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NEWS AND VIEWS

OPINION

Comparisons between Q_{ST} and F_{ST}—how wrong have we been?

PIM EDELAAR,* PABLO BURRACO* and IVAN GOMEZ-MESTRE*†

Estación Biológica de Doñana (EBD-CSIC). Avda. Américo Vespucio s/n, Sevilla E-41092, Spain, †Biodiversity Research Group (CSIC-UO-PA). c/Catedrático Rodrigo Uría s/n, Oviedo E-33071, Spain

Abstract

The comparison between quantitative genetic divergence (Q_{ST}) and neutral genetic divergence (F_{ST}) among populations has become the standard test for historical signatures of selection on quantitative traits. However, when the mutation rate of neutral markers is relatively high in comparison with gene flow, estimates of F_{ST} will decrease, resulting in upwardly biased comparisons of Q_{ST} vs. F_{ST}. Reviewing empirical studies, the difference between Q_{ST} and F_{ST} is positively related to marker heterozygosity. After refuting alternative explanations for this pattern, we conclude that marker mutation rate indeed has had a biasing effect on published Q_{ST}–F_{ST} comparisons. Hence, it is no longer clear that populations have commonly diverged in response to divergent selection. We present and discuss potential solutions to this bias. Comparing Q_{ST} with recent indices of neutral divergence that statistically correct for marker heterozygosity (Hedrick's *G*′st and Jost's *D*) is not advised, because these indices are not theoretically equivalent to Q_{ST}. One valid solution is to estimate F_{ST} from neutral markers with mutation rates comparable to those of the loci underlying quantitative traits (e.g. SNPs). Q_{ST} can also be compared to Φ_{ST} (*Phi*$_{ST}$) of AMOVA, as long as the genetic distance among allelic variants used to estimate Φ_{ST} reflects evolutionary history: in that case, neutral divergence is independent of mutation rate. In contrast to their common usage in comparisons of Q_{ST} and F_{ST}, microsatellites typically have high mutation rates and do not evolve according to a simple evolutionary model, so are best avoided in Q_{ST}–F_{ST} comparisons.

Keywords: divergent selection, F_{ST}, microsatellites, neutral marker mutation rate, Q_{ST}, Φ_{ST}

Correspondence: Pim Edelaar or Ivan Gomez-Mestre, Fax: +34 954 621 125; E-mails: edelaar@ebd.csic.es, igmestre@ebd.csic.es

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Introduction

Determining the relative role of neutral vs. selective processes in the divergence of populations is a major research topic in evolutionary and conservation biology. Several methods have been developed to this end, and one that is commonly applied is the comparison between population divergence in neutral molecular markers (as estimated by F_{ST}) and genetic divergence in functional, quantitative traits (as estimated by Q_{ST}) (reviewed in Whitlock 2008; see Ovaskainen *et al.* 2011 for methodological amendments). Both parameters can vary between 0 and 1 and express variation among populations relative to the total variation. Assuming all genes underlying the phenotypic traits of interest (hereafter, quantitative genetic loci) have equal, additive and independent effects, then Q_{ST} should equal F_{ST} when population divergence is driven only by neutral genetic drift (Merilä & Crnokrak 2001; McKay & Latta 2002; Whitlock 2008). In contrast, Q_{ST} will be larger than F_{ST} if quantitative traits are exposed to diversifying selection across populations, whereas Q_{ST} will be smaller than F_{ST} if selection is stabilizing. F_{ST} estimation is straightforward using frequency data of neutral genetic markers (reviewed in Holsinger & Weir 2009). Q_{ST} is calculated from estimates of within- and between-population additive genetic variance components, preferably obtained from controlled breeding and rearing of large numbers of offspring in a biologically relevant common garden setting. By now, dozens of articles have applied this comparison of Q_{ST} and F_{ST}. Based on meta-analysis, the average and most frequent finding is that $Q_{ST} > F_{ST}$, so it appears that study populations are commonly exposed to divergent selection (Leinonen *et al.* 2008).

There has been ample discussion regarding the potential problems and biases in the estimation of Q_{ST} and its SE (e.g. Merilä & Crnokrak 2001; O'Hara & Merilä 2005; Leinonen *et al.* 2008; Whitlock 2008). From this, it clearly emerges that a critical assumption in Q_{ST} estimation is that within- and among-population variance components be good estimators of additive genetic variance. In practice, few studies do in fact report purely additive genetic variance components, but instead often include some combination of maternal, environmental and/or non-additive genetic effects, inflating the resulting Q_{ST} values. Moreover, epistasis can decrease Q_{ST} values, whereas dominance can substantially increase or decrease Q_{ST} estimates relative to F_{ST}, depending on the model of evolution underlying a given study system (reviewed in Whitlock 2008). Life-history traits, which are often connected to fitness, commonly bear considerable epistatic and dominance components.

Furthermore, the environmental effects on gene expression are often disregarded in this context, assuming that there is little if any genotype by environment interaction. This, however, is not the case, and Q_{ST} estimates strongly depend on the environment in which they are being taken, even if derived from additive genetic variance components under experimental settings (e.g. Gomez-Mestre & Tejedo 2004).

Problems with F_{ST}*?*

Comparatively less attention has been given to sources of bias in F_{ST} calculation. However, recent studies have raised the concern that the comparison of F_{ST} and Q_{ST} might be compromised because of problems with the interpretation of F_{ST} (Hedrick 1999, 2005; Hendry 2002; Jost 2008; Heller & Siegismund 2009; Gerlach *et al.* 2010; Kronholm *et al.* 2010; Meirmans & Hedrick 2010). This is best shown when using G_{ST}, an estimator similar to F_{ST} that is used for multilocus genetic markers (from now on, F_{ST} and G_{ST} will be used interchangeably). $G_{ST} = (H_{TOTAL}–H_{WITHIN})/H_{TOTAL}$, where H_{TOTAL} is the expected heterozygosity of the pooled populations, and H_{WITHIN} is the average expected heterozygosity within populations. Both H_{TOTAL} and H_{WITHIN} have a maximum of 1 (when all individuals are expected heterozygotes). For a highly variable marker, it is not uncommon to find H_{WITHIN} values of, e.g., 0.85. Even assuming that populations are entirely composed of private alleles and H_{TOTAL} approaches its theoretical maximum of 1, then G_{ST} still only has a maximum value of $(1–0.85)/1 = 0.15$. Such a value is frequently interpreted as indicating only mild to moderate differentiation, even though in this case the populations do not share any alleles at all and in that sense are completely genetically differentiated. Hence, for variable markers, F_{ST} does not provide a good measure of genetic differentiation of populations in allelic composition (Jost 2008; Meirmans & Hedrick 2010). It is more useful as a fixation index, which measures differentiation among alleles in population affiliation (when populations are very variable, individual alleles are hardly uniquely associated with particular populations: see Gregorius 2010). If F_{ST} values decrease because of higher H_{WITHIN}, its comparison with Q_{ST} may be highly questionable.

Fortunately, this is not necessarily the case, because Q_{ST} is in fact also a kind of fixation index and responds similarly to F_{ST} to the variability of underlying loci (Edelaar & Björklund 2011). One reason why a genetic locus might be more variable, is that its mutation rate is higher. Under the infinite allele model, in a single isolated population

$$H_{WITHIN} = 4N_e{}^*\mu/(4N_e{}^*\mu + 1), \quad (1)$$

where N_e is the effective population size, and μ is the mutation rate (Hartl & Clark 1997). When μ is very low, H_{WITHIN} will approach zero (fixation), whereas for a very large μ, H_{WITHIN} will approach one (high marker variability). Hence, F_{ST} will decline as mutation rate increases, and correctly so because there will be less fixation of alleles. However, when the quantitative genetic loci have a higher mutation rate, this also results in a decline of Q_{ST}: more genetic variation will now reside within populations relative to variation among populations, and $Q_{ST} = V_{AMONG}/(V_{AMONG} + 2\ V_{WITHIN})$ (for a diploid outbreeding species: McKay & Latta 2002; Whitlock 2008). By means of simulations, Edelaar & Björklund (2011) showed that the quantitative expectation of $F_{ST} = Q_{ST}$ under genetic drift holds, independent of mutation rate.

However, there is one important caveat: this is only true when the effects of mutation rate on F_{ST} and Q_{ST} are comparable, and these effects depend on migration rate. It should be noted that under the island model at equilibrium

$$F_{ST} = 1/[4N^*(m + \mu) + 1], \quad (2)$$

where N is the population size of each deme, m is migration rate and μ is the mutation rate (Hartl & Clark 1997). When $m >> \mu$, F_{ST} is virtually determined by migration only and mutation rate can be neglected. Hence, F_{ST} is virtually independent of mutation rate until the mutation rate becomes high relative to migration. The same is true for Q_{ST} (Edelaar & Björklund 2011). Quantitative genetic loci are thought to have a low mutation rate of around 10^{-6}–10^{-9} (Drake *et al.* 1998; Nachman & Crowell 2000; Roach *et al.* 2010), which will virtually guarantee that in the absence of selection Q_{ST} will be determined by genetic drift and migration alone. In contrast, some neutral genetic markers have much higher mutation rates, up to 10^{-2} (Ellegren 2004), and this may decrease the value of F_{ST}.

To visualize this, we have plotted in Fig. 1 how F_{ST} depends on mutation rate and migration rate, using eqn 2 and assuming a constant population size of $N = 1000$. It is

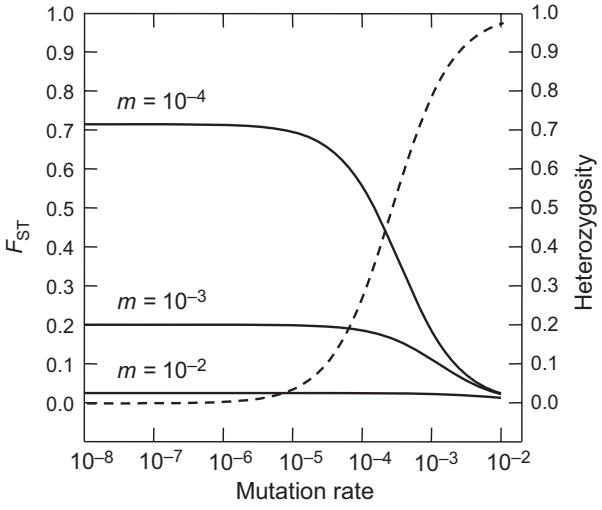

Fig. 1 F_{ST} goes down (continuous line, left *Y*-axis), and heterozygosity goes up (dashed line, right *Y*-axis) with increasing mutation rate. The decline of F_{ST} strongly depends on how high the mutation rate is relative to the migration rate m. Equilibrium values were calculated using eqns 1 and 2, and assuming a population size N of 1000.

clear that a lower migration rate results in a higher F_{ST}. More importantly here, F_{ST} declines when mutation rate becomes relatively high compared to migration rate. Also plotted (Fig. 1) is the positive effect of mutation rate on heterozygosity (using eqn 1), independent of migration rate. Hence, a high mutation rate is indicated by a higher heterozygosity, which empirically is estimated much more easily than mutation rate. Therefore, in Fig. 2, we plotted F_{ST} against heterozygosity using the same equations and parameter values. A high heterozygosity (because of a high mutation rate) does not result in a decline in the estimate of F_{ST} if migration rate is high relative to mutation rate (Fig. 2, lower line). However, for the same high heterozygosity (and same underlying high mutation rate), a serious decline in F_{ST} is observed if migration rate is low relative to mutation rate (Fig. 2, upper line).

Thus, if we compare Q_{ST} with F_{ST} estimates obtained from markers whose mutation rate is too high relative to migration rate, we will obtain a result that is biased towards $Q_{ST} > F_{ST}$ (see Ritland 2000; Hendry 2002; Leinonen *et al.* 2008). Over the years, researchers have actively sought highly variable markers for population genetics studies because these yield more discriminatory power per unit of effort (Kalinowski 2002), resulting in a steady increase in average heterozygosity over time (Fig. 3). In consequence, there is a real possibility that published empirical Q_{ST}–F_{ST} comparisons are biased, and increasingly so (Kronholm *et al.* 2010; Edelaar & Björklund 2011). However, in the absence of empirical estimates of population size, migration rates and mutation rates for each study, we cannot establish theoretically whether such a bias has occurred or not.

Therefore, to determine whether, and to what extent, a bias has occurred, we review here the published empirical comparisons of neutral and quantitative genetic differentiation among populations using a 'Q_{ST} vs. F_{ST}' framework. Because the exact marker mutation rates are not known, we used marker heterozygosity as a proxy for mutation rate (see eqn 1) and test for the expected positive relationship between heterozygosity and the difference Q_{ST}–F_{ST}. Such a positive relationship would support our hypothesis that selecting markers with a high mutation rate and hence high heterozygosity will result in lower estimates of F_{ST}, and subsequently larger, biased values for Q_{ST}–F_{ST}. In addition, we determine whether a quantitative prediction of our hypothesis actually provides a good fit to the observed pattern of Q_{ST}–F_{ST} reported in the literature.

However, some alternative explanations may also fit such a pattern. First, estimates of Q_{ST} and/or F_{ST} could have changed over time for reasons other than increased marker heterozygosity, in which case the correlation would not be causal. For example, the choice of study populations or traits may have been increasingly steered towards those a priori known to be phenotypically divergent and where local adaptation was suspected (another serious and problematic kind of bias, inflating Q_{ST}: see Leinonen *et al.* 2008; Whitlock 2008). We therefore also tested statistically whether the factor 'year' explained part of the variation in Q_{ST}–F_{ST}, independent of the variation in heterozygosity among studies. Second, variation in population size and/or migration rate among studies could create a positive correlation between heterozygosity and Q_{ST}–F_{ST}, as both population size and migration rate could simultaneously

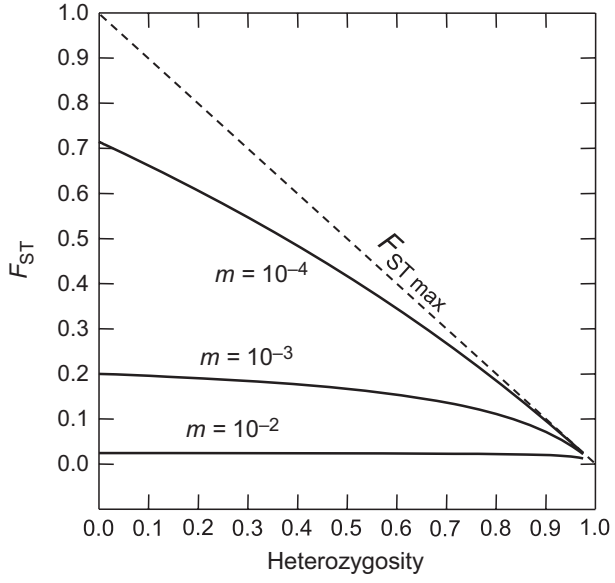

Fig. 2 F_{ST} declines with increasing heterozygosity, but mostly so when migration rate is low. The dotted diagonal line indicates the value of F_{STmax} (= 1 − H_{WITHIN}), which is the upper limit for any F_{ST} value. Note that a reduction in F_{ST} already occurs as soon as heterozygosity > 0 but is much stronger for high heterozygosity. Equilibrium values calculated using eqns 1 and 2, and assuming a population size N of 1000.

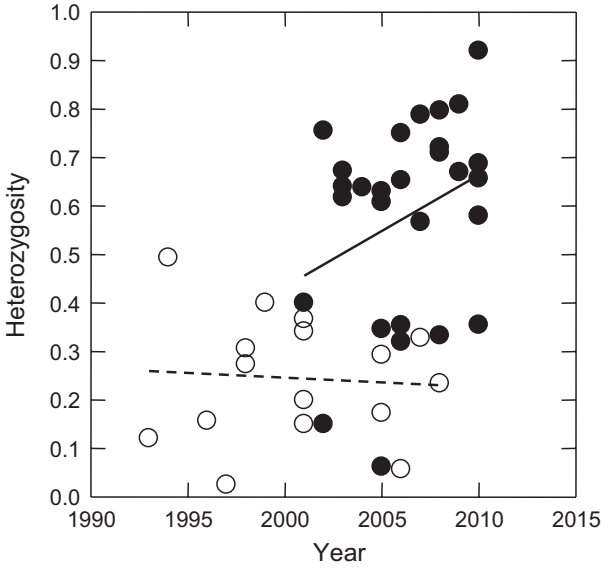

Fig. 3 The heterozygosity of neutral markers used in Q_{ST}–F_{ST} comparisons has increased over time. White dots and dashed regression line are allozymes; black dots and continuous regression line are microsatellites.

increase heterozygosity and decrease F_{ST} (eqns 1 and 2). To differentiate between the effects of mutation rate or population size/migration rate on heterozygosity, we make use of the fact that most studies employed either allozymes or microsatellites as neutral markers, and that microsatellites on average have much higher mutation rates than allozymes (Ellegren 2004). If the variation among studies in heterozygosity is at least partly driven by mutation rate, then heterozygosity should be lower in studies using allozymes. Instead, if variation in heterozygosity is only because of variation in demography among studies, then studies using either allozymes or microsatellites should not differ in heterozygosity, because we have no reason to suspect systematic differences in demography. In all cases we assume that the molecular markers used are neutral (as did the original studies). In principle, selection on molecular markers could also induce a correlation between heterozygosity and Q_{ST}–$F_{ST,}$ but this would require that selection acted on the molecular markers in the majority of studies, so we refute this possibility a priori.

Methods and results

As a starting point for compiling published studies on the topic, we used the latest review of Q_{ST}–F_{ST} comparisons by Leinonen *et al.* (2008). We then further checked all articles that cited Leinonen *et al.* (2008) for relevancy and independently extracted values of F_{ST} and Q_{ST} when possible. Whenever we encountered a difference with values published in Leinonen *et al.* (2008) and the difference could not be resolved in favour of the values published in Leinonen *et al.* (2008), we retained the values extracted by us. In addition, we recorded the published values of within-population expected heterozygosity (H_{WITHIN}) when provided. Most studies used either allozymes or microsatellites (= STR or SSR markers). To allow for a meaningful comparison among marker types with known different average mutation rates, and because the calculation of H_{WITHIN} was sometimes methodologically quite different for certain (dominant) markers, the few studies using other types of markers (AFLP, RAPD, ESTP, RFLP, CAPS) were not further considered here. An overview of the final set of studies and values used in our analyses is presented in Table 1 (n = 44 studies, 40 species).

Figure 4 shows the relationship between the difference between Q_{ST} and F_{ST} and within-population heterozygosity (Fig. 4). The average Q_{ST}–F_{ST} difference across studies clearly lies above the Q_{ST}–F_{ST} = 0 line, supporting the conclusion of Leinonen *et al.* (2008) that Q_{ST} is on average larger than F_{ST}. However, there is also a clear pattern in that the difference Q_{ST}–F_{ST} is positively related to heterozygosity. Dividing the studies into groups with heterozygosities either greater or smaller than 0.5, Q_{ST}–F_{ST} is significantly greater for studies with high heterozygosity (least squares means 0.261 ± 0.042 SE vs. 0.087 ± 0.038 SE; ANOVA: $F_{1,42}$ = 9.34, P = 0.004, R^2 = 0.18). Similarly, regression of Q_{ST}–F_{ST} on heterozygosity yielded a positive slope (β = 0.277 ± 0.123 SE, $F_{1,42}$ = 5.08, P = 0.03, R^2 = 0.11), and a similar pattern was found for studies using microsatellites alone (β = 0.391 ± 0.198 SE, $F_{1,26}$ = 3.90, P = 0.059, R^2 = 0.13).

We calculated a simplified expected relationship between heterozygosity and the Q_{ST}–F_{ST} difference, using the data of these studies. For this, we assumed that the degree of quantitative and neutral divergence is constant across studies, but that the actual value of F_{ST} decreases linearly with increasing heterozygosity. We established that Q_{ST} is indeed not related to heterozygosity (linear regression: $F_{1,42}$ = 0.084, P = 0.77, R^2 = 0.00) and used the mean of Q_{ST} as an estimate for quantitative divergence (0.341 ± 0.033 SE). As an estimate of mean neutral divergence, we used the intercept of the regression of F_{ST} on heterozygosity (0.320 ± 0.056 SE). This predicts the following relationship between heterozygosity and the Q_{ST}–F_{ST} difference: Q_{ST}–F_{ST} = 0.341 − 0.320 * (1 − H_{WITHIN}). This quantitative prediction matched the observed regression slope remarkably well (Fig. 4).

With respect to our first alternative explanation for a relationship between heterozygosity and the Q_{ST}–F_{ST} difference, there was only a mild non-significant temporal trend for the difference Q_{ST}–F_{ST} ($F_{1,42}$ = 1.09, P = 0.30). Moreover, the inclusion of the continuous variable 'year' in the model of the regression of Q_{ST}–F_{ST} on heterozygosity explained virtually no variance in Q_{ST}–F_{ST} (t_{41} = −0.044, P = 0.97), whereas heterozygosity remained a very influential explanatory variable (t_{41} = 1.95, P = 0.058). With respect to our second alternative explanation, mean heterozygosity differed substantially and significantly among studies using either microsatellites or allozymes (Fig. 3; microsatellites: 0.578 ± 0.035 SE, allozymes: 0.245 ± 0.046 SE, ANOVA: $F_{1,42}$ = 33.4, P < 0.0001, R^2 = 0.44).

Discussion

Is there a bias?

Our main hypothesis is that much higher mutation rates in the neutral markers than in the quantitative genetic loci are causing a strong upward bias in Q_{ST}–F_{ST}.

Indeed, we found the positive correlation between heterozygosity and the difference Q_{ST}–F_{ST} that is predicted by this hypothesis. Moreover, the predicted quantitative relationship between heterozygosity and Q_{ST}–F_{ST} based on this hypothesis shows a very good fit with the observed one (Fig. 4), suggesting that this hypothesis is a sufficient explanation for the observed pattern.

We found no significant temporal trend in Q_{ST}–$F_{ST,}$ and time explained virtually no variation in Q_{ST}–F_{ST} independent of the effect of heterozygosity. Thus, the idea that the correlation between heterozygosity and Q_{ST}–F_{ST} is accidentally due to independent temporal trends (e.g. a shift towards markers with higher heterozygosity coinciding with a shift towards systems with higher Q_{ST} values) is not supported.

Variation in population size and/or migration rate among studies could also create a correlation between heterozygosity and Q_{ST}–F_{ST}, independent of variation in mutation rate

Table 1 Overview of studies and values included in our analyses. We only included studies based on allozymes ('allo') or microsatellites ('micro') and that provided estimates of F_{ST} (or a comparable measure of neutral population divergence), Q_{ST} and within-population heterozygosity (H_{WITHIN}) based on the same, wild populations. G'st is the unbiased estimator (G''_{ST}) of Meirmans & Hedrick 2010; k is the number of study populations or regions. Some studies have multiple entries because they included several independent comparisons, e.g. among populations within regions, and among regions. We excluded the studies on *Tigriopus californicus* (Edmands & Harrison 2003) and *Arabidopsis thaliana* (Kuittinen *et al.* 1997) in our statistical analyses because they yielded theoretically impossible values of G'st and/or Jost's $D > 1$

Species	F_{ST}	Q_{ST}	H_{WITHIN}	Jost's D	G'st	k	Year	Marker	References
Apodemus flavicollis	0.045	0.138	0.057	0.003	0.047	10	2006	allo	Wojcik *et al.* (2006)
Arabidopsis thaliana	1.000	0.885	0.000	1.000	1.000	4	1997	allo	Kuittinen *et al.* (1997)
Brassica insularis	0.210	0.080	0.367	0.205	0.332	4	2001	allo	Petit *et al.* (2001)
Carduelis chloris	0.030	0.300	0.025	0.001	0.031	12	1997	allo	Merilä (1997)
Centaurea corymbosa	0.360	0.341	0.200	0.187	0.450	4	2001	allo	Petit *et al.* (2001)
Daphnia obtusa	0.280	0.230	0.121	0.061	0.319	8	1993	allo	Spitze (1993)
Daphnia obtusa	0.290	0.290	0.493	0.455	0.573	8	1994	allo	Lynch & Spitze (1994)
Daphnia pulex	0.310	0.350	0.400	0.318	0.517	17	1999	allo	Lynch *et al.* (1999)
Daphnia pulicaria	0.270	0.526	0.150	0.070	0.318	14	2001	allo	Morgan *et al.* (2001)
Hordeum spontaneum	0.230	0.630	0.173	0.066	0.278	4	2005	allo	Volis *et al.* (2005)
Liatris scariosa	0.210	0.300	0.293	0.120	0.297	12	2005	allo	Gravuer *et al.* (2005)
Littorina saxatilis	0.095	0.253	0.328	0.077	0.141	3	2006	allo	Conde-Padín *et al.* (2006)
Picea glauca	0.014	0.098	0.341	0.009	0.021	6	2001	allo	Jaramillo-Correa *et al.* (2001)
Salix viminalis	0.041	0.070	0.157	0.009	0.049	14	1996	allo	Lascoux *et al.* (1996)
Scabiosa canescens	0.164	0.096	0.273	0.089	0.226	6	1998	allo	Waldmann & Andersson (1998)
Scabiosa columbaria	0.123	0.453	0.306	0.074	0.177	6	1998	allo	Waldmann & Andersson (1998)
Scathophaga stercoraria	0.009	0.144	0.234	0.003	0.012	5	2008	allo	Demont *et al.* (2008)
Amphicarpaea edgeworthii	0.578	0.688	0.333	0.722	0.867	19	2009	micro	Liang *et al.* (2009)
Arabidopsis thaliana	0.600	0.630	0.346	0.866	0.707	12	2005	micro	Le Corre (2005)
Arabidopsis thaliana	0.890	0.820	0.062	0.602	0.949	9	2005	micro	Stenøien *et al.* (2005)
Arabidopsis thaliana	0.639	0.664	0.315	1.221	0.933	3	1997	micro	Kuittinen *et al.* (1997)
Brachionus plicatilis	0.482	0.218	0.355	0.615	0.747	6	2009	micro	Campillo *et al.* (2009)
Bufo calamita	0.030	0.260	0.638	0.068	0.083	5	2004	micro	Gomez-Mestre & Tejedo (2004)
Coregonus lavaretus	0.120	0.250	0.608	0.233	0.306	11	2005	micro	Østbye *et al.* (2005)
Corynopoma riisei	0.025	0.052	0.688	0.060	0.080	18	2010	micro	Arnqvist & Kolm (2010)
Cynopterus sphinx	0.030	0.030	0.755	0.109	0.122	8	2002	micro	Storz (2002)
Daphnia pulicaria	0.390	0.526	0.400	0.459	0.650	14	2001	micro	Morgan *et al.* 2001)
Eucalyptus globulus	0.090	0.129	0.750	0.330	0.360	10	2006	micro	Steane *et al.* (2006)
Ficedula hypoleuca	0.010	0.480	0.809	0.046	0.052	16	2009	micro	Lehtonen *et al.* (2009)
Gasterosteus aculeatus	0.190	0.540	0.653	0.490	0.548	10	2006	micro	Leinonen *et al.* (2006)
Gasterosteus aculeatus	0.080	0.140	0.788	0.369	0.377	8	2007	micro	Raeymaekers *et al.* (2007)
Gasterosteus aculeatus	0.150	0.800	0.710	0.457	0.517	18	2008	micro	Cano *et al.* (2008)
Hirundo rustica	0.001	0.268	0.920	0.017	0.013	3	2010	micro	Santure *et al.* (2010)
Osmerus mordax	0.024	0.550	0.640	0.087	0.067	2	2003	micro	Saint-Laurent *et al.* (2003)
Osmerus mordax	0.013	0.680	0.673	0.054	0.040	2	2003	micro	Saint-Laurent *et al.* (2003)
Primula sieboldii	0.172	0.227	0.670	0.526	0.521	5	2009	micro	Yoshida *et al.* (2009)
Rana arvalis	0.340	0.460	0.354	0.565	0.526	2	2007	micro	Knopp *et al.* (2007)
Rana temporaria	0.235	0.810	0.618	0.596	0.615	6	2003	micro	Palo *et al.* (2003)
Rana temporaria	0.058	0.265	0.580	0.102	0.138	6	2010	micro	Richter-Boix *et al.* (2010)
Salmo trutta	0.065	0.465	0.721	0.239	0.233	4	2008	micro	Jensen *et al.* (2008)
Salvelinus fontinalis	0.153	0.230	0.630	0.615	0.414	2	2005	micro	Perry *et al.* (2005)
Scatophaga stercoraria	0.010	0.144	0.797	0.050	0.049	5	2008	micro	Demont *et al.* (2008)
Thlaspi caerulescens	0.150	0.151	0.567	0.277	0.346	6	2007	micro	Jimenez-Ambriz *et al.* (2007)
Thymallus thymallus	0.100	0.350	0.150	0.029	0.118	3	2002	micro	Koskinen *et al.* (2002)
Tigriopus californicus	0.327	0.130	0.138	0.117	0.379	3	2003	micro	Edmands & Harrison (2003)
Tigriopus californicus	0.811	0.120	0.221	1.826	1.041	3	2003	micro	Edmands & Harrison (2003)
Tigriopus californicus	0.803	0.300	0.180	1.074	0.979	6	2003	micro	Edmands & Harrison (2003)
Tyto alba	0.011	0.353	0.657	0.023	0.032	18	2010	micro	Antoniazza *et al.* (2010)
Vitellaria paradoxa	0.047	0.189	0.320	0.026	0.069	11	2005	micro	Sanou *et al.* (2005)

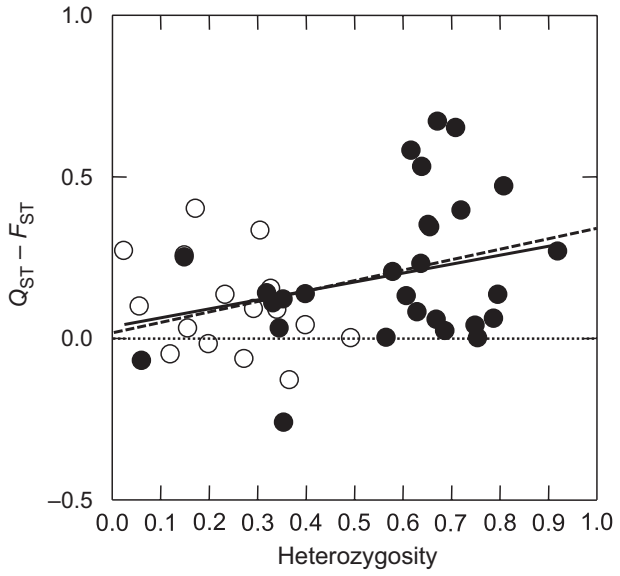

Fig. 4 The difference between Q_{ST} and F_{ST} is positively related to the heterozygosity of the neutral markers used to estimate F_{ST}. White dots are studies using allozymes, black dots are studies using microsatellites. Values above the horizontal dotted line indicate divergent selection on quantitative traits. The continuous line is the regression slope of Q_{ST}–F_{ST} on heterozygosity; the dashed line is the predicted relationship between these same variables assuming that quantitative and neutral divergence are constant across studies, but F_{ST} declines linearly with heterozygosity (see Fig. 2).

of the utilized markers. However, allozymes and microsatellites generally differ in mutation rate (Hartl & Clark 1997; Ellegren 2004). For the studies reviewed here, we found the expected large difference in heterozygosity between these markers, indicating that at least some of the variation in heterozygosity among studies is indeed because of variation in mutation rate, and not just because of variation in demography. Although variation in demography may add to the pattern of Fig. 4, we refute it as being a sufficient explanation. Similarly, any alternative explanation we have not considered here would have to explain why heterozygosity differs systematically between microsatellites and allozymes, and why that alternative is more likely than a difference in mutation rate between them.

It seems thus clear that the usage of more variable neutral markers with higher mutation rates has biased the estimated differences between Q_{ST} and F_{ST}, and that this bias has tended to become increasingly strong over the years. Apparently, by selecting markers with a high heterozygosity, researchers have violated the assumption that migration rates are two or more orders of magnitude larger than mutation rates, such that mutation rate could no longer be ignored and deflated estimates of F_{ST} (see Figs 1 and 2).

Implications

This is a disconcerting conclusion, as it casts considerable doubts on the results of many individual studies. To visualize roughly the extent of the bias, one could move the cloud of data points of the microsatellites on the right-hand side of Fig. 4 towards the left along the regression slope, until it overlaps with the range of heterozygosity of the allozymes (which generally have mutation rates much more comparable to those of quantitative genetic loci so are probably not biased). The result is quite startling. If less variable neutral markers had been used in these studies, it is very likely that many would have found $Q_{ST} < F_{ST}$. Many others might have lost their statistical support for the conclusion that $Q_{ST} > F_{ST}$, because Q_{ST} estimates normally have wide confidence intervals (O'Hara & Merilä 2005; Whitlock 2008).

Similarly, the results of the meta-analyses are compromised, as these did not adequately take this bias into account. Leinonen *et al*. 2008 did recognize the possibility that differences between neutral markers in mutation rate could result in biased F_{ST} values and included the variable 'marker type' in their analyses. They did not find a significant effect on F_{ST} estimates (although their Fig. 4c does show a trend that microsatellites give lower F_{ST} estimates than other markers). We suspect that this is because a fair proportion of the microsatellites used in earlier studies actually have low mutation rates (resulting in low heterozygosities, see Figs 3 and 4), and thus overlapped with allozymes in their effect. In addition, many of the studies using highly variable microsatellites have been published after Leinonen *et al*. (2008), giving our study more statistical power to observe the bias because of marker mutation rate. Overall, the general conclusion from these studies, that populations have commonly diverged because of exposure to divergent selection, has to be substantially moderated. Neutral genetic drift or even stabilizing selection is likely to have a higher relevance for population differentiation than previously inferred.

Potential solutions to biased Q_{ST}–F_{ST} comparisons

Having established that a substantial number of Q_{ST}–F_{ST} comparisons are compromised because of the usage of overly variable neutral markers of course begs the question: what to do next? We briefly discuss three solutions to bias because of use of highly variable markers in Q_{ST}–F_{ST} comparisons: (i) correcting for marker heterozygosity, (ii) using markers with lower mutation rates, and (iii) calculating a measure of neutral population divergence that is not affected by mutation rate.

Potential solution 1: correcting for marker heterozygosity. A first solution to biased Q_{ST}–F_{ST} comparisons might be to correct the effect of marker heterozygosity on F_{ST}, which could be applied to both past and future estimates of divergence in neutral markers. Hedrick (Hedrick 2005; Meirmans & Hedrick 2010) and Jost (2008) have derived estimators of population differentiation that are independent of marker variability, suggesting that perhaps one could avoid bias by calculating the difference between Q_{ST} and these estimators. However, Hedrick's *G'*st and Jost's *D*

are not theoretically equivalent to F_{ST} and measure different aspects of population genetics than F_{ST} and Q_{ST} do (see examples in Meirmans & Hedrick 2010). In addition, *G*'st and *D* are not independent of mutation rate when this is high relative to migration rate (Jost 2008; Whitlock 2011), so it is not clear that values of *G*'st and *D* for markers with high mutation rates are comparable to divergence at quantitative genetic loci with low mutation rates (Kronholm *et al.* 2010; Whitlock 2011). Moreover, any statistical correction for marker heterozygosity such as *G*'st has the problem of deciding for each individual study which proportion of marker heterozygosity is attributable to variation in mutation rate that needs to be corrected for and which part is attributable to variation in population size and migration rate, which have independent effects on Q_{ST}, F_{ST} and heterozygosity and which should not be corrected for. Hence, neither *G*'st nor Jost's *D* seem to be useful as estimators of neutral divergence in the context of comparison with Q_{ST}.

Potential solution 2: using markers with lower mutation rates. A second solution is to abandon the use of highly variable markers, such as microsatellites, for comparisons of Q_{ST} and F_{ST} and use markers with low mutation rates instead, such as SNPs (Edelaar & Björklund 2011). Moreover, the vast majority of SNPs are bi-allelic and can be analysed within the classical F_{ST} framework, because bi-allelic loci can reach their theoretical maximum F_{ST} of 1. Because the information content of single SNPs is rather low, and because evolutionary stochasticity can result in very different F_{ST} estimates for individual SNPs, a decent number of unlinked SNPs would be needed to get a reliable estimate of the mean and variance of F_{ST} across the genome (Whitlock 2008; Edelaar & Björklund 2011). Scoring large numbers of SNPs is now becoming increasingly feasible for any kind of organism (Ouborg *et al.* 2010; Tautz *et al.* 2010). It is even possible to take into account that a small percentage of SNPs might be under divergent selection (Brumfield *et al.* 2003; Glover *et al.* 2010), which otherwise tends to yield higher F_{ST} values (but a more conservative test for divergent selection on the quantitative trait of interest).

Similar suggestions of moving back to markers with low variation have recently been made in the general context of using F_{ST} for the estimation of the demographic parameters driving population differentiation (Meirmans & Hedrick 2010; Whitlock 2011). While highly variable markers can be very useful for some applications, such as assignment of individuals to parents or populations, they appear to be problematic for the interpretation of the demographic history of populations. A more pluralistic usage of marker variability, where marker choice is tailored to the aims of each study, seems called for.

Potential solution 3: calculating a measure of neutral population divergence that is not affected by mutation rate. A third solution would be to compare quantitative genetic divergence of quantitative traits (Q_{ST}) with an equally *quantitative* approach to neutral genetic divergence. It should be noted that virtually all estimators of neutral genetic divergence treat alleles as different by identity only, e.g. in the calculation of heterozygosity. This neglects information about genetic distances among alleles (if this is known). In contrast, Q_{ST} does take the distance among individuals (their genetic breeding values) into account when calculating variance components. As such, Q_{ST} is really a kind of relative genetic *distance* measure, which expresses the difference among populations relative to the difference among individuals within populations. Hence, the best comparison with this seems to be a conceptually similar genetic distance measure for neutral markers that takes into account the genetic distance among alleles. Such a distance measure was described by Excoffier *et al.* (1992) for haplotypes (Φ_{ST}). Likewise, Slatkin (1995) introduced R_{ST}, based on differences in microsatellites allele size, which was later shown by Michalakis & Excoffier (1996) to be comparable to Φ_{ST}. Recently, Kronholm *et al.* (2010) and Whitlock (2011) have shown by simulation that divergence measures which take into account the genetic distance among alleles are independent of mutation rate for any kind of marker, if the mutation process leaves a reliable traceable history of the coancestry of alleles (coalescence information). This condition is not fulfilled by all commonly used neutral markers. For allozymes, it is hard to derive a valid genetic distance among the different alleles. For microsatellites, it is hard to argue that mutations involving several repeats are negligible. The percentage of multistep mutations has been estimated to vary between 11–63% in humans, and between 5–75% in other organisms (Ellegren 2004), so that microsatellite allele size similarity appears to be a poor measure of ancestral similarity (see Balloux & Lugon-Moulin 2002; Li *et al.* 2002; Ellegren 2004). Simulations by Kronholm *et al.* (2010) showed that when 20% of microsatellite mutations were not stepwise, Φ_{ST} did not correct properly for mutation rate. Therefore, only those microsatellite loci that have been confirmed in the study system to adhere to the stepwise mutation model by checking the process of mutation in a large number of pedigreed offspring (Ellegren 2004) could be confidently used to calculate R_{ST} as a measure of neutral genetic divergence. So in practice, this restricts the choice of markers to sequence data of regions other than SSR. Here, the signature of old mutations is only likely to be erased by additional mutations after a long period of evolutionary time and would rarely happen among populations within species. Therefore, any two alleles differing by a greater number of sites are likely to have been evolving independently for longer. In practical terms, such a relative neutral genetic distance measure can be for instance calculated via AMOVA, when implementing a matrix of genetic distances (not identities) among non-recombining haplotypes (Excoffier *et al.* 1992; see also Kronholm *et al.* 2010). The resulting estimate of Φ_{ST} is then the appropriate quantitative measure of relative neutral genetic divergence among populations with which to compare Q_{ST}. (Note that this estimate does not need the correction of Meirmans (2006), which was only proposed for

AMOVA based on allele identities). Because multiple unlinked loci would be required to estimate evolutionary stochasticity among loci (Whitlock 2008), the use of mtDNA would be disqualified because mtDNA normally acts as a single locus. Moreover, mtDNA has a smaller effective population size, has a different mode of inheritance and is exposed to selection. As far as we could establish, a comparison between Q_{ST} and Φ_{ST} using non-SSR sequence data has not yet been made, which is surprising given that AMOVA has been around since the first empirical Q_{ST}–F_{ST} comparisons.

There are two caveats, however, in comparing Q_{ST} with distance-based neutral divergence measures. The first one is that the loci underlying neutral or quantitative genetic divergence may not follow the same mutation model. Earlier, we argued that distance-based measures are independent of mutation rate, if we use the genetic distances among alleles and if these alleles contain sufficient coalescence information. However, for a valid comparison in principle, the same must be true for Q_{ST}. Kronholm *et al.* (2010) and Edelaar & Björklund (2011) showed that when the mutations of quantitative genetic loci are independent (i.e. they do not resemble their ancestral states), Q_{ST} decreases at high mutation rates. In that case, comparing Q_{ST} with a distance-based neutral divergence measure would be inappropriate (although it would provide a conservative test for the hypothesis of selection-driven population divergence). However, at the moment, it appears that the mutation rate of quantitative genetic loci is sufficiently low relative to migration rate that this complication in practice can be ignored, and we do not have to know how quantitative genetic loci mutate. [To the extent that non-Mendelian epigenetic mechanisms of inheritance play a role in divergence (Gilbert & Epel 2009; Herrera & Bazaga 2010), this assumption may need to be reconsidered].

The second caveat is that genetic differentiation estimators based on allele distances can have larger variances than those based on allele identities (Hardy *et al.* 2003). However, Excoffier (2007) suggests this is essentially only true if distances among alleles evolve according to the stepwise mutation model. This is by and large the case for microsatellites: a new mutation may either add or remove a repeat (Li *et al.* 2002; Ellegren 2004). This means that a microsatellite allele of a given length may have evolved from a shorter or from a longer ancestral allele, i.e. identity-by-state is not exactly identity-by-descent, and the coalescent information in allele length variation is reduced. If so, for neutral markers that are closer to the infinite alleles model (multiple mutations at the same site do not occur), the variance in distance-based divergence measures should not be affected.

Conclusions

Usage of neutral genetic markers with high mutation rates has generally led to upwardly biased estimates of the difference between Q_{ST} and F_{ST}. In the absence of estimates of population size and migration rate, the bias for individual studies cannot be determined nor corrected. To obtain unbiased future Q_{ST}–F_{ST} estimates, we suggest the use of neutral markers with mutation rates that are comparable to those of quantitative genetic loci (in the order of 10^{-6} or lower), because this makes it less likely that mutation rate biases neutral genetic divergence. SNPs generally have low mutation rates and can be analysed within the classical F_{ST} framework, so these are a good option. When the neutral marker can be argued to contain much coalescent information (allele distance reliably conveys the degree of shared evolutionary history), we suggest comparing Q_{ST} not to F_{ST} (or its relatives such as G_{ST} or θ) but to measures of genetic divergence such as Φ_{ST} that are based on genetic distances among alleles, because of their conceptual similarity to Q_{ST}. In addition, distance-based measures of neutral genetic divergence are independent of mutation rate, so allow for the use of neutral markers with any mutation rate. Non-recombining stretches of nuclear DNA contain much coalescent information and should be suitable. Microsatellites typically have high mutation rates and contain questionable coalescent information and should be avoided. The use of mtDNA is not advised in view of multiple issues. Other kinds of neutral markers should be judged similarly on their mutation rate and coalescent information content to assess their suitability for Q_{ST}–F_{ST} comparisons.

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P.E. works on the mechanisms that drive or limit adaptation, population differentiation and speciation (currently in the context of biological invasions), with a special focus on the importance of individual variation. P.B. is interested in evolutionary biology and in both genomic and physiological responses to environmental conditions. I.G.M. has a broad interest in evolutionary biology and in the role of phenotypic plasticity in life-history evolution in particular.

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Data accessibility

Please see Table 1.