), F_{ST}s at generation eight were about three times higher under heterogeneous than under uniform selection. In contrast, in small populations, no significant effect of selection was observed ($P=0{\cdot}461$, $N=10$).

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Table 2. *Genetic differentiation of microsatellite markers (F_{ST}) at generation 8*

		Uniform selection		Heterogeneous selection	
Population size	Locus	a	b	a	b
$N=100$	n162	0·039	0·099	0·066	0·225
	n168	0·135	0·104	0·103	−0·054
	n139	0·061	0·035	0·395	0·31
	n106	0·05	0·044	0·184	0·307
	n128	0·117	0·128	0·353	0·33
	Overall	0·077 ± 0·019	0·078 ± 0·019	0·229 ± 0·070	0·259 ± 0·042
$N=25$	n162	0·332	0·2	0·308	0·503
	n168	0·444	0·294	0·57	0·475
	n139	0·222	0·333	0·409	0·445
	n106	0·102	0·21	0·285	0·399
	n128	0·408	0·309	0·374	0·241
	Overall	0·293 ± 0·060	0·262 ± 0·026	0·382 ± 0·040	0·388 ± 0·043
$N=10$	n162	0·663	0·616	0·569	0·456
	n168	0·86	0·384	0·595	0·749
	n139	0·383	0·298	0·623	0·55
	n106	0·684	0·814	0·762	0·537
	n128	0·834	0·786	0·434	0·485
	Overall	0·669 ± 0·079	0·603 ± 0·105	0·600 ± 0·105	0·561 ± 0·063

Overall genetic differentiations were estimated from the five microsatellite markers and are given with their standard error, for the different selection regimes and population sizes. All multilocus values are significantly different from zero ($P<0·001$, except metapopulation 100Ub, $P=0·002$).

Table 3. *ANOVA for genetic differentiation of microsatellites at generation 8 conducted on single-locus F_{ST}s, pooled across the five microsatellite markers*

Source	d.f.	Mean square	*F* value	$P>F$
Population size	2	1·0320	62·25	**<0·0001**
Selection Regime	1	0·0667	4·02	**0·0500**
Regime × Size	2	0·0574	3·46	**0·0385**
Error	54	0·0166		

The genetic differentiation of quantitative traits was also driven by the joint effects of drift and selection heterogeneity (for details see Porcher *et al.*, 2004*a*). The average Q_{ST}s over all traits were significantly higher under small than under large population size and, in large populations only, significantly higher under heterogeneous than under uniform selection (see e.g. Fig. 2). In large populations, we also observed a significant positive relationship between Q_{ST}s and selection heterogeneity (Porcher *et al.*, 2004*a*), which confirms that the genetic differentiation of traits was primarily influenced by selection heterogeneity. Q_{ST}s therefore provided an accurate picture of selection heterogeneity. Note, however, that the confidence intervals associated with Q_{ST} estimates were large in general, sometimes covering the [0, 1] interval.

(iii) *Comparison of the genetic differentiation of quantitative traits and molecular markers*

F_{ST} and Q_{ST} were generally not different from each other, as indicated by overlapping confidence intervals, except in four instances (Fig. 2; Number of fruits, $N=25$, uniform selection; Number of flowering branches, $N=100$, heterogeneous selection; Plant height, $N=25$, heterogeneous selection, Number of fruits, $N=10$, heterogeneous selection). These individual significant values are probably due to numerous comparisons of confidence intervals (105 = 108 traits minus three traits whose Q_{ST} estimates were larger than 1 and for which confidence intervals were not estimated) and therefore may not reflect actual differences between the genetic differentiation of quantitative traits and that of markers.

(iv) *Results of the simulation model*

With initially quasi-independent loci ($D'=0·1$) and large populations ($N=100$), selection influences the genetic differentiation of traits, QTL and markers (Fig. 3). The genetic differentiation of the trait (Q_{ST}) and the differentiation of QTL (F_{ST}) are primarily influenced by selection heterogeneity: after 20 generations, both genetic differentiations are higher under heterogeneous selection than under uniform selection (Fig. 3*A* and *C*). In contrast, the differentiation of molecular markers is primarily influenced by the

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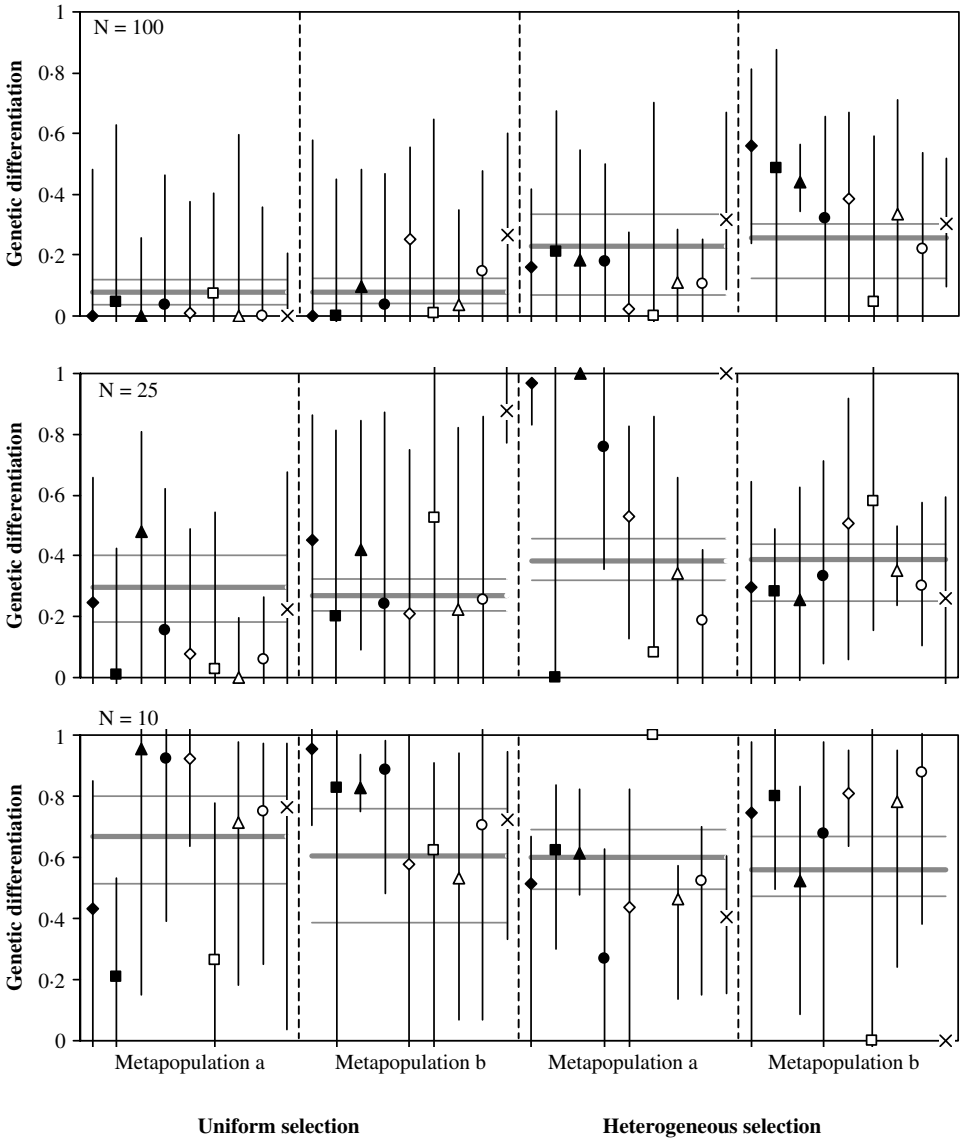

Fig. 2. Comparison of the genetic differentiation of neutral markers and quantitative traits at generation 8. Multilocus F_{ST} estimates (thick grey lines) are plotted with their 95% confidence intervals (thin grey lines). Q_{ST}s for each trait are indicated by different symbols: ◆, plant height; ■, number of primary branches; ▲, number of flowering branches; ●, height of the first branch; ◇, height of the first fruit; □, flowering time; △, number of rosette leaves; ○, duration of rosette stage; ×, number of fruits. Errors bars represent 95% confidence intervals for Q_{ST} estimates. F_{ST}s and Q_{ST}s are considered different when the 95% confidence intervals do not overlap.

strength of selection, not by its heterogeneity (Fig. 3*B* and Le Corre & Kremer, 2003): F_{ST} is highest under uniform moderate selection ($s_1 = s_2 = 0{\cdot}3$), lowest under neutrality, and exhibits intermediate values under heterogeneous selection ($s_1 = 0{\cdot}3$ or 1, $s_2 = 0$). After 20 generations, the influence of selection on the genetic differentiation is much stronger for the selected trait than for the neutral markers. These and further results remain unchanged when the number of QTL is varied ($n_s = 10$ or 50, not shown).

The same influence of the strength and heterogeneity of selection on the genetic differentiation is also observed with smaller population sizes (Fig. 3*D–I*, $N = 25$ and 10). However, the differences across selection regimes are much smaller, due to increasing effects of drift, so that the genetic structure of the selected trait, the underlying loci and the molecular markers are virtually independent of selection in small populations ($N = 10$).

With initial linkage disequilibria among loci, the influence of selection on the genetic differentiation of markers is strengthened and the behaviour of markers becomes more similar to that of QTL. With strong linkage disequilibria ($D' = 1$) and large population size ($N = 100$), F_{ST}s at generation 8 are up to 6 times greater under heterogeneous selection than under neutrality (Fig. 4*A*). In addition, F_{ST}s under uniform selection ($F_{ST} = 0{\cdot}04$, generation 8, $s_1 = s_2 = 0{\cdot}3$; not

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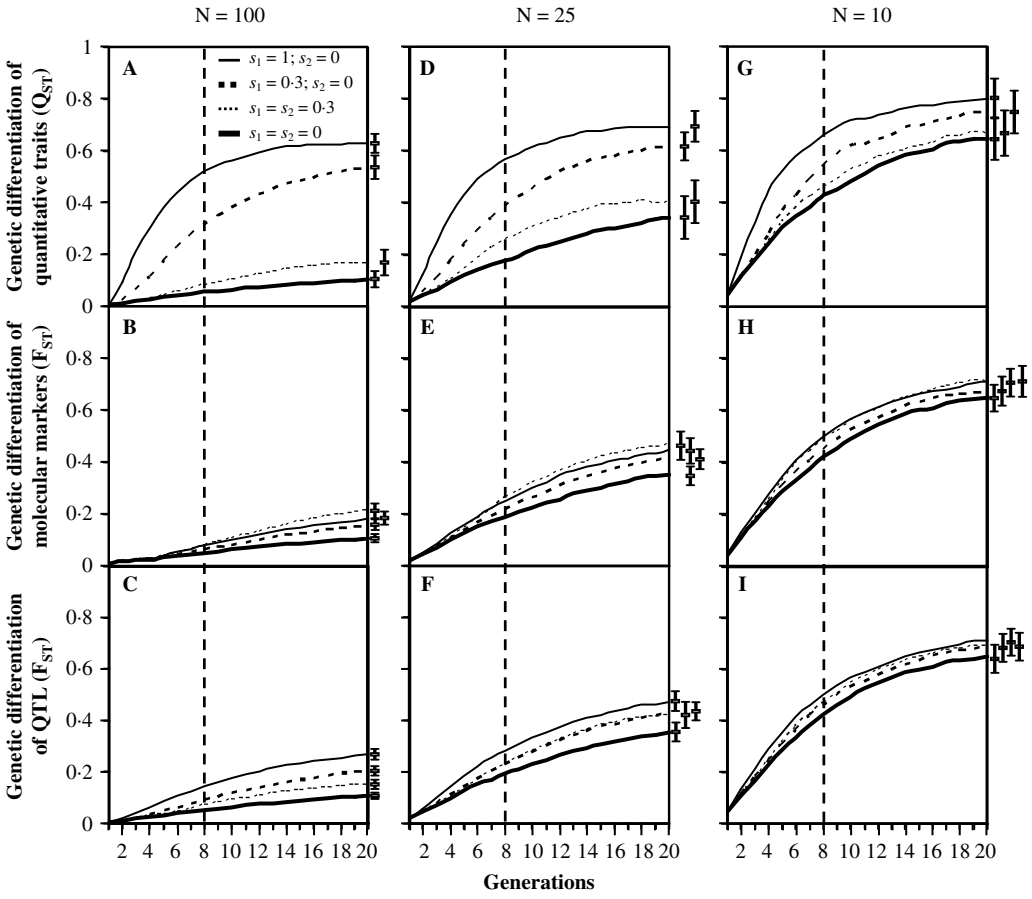

Fig. 3. Influence of selection heterogeneity on population differentiation when loci are initially unlinked. (*A*), (*D*) and (*G*) Differentiation of a single quantitative trait (Q_{ST}). (*B*), (*E*) and (*H*) Multilocus differentiation of five molecular markers (F_{ST}). (*C*), (*F*) and (*I*) Multilocus differentiation of 10 quantitative trait loci (F_{ST}). A metapopulation contains 20 populations with 100 (*A–C*), 25 (*D–F*) and 10 (*G–I*) individuals each; the selfing rate is 90%. Heterogeneous selection is modeled as follows: a polygenic trait (controlled by $n_s = 10$ QTL, 2 alleles each) is submitted to various strengths of selection ($s_1 = 0{\cdot}3$ or 1) in half populations and is neutral ($s_2 = 0$) in the remaining populations. Each generation, the mean of 50 simulations is plotted. The vertical line indicates generation 8, when the genetic differentiation was measured in the experimental metapopulations. Error bars at the right of each graph indicate standard deviations at generation 20.

shown) are somewhat lower than F_{ST}s under neutrality ($F_{ST} = 0{\cdot}05$, generation 8; Fig. 4*A*). Hence, with initial linkage disequilibria among loci, the genetic differentiation of markers is influenced by selection heterogeneity, not by the strength of selection. Initial associations among loci have little effect on the genetic differentiation of the quantitative traits, which remains higher than that of molecular markers (not shown).

The influence of manipulating the initial associations among loci is reduced in smaller populations (Fig. 4*B*, *C*), due to genetic drift, which altogether lessens the effects of selection and creates non-negligible levels of initial linkage across loci, even when the initial alleles are sampled at random.

4. Discussion

We explored the influence of selection on the differentiation of neutral microsatellite loci (F_{ST}) in experimental metapopulations of the predominantly selfing species *A. thaliana* under controlled conditions of drift, migration and selection heterogeneity. We show that when loci are initially linked, the genetic differentiation of neutral markers can be strongly influenced by selection heterogeneity, with larger F_{ST}s under heterogeneous selection. We argue below that this result was not expected from previous theory and is caused by an increased differentiation of the genes underlying traits under selection. Finally, we discuss the consequences of these findings in terms of detection of selection in natural populations.

(i) *Selection heterogeneity influences the differentiation of neutral markers in large populations*

In large metapopulations ($N = 100$), the genetic differentiation of presumably neutral microsatellite markers was significantly larger under heterogeneous selection than under uniform selection. Under

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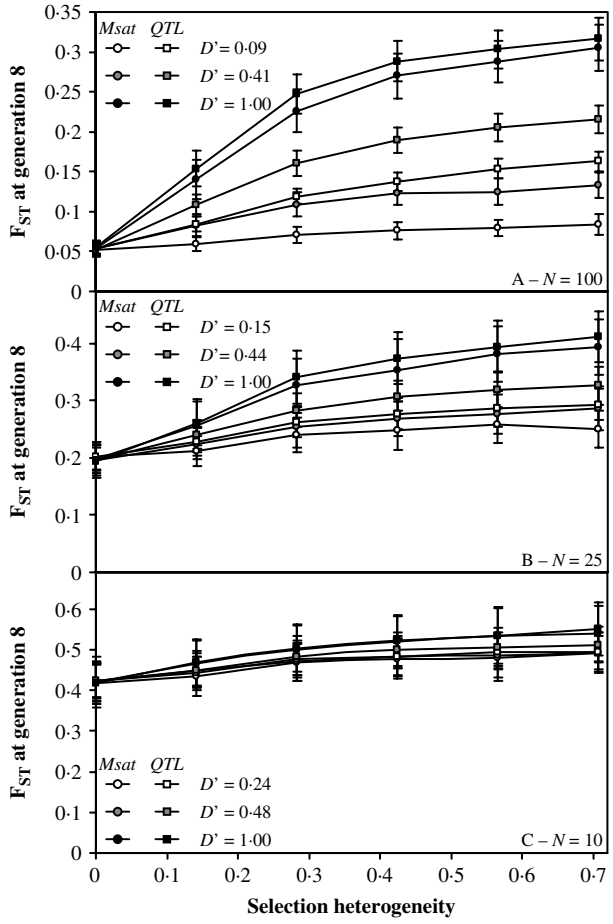

Fig. 4. Influence of initial linkage disequilibria and selection heterogeneity on the genetic differentiation of markers (circles) and quantitative trait loci (squares). Multilocus F_{ST}s at generation 8 are plotted against selection heterogeneity under various initial conditions of linkage disequilibrium (D'; Lewontin, 1964) and population sizes. Except for D', the simulation parameters are as in Fig. 3. Selection heterogeneity is calculated as the standard deviation between two selection differentials ($s_1 = 0$, 0·2, 0·4, 0·6, 0·8 or 1 and $s_2 = 0$). When selection heterogeneity equals zero, the trait is neutral ($s_1 = s_2 = 0$).

heterogeneous selection, observed F_{ST} values were almost 5 times higher than expected from a simple model with no initial linkage disequilibria (mean $\pm$ SE observed $F_{ST} = 0{\cdot}24 \pm 0{\cdot}04$, expected $F_{ST} = 0{\cdot}05$ under neutrality) and 3 times higher than that observed under uniform selection (mean $\pm$ SE $F_{ST} = 0{\cdot}078 \pm 0{\cdot}0005$). In large populations, selection heterogeneity increased the genetic differentiation genome-wide, with all but one loci exhibiting higher F_{ST} under heterogeneous selection (Table 2, $N = 100$, and the same pattern was also found for enzymatic loci, Lavigne *et al.*, 2001). The effect of selection heterogeneity was therefore not due to selection on one of the microsatellite loci, which are unlikely to undergo direct selection (Sunnucks, 2000), but was more likely attributable to the highly selfing mating system creating strong associations between markers and selected loci.

That selection can influence the evolution of a neutral marker is well known and has been thoroughly studied both experimentally and theoretically. One effect of selection is to decrease effective population size at such markers by increasing variance in reproductive success of individuals, thereby increasing the effects of genetic drift (e.g. Kimura & Crow, 1963). This effect alone promotes higher F_{ST} values under selection than neutrality and probably explains to a large extent the influence of selective strength, not selection heterogeneity, on the genetic differentiation of neutral markers in our simulations with no initial associations among loci (e.g. Fig. 3*B*).

When otherwise neutral markers are linked to a selected locus, their genetic diversity can also be considerably influenced by directional (Maynard-Smith & Haigh, 1974), frequency-dependent (Charlesworth *et al.*, 1997), background (Charlesworth *et al.*, 1993) and local (diversifying) selection (Charlesworth *et al.*, 1997). The last study demonstrated that F_{ST}s at neutral markers are increased by diversifying selection on a single locus; high F_{ST} values can be obtained at sites close to a selected locus, or under high selfing rates (Charlesworth *et al.*, 1997). The effect of increased selfing rate is mediated by increased homozygosity, which reduces the efficiency of recombination and creates strong linkage disequilibria between neutral and selected loci (Liu *et al.*, 1998). However, the theoretical studies mentioned above cannot account for the observed F_{ST} values in our experimental approach, because these studies examined the evolution of markers linked to one or two selected loci, which does not accurately describe the evolution of differentiation of the more numerous genes generally controlling a quantitative trait.

Two theoretical studies (Latta, 1998; Le Corre & Kremer, 2003) have explored the joint evolution of genetic differentiation of a quantitative trait and genes controlling this trait in structured populations under diversifying selection. They show that the genetic differentiation of a trait depends on the genetic differentiation of QTL (F_{ST}) but also on within- and between-population covariance among QTL (Le Corre & Kremer, 2003). In outcrossing populations experiencing moderate selection and moderate to high migration, diversifying selection can generate high Q_{ST} values together with a quasi-neutral behaviour of QTL, because the genetic differentiation of a trait is mainly due to between-population covariance (Latta, 1998). This implies that F_{ST}s at neutral markers are not influenced by selection heterogeneity even when tightly linked to QTL, because QTL behave quasi-neutrally. This result holds in highly selfing populations, exhibiting within-population linkage disequilibria, under moderate selection and migration

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($Nm < 10$; Le Corre & Kremer, 2003), which is the case in our experimental metapopulations (average selection differential = 0·28, Porcher *et al*., 2004*a*, and $Nm = 2$ in large populations).

With no initial linkage among loci, our model confirms that selection has a limited influence on the genetic differentiation of both QTL and markers, and shows that the genetic differentiation of markers tends to be influenced by the average strength of selection, affecting effective population size, rather than by its heterogeneity. F_{ST}s at generation 8 under highly heterogeneous selection ($s_1 = 1$, $s_2 = 0$) are 25% higher than under neutrality ($s_1 = s_2 = 0$), and are virtually identical to F_{ST}s under uniform selection ($s_1 = s_2 = 0{\cdot}3$) (Fig. 3). The minor effect of selection heterogeneity on the genetic differentiation of markers, most likely due to reduced effective population size, is not consistent with the large differences between F_{ST}s at markers under uniform and heterogeneous selection that we observed in large populations ($N = 100$, Table 2).

In the experimental setup, QTL for precocity are likely to be initially positively associated, despite the one generation of recombination following initial crosses, because the parental genotypes were chosen for their large variation in flowering time (mean flowering time varied from 36 days (male sterile mutant, ecotype Landsberg) to 56 days (ecotype from the Rhone Valley)). Our model demonstrates that these associations, not incorporated in previous theoretical studies, can strongly influence the evolution of QTL under heterogeneous selection and may partly account for the influence of selection heterogeneity on the genetic differentiation of markers in large populations (Fig. 4). With initial positive associations among QTL and a highly selfing mating system, response to heterogeneous selection occurs via a change in allelic frequencies at QTL, because maladapted genotypes are rapidly removed from populations and highly fit genotypes are retained by selection (i.e. selection is probably initially strong). The genetic differentiation of QTL therefore increases as populations become locally adapted, leading to high differentiation of QTL under heterogeneous selection (Fig. 4*A*). In contrast, when loci are initially unlinked, local adaptation is primarily achieved via creation of positive covariance between populations (Latta, 1998; Le Corre & Kremer, 2003) and QTL are eventually little differentiated. With strong linkage disequilibria, the genetic differentiation of molecular markers increases with that of QTL due to initial associations and high selfing rate. The effect of initial associations on the genetic differentiation of markers is yet likely transitory, especially in our experimental metapopulations where the appreciable outcrossing rate (10%) and effective recombination within initially heterozygous selfing lines rapidly decrease the initial linkage disequilibria. Simulations on longer periods of time suggest that the differentiation of markers depends little on initial associations among loci after 100 generations, although it remains higher under heterogeneous selection than under neutrality (data not shown).

(ii) *Detection of selection via comparisons of F_{ST} and Q_{ST} in highly selfing species with strong within-population linkage disequilibria*

We found no significant differences between F_{ST}s at neutral microsatellite markers and Q_{ST}s for selected traits, measured after an 8-generation evolution from initially unstructured metapopulations of *A. thaliana*. In small populations, similar values of Q_{ST} versus F_{ST} are consistent with genetic drift being the main evolutionary force ($s < 1/2N_e$; Kimura *et al*., 1963). The simulation approach suggests that strong genetic drift has comparable effects on the evolution of quantitative traits and molecular markers, especially in small populations ($N = 10$; Fig. 3), hence yielding identical values of F_{ST} and Q_{ST} on average. We previously observed that genetic differentiation of quantitative traits was mainly driven by genetic drift under small population sizes ($N = 10$ and, to a lesser extent, $N = 25$; Porcher *et al*., 2004*a*).

In large populations ($N = 100$), the absence of significant differences between F_{ST} and Q_{ST} is not consistent with the demonstration of a significant effect of selection heterogeneity on Q_{ST}s (larger Q_{ST}s under heterogeneous selection; Porcher *et al*., 2004*a*), but is not surprising either. The strong influence of selection heterogeneity on the genetic differentiation of markers, discussed above, may be partly responsible for similar values of F_{ST} and Q_{ST}, although the large confidence intervals for Q_{ST} are an equally likely explanation for the absence of detectable differences. Obtaining precise confidence intervals for Q_{ST} is a problematic task. First, quantitative genetics parameters are known to be liable to larger sampling errors than population genetics parameter, due to polygenic determinism and environmental effects (Falconer, 1960). The sample sizes used here for quantitative genetics estimates were unquestionably small regarding the usual requirements, but were not increased due to practical limitations (measurements were conducted in 12 metapopulations). Second, the construction of confidence intervals on variance component ratios is itself not a fully resolved issue. A variety of methods, including bootstrapping over populations or individuals, the delta method (Podolsky & Holtsford, 1995), Bayesian methods (Palo *et al*., 2003) and nonparametric bootstrapping (Gomez-Mestre & Tejedo, 2004), are currently used, but none of them is totally satisfying. Finally, the test based on overlapping of 95% confidence intervals, although commonly used

in statistical comparisons of F_{ST} and Q_{ST} (e.g. Bonnin *et al*., 1996*b*; Widen *et al*., 2002; Saint-Laurent *et al*., 2003), is more conservative than a difference testing with a significance level $\alpha = 5\%$.

Our theoretical approach suggests that tests of detection of selection based on a comparison between Q_{ST} and F_{ST} can be conservative in highly selfing species with strong linkage disequilibria among loci, because the genetic differentiation of markers tends to behave like that of QTL and of quantitative traits. Our observations, revealing no significant differences between Q_{ST} and F_{ST} despite a demonstrated effect of selection on Q_{ST}, agree with these predictions but do not formally demonstrate them, because of the large confidence intervals preventing the detection of differences between Q_{ST} and F_{ST}. In the present experimental approach, confidence intervals may have been reduced with larger sample sizes, notably a larger number of populations, because the large populations ($N = 100$) were little differentiated.

(iii) *Genetic differentiation of quantitative traits and markers in natural populations of selfing species*

Few studies have compared F_{ST}s and Q_{ST}s in natural populations of predominantly selfing species. Bonnin *et al*. (1996*b*) found that most traits experienced diversifying selection, as indicated by high Q_{ST} (0·58) versus F_{ST} (0·33) values, and showed that detecting selection by comparing F_{ST}s and Q_{ST}s was relevant in the highly selfing species *Medicago truncatula*. In these populations, no significant within-population linkage disequilibria were found (Bonnin *et al*., 1996*a*). In contrast, Steinger *et al*. (2002) and Kuittinen *et al*. (1997) measured similar genetic differentiation of quantitative traits and markers in natural populations of *Senecio vulgaris* ($F_{ST} = 0{\cdot}49$, $Q_{ST} = 0{\cdot}51$) and *A. thaliana* ($F_{ST} = 0{\cdot}89$, $Q_{ST} = 0{\cdot}83$), respectively. Natural populations of *A. thaliana* are known to be structured by diversifying selection, notably on flowering time (Mitchell-Olds, 2001), and the absence of significant difference between F_{ST} and Q_{ST} might be due to some extent to strong linkage disequilibria among loci (Nordborg *et al*., 2002). We do not have any information regarding linkage disequilibrium in *S. vulgaris*. These few observations are consistent with the possible important influence of linkage disequilibria on the evolution of both QTL and marker differentiation. Additional data and theoretical studies are, however, required to elucidate whether similar values of F_{ST} and Q_{ST} reflect a predominant effect of drift or whether they are due to strong linkage disequilibria maintained through time, as observed in predominantly selfing species experiencing frequent extinction–recolonization events (Nordborg *et al*., 2002). If such were the case, the test of detection of selection based on the comparison of F_{ST} and Q_{ST} would be conservative in selfing species. The classical conclusion that local selection is ubiquitous in the wild would thus be further reinforced.

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