Statistical Power Analysis of Neutrality Tests Under Demographic Expansions, Contractions and Bottlenecks With Recombination

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ABSTRACT

Several tests have been proposed to detect departures of nucleotide variability patterns from neutral expectations. However, very different kinds of evolutionary processes, such as selective events or demographic changes, can produce similar deviations from these tests, thus making interpretation difficult when a significant departure of neutrality is detected. Here we study the effects of demography and recombination upon neutrality tests by analyzing their power under sudden population expansions, sudden contractions, and bottlenecks. We evaluate tests based on the frequency spectrum of mutations and the distribution of haplotypes and explore the consequences of using incorrect estimates of the rates of recombination when testing for neutrality. We show that tests that rely on haplotype frequencies—especially F_s and Z_{nS}, which are based, respectively, on the number of different haplotypes and on the r^2 values between all pairs of polymorphic sites—are the most powerful for detecting expansions on nonrecombining genomic regions. Nevertheless, they are strongly affected by misestimations of recombination, so they should not be used when recombination levels are unknown. Instead, class I tests, particularly Tajima's D or R_2, are recommended.

A N increasing number of statistical tests (Tajima 1989a; Fu and Li 1993; Fu 1997; Fay and Wu 2000; Ramos-Onsins and Rozas 2002) have been developed to detect departures of DNA sequence variability from the expectations of the neutral theory of evolution (Kimura 1968). Most of the research in this area is based upon the Wright–Fisher model (Fisher 1930; Wright 1931; Hein *et al.* 2005), which assumes populations of constant size that are panmictic and nonrecombining. Moreover, the Wright–Fisher model provided the founding of coalescent theory (Kingman 1982a,b, 2000; Hudson 1990; Donnelly and Tavare 1995; Fu and Li 1999), which was fundamental for developing neutrality tests and furthering their study. Even if these models are quite prone to mathematical treatment, analytic derivations are often unreachable and the significance of departures from neutrality and the statistical power of the tests are estimated by computer simulations based on the coalescent process (Wall 1999; Ramos-Onsins and Rozas 2002; Depaulis *et al.* 2003).

The detection of departures from the null hypothesis of neutrality points to the violation of one or more of its assumptions. These deviations can be due to selective and/or demographic events. For example, selective sweeps or population growth can produce longer external branches in the genealogy that result in an excess of recent mutations over neutral predictions. In contrast, population subdivision or balancing selection will result in longer internal branches and, consequently, in an excess of old over recent mutations. In summary, different kinds of processes can produce similar genealogies and therefore confound the interpretation of tests.

Much effort has been devoted to ascertaining the power of different statistical tests to reject the null hypothesis of neutrality when it is actually false, as well as to defining properly which tests perform best in each scenario. Ramos-Onsins and Rozas (2002) studied the power of 17 statistical tests under sudden or logistic population-expansion models. The tests were classified in three categories on the basis of the information they used. Class I tests are based on the frequency spectrum of mutations, class II on the haplotype distribution, and class III on the distribution of pairwise differences. Depaulis *et al.* (2003) studied the power of seven statistics under bottlenecks (both severe and moderate) and hitchhiking with positively selected mutations.

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More recently, the power of several tests has been studied under exponential population growth and bottlenecks (SANO and TACHIDA 2005) and population structure and hitchhiking (JENSEN *et al.* 2005). However, the effect of intragenic recombination has been considered in only a limited number of studies, and, in particular, the joint effect of recombination and population expansions on the statistical power of neutrality tests has not, to the best of our knowledge, been explored.

The neutral model with no recombination, which is commonly used as the null hypothesis, has larger variance in genealogy length than the same model including recombination (HEIN *et al.* 2005). Such larger variance makes the assumption of no recombination a conservative assumption for many statistical tests. In particular, tests based on the frequency spectrum of mutations are likely to be conservative on recombining regions (TAJIMA 1989a; FU and LI 1993; FU 1996). On the other hand, tests based on haplotype or linkage disequilibrium (LD) are expected to be strongly affected by recombination, since it will break down existing haplotypes and generate new ones, thus decreasing LD. Moreover, as recombination can also smooth the mismatch distribution, it is likely that statistical tests based on this distribution will have little power (RAMOS-ONSINS and ROZAS 2002). Finally, recombination can mimic the effect of some demographic models, such as population growth. It is therefore of general interest to distinguish the individual effects of recombination and population growth on DNA sequence variation and on neutrality tests (SCHIERUP and HEIN 2000).

The study of population expansions is also of great interest since their effects on genealogies (and, thus, on many neutrality statistics) are similar to those of other selective or demographic events. Among the former, selective sweeps caused by positively selected variants, as well as background selection against deleterious mutations, lead to an excess of low-frequency variants (CHARLESWORTH *et al.* 1993; PRZEWORSKI 2002). On the other hand, recent bottlenecks can also mimic the effects of an expansion (TAJIMA 1989a,b), so these phenomena can be quite difficult to disentangle. In spite of such difficulty, considerable progress is being made to distinguish between expansions and selective sweeps (JENSEN *et al.* 2005; WILLIAMSON *et al.* 2005) or between bottlenecks and positive selection (HADDRILL *et al.* 2005).

Here we use coalescent simulations to test the power of 16 statistical tests to detect population expansions, contractions, and bottlenecks under different recombination levels. The selected tests belong to the first two categories described by RAMOS-ONSINS and ROZAS (2002). We pay special attention to the problem of misestimation of recombination rates and study how the use of incorrect recombination rates when simulating neutral samples can affect the power and the false-positive rates of tests. We have found that statistics based on haplotype diversity are the most powerful tests for detecting pop-

ulation expansions on nonrecombining regions. In contrast, since they are very sensitive to recombination, their use should be avoided when there is recombination.

MATERIALS AND METHODS

Statistics: We have considered two classes of statistics: statistics based on the frequency spectrum of mutation (class I) and statistics based on linkage disequilibrium and haplotype distribution (class II). No statistics based on the distribution of pairwise differences (*e.g.*, the mismatch distribution) have been used, as they were shown to perform very poorly in the study by RAMOS-ONSINS and ROZAS (2002). A summary of all statistics can be found in Table 1.

Class I statistics: Class I statistics use information on the frequency of mutations and are based on the differences between estimators of the population mutation rate $\theta = 4N\mu$, where N is the effective population size and μ is the mutation rate. From this class, we present results for Tajima's D (TAJIMA 1989a), Fu and Li's D, F, D^*, and F^* (FU and LI 1993), and Fay and Wu's H (FAY and WU 2000). We have also included the R_2 statistic (RAMOS-ONSINS and ROZAS 2002), which is based on the difference between the number of singletons per sequence and the average number of nucleotide differences.

Class II statistics: Class II includes statistics based on the haplotype distribution. They are expected to be the most affected by recombination. Within this class, we have studied the following statistics: Fu's F_s (FU 1997), the unbiased haplotype diversity estimate Dh (NEI 1987, Equation 8.5), Wall's B and Q (WALL 1999), Kelly's Z_{nS} (KELLY 1997), Rozas' Z_A and ZZ (ROZAS *et al.* 2001), and two statistics based on the *extended haplotype homozygosity* (EHH) (SABETI *et al.* 2002).

EHH statistics are a complex family of heuristic methods for which no consensus summary statistic has yet been developed. We have computed two EHH-based statistics by taking the first three SNPs of each sequence as a core haplotype (that is, as the locus of interest) and then considering the distance from each core at which EHH decays to ≤ 0.5. Two values are given: (1) the EHH average, corresponding to the weighted average for all core haplotypes of the distance at which EHH decays to ≤ 0.5 and (2) the EHH maximum, the distance corresponding to the core haplotype that decays to ≤ 0.5 at a greater distance. If a simulated segment finishes without EHH reaching a value ≤ 0.5, taking the chromosome length as L, we arbitrarily consider that the position will be at $2L$.

Coalescent simulations: We tested the statistical power of the statistics under different demographic models by running neutral coalescent simulations using the algorithm described by HUDSON (1990) and implemented in the ms package (HUDSON 2002). This program generates coalescent trees for a given sample size, recombination rate, and a demographic scenario, implementing an infinite-sites mutation model that leads to biallelic sites.

There is an intense debate on how to best perform simulations and, specifically, on the suitability of running coalescent simulations by fixing either the number of segregating sites (S) or the population mutation rate $\theta = 4N\mu$ (HUDSON 1993; WALL and HUDSON 2001; DEPAULIS *et al.* 2001, 2005). Conditioning on θ has the disadvantage that its value has to be estimated. Furthermore, even if its true value could be known, it produces broader confidence intervals, thus reducing the power of tests (DEPAULIS *et al.* 2005). On the other hand, although forcing a given number of segregating sites in all trees without considering their particularities (such as branch length) is also unrealistic, conditioning on the number of segregating sites has the advantage that S is a

TABLE 1

Definition of the neutrality statistics used

Test	Definition	Reference
Class I		
Tajima's D	Comparison of estimates of the no. of segregating sites and the mean pairwise difference between sequences	Tajima (1989a)
Fu and Li's D (D^F) and D^*	Comparison of the number of derived singleton mutations and the total number of derived nucleotide variants (the asterisk indicates "without an outgroup")	Fu and Li (1993)
Fu and Li's F and F^*	Comparison of the number of derived singleton mutations and the mean pairwise difference between sequences (the asterisk indicates "without an outgroup")	Fu and Li (1993)
Fay and Wu's H	Comparison of the number of derived segregating sites at low and high frequencies and the number of variants at intermediate frequencies	Fay and Wu (2000)
R_2	Comparison of the difference between the number of singleton mutations and the average number of nucleotide differences	Ramos-Onsins and Rozas (2002)
Class II		
Fu's F_s	Based on Ewens' sampling distribution, taking into account the number of different haplotypes in the sample	Fu (1997)
Dh	Based on the number of different haplotypes in the sample	Nei (1987)
EHH average	Weighted average for all core haplotypes of the position at which the haplotype homozygosity decays to ≤ 0.5	Based on Sabeti et al. (2002)
EHH maximum	The position corresponding to the core haplotype that decays to ≤ 0.5 at a greater distance	Based on Sabeti et al. (2002)
Wall's B	Counts the number of pairs of adjacent segregating sites that are congruent (if the subset of the data consisting of the two sites contains only two different haplotypes)	Wall (1999)
Wall's Q	Adds the number of partitions (two disjoint subsets whose union is the set of individuals in the sample) induced by congruent pairs to Wall's B	Wall (2000)
Kelly's Z_{nS}	Average of the squared correlation of the allelic identity between two loci over all pairwise comparisons	Kelly (1997)
Rozas' Z_A	Average of the squared correlation of the allelic identity between two loci over adjacent pairwise comparisons	Rozas et al. (2001)
Rozas' ZZ	Comparison between Z_{nS} and Z_A	Rozas et al. (2001)

parameter that can be observed in the sample. To solve this problem, several strategies have been proposed to obtain realistic samples conditioning on both θ and S (Hudson 1993; Depaulis et al. 2001, 2003, 2005; Wall and Hudson 2001; Przeworski 2002). Different authors agree that simulated parameters are more accurate if the simulations are conditioned on S taking into account the uncertainty of θ (Tavare et al. 1997; Pritchard et al. 1999).

However, obtaining neutral models fixing the number of segregating sites—which can be directly obtained from the sample—or estimating θ from S are still widely used by researchers (Macdonald and Long 2005; Soejima et al. 2005; Stajich and Hahn 2005; Tarazona-Santos and Tishkoff 2005). Taking into account this popularity, we have conditioned our simulations on S and the θ estimator θ_W after proper validation of this approach (see below). For simulations conditioned on the number of segregating sites, S values were set to 10, 100, and 400. This corresponds to the rounded minimum, average, and maximum segregating sites found in the genes resequenced by SeattleSNPs (http://pga.gs.washington.edu/; Crawford et al. 2005), the largest ongoing human resequencing project, which currently contains sequences of a length of 3.5–71 kb for >300 genes obtained from 23 European–American and 24 African–American individuals. For simulations

conditioned on θ_W (Watterson 1975), θ_W values correspond to the S values used in the previous simulations. All the values have been fixed assuming a panmictic and stationary neutral population, which could cause incorrect power estimations for statistics, depending on the number of segregating sites.

To ascertain the validity of our approach, results for simulations fixing S in expansions have been compared with results obtained considering the uncertainty of θ and using the rejection algorithm (Tavare et al. 1997). Comparisons have been performed for all S values studied and for the minimum (0) and maximum (10^{-7}) recombination values. Comparison shows that the differences in estimates of nominal rejection level between the two methods are very small. In fact, in 96% of the cases they are <5%, and in no case do they reach values >15%. Moreover, these differences become even smaller with increasing recombination rates (results not shown). In summary, we use a methodology that is accurate for neutral simulations (Depaulis et al. 2001; Wall and Hudson 2001; Ramos-Onsins et al. 2007) and for all our expansion models (Ramos-Onsins et al. 2007; materials and methods). However, our approximate method can produce deviations when computing statistical power under other alternative models, such as the contraction and bottleneck models (Ramos-Onsins et al. 2007). The magnitude of these deviations depends on the

particular statistic and the parameters of the model. Deviation from the exact strategy has been evaluated partially, and our results indicate that, in the studied cases, the deviation in the statistical power is not large.

Recombination: Recombination rates were set to $r = 10^{-10}$, $r = 10^{-8}$, and $r = 10^{-7}$ per nucleotide pair; as simulations are scaled in units as $4N$ generations, assuming $N_e = 10,000$ for humans (Takahata *et al.* 1995), this would correspond to population recombination rates of $R = 4Nr$ equal to 4×10^{-6}, 4×10^{-4}, and 4×10^{-3} per nucleotide, respectively. These values correspond to the rounded minimum, average, and maximum values estimated by Kong *et al.* (2002) for the human genome. Simulations without recombination were also performed. To calculate the crossover probability, we have assumed sequence lengths of 3000, 21,000, and 72,000 bp, as these lengths correspond to the minimum, average, and maximum rounded lengths of the resequenced human genes in SeattleSNPs, and $N_e = 10,000$.

Demography: We have simulated four basic demographic models: stationarity, sudden population growth, sudden population contraction, and population bottleneck, with emphasis on the two first cases. All scenarios consider different recombination rates. For each scenario, we ran 10,000 simulations. The significance level was set to 0.05, and the critical value for each statistic was obtained from the empirical distribution of the corresponding neutral model. As mutations were simulated under an infinite-sites model (which implies no recurrent mutation), the outgroup (for those statistics that require it) was set to a string of 0's, where 0 is the ancestral state as coded by ms.

The sudden population growth model (Rogers and Harpending 1992) assumes that a population of size N_0 in equilibrium experienced a sudden growth and reached maximum size (N_{max}) T_e generations before present. In the simulations, time is scaled in units of $4N_e$ generations. Changes in population size are performed according to the standard procedures in the ms program. Expansion times have been set to range from 0.0075 to 0.2. In humans, taking again $N_e = 10,000$ and a generation time of 20 years, the simulated expansion times would range from 6000 to 160,000 years ago. The latter can be taken as the earliest estimate for a human expansion (Jobling *et al.* 2004). Since some statistics showed significant power at that earlier expansion time, and to produce results applying to other species, some additional expansion times were added at $T_e = 0.4$, $T_e = 0.6$, $T_e = 0.8$, and $T_e = 1$ (equivalent, respectively, to 16,000, 24,000, 32,000, and 40,000 generations and, in human terms, 320,000, 480,000, 640,000, and 800,000 years ago). The degree of expansion ($D_e = N_{max}/N_0$) has been set to $D_e = 10$ and $D_e = 100$.

The sudden contraction model considers a population of constant size N_0, which has instantaneously contracted to a size N_{min}; the contraction degree is defined as $D_e = N_{min}/N_0$. This model has been simulated as the opposite of our expansion model and thus the same N_e and T_e have been used. Contraction degree has been set to $D_c = 0.1$ and $D_c = 0.01$. We have performed simulations for $S = 10$ and $S = 100$.

The bottleneck model has been simulated as in Voight *et al.* (2005). It assumes a population of size N_A, which has been suddenly reduced to a second size, $b \cdot N_A$ for T_{dur} generations T_{start} generations ago, where b is the bottleneck severity. Immediately afterward, the population has instantaneously recovered its original size, N_A. As in the expansion model, N_A has been set to 10,000. According to the results by Voight *et al.* (2005), we have simulated five bottleneck severities (b): 0.4, 0.1, 0.05, 0.01, and 0.005, and for each we have simulated durations of 0.01, 0.02, 0.03, and 0.04 (400, 800, 1200, and 1600 generations). T_{start} has been set to 0.02, 0.04, 0.08, and 0.12 (800, 1600, 3200, and 4800 generations, respectively).

Additional points have been simulated to better illustrate this model in figures.

RESULTS

Exploring parameter space: We have tested the power of 16 statistics under the different conditions of sample size (n), number of segregating sites (S), neutral mutation parameter (θ), demography, and recombination rates. Although a wide range of parameters have been studied, only the most interesting cases are shown. Full results are provided as supplemental data A–D. Supplemental data A and B show all results with correct recombination rates in the neutral model for the expansion model (A) and the contraction and bottleneck (B). Supplemental data C and D show results for mispecified recombination rates in the neutral model for expansions (C) and contractions and bottlenecks (D). As expected, larger sample sizes improve the power of the statistics, so we have plotted all power curves with $n = 100$; curves for other sample sizes can be found in the supplemental data files. Also, we have fixed the degree of expansion, D_e, to 10 in all expansion figures, as $D_e = 100$ produced uniformly maximal power for all statistics. For figures involving contraction, the degree of contraction, D_c, has been fixed to 0.1, and bottlenecks are shown with a severity of $b = 0.01$. Other parameter values fixed in some figures have also been chosen to avoid saturation.

The power of statistics has been shown for the tail for which each statistic shows power under its corresponding model. Since a population-growth event will cause an excess of derived low-frequency nucleotide variants and of the number of haplotypes and a reduction of linkage disequilibrium values, this tail corresponds in expansions to the left one for all statistics except for Dh (in the simulations fixing S) and Fay and Wu's H. The same happens for most bottlenecks, but not for population contractions or recently finished bottlenecks where tests show power at the right tail of the distribution (except Dh and H). Only some of the statistics have been represented to make graphs clearer. Of all Fu and Li's statistics, only F^* is shown, as it is the one with greatest power (although for simulations based on S, it has the same power as F). However, considering that in the contraction model D and D^* are more powerful, D has been chosen for these simulations. Of Wall's B and Q, we represent only the statistic that shows more power under each circumstance, as with the EHH average and EHH maximum. We also leave ZZ out, as it is simply the difference between Z_{nS} and Z_A and therefore can be easily inferred from them. In expansions, only T_e from 0.0075 to 0.2 and $T_e = 1$ has been represented, as in general at $T_e = 0.4$ tests have reached a power of ~ 0.5.

Statistical power for different demographic events: Figure 1 shows the statistical power of the tests under study in the absence of recombination and for different
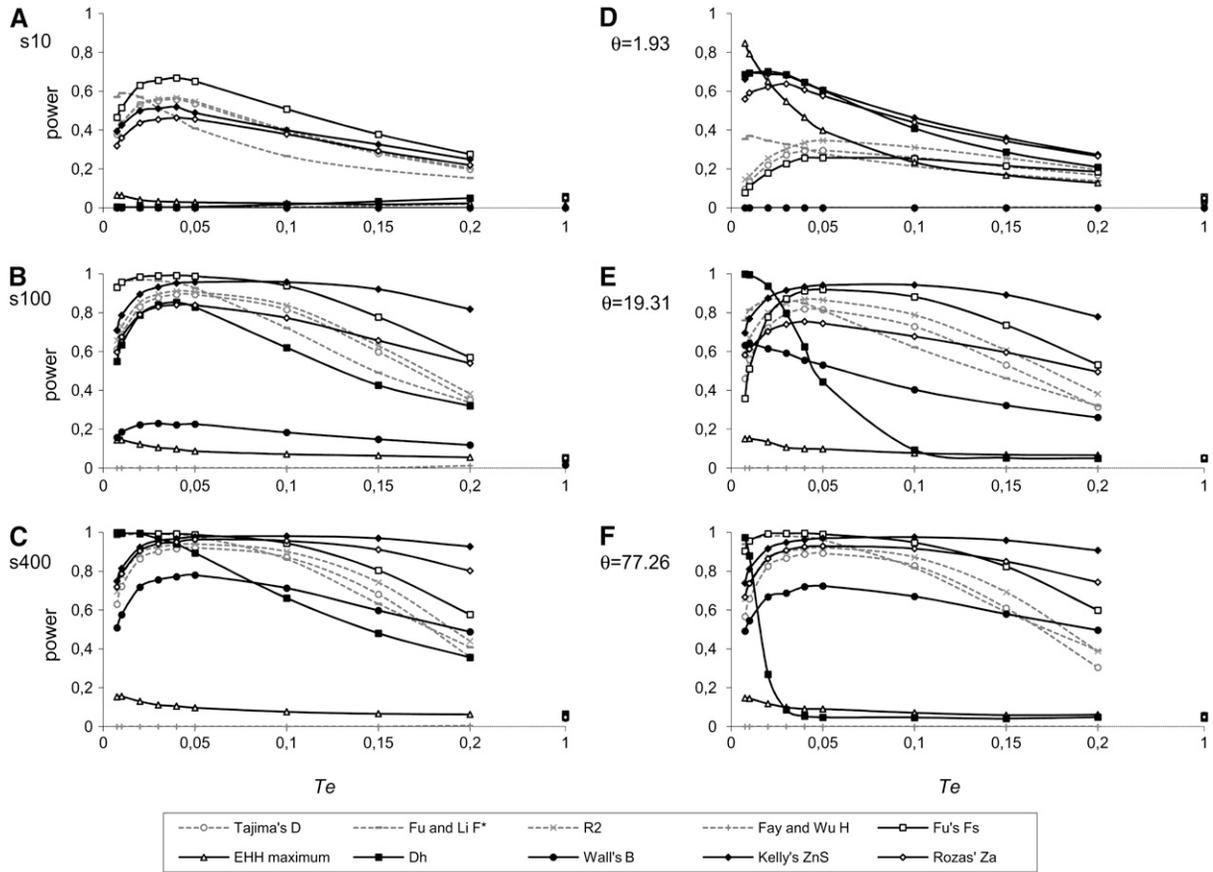
FIGURE 1.—Power of the test depending on the time elapsed since the expansion, without recombination. $N = 100$, $D_e = 10$. (A) $S = 10$. (B) $S = 100$. (C) $S = 400$. (D) $\theta = 1.93$. (E) $\theta = 19.31$. (F) $\theta = 77.26$.
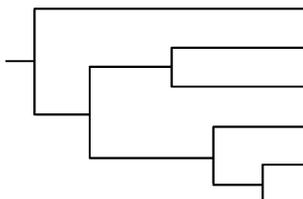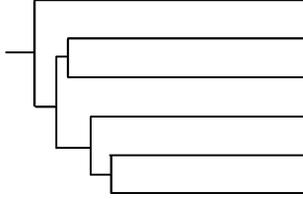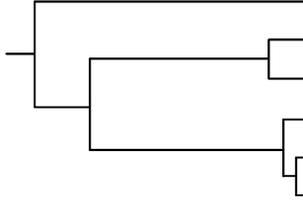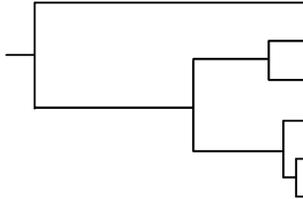
times since the expansion. Expansion ages range from $T_e = 0.0075$ (300 generations) to $T_e = 1$ (40,000 generations). Our results reproduce those from RAMOS-ONSINS and ROZAS (2002). For all statistics, power increases with the number of segregating sites (Figure 1, A–C), especially in class II statistics, which are based on haplotype structure. In fact, as shown in RAMOS-ONSINS and ROZAS (2002), Fu's F_s is the best-performing statistic for large sample sizes; however, Z_{nS} and Z_A statistics become more powerful when considering 100 or more segregating sites, especially to detect expansions older than $T_e = 0.05$ (2000 human generations). This can be explained by a saturation effect, such as the saturation of the number of haplotypes due to a high number of segregating sites with respect to n (NEI 1987). Figure 1, D–F, shows the effect of increasing θ upon statistical power. Simulations based on θ show the same patterns as when conditioning on S; the main differences reside in the behavior of Dh, which does not show power at the right tail of the distribution (as we would expect; see Table 2), but rather at the left tail. Moreover, the pattern of Dh's power is also particular, as it has its maximum at very recent times and a decay that becomes more pronounced as θ increases. For very low values of θ, most genealogical trees have zero or only one mutation, which greatly affects the power of any

haplotype-based statistic. In both kinds of simulations (fixing either S or θ), the lowest values ($S = 10$ and $\theta = 1.93$) show power curves that are different from those obtained for a higher polymorphism level, the reason for this behavior being that low polymorphism leads to imprecise statistics.

As for the effect of the elapsed time since the expansion, in both normal and high-polymorphism scenarios most well-performing statistics (statistics with powers that are normally >0.4, which excludes EHH estimators and Fay and Wu's H) reach their maximum power between $T_e = 0.04$ and $T_e = 0.05$. Exceptions to this rule are Fu and Li's tests (D, F, D^*, and F^*; only F^* is shown in Figure 1), which have maximum power at shorter times ($T_e = 0.01$–0.02), and Z_{nS}, which does not reach its maximum power until $T_e = 0.1$. Power decays with time since the expansion, but does it differently for class I and class II statistics: while class I statistics decay rapidly after reaching their maximum power, class II statistics have gentler slopes (again considering only well-performing statistics in medium and high-polymorphism scenarios). However, Fu's F_s and R_2 always decay in a very similar way. At $T_e = 1$ all statistics tend to 0.05 for simulations based on S, thus reaching the nominal type I error rate.

As expected (see Table 2), in the sudden contraction model the power of the neutrality tests behaves oppo-

TABLE 2

Description of tree topologies in different demographic scenarios

Scenario	Characteristics	Tree topology
Neutrality	Trees have regular neutral topologies.	
Expansion	Trees have a star-like topology, with long external branches which accumulate a greater number of low-frequency mutations than expected. This leads to a deficit of (a) most class I statistics and (b) LD structure, including en excess of haplotypes. In this case, most statistics are expected to show power at the right tail of the distribution.	
Contraction	Trees have long internal branches, which results in a greater number of intermediate-frequency mutations than expected. This leads to an excess of (a) most class I statistics and (b) LD structure that produces a deficit of haplotypes. In this case, most statistics are expected to show power at the left tail of the distribution.	
Weak bottleneck	Several lineages can escape the bottleneck without coalescing and thus provide trees with long internal branches. This produces an excess of (a) most class I statistics and (b) LD structure. Most tests how power at the left tail of the distribution.	
Strong bottleneck	It is most likely that no lineages survive the bottleneck without coalescing, resulting in star-like trees. This leads to a deficit of (a) most class I statistics and (b) LD structure. Most statistics will show power at the right tail of the distribution.	
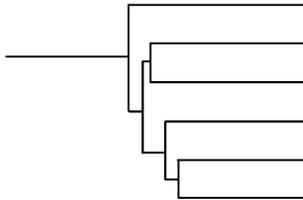
site than in expansions. The most powerful tests are Fu and Li's D^* and F_s. Maximum power to detect population contractions is influenced mainly by the number of segregating sites and can be found between $T_c = 0.1$ ($S = 100$) and $T_c = 0.4$ ($S = 10$). More details can be found in the supplemental Results and supplemental Figure 1.

Bottlenecks have different outcomes depending on whether or not several lineages have survived the bottleneck stage without coalescing. Thus, the effects of severity and the age of the perturbation are non-monotonic and tests have power at both tails of the distribution (see Table 2). For old ($T_{start} = 0.04$–0.08), strong ($b = 0.05$ or stronger), and long-lasting bottlenecks (after which most lineages will have coalesced), the most powerful statistics are R_2, F_s, and Z_{nS}. However,

they lack the power to detect bottlenecks that have just finished; as in this case, the fact that all sequences have coalesced during the bottleneck produces a very shallow genealogy and thus the statistics will behave similarly. On the contrary, in the case of weak, recent ($T_{start} = 0.02$–0.04) and recently finished bottlenecks, the most powerful tests are class I tests and Fu's F_s. More details can be found in the supplemental Results and in supplemental Figure 2.

The very different genealogy shapes that can be produced by bottlenecks (Table 2) dramatically affect the variance of the power of certain tests, especially in intermediate bottlenecks, which, depending on the run, can lead to either shallow or deep genealogies. To study this effect, we have calculated the variance of the statistics for each set of bottleneck parameters. We
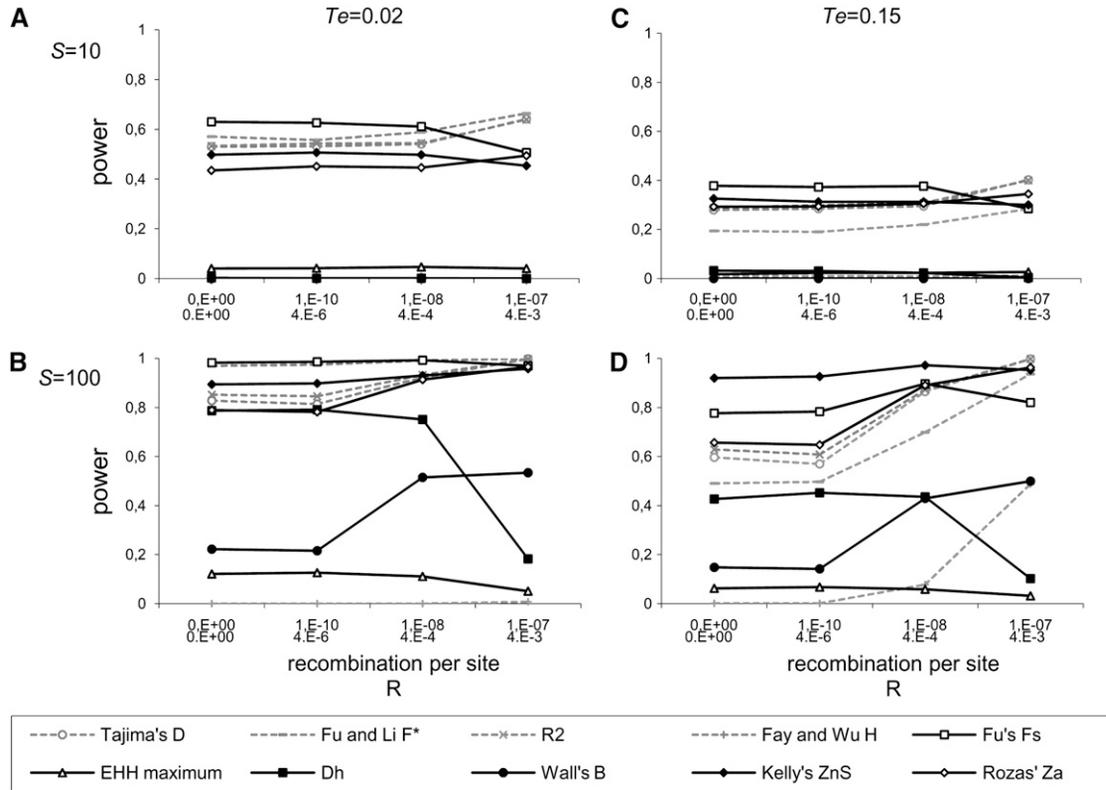
FIGURE 2.—Power of the test in the expansion model depending on recombination rates. $N = 100$, $D_e = 10$. (A) $S = 10$, $T_e = 0.02$. (B) $S = 100$, $T_e = 0.02$. (C) $S = 10$, $T_e = 0.15$. (D) $S = 100$, $T_e = 0.15$.

focused on bottleneck severity and studied how it modifies variance when other parameters are equal (supplemental data E). Variance patterns are quite similar for all n and S simulated values, and in general take maximum values around severities of $b = 0.05$, ranging from $b = 0.01$ to $b = 0.1$. This moment of maximum variance depends mainly on the time of onset and duration of the bottleneck, approaching $b = 0.01$ in recent and short bottlenecks and $b = 0.1$ in old and long-lasting ones. Moreover, some tests are more sensitive to changes in the bottleneck parameters T_{start} and T_{dur}. Fu and Li's tests and F_s tend to reach maximum variance values for less severe bottlenecks later than other tests (that is, for older and more long-lasting bottlenecks), while R_2 and Fay and Wu's H behave in an opposite way. Variance patterns seem to correlate inversely to the power of the statistics seen for the different bottleneck severities, as tests show maximum powers for severities ≥ 0.05 while maximum variances correspond to severities ≤ 0.05. This could be due to the fact that less severe bottlenecks leave a much weaker signature.

Recombination: Figure 2 shows the effects of recombination rate on statistical power in population expansions. While for $S = 10$ recombination has hardly any effect, for higher values of S the power increases dramatically between low recombination levels (0 and 10^{-10}) and high recombination levels (10^{-8} and 10^{-7}). For low recombination values (Figure 2), in most cases

power is not affected, although under some circumstances (*e.g.*, Fu and Li's D and D^* for $S = 100$; data not shown) power can be lower for $r = 10^{-10}$. In contrast, most statistics improve their power under high recombination, with the exceptions of Dh, EHH, and, for ancient expansions, of F_s and Z_{nS}, which have a tendency to decrease their power in the interval between $r = 10^{-8}$ to $r = 10^{-7}$. It is noteworthy that, for ancient expansions and high recombination values, Fay and Wu's H shows increased power, reaching values >0.9 for $S = 400$ (results not shown). Simulations based on θ show similar patterns to those based on S (results not shown). As for population contractions and bottlenecks, the changes in the power of the tests in the presence of recombination are very similar to those found in expansions (more details in the supplemental Results and supplemental Figures 3 and 4, respectively).

Recombination shuffles nucleotide variation, increasing the number of haplotypes through the creation of new recombinant ones. Thus, it is expected to strongly affect the mean of haplotype-based tests and also to reduce the variance of both kinds of statistics (Figure 3A). Changes in the power curve reflected in Figure 2 may therefore be due to two nonmutually exclusive factors: (i) an actual increase in the amount of information in the sequence that detects population expansions when recombination is acting and (ii) recombination shifting the distribution of neutrality
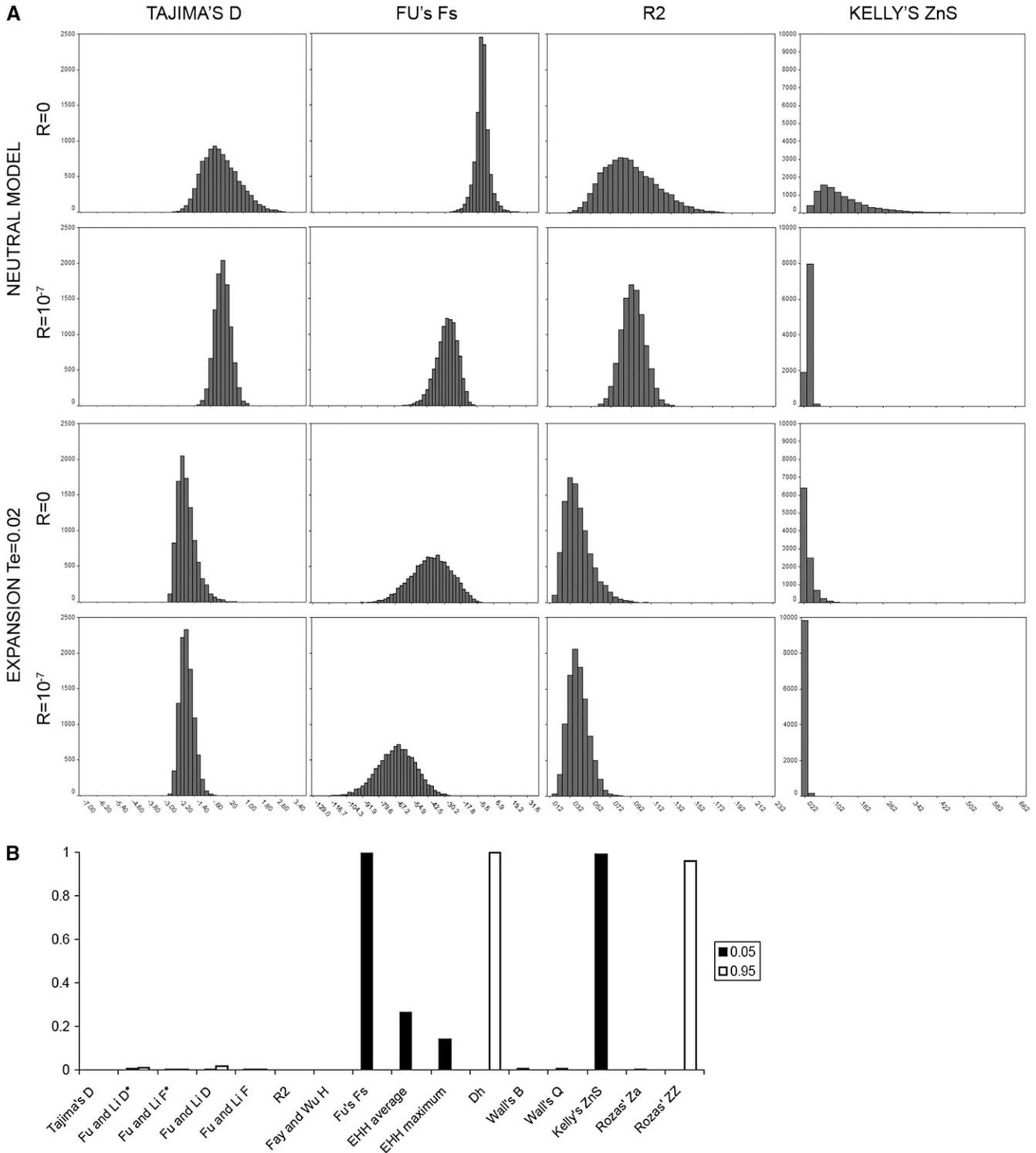
FIGURE 3.—(A) distribution of the values of Tajima's D, Fu and Li's F, R_2, and Kelly's Z_{nS} under the neutral model and the expansion model at $T_e = 0.02$ without recombination and with $r = 10^{-7}$. (B) Power of the tests to detect recombination in the null model for the right and left tails of the distribution. (A and B) $S = 100$, $n = 100$.

statistics. To assess the relative weights of these two explanations, we have compared the constant-population model without recombination with the constant-population model with high recombination. As shown in Figure 3B (see also supplemental data F), F_s and Z_{nS} are very sensitive to recombination (left tail of the

distribution), while Dh and ZZ are sensitive at the right tail. Both EHH estimators are also able to detect recombination (left tail) but with less power (<0.3). Thus, these statistics are liberal for detecting a population expansion acting on a recombining sequence and may often produce false positives.
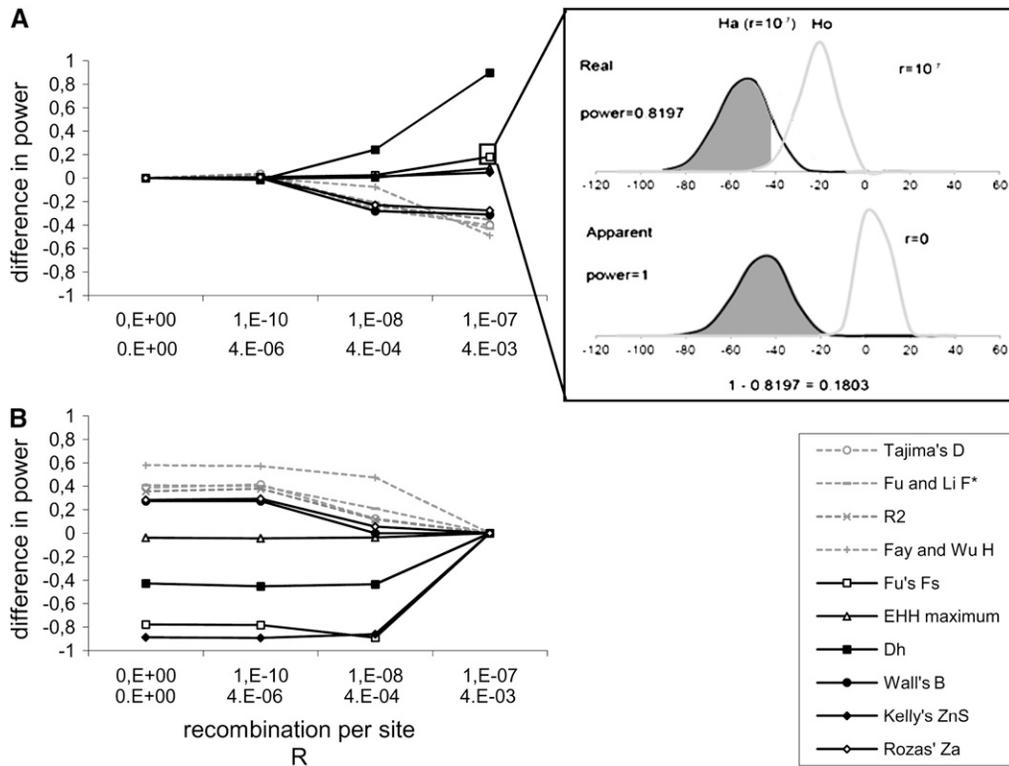
FIGURE 4.—Error made by statistics when testing for expansions when recombination is under- or overestimated in the null model. $S = 100$, $n = 100$, $D_e = 10$, $T_e = 0.15$. The cartoon shows how this error is calculated: first, we calculated the real power of the statistic, comparing the null hypothesis with the alternative hypothesis with the same recombination values. Afterward, we calculated the probability of rejecting the null hypothesis when recombination has been erroneously estimated, that is, when the recombination rate used to generate the null hypothesis is different from that of the alternative hypothesis. The error made is the latter (apparent power) minus the real power. (A) The apparent power of the null hypothesis was produced without recombination. (B) The apparent power of the null hypothesis has a recombination rate of 10^{-7}.

Unknown or misspecified recombination: Under some circumstances, recombination rates cannot be taken into account when testing for demographic or selective events. Indeed, the default option in Arlequin 3.0 (EXCOFFIER *et al.* 2005) or DnaSP (ROZAS *et al.* 2003), the two standard software packages for genetic analysis, is to compute the significance of neutrality tests without recombination, although DnaSP will also produce null distributions with any population recombination rate supplied by the user. When estimates of recombination rates are available, it is important to consider that they may be over- or underestimates. For example, KONG *et al.* (2002) measured recombination rates in human pedigrees at intervals of median length ∼350 kb. Considering that it has been estimated that there is a hotspot every 50 kb (MYERS *et al.* 2005), such intervals will most likely contain recombination hotspots, and therefore the recombination rate estimated for the whole interval will be much greater than the real one for most parts of the region. On the other hand, hotspots may also result in a recombination reaching saturation and thus to an underestimate of the average recombination rate in the region.

To investigate the potential errors caused by over- or underestimation of recombination, we compared the statistical power of tests when true recombination values were assumed *vs.* the power of the same tests assuming erroneous rates. Figure 4A shows the difference between the apparent power of a test (that is, comparing the constant-size null hypothesis without recombination with the population-expansion alternative hypothesis using the true recombination value) and its real power (that is, comparing the null hypothesis and the alternative hypotheses using the true recombination value). When actual recombination rates are small (10^{-10}) and thus underestimation is not too serious (*e.g.*, using as a null hypothesis the constant nonrecombining model when the actual rate is $r = 10^{-10}$), the true and the apparent statistical power are not appreciably different. However, for larger underestimates (when the real rates are $r = 10^{-8}$ or 10^{-7}), class I statistical tests become conservative (with an increase in type II error) whereas some class II statistics (Dh, Fu's F_s, EHH, and Z_{nS}) become liberal (with an increase in type I error). In population contractions, class I statistics behave as in expansions, while most class II statistics are liberal. As expected, strong bottlenecks tend to behave as expansions while weak ones tend to behave as contractions (see Table 2; more details are in the supplemental Results and supplemental Figures 5 and 6 for contractions and bottlenecks, respectively).

Figure 4B shows the difference between the apparent power of the tests when assuming an overestimated recombination of 10^{-7} on the constant-size null hypothesis and their true power (that is, using true recombination values to compare the null hypothesis and

TABLE 3

Sudden expansion model decision table

| T_e | No recombination | | Recombination (10^8) | |
	Strong ($D_e = 0.01$)	Weak ($D_e = 0.1$)	Strong ($D_e = 0.01$)	Weak ($D_e = 0.1$)
	Small S (≈ 10), small n (≈ 20)			
≤ 0.01	F_s, R_2, D^*	F_s, R_2, Z_{nS}, D^*	R_2, F, D^*, F^*	R_2
0.01–0.15	R_2, F_s	F_s, R_2, Z_{nS}	R_2	R_2
≥ 0.15	F_s, R_2, Z_{nS}	F_s, R_2, Z_{nS}	R_2	R_2
	Small S (≈ 10), large n (≈ 100)			
≤ 0.01	F_s, R_2, D, F, F^*	F and F^*	R_2, D, F, D^*, F^*	D^F
0.01–0.05	F_s, R_2, D	F_s	R_2, D	D^F
≥ 0.05	F_s	F_s	R_2, D	D^F
	Large S (≈ 100 or more), small n (≈ 20)			
≤ 0.01	$F_s, R_2, D, D^F, F, D^*, F^*, Z_{nS}, Z_A, Dh^a$	F_s, Dh^a	R_2, D, D^F, F, D^*, F^*	R_2
0.01–0.03	$F_s, R_2, D, D^F, F, D^*, F^*, Z_{nS}, Z_A$	F_s	R_2, D, D^F, F, D^*, F^*	R_2
0.03–0.05	R_2, D, Z_{nS}	R_2, Z_{nS}	R_2, D, D^F, F, D^*, F^*	R_2
>0.05	Z_{nS}	Z_{nS}	R_2	R_2
	Large S (≈ 100 or more), large n (≈ 100)			
≤ 0.02	$F_s, R_2, D, D^F, F, D^*, F^*, Z_{nS}, Z_A$	$F_s, D^F, F, D^*,$ and F^*	R_2, D, D^F, F, D^*, F^*	D^F, F, D^*, F^*
0.02–0.05	$F_s, R_2, D, D^F, F, D^*, F^*, Z_{nS}$	F_s	R_2, D, D^F, F, D^*, F^*	D^F, F, D^*, F^*
0.02–0.15	F_s, R_2, D, Z_{nS}	F_s, Z_{nS}	R_2, D	R_2, D
≥ 0.15	Z_{nS}	Z_{nS}	R_2, D	R_2, D

Power is at the left tail of the distribution unless otherwise indicated. D^F, Fu and Li's D.

[a] Power is at the right tail of the distribution.

alternative hypotheses). The effect of recombination overestimation shows the opposite pattern, and therefore all tests are liberal with the exception of Dh, Fu's F_s, EHH, and Z_{nS}, which become conservative. The difference in behavior between these class II and class I statistics (Figure 3) can be due to the fact that the former are highly dependent on the actual recombination rates (Wall 2000). As in the case of under-

TABLE 4

Sudden contraction model decision table

| T_c | No recombination | | Recombination (10^8) | |
	Strong ($D_c = 0.01$)	Weak ($D_c = 0.1$)	Strong ($D_c = 0.01$)	Weak ($D_c = 0.1$)
	Small S (≈ 10), small n (≈ 20)			
≤ 0.10	D^*	D^*	D^*	D^*
0.10–0.40	F_s, D^*	F_s, D^*	D^*	D^*
0.40–0.80	F_s, Z_{nS}, Z_A	F_s, Z_{nS}, Z_A	D^*	D^*
>0.80	F_s, Z_{nS}, Z_A, B, Q	$F_s, Z_{nS}, Z_A, B, Q, Dh^a$	D^*	D^*
	Small S (≈ 10), large n (≈ 100)			
≤ 0.20	D^F	D^F	D^F	D^F
0.20–0.60	Z_{nS}	Z_{nS}, B	D^F	D^F
≥ 0.60	$Z_{nS}, Z_A, B, Q, F_s, D^F$	$Z_{nS}, Z_A, B, Q, F_s, D^F$	D^F	D^F
	Large S (≈ 100 or more), small n (≈ 20)			
≤ 0.10	F_s, Dh^a	F_s, Dh^a	D^F	D^F
0.10–0.40	F_s, Dh^a	F_s, Dh^a	D^F, F, F^*	D^F, F, F^*
≥ 0.40	Dh^a	Dh^a	D^F, D^*	D^*
	Large S (≈ 100 or more), large n (≈ 100)			
≤ 0.02	D^F, D^*	D^F, D^*	D^F, D^*	D^F, D^*
0.02–0.10	F_s	F_s	F, F^*	F, F^*
0.10–0.40	F_s, Dh^a	F_s	F, F^*, R_2, D	F, F^*
0.40–0.80	$F_s, Z_{nS}, Z_A, B, Q, Dh^a$	Z_{nS}, Z_A	D^F, D^*	D^F
>0.80	$F_s, D^F, D^*, Z_{nS}, Z_A, B, Q$	Z_{nS}, Z_A, B, Q	D^F, D^*	F, F^*

Power is at the right tail of the distribution unless otherwise indicated. D^F, Fu and Li's D.

[a] Power is at the left tail of the distribution.

TABLE 5

Bottleneck decision table

T_{start}	No recombination		Recombination (r^8)	
	Strong ($b = 0.01$)	Weak ($b = 0.1$)	Strong ($b = 0.01$)	Weak ($b = 0.1$)
	Small S (≈ 10), small n (≈ 20)			
0.02	D, F, D^*, F^*, R_2, F_s	D^{*a}, F_s^{a}	$D^a, F^a, D^{*a}, F^{*a}, R_2^{a}$	$D^a, F^a, D^{*a}, F^{*a}, R_2^{a}$
0.04	D, R_2, F_s	$F_s^{a}, Z_{nS}^{a}, Z_A^{a}$	D^a, R_2^{a}	$D^a, F^a, D^{*a}, F^{*a}, R_2^{a}$
0.08–0.12	R_2, F_s	$D, D^*, F^*, R_2, F_s, Z_{nS}$	R_2	D, R_2
	Small S (≈ 10), large n (≈ 100)			
0.02	D, F, F^*, R_2, F_s, Dh	D^a, D^{Fa}, F_s^{a}	D^*, Dh	D^a, D^{Fa}, R_2^{a}
0.04	D, R_2, F_s	$F_s^{a}, B^a, Z_{nS}^{a}, Z_A^{a}$	D, D^*, R_2	$D^a, D^{Fa,b}, R_2^{a}$
0.08	D, R_2, F_s	D, F, R_2, F_s, Dh	D, R_2	D^F
0.12	F_s	D, R_2, F_s	D, R_2	D^F
	Large S (≈ 100 or more), small n (≈ 20)			
0.02	$D, D^F, F, D^*, F^*, R_2, F_s$	D^{*a}, F_s^{a}	$D^a, D^{Fa}, F^a, D^{*a}, F^{*a}, R_2^{a}$	$D^{Fa}, F^a, D^{*a}, F^{*a}$
0.04	$D, D^F, F, D^*, F^*, R_2, F_s, Z_{nS}, Z_A$	$F_s^{a,b}, B^a, Q^a, Z_{nS}^{a}, Z_A^{a}$	$D^a, D^{Fa}, F^a, D^{*a}, F^{*a}, R_2^{a}$	$D^a, D^{Fa}, F^a, F^{*a}, R_2^{a}$
0.08	D, R_2, Z_{nS}, Z_A	R_2, F_s	D, D^F, F, D^*, F^*, R_2	D, R_2
0.12	R_2, Z_{nS}	R_2	D, R_2	D, R_2
	Large S (≈ 100 or more), large n (≈ 100)			
0.02	D, F, D^*, F^*, R_2	D^{*a}, F_s^{a}	D^{Fa}, D^{*a}	$D^a, D^{Fa,b}, F^{a,b}, D^{*a,b}, F^{*a,b}, R_2^{a}$
0.04	$D, D^F, F, D^*, F^*, R_2, F_s, Z_{nS}, Z_A$	$F_s^{a}, B^a, Q^a, Z_{nS}^{a}, Z_A^{a}$	$D^a, D^{Fa}, F^a, D^{*a}, F^{*a}, R_2^{a}$	$D^a, D^{Fa,b}, F^{a,b}, D^{*a,b}, F^{*a,b}, R_2^{a}$
0.08	$D, D^F, F, D^*, F^*, R_2, Z_{nS}, Z_A$	F_s	D, D^F, F, D^*, F^*, R_2	D^F, F, D^*, F^*, R_2
0.12	D, R_2, Z_{nS}	F_s	D, F, F^*, R_2	D, F, F^*, R_2

Power is at the left tail of the distribution unless otherwise indicated. D^F, Fu and Li's D.

[a] Power is at the right tail of the distribution.

[b] Only for finished bottlenecks.

estimation of recombination, when recombination is overestimated in a scenario of population contraction, class I tests behave similarly than in expansions. This is not the case for class II tests, which became liberal. Again, strong bottlenecks behave similarly to expansions while weak ones behave more like contractions (Table 2; more details are in the supplemental Results and supplemental Figures 5 and 6 for contractions and bottlenecks, respectively).

DISCUSSION

Power of neutrality tests: We have examined statistical power in detecting a sudden population expansion, a sudden contraction, or a bottleneck analyzing DNA polymorphism data by means of a wide range of statistics. The most powerful tests are those belonging to class II, that is, those based on haplotype frequencies. Within those, Fu's F_s and Z_{nS} perform best although, for small sample sizes, R_2 is also recommended. Class II tests, however, are strongly affected by recombination, particularly F_s and Z_{nS}, which not only lose power under high recombination rates, but also become significant with recombination events when no expansion has taken place and the population has remained constant. For this reason, when recombination is suspected to be acting on a sample, and especially if there is risk of over-

or underestimating it, it is not advisable to use class II tests and, thus, R_2, Tajima's D or Fu and Li's tests should be used instead. A similar situation, with class II statistics being more powerful than class I, can be found for contractions, although tests have power at the opposite tail of the distribution and they start to perform well at larger times and for longer S. However, in the case of bottlenecks, class I tests are best as a general rule, with the exception of F_s and Z_{nS} in some particular cases.

Combinations of the different tests and the tail at which they show power can therefore provide a rough idea about the demographic event acting over a population and about the time and strength of this event, as well as about other factors such as recombination.

The effect of recombination on the power of the tests: As discussed above, recombination is expected to have some effects on the power of statistical tests based on the allele frequency spectrum and to reduce the power of tests that rely on haplotype-based statistics (see, for example, QUESADA *et al.* 2006 and references therein). We performed a detailed series of simulations suggesting that high recombination rates generally improve the power of most statistical tests, although some class II tests (Dh, EHH, F_s, and Z_{nS}) lose power for the maximum recombination rates considered in this study, especially for old expansions. Furthermore, even when recombination is considered in the null constant-population

models, those tests have great power to "detect recombination" in a sample. This efficiency in recognizing recombination can be easily explained as recombination modifies the mean and reduces the width of the distribution of class II statistics. Therefore, careful attention should be paid to the interpretation of those tests when recombination is suspected to have shaped the genealogy of the sampled sequences. We have also examined the effect of using mistaken recombination rates, because their estimation can be a problem for most organisms. In the case of humans, actual recombination maps made through the genotyping of 5136 microsatellite markers for 146 families, with a total of 1257 meiotic events, are available at an $\sim$350-kb resolution (Kong *et al.* 2002). Beyond that level, current efforts are aimed at pinpointing hotspots and recombination deserts as inferred from linkage disequilibrium patterns (McVean *et al.* 2004; Fearnhead and Smith 2005; Myers *et al.* 2005). Wall (1999) observed that larger sequence sizes with high recombination decreased the power of tests and suggested that this was due to the difference between the recombination rates of the null and the alternative hypotheses. Our results show that severe over- or underestimations of recombination have large impacts on power. When recombination is underestimated, the power of tests decreases in expansion and bottlenecks, supporting the results in Wall (1999). In contrast, when recombination is overestimated, type I errors increase, leading to a spurious gain in the power of the tests. Since class II Z_{nS}, Fu's F_s, Dh, and EHH statistics are very sensitive to recombination, they have an opposite behavior. Considering all recombination results together, a conservative recombination rate should be used in the null hypothesis when using class II statistics—if recombination could be acting over the sample, for example, it would be recommended to use a lower bound or no recombination if there is a deficit of haplotypes or an upper bound if there is an excess.

Recommendations for test usage arising from our results are summarized in Tables 3–5. We hope that they help to produce guidelines for a rational choice among the wide variety of neutrality tests available.

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