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Revisiting F_{IS}, F_{ST}, Wahlund Effects, and Null Alleles

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Abstract

Null alleles and Wahlund effects are well known causes of heterozygote deficits in empirical population genetics studies as compared to Hardy–Weinberg genotypic expectations. Some authors have theoretically studied the relationship of Wright's F_{IS} computed from subsamples displaying a Wahlund effect and F_{ST} before the Wahlund effect, as can occasionally be obtained from populations of long-lived organisms. In the 2 subsample case, a positive relationship between these 2 parameters across loci would represent a signature of Wahlund effects. Nevertheless, for most organisms, getting 2 independent subsamples of the same cohort and population, one with a Wahlund effect and the other without, is almost never achieved and most of the time, empirical population geneticists only collect a single sample, with or without a Wahlund effect, or with or without null alleles. Another issue is that null allele increase F_{IS} and F_{ST} altogether and thus may also create such correlation. In this article, I show that, for organisms collected in a single sample, which corresponds to the most common situation, Wahlund effects and null alleles affect the values of both F_{IS} and F_{ST} though in the opposite direction. I also show that Wahlund effect produces no or weak positive correlation between the 2 *F*-statistics, while null alleles generate a strong positive correlation between them. Variation of these *F*-statistics is small and even minimized for F_{ST} under Wahlund effects as compared to null alleles. I finally propose a determination key to interpret data with heterozygote deficits.

Subject area: Population structure and phylogeography
Key words: differentiation, *F*-statistics, genetic identities, inbreeding

Empirical population genetics is the study of the population biology of natural populations through the analysis of spatio-temporal variation of molecular markers (e.g., allozymes, microsatellite loci, SNPs, etc.). Hardy–Weinberg equilibrium and its expected genotypic proportions are central in such studies, because any deviation from these expected proportions can provide clues for inferences on the functioning of the targeted populations (De Meeûs et al. 2007). Many tools are currently available but some of the most widely used are Wright's (Wright 1965) *F*-statistics (Nagylaki 1998): F_{IS}, is a measure of deviation from panmixia at local scales (e.g., within subsamples); F_{ST} is a measure of subdivision; F_{IT} is a measure of deviation from panmixia at the whole sample scale and actually corresponds to the combined effect of the 2 first ones: $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$ (De Meeûs et al. 2007).

In a recent article, Waples (2015) reviews the different causes of deviations from Hardy–Weinberg genotypic expected proportions and linkage disequilibrium, with some attention, among other factors, to Wahlund effect and null alleles. Wahlund effect occurs when

genotypic proportions are computed from heterogeneous samples where individuals belonging to genetically differentiated entities are pooled. These entities can represent members of different (and genetically distant) subpopulations, different cohorts, or different cryptic species. This typically happens when the real scale at which population structure occurs is unknown, resulting in an inaccurate sampling design. Null alleles occurs when the genotyping technique used fails to unveil the presence of true alleles that thus appear as missing data if homozygous or as apparent homozygous when heterozygous with a visible allele. This typically arises in microsatellite markers when a mutation event alters one of the flanking sequences where primers are to attach to initiate DNA amplification (PCR). If this mutation prevents the primer annealing to template DNA during amplification of the microsatellite locus by PCR, this results in a null allele. Wahlund effect and null alleles both increase F_{IS} and thus produce apparent heterozygote deficits. In his article, Waples (2015) offered a criterion to discriminate a Wahlund effect from other causes as null alleles, in a 2 populations and 2 alleles context, which was generalized by Zhivotovsky (2015) for multiple alleles. In these models, the F_{ST} before Wahlund effect must be known from an independent sample and F_{IS} measured in another sample with Wahlund effect. I will label the Wahlund effect on F_{IS} as F_{IS_W}. The main result of these works is that there is an expected positive correlation between F_{IS_W} and F_{ST} across loci, which can be used as a criterion as opposed to null alleles where such correlation is believed not to happen. It was also stated that because of this correlation, the criterion that is often found in the literature of stability of F_{IS} across loci under a Wahlund effect, as compared to the effect of null alleles, is void. It is important to specify that in these models, the true F_{ST} (without Wahlund effect) must be known and the regression undertaken between this true F_{ST} and the F_{IS} is measured after the Wahlund effect (F_{IS_W}). Such correlation between F_{ST} and F_{IS_W} can also be easily (and with more generality) derived from the famous Wright's equation $(1 - F_{IT}) = (1 - F_{IS})\ (1 - F_{ST})$ because in a sample with a Wahlund effect, and by definition of those *F*-statistics, what is measured as F_{IS_W} is necessarily strongly connected to the initial (i.e., "true") F_{IT} and is equal to it when the final sample is a random collection of existing real subpopulations.

The knowledge of true F_{ST} is a rare situation that I only know of from a few studies on fairly long-lived mammal species: the Leadbeater's possum in Australia (Waples 2015), and the North Pacific minke whale (Waples 2011). Long-lived organisms indeed allow sampling the same cohort after several years or months so that each locus keeps the same characteristic over time between 2 sampling campaign, one of which displays a Wahlund effect, and the other displays the true population subdivision. For most organisms, in particular small ones for which population genetics tools represent the only mean to study their population biology (De Meeûs et al. 2007), initial (true), F_{ST} and Wahlund F_{IS_W} resulting from inaccurate sampling cannot be known together: either the sampling is accurate and F_{IS} is not affected by a Walund effect, or sampling is not accurate and F_{IS} and F_{ST} are both affected. Because of short generation times, even if 2 samples are available, one of which with true F_{ST} and the other with a Wahlund effect (a case that I have never met except for the 2 examples cited above), the correlation would have little chance to survive the redistribution of per locus statistics with genetic drift. A Wahlund effect typically occurs for organisms for which population subdivision is unknown, if subsamples are collected at larger scales than the actual subdivision unit. In that case, sampling contains a Wahlund effect that affects all statistics all together. Several such examples and discussions can be found in Ravel et al. (2007), Bouyer et al. (2009), Kempf et al. (2010), Prugnolle and De Meeûs (2010), Solano et al. (2010), and Rougeron et al. (2015). It is also known that null alleles also increase F_{ST} (Chapuis and Estoup 2007). A positive correlation between F_{IS} and F_{ST} can thus be predicted in that case. This thus can lead to confusion. If both F_{ST} and F_{IS} observed in the same sample are regressed and a positive correlation found, concluding to a Wahlund effect signature, as can be seen in some studies (Criscione et al. 2011; Bohling et al. 2016), may be wrong. In "classic" (i.e., single sample) samples, it can also be suspected that Wahlund effect also affects F_{ST} computations.

In this article, I analyze the most common situation where *F*-statistics are estimated from the same sample. I show that null alleles increase both F_{IS} and F_{ST} and that Wahlund effect increases F_{IS} but decreases F_{ST}. Simulations show that when sampling and genotyping are accurate (no Wahlund effect and no null alleles), a negative correlation is expected between F_{IS} and F_{ST} most of the time and that a Wahlund effect will weakly change this tendency. I also show that a strong positive correlation links F_{IS} and F_{ST} in presence of null alleles with a maximal variation of these statistics across loci in that case, while Wahlund effect weakly (if any) affects F_{IS} variation and minimize F_{ST} variance across loci. I finally propose a determination key to interpret data with heterozygote deficits. Because I am focusing on heterozygote deficits, I will not discuss selective processes. The only selective process that can cause heterozygote deficits is underdominance, a very unstable situation that should be met extremely rarely (De Meeûs et al. 2007). The only possible example could be the African butterfly *Pseudacraea eurytus*. But I could only find it in Wikipedia. A more convincing example corresponds to the rhesus system in human populations (De Meeûs et al. 2007; Abbey et al. 2011). Multilocus underdominance might happen in hybrid zones. Nevertheless, gathering different cryptic species (or subspecies) into single subsamples will more correspond to more or less extreme "classic" Wahlund effects, as treated in the present article.

Methods

Definitions

In this article, I will use *F*-statistics definitions according to Cockerham (1969, 1973) and Rousset (1996, 2004) and a notation that I find easier to follow as in De Meeûs et al. (2007). In a 3 hierarchical (i.e., nested) level of sampling structure, with individuals in subsamples and total sample, we can define 3 identity parameters:

- Q_I is the probability to twice sample the same allele in an individual;
- Q_S is the probability to twice sample the same allele from 2 individuals from the same subsample;
- Q_T is the probability to twice sample the same allele from 2 different subsamples.

From there, we can define Wrights *F*-statistics:

$$\begin{cases} F_{IS} = \dfrac{Q_I - Q_S}{1 - Q_S} \\ F_{ST} = \dfrac{Q_S - Q_T}{1 - Q_T} \\ F_{IT} = \dfrac{Q_I - Q_T}{1 - Q_T} \end{cases} \quad (1)$$

Note that $1 - Q_S$ and $1 - Q_T$ are the probabilities to sample different alleles in one subsample or in the total sample, respectively, and are thus equal or close to subsample and total sample genetic diversities H_S and H_T, respectively.

I will use the above notation for the "true" parameters, that is, the ones that would be measured if the population investigated would have been accurately sampled (no Wahlund effect) and the marker used immune from genotyping errors (no null alleles, dropouts, or stuttering).

Q_{I_W}, Q_{S_W}, and Q_{T_W} are the probabilities of identity measured in subsamples with Wahlund effects and F_{IS_W}, F_{ST_W}, and F_{IT_W} are the corresponding *F*-statistics.

W_S and W_T parameters describe the intensity of Wahlund effect on Q_S and Q_T, respectively. These parameters are a function of real allele frequencies in the different subpopulations and of the importance and modality of admixture of individuals from different subpopulations in the actual sample. These parameters are thus functions of F_{ST}.

Q_{I_N}, Q_{S_N}, Q_{T_N}, F_{IS_N}, F_{ST_N}, and F_{IT_N} are the same as above but when the genetic marker is affected by null alleles.

Q_{Ii}, Q_{Si}, and F_{ISi} are the true corresponding parameter values in subpopulation *i*.

In the bi-allelic case, p_i and q_i are the "true" allele frequencies of the 2 visible alleles of the genetic marker in subpopulation *i* and p_{Ni} is the frequency of null alleles in subpopulation *i*.

The bar above a parameter will mean the average value across all subsamples: for instance, the average null allele frequency across *n* subsamples will be:

$$\overline{p_N} = \frac{1}{n}\sum_{i=1}^{n} p_{Ni} \quad (2)$$

Two population structure models, with *n* subpopulations of size *N* and immigration rate *m* were explored: the Island model, where each subpopulation, at each generation, is composed of $(1 - m)N$ residents and mN immigrants coming from any of the $n - 1$ other subpopulations (no spatial structure); the 2 dimensional stepping stone model, where immigrants come from direct neighbors (4 neighbors in central subpopulations, 2 in corner subpopulations, and 3 for other marginal subpopulations). Stepping stone models thus result into isolation by distance (spatial population structure).

Different sampling errors lead to a Wahlund effect. A Wahlund effect can be spatial and/or temporal. Spatial Wahlund effect can itself result from several kinds of sampling errors, some of which are illustrated in Figure 1. Each kind gathers individuals that belong to different subpopulations (with different allele frequencies). For the unshared Wahlund effect, each subsample contains individuals from several subpopulations that are not shared with other subsamples (Figure 1D). For the shared Wahlund effect, each subsample shares common origins with other subsamples with respect to subpopulations that are pooled (Figure 1B,F). A third sampling gathers heterogeneous admixtures (unbalanced Wahlund effect, Figure 1E). Wahlund effects arise from a lack of knowledge of the true structure of a population. Hence, "shared" and/or "unbalanced" samples are probably the most frequent causes of Wahlund effects. For populations with isolation-by-distance migration models (2-dimensional stepping stone models), the closest subpopulations are genetically the most similar. For Island models of migration with no spatial structure of genetic information, genetic differentiation is uncorrelated with subpopulation position.

Temporal effects occur when members of different cohorts are pooled in the same subsamples. This is a classical cause of biased

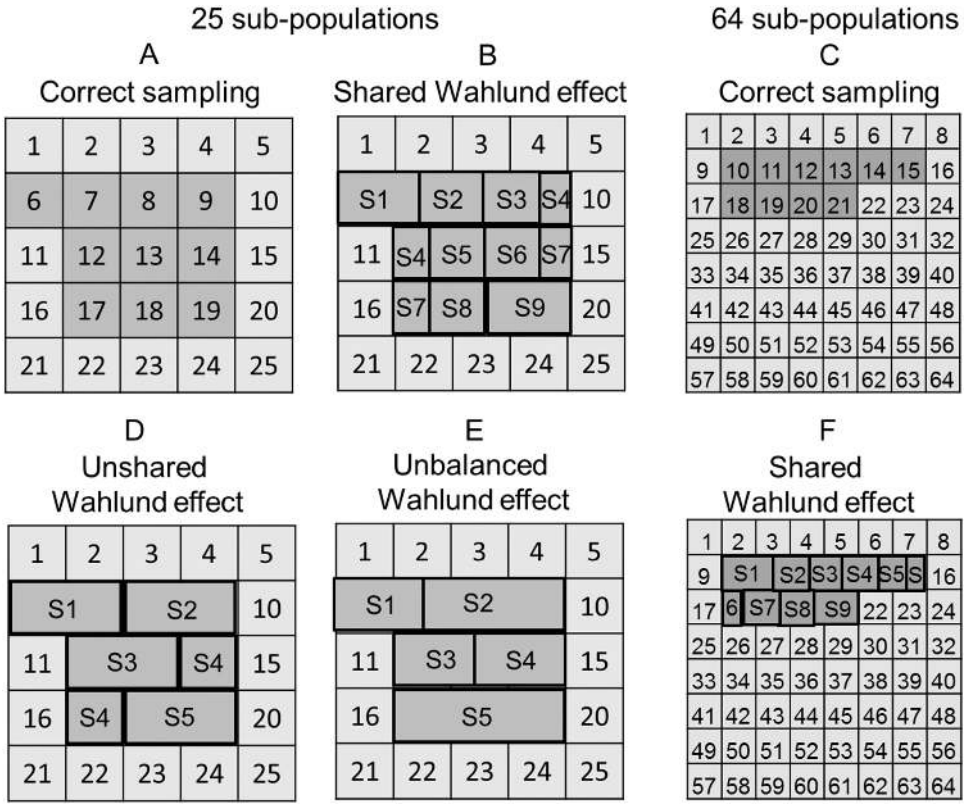

Figure 1. Different sampling designs that can lead to a Wahlund effect for simulations with 25 (**A**, **B**, **D**, **E**) or 64 subpopulations (**C**, **F**). Subsamples are represented in dark grey. Correct sampling (**A**, **C**) illustrates how subpopulations were initially sampled. For unshared Wahlund effect (**D**), subsamples gather individuals from different subpopulations but each subsample does not share individuals from the same subpopulation with other subsamples. In shared Wahlund effect (**B**, **F**), subsamples gather individuals from different subpopulations that are also shared between subsamples. For this case, subsamples 1 and 9 contain unbalanced representation of 2 subpopulations. In unbalanced Wahlund effect (**E**), the variance of representation of each initial subpopulation is maximized as compared to shared Wahlund effect (**B**, **F**).

parameter estimates and statistics in microorganisms for which strains from different years are often pooled, most of the time to increase subsample sizes. Nevertheless, the gain in size is made at the expense of accuracy and power. Interested readers can find some more specific comments about population genetics of *Leishmania* parasites in Rougeron et al. (2015). I will only treat temporal effects in the discussion in the light of spatial Wahlund effects results.

Simulations

To check for analytical and simplification accuracy, I have undertaken several simulations. All simulations were undertaken with Easypop 2.0.1 (Balloux 2001) and analyzed with Fstat 2.9.4, updated from Goudet (1995). All simulations consisted of 25 or 64 subpopulations of 200 or 100 individuals with balanced sex ratio in Island or 2-dimensional stepping stone models with migration rates of m = 0.01, 0.05, 0.2, or 0.5, for 10 000 discrete generations, 20 loci with a KAM mutation rate of $u = 10^{-4}$ and K = 99 possible alleles. In some instances, I also simulated heterogeneous loci with various mutation rates and K: Loci 1–5, $u = 10^{-9}$, K = 2; Loci 6–10, $u = 10^{-6}$, K = 10; Loci 11–15, $u = 10^{-5}$, K = 20; Loci 16–20, $u = 10^{-4}$, K = 40. Hermaphrodites with a selfing rate of s = 0.5 were also simulated for several parameter sets, some of which (the most significant ones) are presented in the Results section. Each parameter set was replicated 10 times. For each replicate, I sampled 20 individuals in 10 subpopulations (Figure 1).

Null alleles were generated as in Séré et al. (2014, 2017). For each locus, alleles 1–10 (10% of null alleles), 1–20 (20%), and 1–50 (50%) were recoded as null alleles. Hence, individuals harboring one of these alleles were recoded as homozygous for the other allele harbored, individuals harboring 2 of these alleles were recoded as missing data and individuals harboring none of these alleles were not recoded. At the end of each simulation, some alleles are lost by drift. This way, the same process (mutation and drift) affects all alleles (that are all present at generation 1) at all loci homogeneously, resulting in (approximately) 10%, 20%, or 50% of null allelic states among the K possible ones. All alleles (including null alleles) are then submitted to genetic drift and mutation like any other locus. This produces a large variation in null allele frequencies across loci and replicates (absence, weak, medium, or substantial frequency) as it is expected in real data with null alleles (De Meeûs et al. 2007; Séré et al. 2014, 2017). These proportions of null alleles (10%, 20%, and 50%) actually correspond to the proportions of allelic states that are null and not the actual real null allele frequencies which vary considerably across subpopulations and across replicates. For the sake of simplification, I will call these "proportion of null alleles."

Wahlund effects were produced as in Figure 1. Merging adjacent subpopulations (e.g., 6 with 7, 8 with 9, etc.) resulted in 5 subsamples with unshared Wahlund effect (see definitions and Figure 1D). In that case, all subsamples contain a balanced admixture of 2 subpopulations. For Shared Wahlund effect, subsample 1 (subsample S1, Figure 1) is built with 20 individuals from subpopulation 6 and 10 individuals from subpopulation 7 (for n = 25, Figure 1B) or, for n = 64 (Figure 1F), with 20 individuals from subpopulation 10 and 10 individuals from subpopulation 11. Subsequent subsamples are then built with 10 remaining individuals from the previous subpopulation and the 10 first of the following one. The last subsample (subsample S9, Figure 1B,F) is finally composed of 10 individuals from subpopulation 18 (Figure 1B) or 20 (Figure 1F) and 20 individuals from subpopulation 19 (Figure 1B) or 21 (Figure 1F). Here, the first and last subsamples contain unbalanced admixtures of 2 subpopulations while others contain balanced admixtures. Finally, the unbalanced Wahlund effect was generated through heterogeneous admixtures in subsamples (Figure 1E). For stepping-stone models, Wahlund effects are expected weaker than for Island models since for the first, merged subpopulations are neighbors and hence are genetically relatively closed even if m is small. For Island model, merged subpopulations are genetically similar or distant, depending on m.

Spearman's rank correlation test was undertaken with the package Rcmdr (Fox 2005, 2007) for R 3.3.2 (R Development Core Team 2016) and 95% confidence intervals for different statistics were estimated using the variation of these statistics across replicates of the same simulation. When indicated, 95% confidence intervals of F_{IS} and F_{ST} were computed using the standard error of jackknife over subsamples (for each locus) or using 5000 bootstraps over loci (for the average across loci). These were computed with Fstat. More detailed explanations on how to compute confidence intervals can be found in De Meeûs et al. (2007). The correlation between F_{ST} and F_{IS} across loci was measured with Pearson's correlation coefficient.

Results

Wahlund Effects

In the case of a Wahlund effect, the total sample is composed of several subsamples that contain admixtures of individuals from different real subpopulations in proportions that are unknown and variable from one subsample to the other. We can write identity probabilities as (Equation 1):

$$\begin{cases} Q_{I\_W} = Q_I \\ Q_{S\_W} = Q_S - W_S \\ Q_{T\_W} = Q_T + W_T \end{cases} \quad (3)$$

There is indeed no reason why a Wahlund effect would affect the probability of homozygosity Q_I. On the contrary, the presence of new alleles from different subpopulations will tend to decrease the probability of identity between individuals in each subsample, and hence $0 \leq W_S \leq Q_S$. W_S will more depend on the level of differentiation between gathered subsamples (F_{ST}) than on sampling design itself. The value of W_T will depend more on the sampling design. In "unshared subsamples," its value should be close to 0. In "shared" or "unbalanced" subsamples, the probability to sample the same allele from different subsamples should increase more, but the difference might be very small in fact. Hence, $0 \leq W_T \leq 1 - Q_T$. High values should not be frequently met in real situations.

Temporal Wahlund effects will be similar though less predictable, in particular, on W_T. With very heterogeneous cohort composition from one site to the other, it might happen that $W_T < 0$, particularly so in short generation time species with small effective population sizes (e.g., microbes) that can experience swift divergences between different cohorts. This might be clearer with a caricatured example. If subsample 1 at site 1 is composed of cohorts 1 and 4, and subsample 2 at site 2 is composed of cohorts 7 and 10, in a short lived species with small effective population sizes, drift will be quick and genetic differentiation between cohorts 4 and 7 might be bigger than between site 1 cohort 1 and site 2 cohort 1. Nevertheless, this last situation probably occurs only rarely.

Under a Wahlund effect, and combining Equation 1 and 3, F_{IS} and F_{ST} become:

$$\begin{cases} F_{IS\_W} = \dfrac{Q_I - Q_S + W_S}{1 - Q_S + W_S} \\ F_{ST\_W} = \dfrac{Q_S - W_S - Q_T - W_T}{1 - Q_T - W_T} \end{cases} \quad (4)$$

It can easily be shown that, whatever initial identity probabilities, $F_{IS\_W} \geq F_{IS}$ and $F_{ST\_W} \leq F_{ST}$. Please, note that, as seen above, special temporal Wahlund effects might end up with $F_{ST\_W} > F_{ST}$. This situation is probably exceptional because it requires that $W_T < 0$ and $|W_T| > |W_S|$. We can rearrange Equation 4 into:

$$\begin{cases} F_{IS\_W}\left[1-Q_S+W_S\right]=Q_I-Q_S+W_S \\ F_{ST\_W}=\dfrac{Q_S-W_S-Q_T-W_T}{1-Q_T-W_T} \end{cases}$$
$$\Leftrightarrow$$
$$\begin{cases} -\left(Q_I-Q_S\right)+F_{IS\_W}\left(1-Q_S\right)=W_S\left[1-F_{IS\_W}\right] \\ F_{ST\_W}=\dfrac{Q_S-W_S-Q_T-W_T}{1-Q_T-W_T} \end{cases}$$
$$\Leftrightarrow$$
$$\begin{cases} W_S=\dfrac{F_{IS\_W}\left(1-Q_S\right)-\left(Q_I-Q_S\right)}{1-F_{IS\_W}} \\ F_{ST\_W}=\dfrac{Q_S-\dfrac{F_{IS\_W}\left(1-Q_S\right)-\left(Q_I-Q_S\right)}{1-F_{IS\_W}}-Q_T-W_T}{1-Q_T-W_T} \end{cases} \quad (5)$$
$$\Leftrightarrow$$
$$\begin{cases} W_S=\dfrac{F_{IS\_W}\left(1-Q_S\right)-\left(Q_I-Q_S\right)}{1-F_{IS\_W}} \\ F_{ST\_W}=+\dfrac{Q_S-Q_T-W_T}{1-Q_T-W_T}+\dfrac{Q_I-Q_S}{1-F_{IS\_W}}\times\dfrac{1}{1-Q_T-W_T} \\ \qquad -\dfrac{F_{IS\_W}}{1-F_{IS\_W}}\times\dfrac{1-Q_S}{1-Q_T-W_T} \end{cases}$$

In most situations we expect W_T to be small so that we could rewrite Equation 5 as:

$$\begin{cases} W_S=\dfrac{F_{IS\_W}\left(1-Q_S\right)-\left(Q_I-Q_S\right)}{1-F_{IS\_W}} \\ F_{ST\_W}\approx\dfrac{Q_S-Q_T}{1-Q_T}+\dfrac{Q_I-Q_S}{1-F_{IS\_W}}\times\dfrac{1}{1-Q_T}-\dfrac{F_{IS\_W}}{1-F_{IS\_W}}\times\dfrac{1-Q_S}{1-Q_T} \end{cases}$$
$$\Leftrightarrow \quad (6)$$
$$\begin{cases} W_S=\dfrac{F_{IS\_W}\left(1-Q_S\right)-\left(Q_I-Q_S\right)}{1-F_{IS\_W}} \\ F_{ST\_W}\approx F_{ST}+\dfrac{Q_I-Q_S}{1-F_{IS\_W}}\times\dfrac{1}{1-Q_T}-\dfrac{F_{IS\_W}}{1-F_{IS\_W}}\times\dfrac{1-Q_S}{1-Q_T} \end{cases}$$

If we assume local panmixia ($Q_S = Q_I$). In that case Equation 6 becomes:

$$\begin{cases} W_S=\dfrac{F_{IS\_W}}{1-F_{IS\_W}}\left(1-Q_I\right) \\ F_{ST\_W}\approx F_{ST}-\dfrac{F_{IS\_W}}{1-F_{IS\_W}}\times\dfrac{1-Q_I}{1-Q_T} \end{cases} \quad (7)$$

or

$$\begin{cases} W_S=\dfrac{F_{IS\_W}}{1-F_{IS\_W}}H_I \\ F_{ST\_W}\approx F_{ST}-\dfrac{F_{IS\_W}}{1-F_{IS\_W}}\times\dfrac{H_I}{H_T} \end{cases}$$

where H_I is the proportion of heterozygotes in the total sample (or its average across subsamples) and H_T is the total genetic diversity of the sample, which is approximately the same as in the real population if W_T is small.

Equation 7 can be rearranged into:

$$\begin{cases} W_S=\dfrac{F_{IS\_W}}{1-F_{IS\_W}}H_I \\ F_{ST}\approx F_{ST\_W}+\dfrac{F_{IS\_W}}{1-F_{IS\_W}}\times\dfrac{H_I}{H_T} \end{cases} \quad (8)$$

which provides a rough proxy of the true F_{ST}, assuming local panmixia and small impact of the Wahlund effects on total genetic identity. Please note that this proxy will be very bad if the subpopulations are not panmictic and the impact of the Wahlund effect on Q_T is substantial. It might seem from Equation 7 that F_{IS_W} and F_{ST_W} are negatively correlated but this is compensated by the fact that F_{IS_W} is more strongly correlated to F_{ST} (and positively so) than to F_{ST_W} (negatively so).

Null Alleles

This case is in fact more difficult to track analytically. I will thus directly assume panmixia in each subsample with 3 alleles (1, 2, and null). In that case the (unseen) real genotypic proportions in subsample i are p_i^2, $2p_iq_i$, q_i^2, $2p_ip_{Ni}$, $2q_ip_{Ni}$, and p_{Ni}^2 for genotypes 11, 12, 22, 1N, 2N, and NN, respectively. We can also express Q_{Ii} and Q_{Si}:

$$Q_{Ii}=Q_{Si}=p_i^2+q_i^2+p_{Ni}^2 \quad (9)$$

We can now compute the perceived allele frequency of allele 1 in subsample i, remembering that 1N and 2N individuals are erroneously interpreted as homozygous 11 and 22, respectively:

$$p_{i\_N}=\frac{p_i^2+2p_ip_{Ni}+\frac{1}{2}\times 2p_iq_i}{1-p_{Ni}^2}$$
$$\Leftrightarrow$$
$$p_{i\_N}=p_i\frac{p_i+2p_{Ni}+q_i}{1-p_{Ni}^2}=p_i\frac{1+p_{Ni}}{1-p_{Ni}^2}=p_i\frac{1+p_{Ni}}{\left(1-p_{Ni}\right)\left(1+p_{Ni}\right)} \quad (10)$$
$$\Leftrightarrow$$
$$\begin{cases} p_{i\_N}=\dfrac{p_i}{\left(1-p_{Ni}\right)} \\ q_{i\_N}=\dfrac{q_i}{\left(1-p_{Ni}\right)} \end{cases}$$

From there, the observed within individual genetic identity in subsample i with null alleles can be written:

$$Q_{I\_Ni}=\frac{p_i^2+q_i^2+2p_{Ni}\left(p_i+q_i\right)}{1-p_{Ni}^2} \quad (11)$$

We can rearrange Equation 11 and combine it with Equation 9:

$$Q_{I\_Ni}=\frac{Q_{Si}-p_{Ni}^2+2p_{Ni}\left(1-p_{Ni}\right)}{1-p_{Ni}^2} \quad (12)$$

To understand the possible values taken by Q_{I_Ni}, it is useful to compute the minimum and maximum possible values for Q_S, with a null allele frequency p_{Ni}. Let us call these quantities $Q_{Si\_min/N}$ and $Q_{Si\_max/N}$. Probability of identity between alleles 1 and 2 will be minimal when both share the same allele frequency $(1 - P_{Ni})/2$ and will be maximal when one is very close to 0 and the other with frequency close to $1 - p_{Ni}$. We can thus write:

$$\begin{cases} Q_{Si\_min/N}=2\times\left[\dfrac{1}{2}\left(1-p_{Ni}\right)\right]^2+p_{Ni}^2 \\ Q_{Si\_max/N}=\left(1-p_{Ni}\right)^2+p_{Ni}^2 \end{cases} \quad (13)$$
$$\Leftrightarrow$$
$$\begin{cases} Q_{Si\_min/N}=\dfrac{1}{2}\left(1-p_{Ni}\right)^2+p_{Ni}^2 \\ Q_{Si\_max/N}=\left(1-p_{Ni}\right)^2+p_{Ni}^2 \end{cases}$$

We can use this to check the range of variation of Q_{I_Ni} for a given p_{Ni} combining Equations 11 and 13. Its extremum values are then:

$$\begin{cases} Q_{I\_Ni\_min/N} = \dfrac{\frac{1}{2}(1-p_{Ni})^2 + p_{Ni}^2 - p_{Ni}^2 + 2p_{Ni}(1-p_{Ni})}{1-p_{Ni}^2} \\ Q_{I\_Ni\_max/N} = \dfrac{(1-p_{Ni})^2 + p_{Ni}^2 - p_{Ni}^2 + 2p_{Ni}(1-p_{Ni})}{1-p_{Ni}^2} \end{cases}$$

$$\Leftrightarrow$$

$$\begin{cases} Q_{I\_Ni\_min/N} = (1-p_{Ni})\dfrac{\frac{1}{2}(1-p_{Ni}) + 2p_{Ni}}{(1-p_{Ni})(1+p_{Ni})} \\ Q_{I\_Ni\_max/N} = (1-p_{Ni})\dfrac{(1-p_{Ni}) + 2p_{Ni}}{(1-p_{Ni})(1+p_{Ni})} \end{cases}$$

Since $p_{Ni} > 0$ we can write:

$$\begin{cases} Q_{I\_Ni\_min/N} = \dfrac{1}{2}\left(\dfrac{1+3p_{Ni}}{1+p_{Ni}}\right) \\ Q_{I\_Ni\_max/N} = \dfrac{1+p_{Ni}}{1+p_{Ni}} \end{cases}$$

$$\Leftrightarrow \quad (14)$$

$$\begin{cases} Q_{I\_Ni\_min/N} = \dfrac{1}{2}\left(1+\dfrac{2p_{Ni}}{1+p_{Ni}}\right) \\ Q_{I\_Ni\_max/N} = 1 \end{cases}$$

It can easily be seen that the upper term of Equation 14 is minimized when null allele frequencies are very small (and their effect negligible) and then equals ½ (as expected for the bi-allelic case). We can thus write $½ \le Q_{I\_Ni} \le 1$.

We now need to derive equations for Q_{S_Ni}. Combining Equations 9 and 10, we obtain

$$Q_{S\_Ni} = \left(\frac{p_i}{1-p_{Ni}}\right)^2 + \left(\frac{q_i}{1-p_{Ni}}\right)^2 = \frac{1}{(1-p_{Ni})^2}\left(p_i^2 + q_i^2\right) \quad (15)$$

In subsample i, combining Equations 1, 12, and 15, F_{IS_Ni} will have the same sign as:

$$\Delta Q_{IS\_N} = Q_{I\_Ni} - Q_{S\_Ni} = \frac{Q_{Si} - p_{Ni}^2 + 2p_{Ni}(1-p_{Ni})}{1-p_{Ni}^2} - \frac{1}{(1-p_{Ni})^2}\left(Q_{Si} - p_{Ni}^2\right)$$

$$\Leftrightarrow$$

$$\Delta Q_{IS\_Ni} = \frac{(1-p_{Ni})\left[Q_{Si} - p_{Ni}^2 + 2p_{Ni}(1-p_{Ni})\right] - (1+p_{Ni})\left(Q_{Si} - p_{Ni}^2\right)}{(1+p_{Ni})(1-p_{Ni})^2}$$

$$\Leftrightarrow \quad (16)$$

$$\Delta Q_{IS\_Ni} = 2p_{Ni}\frac{p_{Ni}^2 + (1-p_{Ni})^2 - Q_{Si}}{(1+p_{Ni})(1-p_{Ni})^2}$$

$$\Leftrightarrow$$

$$\Delta Q_{IS\_Ni} = 2p_{Ni}\frac{p_{Ni}^2 + (1-p_{Ni})^2 - Q_{Si}}{(1+p_{Ni})(1-p_{Ni})^2}$$

$$\Leftrightarrow$$

$$\Delta Q_{IS\_Ni} = 2p_{Ni}\frac{1-\left[2p_{Ni}(1-p_{Ni}) + Q_{Si}\right]}{(1+p_{Ni})(1-p_{Ni})^2}$$

This quantity is always positive (or null when $Q_{I\_Ni} = Q_{S\_Ni} = 1$, no polymorphism), because Q_{Si} is the sum of true homozygote frequencies and hence Equation 16 can be rearranged as:

$$\Delta Q_{IS\_Ni} = 2p_{Ni}\frac{1-(1-2p_iq_i)}{(1+p_{Ni})(1-p_{Ni})^2}$$

$$\Leftrightarrow$$

$$\Delta Q_{IS\_Ni} = 2p_{Ni}\frac{2p_iq_i}{(1+p_{Ni})(1-p_{Ni})^2}$$

As expected, this quantity is always positive or null and then $F_{IS\_Ni} \ge 0$.

Over the n subsamples:

$$\begin{cases} \overline{Q_{I\_N}} = \dfrac{1}{n}\sum_{i=1}^{n} Q_{I\_Ni} \\ \overline{Q_{S\_N}} = \dfrac{1}{n}\sum_{i=1}^{n} Q_{S\_Ni} \end{cases} \quad (17)$$

and

$$\overline{F_{IS\_N}} = \frac{\overline{Q_{I\_N}} - \overline{Q_{S\_N}}}{1-\overline{Q_{S\_N}}} \quad (20)$$

If some polymorphism is maintained in some of the subsamples, then the quantity defined in Equation 20 is always positive.

The real (unseen) total identity probability can be computed as:

$$Q_T = \overline{p}^2 + \overline{q}^2 + \overline{p_N}^2 \quad (21)$$

The seen total genetic identity, with null alleles at frequency p_{Ni} in subsample i, and combining Equations 10 and 21, can be written as:

$$Q_{T\_N} = \left(\frac{1}{n}\sum_{i=1}^{n}\frac{p_i}{1-p_{Ni}}\right)^2 + \left(\frac{1}{n}\sum_{i=1}^{n}\frac{q_i}{1-p_{Ni}}\right)^2$$

$$\Leftrightarrow$$

$$Q_{T\_N} = \frac{1}{n^2}\left[\left(\sum_{i=1}^{n}\frac{p_i}{1-p_{Ni}}\right)^2 + \left(\sum_{i=1}^{n}\frac{q_i}{1-p_{Ni}}\right)^2\right]$$

From there some numerical explorations can lead to simple rules that will simplify the algebra.

$$Q_S \le Q_T$$

$$\begin{cases} Q_{I\_N} = Q_I + \nu_I \\ Q_{S\_N} = Q_S + \nu_S \\ Q_{T\_N} = Q_T + \nu_T \end{cases}$$

where $0 \le \nu_T \le \nu_S < \nu_I \le 1 - Q_{I\_N}$.

Indeed, the effect of null alleles will always be much greater on homozygosity than on identity between individuals and the effect of null alleles on identity between individuals will generally tend to be slightly higher than on identity between subsamples, especially so if the population is substantially subdivided. For instance if $Q_T \sim 0$, then the effect of null alleles will be very weak (if any) on this parameter ($\nu_T \sim 0$), while $Q_S = Q_T$ (no subdivision), necessarily means that $\nu_T = \nu_S$.

We can thus write:

$$\begin{cases} F_{IS\_N} = \dfrac{Q_I + \nu_I - Q_S - \nu_S}{1 - Q_S - \nu_S} \\ F_{ST\_N} = \dfrac{Q_S + \nu_S - Q_T - \nu_T}{1 - Q_T - \nu_T} \end{cases} \quad (22)$$

It is not difficult to show that $F_{IS\_N} \ge F_{IS}$ and $F_{ST\_N} \ge F_{ST}$.

Here, because both statistics are positively correlated to null allele frequencies, we may expect a positive correlation between F_{IS_N} and F_{ST_N}.

For more precisions on how to compute null allele frequencies I encourage readers to have a look at classic papers and reference therein (Brookfield 1996; Van Oosterhout et al. 2004; David et al. 2007; Guillot et al. 2008; Chybicki and Burczyk 2009; Robledo-Arnuncio and Gaggiotti 2017).

Simulations

With correct sampling and unaffected genotypes (no null alleles), we can see from Figure 2 that there is a strong (but not systematic) tendency for a negative correlation between F_{IS} and F_{ST} across loci as measured by Pearson's correlation coefficient. This tendency seems less pronounced for substantially inbred subpopulations (s = 0.5), small subpopulations (N = 100) and for heterogeneous loci (variable K and u). This is probably connected to the level of polymorphisms that can be maintained in subpopulations as illustrated by a highly significant negative correlation with H_S (Figure 3).

It can be seen from Figure 2 that Wahlund effects weakly increases the average correlation between F_{IS} and F_{ST}, which stays below 0 most of the time, except in strongly subdivided populations. Even in this case, this correlation hardly exceeds 0.1 and displays an important variance.

As expected with null alleles, correlation between F_{IS} and F_{ST} is always positive (Figure 2), except in weakly subdivided populations with high null allele frequencies (p_N = 0.5) (Supplementary Figure S1). Nevertheless, if loci with no heterozygosity (H_I = 0) are removed from the data, all simulations with null alleles showed a strong positive correlation between the 2 statistics, especially so for strongly subdivided populations (Figure 2). Figure 2 presents a subset of all simulations undertaken with the most significant results. Other results can be found in the Supplementary File S1.

The variation of F_{IS} and F_{ST} across loci was measured with the standard error of jacknife over loci as computed in Fstat (StrdErrFIS and StrdErrFST, respectively). The results are presented in Figure 4. It can be seen that F_{ST} variation across loci tends to decrease from correct sampling (no Wahlund) to unshared Wahlund effect and shared Wahlund effect while it remains low and unchanged for F_{IS} (no effect of sampling). A higher impact can be observed with loci presenting heterogeneous mutation models or smaller subpopulations (N = 100). For both statistics, for 10% of null alleles, the variation across loci jumps to much higher values. This is particularly true for F_{IS} for which the standard error obtained with null alleles has nothing in common with simulations without null alleles of any kind. When there is no Wahlund effect, it can be seen that StrdErrFIS ≈ StrdErrFST, including simulations with selfing s = 0.5. In other situations, we can see that StrdErrFIS > StrdErrFST (Wahlund effects) or StrdErrFIS >> StrdErrFST (null alleles).

Discussion

The tendency for negative correlation across loci that links F_{IS} and F_{ST} under the null hypothesis (no Wahlund and no null alleles) was unexpected (at least by me). It probably comes from the dependency, in an opposite direction, of both statistics on Q_S. Nevertheless, this correlation displays a strong variance around the average. There is thus little hope that this can often offer a useful criterion, especially when polymorphism is weak (H_S<0.5). A strong negative correlation, more likely when H_S > 0.6, will however represent a strong argument against the presence of null alleles.

Contrary to what might be understood from the literature (Criscione et al. 2011; Waples 2015; Zhivotovsky 2015; Bohling et al. 2016), the correlation across loci between F_{IS} and F_{ST} under a Wahlund effect will be very weak and most of the time even negative, while this correlation is always positive in the presence of null alleles, except in extreme situations with very high null allele frequencies, weak subdivision and if extreme loci displaying very low heterozygote frequencies are kept.

The variation across loci for F-statistics does not tend to increase with Wahlund effect but at best remains unchanged or even decreases. On the contrary, with null alleles, this variation significantly increases, even for small null allele proportions (Figure 4). For F_{IS}, variation across loci remains strictly the same under Wahlund effects except when the mutation model varies considerably across loci or in smallest subpopulations. For F_{ST}, even selfing generates more variation than Wahlund effect (Figure 4). But again, these variations are very modest as compared to those generated by null alleles.

Temporal Wahlund effect should have similar consequences as geographic Wahlund effect though it may affect total genetic identity more strongly, depending on the cohort composition of each subsample and drift speed (hence population structure). In case of highly

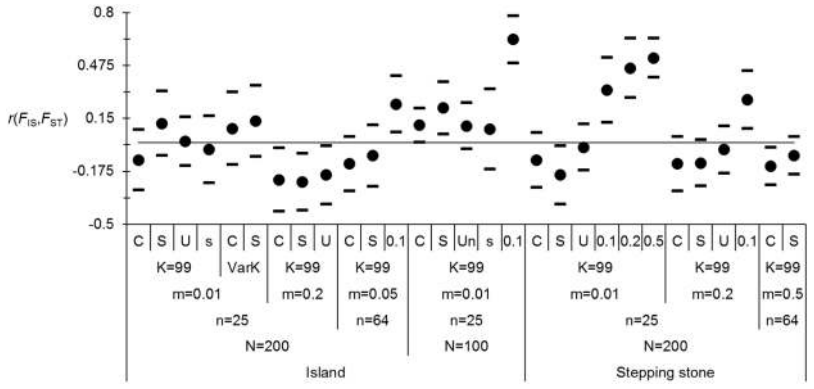

Figure 2. Average Pearson's correlation coefficient [$r(F_{IS}, F_{ST})$] between F_{IS} and F_{ST} across loci and 95% confidence intervals for simulations in Island or 2-dimensional stepping stones models (stepping stone) with different subpopulation sizes (N), number of subpopulations (n), migration rates (m), and different models of mutation: K = 99 for 99 alleles and mutation rate $u = 10^{-4}$ and VarK for variable K and u as defined in the text. The results obtained for correct data (no Wahlund effect, no null alleles) (C), for data with unshared (U), shared (S), or unbalanced (Un) Wahlund effects, as defined in the text and with 50% selfing (s), different null allele frequencies (0.1, 0.2, 0.5) or random mating without null alleles (all others) are presented. Note that, for the sake of comparison, Wahlund, null alleles, or selfing always occur alone (no combination). The straight plain line in the middle represents the 0 correlation value.

heterogeneous cohort compositions across subsamples, an increase of perceived F_{ST} might happen, but this should not be the most frequent situation.

Different phenomena can affect heterozygosity all together in a single sample. Selfing and/or null alleles and/or Wahlund effect will then have different contribution that will be difficult to identify specifically. The classic Wright's equation $(1 - F_{IT}) = (1 - F_{IS})\ (1 - F_{ST})$ (e.g., De Meeûs et al. 2007) gives the relationship between the different *F*-statistics. F_{IT} represents the combination of Wahlund effect (F_{ST}) and other causes (null alleles or deviation from panmixia) (F_{IS}). The true F_{ST} can sometimes be known as described in Waples (2011, 2015) for long-lived mammals, in which case the true F_{IS} might be extrapolated easily. Most of the time, such information is not available and adjusting data for the true F_{IS} will be necessary. This can be made, for instance, by computing the contribution of null alleles to the F_{IS}. A proxy can be found with the determination coefficient of the regression between missing genotypes counts and per locus F_{IS} (if the correlation is good) (e.g., Melachio et al. 2011). Cross experiments can also be useful at determining possible range for selfing or sib mating rates, when such experiments are possible.

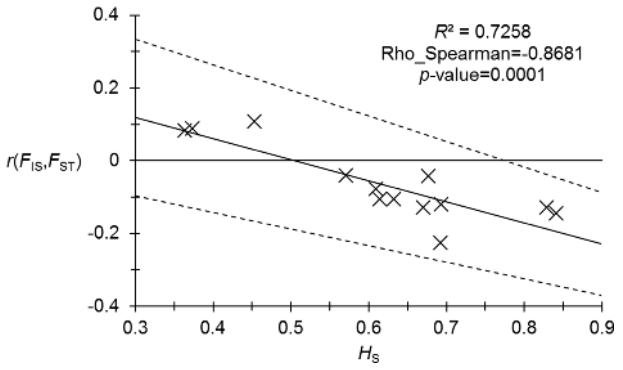

Figure 3. Relationship between $r(F_{IS}, F_{ST})$ (Pearson's correlation between F_{IS} and F_{ST}) and local genetic diversity (H_S) averaged across 10 replicates for each parameter sets with correct data (no Wahlund effect, no null alleles) as described in the text. Regressed 95% confidence intervals computed from variation across replicates are indicated as dotted lines. The regression determination coefficient (R^2), Sperman's rho and its corresponding *P*-value are also provided. The straight plain line in the middle represents the 0 correlation line.

Wahlund effect results from a poor knowledge of the actual structure of a given population. There is thus little chance that real F_{ST} and F_{IS} can be known in real situations dealing with a Wahlund effect. As shown elsewhere (Chapuis and Estoup 2007) and in the present study, null alleles and Wahlund effects alter both F_{IS} and F_{ST}, though in a different direction. Null alleles produce an increase of both statistics; while Wahlund effects increase F_{IS} and, in most situations, decrease F_{ST} (F_{ST} might increase with temporal Wahlund effect with extreme heterogeneity of cohort composition).

There will always be variation of *F*-statistics across loci, even under the null hypothesis, with some loci significantly deviating from panmictic proportions by chance only [for an excellent discussion on this issue see Waples (2015)]. This is why only a global significance across loci and subsamples represents the best clue that something is happening in the population under study (e.g., deviation from random mating, Wahlund effect, genotyping miscoring). In case of global significant deviation from Hardy–Weinberg proportions, excessive variation across loci of *F*-statistics represents an accurate signature of null alleles, or at least of locus-specific genotyping problems. For instance, it is known that allelic dropouts have similar effects as null alleles (Séré et al. 2014). On the contrary, Wahlund effects have no or very weak influence on this variation. Additionally, null alleles also produce strong positive correlations between F_{IS} and F_{ST}, while Wahlund effects or selfing seldom do. To this respect, high variation of *F*-statistics across loci with a positive correlation between them will always represent a hallmark of locus-specific amplification problems (null alleles, dropouts, and stuttering). The absolute value of the jacknife standard error of F_{IS} and F_{ST} may not be very useful as other factors as sample size and number of loci and their polymorphism may produce relatively high values. Nevertheless, plotting the variation of F_{IS} and F_{ST} as in Figures 5 and 6 is always useful. These figures provide examples of what kind of variations can be attributed to null alleles or not (especially so for F_{IS}). The presence of missing data (putative null homozygotes) can also helpfully add arguments in favor of null alleles, particularly so when the regression between F_{IS} across loci as a function of the number of observed missing genotypes is significant (e.g., Melachio et al. 2011). Alternatively, relatively stable *F*-statistics with a correlation $r(F_{IS}, F_{ST})$ around 0 or just above, and of course a significant positive F_{IS} should strongly suggest a Wahlund effect, though this pattern will sometimes be hard

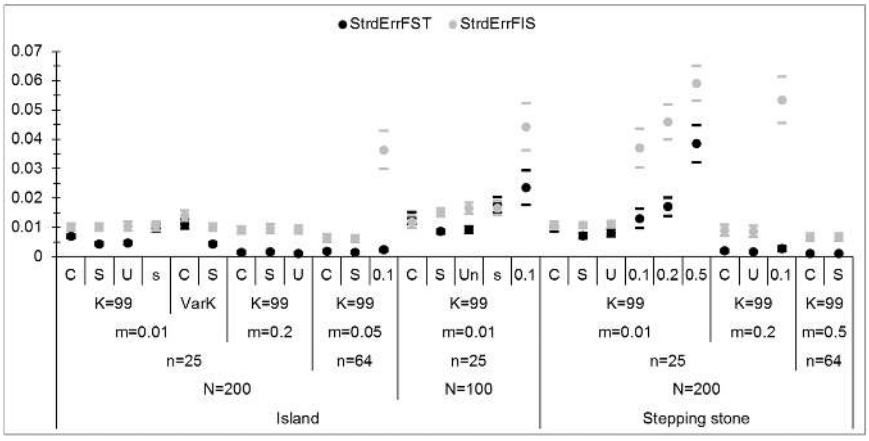

Figure 4. Standard error of F_{IS} (grey) and F_{ST} (black) from jacknife over loci as computed by Fstat and 95% confidence intervals. The results presented corresponds to 2-dimensional stepping stone or Island models with different subpopulation sizes (*N*), number of subpopulations (*n*), migration rates (m), homogeneous mutation models (with 99 alleles and 10^{-4} mutation rate) ($K = 99$), heterogeneous mutation models (VarK) and different situations: Correct sampling and genotyping (C), unshared (U), Shared (S), or unbalanced (U) Wahlund effects, 10% of null alleles (0.1) and 50% selfing rate (s). Note that, for the sake of comparison, Wahlund, null alleles, or selfing always occur alone (no combination).

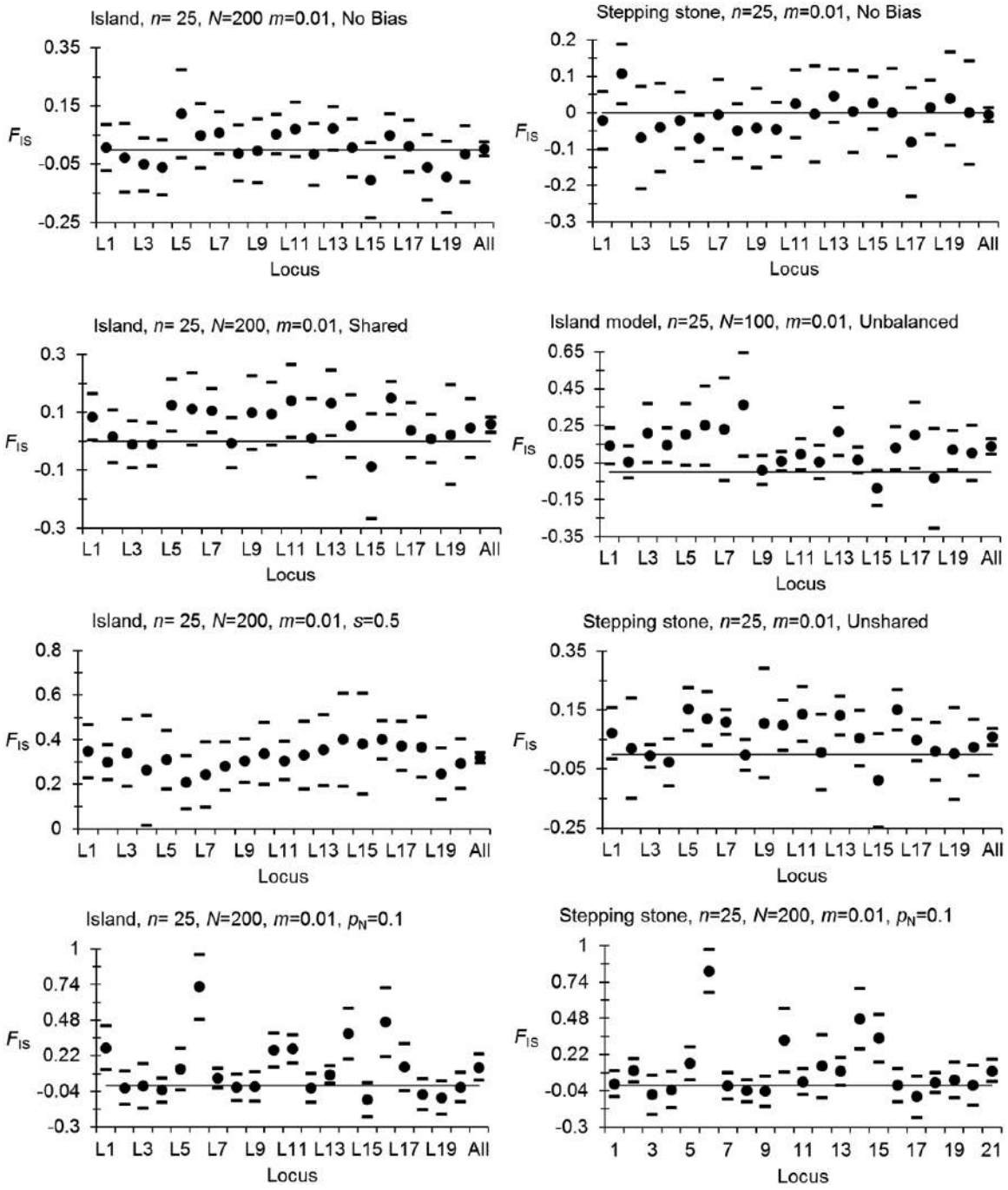

Figure 5. Example of the variation of F_{IS} across loci, with 95% confidence intervals of jackknife over subsamples (each locus) or of bootstrap over loci (all) in different population models (Island or 2-dimensional stepping stone), one mutation model ($K = 99$, $u = 10^{-4}$ for all loci), subpopulation numbers and size (n and N), with shared, unshared, or unbalanced Wahlund effects or with $p_N = 0.1$ (proportion of null alleles). The straight plain line in the middle represents the 0 value.

to distinguish from the effect of local deviation from panmixia (selfing or sib-mating). Nevertheless, under ideal conditions, StrdErrFIS > StrdErrFST should correspond to Wahlund effects more than to selfing or sib-mating. I thus propose the following determination key for data with global (across loci and subsamples) significant heterozygote deficits:

- $r(F_{IS}, F_{ST}) >> 0$ when excluding loci with $H_I \approx 0$ (if any), high variations of F_{IS} and F_{ST} across loci, StrdErrFIS >> StrdErrFST and occurrence of missing data that explains F_{IS} variation across loci: null alleles are affecting the data and the frequency of theses null alleles can be estimated with (for instance) MicroChecker (Van Oosterhout et al. 2004) and F_{ST} can be corrected with the ENA algorithm (Chapuis and Estoup 2007); additionally, if panmixia is never or rarely met for any locus, an additional phenomenon (Wahlund effect, selfing) might be invoked;
- $r(F_{IS}, F_{ST})$~0 or even moderately positive, very small variations of F_{ST} across loci, moderate variations of F_{IS} across loci, StrdErrFIS

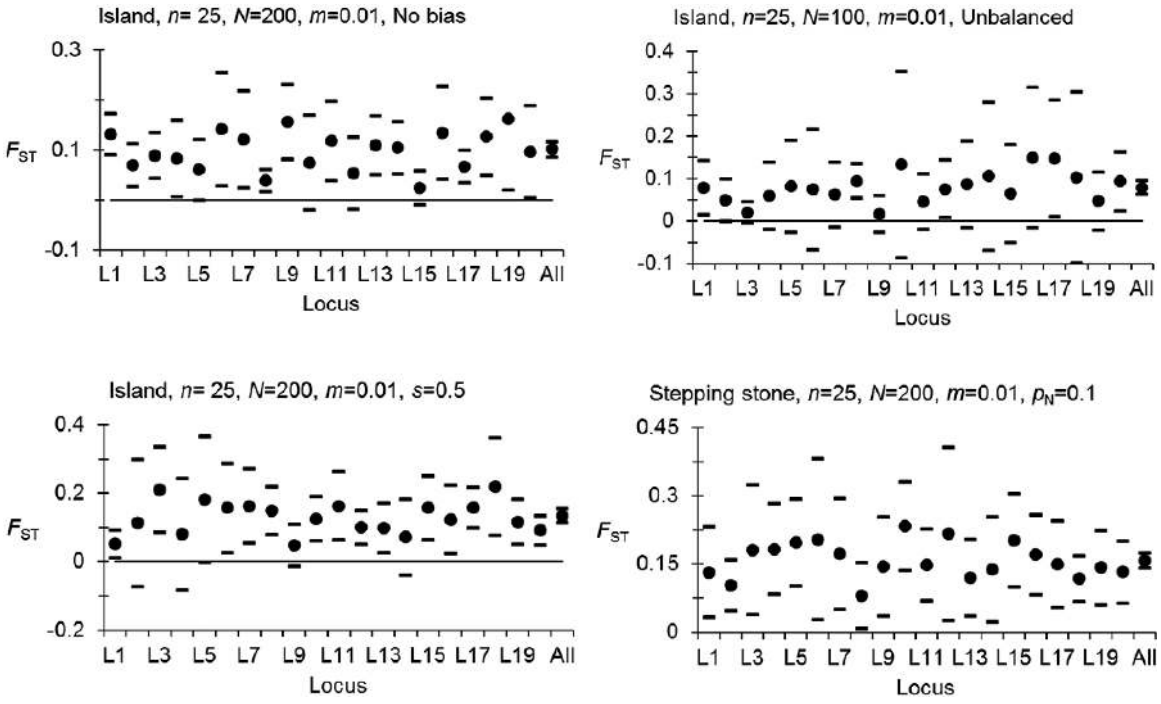

Figure 6. Example of the variation of F_{ST} across loci, with 95% confidence intervals of jackknife over subsamples (each locus) or of bootstrap over loci (all) in different population models (Island or 2-dimensional stepping stone), one mutation model ($K = 99$, $u = 10^{-4}$ for all loci), subpopulation numbers and size (n and N), with shared, unshared, or unbalanced Wahlund effects or with $p_N = 0.1$ (proportion of null alleles). The straight plain line in the middle represents the 0 value.

> StrdErrFST and no or very rare missing data: Wahlund effect between subpopulations with an approximate F_{ST} given by Equation 8 (assuming local panmixia);

- $r(F_{IS}, F_{ST}) \approx 0$, moderate variance in F_{IS} and F_{ST} across loci, StrdErrFIS = StrdErrFST and no or very rare missing data: selfing or sib mating might better explain the data, the rate of which can be estimated from F_{IS} (e.g., De Meeûs 2012 p. 42 and 309).

One additional criterion may help decision making. It uses the regression of per locus F_{IS} (averaged over subsamples) as a function of missing genotypes observed in all subsamples. A significant positive relationship is a very sound indication that null alleles explain all or part of observed F_{IS}. In that case, the determination coefficient (R^2) will roughly indicate how much of variance of F_{IS} across loci is explained by null alleles (Melachio et al. 2011).

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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Data Availability

All detailed simulation results, with corresponding graphics and simulation output files are available as Supplementary File S1. The genuine file can be downloaded from http://t-de-meeus.fr/Data/DeMeeusSimulationResultsNuls&WahlundSupFileS1.xlsx. Simulation results represent more than 1200 files. These files can easily be generated again with appropriate settings but the author is ready to share all or part of it with the community on request at thierry.demeeus@ird.fr.

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