

Note

On the Distribution of Temporal Variations in Allele Frequency: Consequences for the Estimation of Effective Population Size and the Detection of Loci Undergoing Selection

Isabelle Goldringer^{*,1} and Thomas Bataillon[†]

^{*}UMR de Génétique Végétale, INRA UPS INA-PG CNRS, Ferme du Moulon, 91190 Gif sur Yvette, France and [†]UMR 1097 Diversité et Génomes des Plantes Cultivées, INRA Domaine de Melgueil, 34130 Mauguio, France

Manuscript received December 17, 2003

Accepted for publication June 7, 2004

ABSTRACT

The effective population size (N_e) is frequently estimated using temporal changes in allele frequencies at neutral markers. Such temporal changes in allele frequencies are usually estimated from the standardized variance in allele frequencies (F_c). We simulate Wright-Fisher populations to generate expected distributions of F_c and of \bar{F}_c (F_c averaged over several loci). We explore the adjustment of these simulated \bar{F}_c distributions to a chi-square distribution and evaluate the resulting precision on the estimation of N_e for various scenarios. Next, we outline a procedure to test for the homogeneity of the individual F_c across loci and identify markers exhibiting extreme F_c -values compared to the rest of the genome. Such loci are likely to be in genomic areas undergoing selection, driving F_c to values greater (or smaller) than expected under drift alone. Our procedure assigns a P -value to each locus under the null hypothesis (drift is homogeneous throughout the genome) and simultaneously controls the rate of false positive among loci declared as departing significantly from the null. The procedure is illustrated using two published data sets: (i) an experimental wheat population subject to natural selection and (ii) a maize population undergoing recurrent selection.

THE effective population size (N_e), defined as the size of an ideal Wright-Fisher population undergoing the same rate of genetic change as the population under study, is an essential parameter to predict the evolution of a population due to genetic drift in terms of rates of loss of genetic variation, fixation of deleterious alleles, or inbreeding (WRIGHT 1969). However, obtaining direct estimates of N_e from demographic data has often proved difficult. An alternative is to use indirect methods, for instance, those based on the measurement of temporal changes in allele frequencies at neutral markers (KRIMBAS and TSAKAS 1971; WAPLES 1989a). The foundation of these methods is that the variance of allele frequency due to drift from parents to offspring, $V(P_t)$, depends on N_e as follows: $V(P_t) = P_0(1 - P_0)/2N_e$, where P_0 is the frequency in the parental population. After t generations of drift, the expected frequency of the allele is $E(P_t) = P_0$ and the variance of the allele frequency, $V(P_t) = E(P_t - P_0)^2$, can be written as a function of N_e : $V(P_t) = P_0(1 - P_0)[1 - (1 -$

$1/2N_e)^t]$ (CROW and KIMURA 1970). If t is not too large ($t \ll N_e$), N_e can be approximated by $N_e \approx (P_0(1 - P_0)t)/(2V(P_t))$ and therefore an estimator for N_e based on the standardized variance in allele frequency is $(V(P_t))/(P_0(1 - P_0))$. NEI and TAJIMA (1981) proposed estimating the standardized variance in allele frequency between generation t_x and t_y for each locus l with K_l alleles as

$$\hat{F}_{c,l} = \left(\frac{1}{K_l} \right) \sum_{i=1}^{K_l} \frac{(p_{x(i,l)} - p_{y(i,l)})^2}{(p_{x(i,l)} + p_{y(i,l)})/2 - p_{x(i,l)}p_{y(i,l)}}, \quad (1)$$

where $p_{x(i,l)}$ [respectively $p_{y(i,l)}$] represents the frequency of allele i at locus l in the sample of S_x individuals drawn at generation t_x (respectively S_y individuals at t_y). A weighted mean of $\hat{F}_{c,l}$ -values across several loci,

$$\hat{F}_c = \frac{\sum_l K_l \hat{F}_{c,l}}{\sum_l K_l}, \quad (2)$$

is then typically used to estimate N_e via

$$\hat{N}_e = \frac{t_y - t_x}{2(\hat{F}_c - 1/(2S_x) - 1/(2S_y))} \quad (3)$$

(WAPLES 1989a). Note that Equation 1 assumes that allele frequencies are estimated from samples taken prior to reproduction (so-called plan II sampling scheme).

¹Corresponding author: UMR de Génétique Végétale, INRA UPS INA-PG CNRS, Ferme du Moulon, 91190 Gif sur Yvette, France.
E-mail: isa@moulon.inra.fr

We use that sampling in the remainder of this article; allowing for an alternative sampling scheme is straightforward.

Recently, renewed interest in estimating N_e has led to the development of numerous methods using allele frequencies observed in a series of temporally spaced samples of a population. WILLIAMSON and SLATKIN (1999) and ANDERSON *et al.* (2000) introduced a maximum-likelihood approach to estimate N_e . BERTHIER *et al.* (2002) proposed a coalescent-based likelihood approach to estimate N_e and WANG (2001) devised a faster approximate version using a pseudo-maximum-likelihood method. These methods, tested by the authors for some values of population parameters (N_e , l , K_b and $p_{s(i)}$), have proved to be slightly more accurate than the F_c method, but are much more computationally intensive. Hence F_c -based estimators of N_e remain frequently used in practice (FUJIIO *et al.* 1999; TURNER *et al.* 1999; GOLDRINGER *et al.* 2001; SHIKANO *et al.* 2001). Properties of F_c -based estimators of N_e and the quality of the confidence intervals around such estimates depend critically on the distribution of \bar{F}_c -values. Confidence intervals around N_e have been based on the fact that $n\bar{F}_c/E(\bar{F}_c)$, with $n = \sum_i(K_i - 1)$, is distributed approximately as a chi-square with n d.f. (LEWONTIN and KRAKAUER 1973). Hence, assessing the adjustment of the actual \bar{F}_c distribution to a chi-square distribution is important. The chi-square approximation has been studied for some special cases, but the effects of initial allele frequencies, of the number of alleles, of the number of loci, and of the number of generations as well as the “true” effective population size on the distribution of \bar{F}_c -values and on \hat{N}_e are still poorly known (WAPLES 1989a).

Before averaging estimates of F_c obtained at individual loci to obtain \bar{F}_c and an estimate of N_e , it is desirable to test whether all loci used for that study have experienced the same effective population size. This implicit assumption, which underlies all methods of estimation mentioned above, is rarely tested. Several factors can modify the local effective size at a given locus. The recurrent elimination of deleterious variants linked to a marker locus, known as “background selection,” will reduce the effective size locally; this effect depends on the local recombination rates and on genome-wide parameters describing spontaneous mutation and their effect on fitness (CHARLESWORTH *et al.* 1993). Hitchhiking will also drive higher than expected the temporal variance in allele frequency of markers linked to a positively selected variant (WIEHE and STEPHAN 1993).

The remainder of this note is organized as follows. In the first part, we study the actual \bar{F}_c distribution and the quality of the chi-square approximation. The actual distribution of \bar{F}_c , its divergence from a chi-square distribution, and the quality of the N_e estimation based on F_c are studied under various scenarios by varying the initial allele frequencies, the number of loci, the number of generations, the sample size at both generations,

and the true effective population size. In the second part, we outline a procedure to identify loci with “extreme” individual $\hat{F}_{c,i}$ -values. We illustrate our approach by reanalyzing two experimental data sets: temporal variation in allele frequencies at 29 markers in an experimental wheat population under natural selection and frequencies at 82 markers in a maize population under recurrent selection.

The actual distribution of \bar{F}_c and its consequences for estimating N_e : To investigate the actual distribution of \bar{F}_c , we simulated Wright-Fisher populations using an exact multinomial sampling scheme. We generated expected distributions of temporal variations in allele frequencies conditional on initial allele frequencies. Distributions were based on 3000 independent replicates. In each replication, several loci with the same initial allele frequencies were simulated and \bar{F}_c was computed. All simulations were carried out using *Mathematica* (WOLFRAM 1996). Our simulations show that substantial departure of the actual distribution of \bar{F}_c from a chi-square distribution, as measured through KULLBACK’s (1968) symmetric measure of divergence between both distributions (see Table 1), can be observed under a variety of conditions depending on the parameter values chosen (Table 1). The actual \bar{F}_c distribution is closest to a chi-square and thus N_e is best estimated when biallelic marker loci with equal frequencies are used. Conversely, the discrepancy between the actual \bar{F}_c distribution and the chi-square approximation is large when allele frequencies are strongly unbalanced ($P_0 < 0.1$ for at least one allele), when the number of alleles per locus is large ($K \geq 5$) such as for microsatellite markers, or when the number of generations increases ($\delta T = t_y - t_x > 15$ when $N_e = 100$ is assumed). Increasing the sample sizes up to 200 or 500 individuals does not diminish the discrepancy, especially for a high number of alleles (data not shown). In most cases, the distribution of simulated values is shrunk compared to the chi-square approximation. In addition, the actual distribution is a bit skewed toward higher values of F_c . As a consequence, the distribution of \hat{N}_e in the simulations is often more narrow than the one based on the chi-square approximation. Confidence intervals at the 95% level based on either the chi-square approximation or the actual \bar{F}_c distribution are given in Table 1. These are exactly the intervals that would be computed in experimental studies. Chi-square confidence intervals are often wider than the confidence intervals based on the simulated \bar{F}_c (Table 1). The width of this interval, which is connected to the precision of the estimation, depends mainly on the number of independent alleles used [$L(K - 1)$]. Note that the product $L(K - 1)$ is also the number of degrees of freedom of the chi-square used in previous approximations. Confidence intervals derived from simulated data are reduced by 10–25% relative to chi-square-based confidence intervals for scenarios involving unbalanced initial allele frequencies with $5 \leq L(K - 1) \leq$

TABLE 1
Distribution of \bar{F}_c : N_c estimation and divergence from chi-square distribution according to different parameters

δT	L	K	P_0 vector	N_c	$S_x = S_y$	\bar{F}_c^*	\hat{N}_c^*	n (d.f.)	Kullback's divergence	Confidence intervals on \hat{N}_c	
										Chi-square	Simulations
					Varying no. of loci L						
10	5	3	0.4/0.3/0.3	100	100	0.0586	103.0	10	0.011	29-269	31-250
10	10	3	0.4/0.3/0.3	100	100	0.0587	102.6	20	0.017	44-205	46-196
10	20	3	0.4/0.3/0.3	100	100	0.0583	103.6	40	0.022	53-164	59-165
					Varying no. of alleles and initial frequencies (P_0)						
10	5	3	0.7/0.2/0.1	100	100	0.0572	105.9	10	0.017	30-279	32-273
10	5	3	0.85/0.1/0.05	100	100	0.0538	114.0	10	0.023	32-307	33-280
10	5	2	0.5/0.5	100	100	0.0590	102.1	5	0.008	14-385	17-366
10	5	2	0.95/0.05	100	100	0.0516	120.1	5	0.124	17-494	23-379
10	5	5	0.2/0.2/0.2/0.2/0.2	100	100	0.0578	104.5	20	0.022	45-210	48-198
10	5	5	0.5/0.15/0.15/0.15/0.05	100	100	0.0568	106.8	20	0.031	46-215	45-199
10	5	10	0.1/0.1/0.1/...	100	100	0.0561	108.3	45	0.048	58-168	63-155
10	5	10	0.3/0.3/0.05/0.05/0.05/...	100	100	0.0529	116.7	45	0.076	62-183	62-160
					Varying sample size (S_x and S_y)						
10	5	3	0.3/0.3/0.4	100	50	0.0681	104.0	10	0.017	26-378	28-346
10	5	3	0.3/0.3/0.4	100	200	0.0538	102.4	10	0.007	31-235	32-240
					Varying no. of generations ($\delta T = t_y - t_x$)						
5	5	3	0.4/0.3/0.3	100	100	0.0348	100.7	10	0.009	26-357	27-354
15	5	3	0.4/0.3/0.3	100	100	0.0813	105.2	10	0.021	31-253	35-230
30	5	3	0.4/0.3/0.3	100	100	0.1468	109.6	10	0.053	34-243	38-219
50	5	3	0.4/0.3/0.3	100	100	0.2260	115.7	10	0.143	36-249	41-210
					Varying true effective size (N_c)						
10	5	3	0.4/0.3/0.3	50	100	0.1041	53.1	10	0.029	16-122	19-112
10	5	3	0.4/0.3/0.3	75	100	0.0740	78.1	10	0.019	23-191	25-178
10	5	3	0.4/0.3/0.3	200	100	0.0344	204.7	10	0.007	52-734	53-715

The sampling process over $\delta T = t_y - t_x$ generations was simulated using multinomial sampling of N_c individuals ($2 \times N_c$ genes) within the vector of allele frequencies at the previous generation. Varying parameters are N_c , the number of loci (L), the number of alleles per locus (K), and the set of initial allele frequencies (P_0 vector). In each replicate, \bar{F}_c , the standardized variance of allele frequencies (NEI and TAJMA 1981) over L loci (each with K alleles and identical P_0) is computed between generation t_x and generation t_y ($\delta T = t_y - t_x$). The distribution of simulated \bar{F}_c -values is generated from 3000 replications. \bar{F}_c^* is the average of \bar{F}_c over the 3000 replicates. \hat{N}_c^* is the average estimated effective size calculated as $\hat{N}_c^* = \delta T / (2(\bar{F}_c^* - 1/2S_x - 1/2S_y))$. The distance between the simulated distribution of \bar{F}_c -values and its chi-square approximation is measured through Kullback's symmetric divergence (KULLBACK 1968). Note that this is not a P -value but rather a way to assess which parameters lead to a poorer approximation of the actual \bar{F}_c distribution by a chi-square. The chi-square 95% confidence intervals on $E(\hat{N}_c)$ are derived by setting the experimental estimated value (\hat{N}_c) to \hat{N}_c^* and assuming a chi-square distribution for \bar{F}_c , according to WAPLES (1989a, Equation 16). Simulation-based confidence intervals are: the lower (respectively upper) bound is computed as the lowest (respectively highest) N_c -value (size of the simulated populations) for which at most 2.5% of estimated \hat{N}_c -values (from 3000 simulations) are larger (respectively lower) than \hat{N}_c^* .

10, or very large number of alleles ($K = 10$), sample sizes < 100 , $\delta T > 10$, or $N_e < 75$. Otherwise, they are quite close to the chi-square-based intervals (reduction is $< 10\%$). Similar results are found with larger sample sizes (200 and 500 individuals).

Except for $\delta T = 5$ generations, \hat{N}_e^* , computed from \bar{F}_c^* (an average of \bar{F}_c over 3000 independent replicates), is always higher than the population size N_e used in simulations. This indicates that \bar{F}_c -based estimation tends to return overestimated values of N_e . RICHARDS and LEBERG (1996) and LUIKART *et al.* (1999) argued that the overestimation of N_e using \bar{F}_c [or POLLAK's (1983) estimator, \hat{F}_k] is due mainly to the loss of alleles in early generations, suggesting that the bias would be greater with increasing drift and when there are rare alleles. LUIKART *et al.* (1999) focused on the estimation of N_e after very strong bottlenecks ($N_e = 4\text{--}40$), which were not considered here. Whereas our results confirm the existence of a greater bias for rare alleles, for the range of N_e we considered, population size does not appear as critical; however, increasing the number of generations between samples leads to overestimation of N_e . Hence, to improve precision on the F_c -based estimation of N_e , we recommend generating the actual \bar{F}_c distribution using simulations based on the estimated value \hat{N}_e and obtaining a confidence interval directly on \hat{N}_e . With such a gain in accuracy, the performance of the F_c -based estimator of N_e becomes close to those of likelihood-based estimators.

Distribution of F_c at individual loci and the detection of loci departing from pure drift: Once N_e has been estimated on the basis of \bar{F}_c by averaging over several marker loci, it is desirable to test whether all loci considered have undergone the same rate of change in allele frequency. Heterogeneity in individual F_c -values might be used as evidence for selection since “*while natural selection will operate differently for each locus and each allele at a locus, the effect of breeding structure (migration, genetic drift, inbreeding) is uniform over all loci*” (LEWONTIN and KRAKAUER 1973, pp. 176–177). Loci with significantly high $\hat{F}_{c,l}$ -values should be discarded before (re)computing \bar{F}_c to yield a more reliable estimate of N_e .

We propose a way to identify, in a series of experimental $\hat{F}_{c,l}$ measurements, markers exhibiting $\hat{F}_{c,l}$ -values significantly higher than expected under pure drift based on \hat{N}_e . To do so, each $\hat{F}_{c,l}$ -value should be compared to an expected distribution based on a genome-wide effective size estimated from the remaining loci and the trajectory of allele frequencies at this locus. We exemplify below our method with two published data sets. First we consider individual $\hat{F}_{c,l}$ -values estimated from temporal variations of allele frequencies in an experimental composite wheat population undergoing natural selection. A total of 250 and 213 individuals were sampled at generations 1 and 10, respectively, and

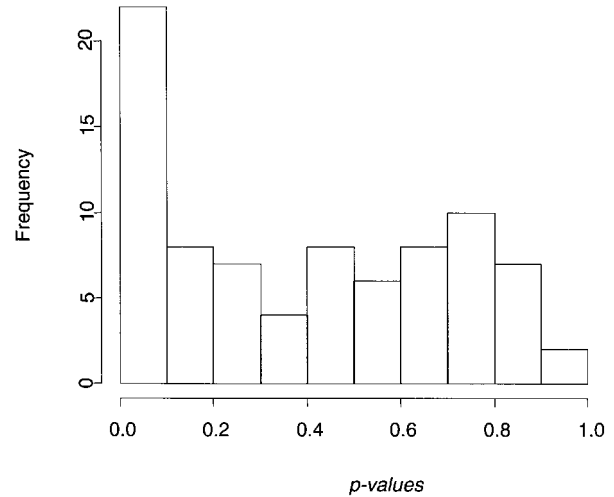


FIGURE 1.—Distribution of P -values for the 82 loci in the maize data set of LABATE *et al.* (1999).

genotyped at 29 RFLP loci (GOLDRINGER *et al.* 2001). For each locus l , we pooled the remaining 28 loci to obtain a global \bar{F}_c estimate and an \bar{F}_c -based estimate of N_e (described hereafter as the genome-wide average estimate). The expected distribution of F_c at locus l was then obtained (using typically 3000–5000 independent simulations) conditional on \hat{N}_e (excluding locus l) and the observed initial allele frequencies (at locus l). We then tested whether the observed temporal variance of allele frequencies at locus l , $\hat{F}_{c,l}$, was significantly larger than the genome-wide average variations by computing p , the probability for $\hat{F}_{c,l}$ to be greater than or equal to the observed value at this locus on the basis of the simulated distribution described above. Note that one could also test for the presence of loci exhibiting smaller than expected variations in allele frequency. Some loci exhibited some “excess drift” relative to the rest of the loci and accordingly fairly small P -values: *Fba242-C* ($P = 0.021$), *Fba280-C* ($P = 0.042$), *Fba65-D* ($P = 0.085$), and *Fba204-A* ($P = 0.09$). However, the distribution of P -values was fairly uniform (data not shown) and to take into account the fact that multiple loci were examined we computed the expected false discovery rates, also known as q -values, using the distribution of P -values (see STOREY and TIBSHIRANI 2003 for details). The q -values were calculated using the package QVALUE (<http://faculty.washington.edu/jstorey/qvalue/index.html>). This analysis suggests that declaring only *Fba242-C* and *Fba280-C* as “significant” for excess of drift would still yield an estimated rate of false positives of $\sim 40\%$ among these two loci.

Next we consider the study published by LABATE *et al.* (1999), where temporal variations in allele frequencies were surveyed at 82 RFLP loci after 12 generations in maize populations undergoing recurrent selection. A P -value was calculated at each locus (Figure 1), using the method described above. The distribution of P -val-

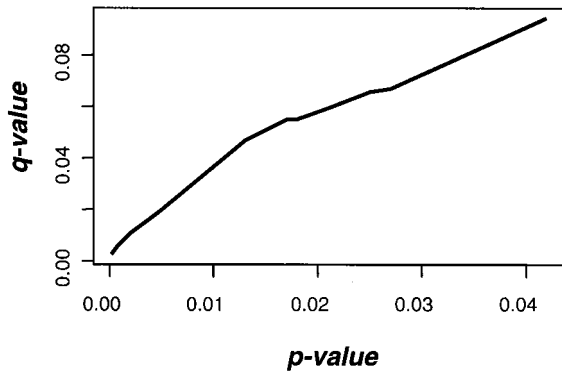


FIGURE 2.—Expected proportion of false positives (q -values) among the loci found to be departing significantly from the genome-wide average effective size in the maize data set as a function of the individual threshold used for significance (P -value). Calculations of the q -values were done using the procedure proposed by STOREY and TIBSHIRANI (2003).

ues was then used to calculate corresponding q -values (Figure 2). In contrast with the previous case, the distribution of P -values is clearly L-shaped (Figure 1) and choosing a q -value cutoff of 0.05 yields 10–11 loci exhibiting significant departures from the genome-wide level of drift. This method proved to be somewhat more conservative than the one used by the authors, who declared 14 loci as outliers (LABATE *et al.* 1999). Discarding those outlier loci, one can compute a new genome-wide effective size and check that no more loci exhibit F_c -values departing significantly from the null hypothesis of homogeneous drift (data not shown).

WAPLES (1989b) proposed a method based on the chi-square test of homogeneity to test the hypothesis that observed changes in allele frequencies can be satisfactorily explained by drift alone. This allows one to examine the variation of a particular allele according to the range of possible N_e -values for the population under study. Yet, this test has not been widely used in experimental studies. Indeed, it is rather complicated to implement, particularly in cases of multiple alleles since it is necessary to consider covariances of frequencies for different alleles sampled at different times. LEWONTIN and KRAKAUER (1973) provided the theoretical grounds for homogeneity tests of variation in allele frequencies, but they emphasized more spatial variation, and the test proposed for temporal variation, which again relies on the assumption of a chi-square distribution of individual $F_{c,i}$ -values, is much too restrictive [see BEAUMONT and NICHOLS (1996) and VITALIS *et al.* (2001) for the case of spatial variation in allele frequencies]. The use of simulation-based distributions provides a robust method to test for homogeneity of $\hat{F}_{c,i}$ -values across loci before pooling estimates. Our procedure yields a more reliable genome-wide estimate of the realized N_e and can be used to detect markers exhibiting

F_c -values significantly higher than expected on the basis of mean N_e , thereby providing a (formal) way of assessing if selection is operating on any given genomic segment (see also LUIKART *et al.* 2003 for a review of available methods for population structure). One potential caveat of our method is that the distribution of F_c under the null hypothesis is generated using information from the data (to estimate the genome-wide N_e). We verified through simulations (see online supplementary material at <http://www.genetics.org/supplemental/>) that our procedure is actually fairly robust to uncertainty in the estimation of the genome-wide N_e . A program generating the expected individual or mean F_c distributions used in this note is available upon request as a *Mathematica* notebook from the authors.

We thank F. Hospital, I. Bonnin, and C. Dillmann for helpful discussions and A. Tsitrone, R. Waples, and an anonymous reviewer for their comments on earlier versions of this article. We thank O. Martin for correcting and improving the English of this article.

LITERATURE CITED

- ANDERSON, E. C., E. G. WILLIAMSON and E. A. THOMPSON, 2000 Monte Carlo evaluation of the likelihood for N_e from temporally spaced samples. *Genetics* **156**: 2109–2118.
- BEAUMONT, M. A., and R. A. NICHOLS, 1996 Evaluating loci for use in the genetic analysis of population structure. *Proc. R. Soc. Lond. Ser. B* **263**: 1619–1626.
- BERTHIER, P., M. A. BEAUMONT, J. M. CORNUET and G. LUIKART, 2002 Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: a genealogical approach. *Genetics* **160**: 741–751.
- CHARLESWORTH, B., M. T. MORGAN and D. CHARLESWORTH, 1993 The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**: 1289–1303.
- CROW, J. F., and M. KIMURA, 1970 *An Introduction to Population Genetics Theory*. Burgess Publishing, Minneapolis.
- FUJIO, Y., M. NAKAJIMA and A. A. BARINOVA, 1999 Decrease of the effective population size during maintenance of the guppy strain. *Fish. Sci.* **65**: 362–366.
- GOLDRINGER, I., J. ENJALBERT, A.-L. RAQUIN and P. BRABANT, 2001 Strong selection in wheat populations during ten generations of dynamic management. *Genet. Sel. Evol.* **33** (Suppl. 1): 441–463.
- KRIMBAS, C. B., and S. TSAKAS, 1971 The genetics of *Dacus oleae*: changes of esterase polymorphism in natural population following insecticide control: Selection or drift? *Evolution* **25**: 454–460.
- KULLBACK, S., 1968 *Information Theory and Statistics*. Dover, New York.
- LABATE, J. A., K. R. LAMKEY, M. LEE and W. L. WOODMAN, 1999 Temporal changes in allele frequencies in two reciprocally selected maize populations. *Theor. Appl. Genet.* **99**: 1166–1178.
- LEWONTIN, R. C., and J. KRAKAUER, 1973 Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics* **74**: 175–195.
- LUIKART, G., J. M. CORNUET and F. W. ALLENDORF, 1999 Temporal changes in allele frequencies provide estimates of population bottleneck size. *Conserv. Biol.* **13**: 523–530.
- LUIKART, G., P. R. ENGLAND, D. TALLMON, S. JORDAN and P. TABERLET, 2003 The power and promise of population genomics: from genotyping to genome typing. *Nat. Rev. Genet.* **4**: 981–994.
- NEI, M., and F. TAJIMA, 1981 Genetic drift and estimation of effective population size. *Genetics* **98**: 625–640.
- POLLAK, E., 1983 A new method for estimating the effective population size from allele frequency changes. *Genetics* **104**: 531–548.
- RICHARDS, C., and P. L. LEBERG, 1996 Temporal changes in allelic frequencies and a population history's severe bottlenecks. *Conserv. Biol.* **10**: 832–839.
- SHIKANO, T., T. CHIYOKUBO and N. TANIGUCHI, 2001 Temporal

- changes in allele frequency, genetic variation and inbreeding depression in small populations of the guppy, *Poecilia reticulata*. *Heredity* **86**: 153–160.
- STOREY, J. D., and R. TIBSHIRANI, 2003 Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci. USA* **100**: 9440–9445.
- TURNER, T. F., L. R. RIDCHARDSON and J. R. GOLD, 1999 Temporal genetic variation of mitochondrial DNA and the female effective population size of red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico. *Mol. Ecol.* **8**: 1223–1229.
- VITALIS, R., K. DAWSON and P. BOURSOT, 2001 Interpretation of variation across marker loci as evidence of selection. *Genetics* **158**: 1811–1823.
- WANG, J., 2001 A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genet. Res.* **78**: 243–257.
- WAPLES, R. S., 1989a A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**: 379–391.
- WAPLES, R. S., 1989b Temporal variation in allele frequencies: testing the right hypothesis. *Evolution* **43**: 1236–1251.
- WIEHE, T. H. E., and W. STEPHAN, 1993 Analysis of a genetic hitchhiking model, and its application to DNA polymorphism data from *Drosophila melanogaster*. *Mol. Biol. Evol.* **10**: 842–854.
- WILLIAMSON, E. G., and M. SLATKIN, 1999 Using maximum likelihood to estimate population size from temporal changes in allele frequencies. *Genetics* **152**: 755–761.
- WOLFRAM, S., 1996 *The Mathematica Book*, Ed. 3. Cambridge University Press, Cambridge/London/New York.
- WRIGHT, S., 1969 *Evolution and the Genetics of Populations, Vol. 2: The Theory of Gene Frequencies*. University of Chicago Press, Chicago.

Communicating editor: O. SAVOLAINEN