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HW_TEST PROGRAM USER'S GUIDE

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The present program, adapted and updated from the original VISUAL BASIC (© Microsoft Inc.) version (Santos and Otto, 2005; Santos, 2006), was developed in Liberty BASIC v. 4.04, a dialect of BASIC language (© Shoptalk Systems 1992-2010, www.libertybasic.com) that runs in the PC Windows environment. The zipped program can be obtained free of charge by email from the authors (lemes.rb@usp.br or otto@usp.br) or directly from the [github repository](https://github.com/Lemes-RenanB/HardyWeinbergTesting) <https://github.com/Lemes-RenanB/HardyWeinbergTesting>. The program is the intellectual property of its authors, and as such, any use of it or of the materials included in it must contain an explicit reference to their origin. Feedback from users is welcome and will be used to improve the program and to correct unforeseen flaws. The program is free and as such it comes with no warranty.

Downloading the program

The instructions on how to download the program are detailed on the above github repository. After unzipping the downloaded zip file in any location of the user's computer, a folder named HW_TEST will be available. This folder will contain the executable (compiled) file HW_TEST.exe, the corresponding application distribution (tokenized) file HW_TEST.tkn, and a set of static and dynamic link library files necessary to run the compiled program (vbas31w.sll, vgui31w.sll, voflr31w.sll, vthk31w.dll, vtkl631w.dll, vtk3231w.dll, vvm31w.dll, and vvmt31w.dll). A pdf user's manual and a formatted text archive with the source BASIC code of the program can also be obtained from the same site.

Running the program and entering data

When the executable file HW_TEST.exe is activated, the following interface graphic window (Figure 1) will be displayed in the computer's screen:

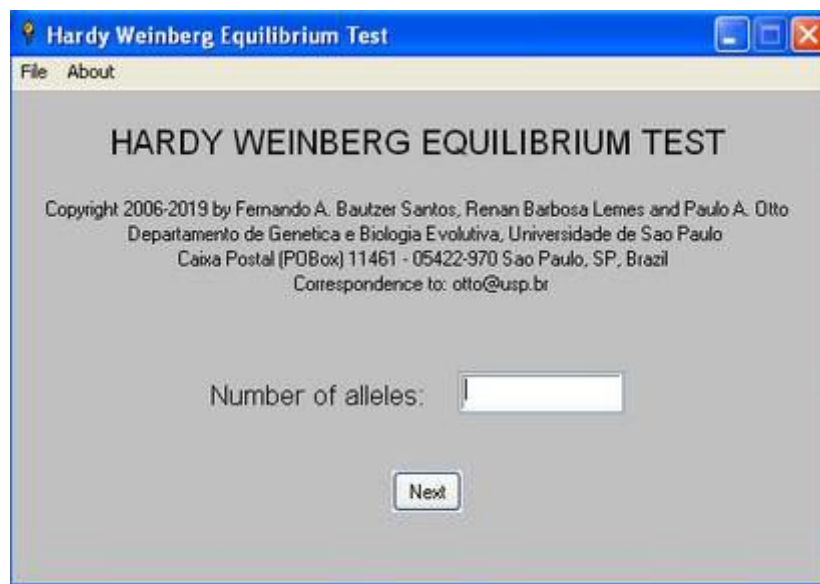


Figure 1 - Initial prompting window used for entering the number of alleles ($2 \leq k \leq 12$) present in the genotype sample to be analyzed.

(1) THE CASE OF TWO ALLELES

If the number 2 is entered (two-allele case), the user is prompted with the window shown in Figure 2. The text that follows corresponds to this case. The generalized case of n alleles will be dealt with at the end of this section, with the corresponding text identified by the caption (2) THE GENERALIZED CASE OF k ALLELES.



Figure 2 - Prompting window for choosing options and entering genotype data in the two-allele case.

The user should select, from the leftsided list on the window of Figure 2, the tests to be performed. The option 'Chi-square without correction' is preselected and will always be performed by the program. By clicking the bar with the message 'Select all' the user can select all options on the list. The user should then enter on the rightsided genotype fields the absolute frequencies of observed genotypes to be tested. The program will not accept total sample sizes of observed data less than 5 or two null entries. There is no sense, either, in testing sample sizes of the order of 20 or less, because rarely the null hypothesis of HW ratios is rejected with such small sample sizes. Also, if the HW null hypothesis is rejected with sample sizes of this order of magnitude the possibility of genotype misclassification typing errors should be seriously considered. And for any sample size, obtained test probability values of the order of less than 10^{-6} should be considered cautiously for the same reason.

WHAT THE PROGRAM DOES IN THE TWO-ALLELE CASE

In the two-allele case the program performs the tests listed in the text below, which was adapted/updated/corrected from Bautzer Santos (2006) and Otto (2008). All topics discussed in this user's manual can also be found or complemented in Thomson et al. (2009).

(1) Chi-squared HW tests with and without continuity correction

Hardy-Weinberg (HW) equilibrium is usually a null hypothesis $\{H_0: P(AA)=p^2, P(Aa)=2pq, P(aa)=q^2\}$ tested by Pearson's non-parametric chi-squared statistics:

	AA	Aa	aa	Total
abs. obs. freq. (o_i)	$D = o_1$	$H = o_2$	$R = o_3$	N
abs. exp. freq. (e_i)	$Np^2 = e_1$	$2Npq = e_2$	$Nq^2 = e_3$	N

where $p = (2D+H)/(2N) = d + \frac{1}{2}h$, $q = (H+2R)/(2N) = \frac{1}{2}h + r$. The number of degrees of freedom (d.f.) is 1 because the expected values are calculated using the gene frequency estimated from the data being tested. The chi-squared test value is obtained from the following formula:

$$\chi^2 = \sum(o_i - e_i)^2/e_i = \sum(o_i^2 / e_i) - N = D^2 / Np^2 + H^2 / 2Npq + R^2 / Nq^2 - N.$$

For small samples, especially for those in which at least an e_i is smaller than 5, commonly the test is corrected by subtracting $\frac{1}{2}$ from every absolute frequency $o_i > e_i$ and adding $\frac{1}{2}$ to each absolute frequency $o_i < e_i$.

Since the frequencies of AA, Aa, and aa individuals in a population with any possible mating system are given by $P(AA) = d = p^2 + Fpq$, $P(Aa) = h = 2pq(1-F)$, and $P(aa) = r = q^2 + Fpq$, the testing of $F = 0$ is equivalent to Hardy-Weinberg testing. If we make

	AA	Aa	aa
abs. obs. freq. (o_i)	$N(p^2 + Fpq)$	$2Npq(1-F)$	$N(q^2 + Fpq)$
abs. exp. freq. (e_i)	Np^2	$2Npq$	Nq^2

it comes out that

$$\begin{aligned}\chi^2 &= \sum(o_i - e_i)^2/e_i = N^2F^2p^2q^2/Np^2 + 4N^2F^2p^2q^2/2Npq + N^2F^2p^2q^2/Np^2 \\ &= NF^2(q^2+2pq+p^2) = NF^2, \text{ where } F = 1 - h/2pq.\end{aligned}$$

There exists an obvious correspondence between the formula $\chi^2 = \sum(o_i - e_i)^2/e_i$ and the one obtained from the contingency table:

D	H/2	Np
H/2	R	Nq
Np	Nq	N

since $e_1 = Np \times Np / N = Np^2$, etc, the formula $\chi^2 = \sum(o_i - e_i)^2/e_i$ can be easily rearranged algebraically as:

$$\begin{aligned}\chi^2 &= (H^2/4 - DR)^2N / [(D + H/2)^2(H/2 + R)^2] \\ &= (H^2 - 4DR)^2N / [(2D + H)^2(H + 2R)^2].\end{aligned}$$

With Yates' continuity correction (Yates, 1934) the formula for χ^2 becomes

$$\begin{aligned}\chi^2 &= [|H^2/4 - DR|^2 - N/2]^2N / [(D + H/2)^2(H/2 + R)^2] \\ &= [|H^2 - 4DR|^2 - 2N]^2N / [(2D + H)^2(H + 2R)^2].\end{aligned}$$

Since the data can be rearranged as the above table, we can use other tests that make use of the same contingency table, such as the G

(likelihood ratio) test with or without correction and **Fisher's exact test**.

(2) G (log-likelihood) tests with and without continuity correction

In the case of the **G** test, which has approximately a chi-squared distribution, the formula is

$$G = 2[\sum o_i \cdot \log(o_i/e_i)] = 2[\sum o_i \cdot \log(o_i) - \sum o_i \cdot \log(e_i)] ,$$

which in the case of a contingency table takes the form

$$G = 2[\sum_i \sum_j o_{ij} \cdot \log(o_{ij}) - \sum R_i \cdot \log(R_i) - \sum C_j \cdot \log(C_j) + N \cdot \log(N)] ,$$

where C_j is the marginal total of column j and R_i is the marginal total of row i . For the case of the contingency table used in the chi-squared test seen above, the formula becomes

$$\begin{aligned} G &\approx \chi^2 \approx 2[D \log(D) + H/2 \log(H/2) + H/2 \log(H/2) + R \log(R) \\ &\quad - (D+H/2) \log(D+H/2) - (R+H/2) \log(R+H/2) + N \log(N)] \\ &\approx 2\{D \log(D) + H \log(H) + R \log(R) - H \log(2) \\ &\quad - 2N[p \log(p) + q \log(q)] - N \log(N)\} . \end{aligned}$$

The same formula is obtained by letting

$$G = 2 \cdot \log(P_1/P_0) ,$$

where

$$\begin{aligned} P_1 &= N! / (D!H!R!) \cdot (D/N)^D (H/N)^H (R/N)^R \\ &= N! / (D!H!R!) \cdot D^D \cdot H^H \cdot R^R / N^N \end{aligned}$$

and

$$\begin{aligned} P_0 &= N! / (D!H!R!) \cdot (p^2)^D (2pq)^H (q^2)^R = N! / (D!H!R!) \cdot p^{2D+H} q^{H+2R} 2^H \\ &= N! / (D!H!R!) \cdot [(2D+H)/2N]^{2D+H} [(H+2R)/2N]^{H+2R} \cdot 2^H \\ &= N! / (D!H!R!) \cdot (2D+H)^{2D+H} (H+2R)^{H+2R} \cdot 2^H / (2N)^{2N} \end{aligned}$$

so that

$$G = 2 \cdot \log\{[(2N)^{2N} \cdot D^D \cdot H^H \cdot R^R] / [N^N \cdot (2D+H)^{2D+H} \cdot (H+2R)^{H+2R} \cdot 2^H]\} .$$

The **G** test with continuity correction is obtained from the formula above as follows: first we should verify if $DR \geq H^2/4$ or $DR < H^2/4$. If $D < H^2/4$, the values D , $H/2$ and R are replaced respectively with $D + 0.5$, $H/2 - 0.5$ and $R + 0.5$; otherwise ($DR \geq H^2/4$), the values D , $H/2$ and B are replaced with $D - 0.5$, $H/2 + 0.5$ and $R - 0.5$.

(3) Fisher's exact test

Still considering the contingency table

$a = D$	$b = H/2$	$a + b$
$c = H/2$	$d = R$	$c + d$
$a + c$	$b + d$	N

with fixed marginal values $[(a + c), (b + d), (a + b), (c + d) \text{ and } N]$, Hardy-Weinberg equilibrium can be verified through Fisher's exact test. After this test, based on the hypergeometric distribution, the probability of occurrence of the observed table, under the null hypothesis of no association, is

$$P(a,b,c,d) = [(a+b)! (c+d)! (a+c)! (b+d)!] / (a!b!c!d!N!).$$

The method calculates the probabilities corresponding to all possible tables with the same marginal values $(a + b), (c + d), (a + c), (b + d)$ and N . The two-tailed test probability is obtained adding all probability values equal or less than the probability value of the observed table. For the case in which H (observed number of heterozygotes) is odd, the values of the cells in the secondary diagonal of the table $(b = c = H/2)$ are replaced with $(H+1)/2$ and $(H-1)/2$.

There exists in the literature a number of tests that were developed to cope with population samples with reduced number of individuals, the more frequently used being the tests proposed by Hogben (1946) and Levene (1949), Haldane (1954), and Cannings and Edwards (1969). Haldane's test corresponds to the exact test that lists all possible samples with the same allele frequency, to be discussed in detail separately.

The reasoning used by Hogben, Levene and Cannings & Edwards is simple: in a sample small in number, if we take any gene (a_i) from it, the probability of a second gene being of the same type automatically decreases, that is, the probability of formation of homozygotes $a_i a_i$ becomes less than p_i^2 , which can be expressed by the inequality $P(a_i a_i) < P(a_i) \times P(a_i)$. The conceptual difference between the tests of Hogben (1946) / Levene (1949) and Cannings & Edwards (1969) is that the first considers the formation of a genotype $a_i a_j$ from a single gene pool that contains both types of gametes carrying alleles a_i and a_j , whereas the second considers the formation of individuals from the combination of gametes from two distinct sets of gametes produced by males and females.

(4) Hogben/Levene's chi-squared method

In the method of Hogben / Levene, the expected numbers of **AA**, **Aa** and **aa** individuals are calculated respectively after $(2D + H)(2D + H - 1) / [2(2N - 1)]$, $(2D + H)(H + 2R) / (2N - 1)$ and $(H + 2R)(H + 2R + 1) / [2(2N - 1)]$. The corresponding chi-squared formula then simplifies after

$$\begin{aligned} \chi^2 = & 2D^2(2N - 1) / [(2D + H)(2D + H - 1)] \\ & + 2H^2(2N - 1) / [(2D + H)(H + 2R)] \\ & + 2R^2(2N - 1) / [(2R + H)(H + 2R - 1)] - N. \end{aligned}$$

(5) Cannings & Edwards chi-squared method

In the method proposed by Cannings & Edwards, the expected numbers of **AA**, **Aa**, and **aa** individuals are respectively calculated after $[(2D+H)^2 - H]/4N$, $[(2D+H)(H+2R)+H]/2N$ and $[(H+2R)^2 - H]/4N$. The formula of the corresponding chi-squared test reduces then to:

$$\begin{aligned} \chi^2 = & 4ND^2 / [(2D + H)^2 - H] \\ & + 2NH^2 / [(2D + H)(H + 2R) + H] \\ & + 4NR^2 / [(2R + H)^2 - H] - N. \end{aligned}$$

(6) Haldane's exact test

The classical example of exact test in population genetics is given by the panmixia test applied to the case of two autosomal alleles without dominance. Given that $nAA = D$ AA individuals, $nAa = H$ Aa individuals and $naa = R$ aa individuals were observed out of a total of $N = nAA + nAa + naa$ sampled individuals and that the binomially distributed sample allelic frequencies are $p = P(A) = nA / (nA + na) = (2nAA + nAa) / 2N$ and $q = P(a) = 1 - p$, the probability of occurrence of the sample under the hypothesis of panmixia is given by

$$P_0 = P\{[nAA=Np^2, nAa=2Npq, naa=Nq^2] | [nA=2Np=(2nAA+nAa)/2, na=2Nq=(nAa+2naa)/2]\} \\ = P(nAA, nAa, naa) / P(nA, na) = N! / (2N)! \cdot (2D+H)! (H+2R)! 2^H / (D! H! R!)$$

The test lists all possible samples with same size and allele frequencies and calculates the probabilities of occurrence of each one of them under the hypothesis of panmixia. Each one of these probabilities (P_i) is then compared to P_0 ; if $P_i \leq P_0$, its value is added to $P = \sum P_i$, whose final value is therefore the probability of occurrence of the observed sample (P_0) and of all samples with probability P_i less than P_0 . This is the so-called exact probability favoring the hypothesis of the sampled genotypes being in Hardy-Weinberg ratios $p^2 : 2pq : q^2$. Haldane, who originally proposed this test, noticed also that for a fixed value of p or q all possible populations can be expressed as function of N and the number of heterozygotes (H), since $p = 1-q = (2D+H)/2N$, $D = Np-H/2 = N(1-q)-H/2$ and $q = 1-p = (H+2R)/2N$, $R = Nq-H/2 = N(1-p)-H/2$.

The exact test just examined has a severe limitation: the maximum number of possible populations with the same gene frequencies increases dramatically with the number of sampled individuals and the number of alleles. To circumvent this problem, exact Hardy-Weinberg tests are generated through computer simulation, a topic we discuss in the lines that follow.

(7) Exact tests based on computer simulations

The program starts by extracting the allele frequencies p and q from the set of observed data ($D = nAA$, $H = nAa$, $R = naa$, $D+H+R = N$) and calculates the probability of occurrence of the sample under the hypothesis of Hardy-Weinberg equilibrium:

$$P_0 = N! / (2N)! \cdot (nA! na! 2^{nAa}) / (nAA! nAa! naa!) \\ = N! / (2N)! \cdot (2D+H)! (H+2R)! 2^H / (D! H! R!)$$

The program generates a normalized random number between 0 and 1; if the number is smaller than or equal to $p^2 = [(2D+H)/2N]^2$, this indicates that an AA homozygous genotype was obtained among the N of the sample; if the random number is larger than p^2 , but smaller than $p^2 + 2pq = 1 - q^2 = 1 - [(H+2R)/2N]^2$, this indicates that a heterozygous genotype Aa was generated; and, finally, if the random number is larger than $1 - q^2$, the genotype is aa. The process is then repeated $N-1$ times, and in each instance the random number generated is compared to p^2 and $1 - q^2$. When the computer generates the N individuals of the sample, the frequencies p and q of A and a alleles are calculated from the numbers of AA, Aa and aa generated individuals. The computer repeats this process t times (t , the

number of simulations is a number of the order of magnitude of **1,000** to **10,000**; this program generates 1,000 simulations). After each simulation the computer calculates the value of the probability P_i of occurrence of the sample under the hypothesis of Hardy-Weinberg equilibrium:

$$P_i = N! / (2N)! \cdot (2D_i' + H_i')! (H_i' + 2R_i')! 2^{H_i'} / (D_i'! H_i'! R_i'!).$$

This probability P_i is then compared to P_0 , the probability of occurrence of the observed sample under the hypothesis of HW equilibrium. The exact probability P , obtained after t simulations, is given by $P = T/t$ (our program uses $t = 1000$ and $P = T/1000$), where T is the number of times in which P_i is smaller than or equal to P_0 ($P_i \leq P_0$).

Besides generating 1,000 populations in expected HW proportions, the program simulates also 1,000 populations with frequencies $d = D/N$, $h = H/N$ and $r = R/N$.

Our program calculates also exact confidence intervals for genotype frequencies, based on algorithms that use random numbers to simulate genetic populations. The 95% probability confidence intervals using computer simulations (bootstrap column) are "exact" and are calculated as follows: (a) for the items labelled as observed, by excluding 2.5% of the smaller and 2.5% of the larger $t = 1000$ frequency values generated by the program for each genotypic class; (b) for the items labelled as expected, the genotype frequencies are calculated by squaring the allele frequencies ($p^2, 2pq, q^2$) generated for each simulation cycle and then excluding 2.5% of the smaller and 2.5% of the larger values out of the total set of $t = 1000$ calculated frequencies for each possible genotype. The normal approximations to these confidence intervals are constructed: (a) for the items labelled as observed the confidence intervals are obtained directly from the sampled observed values of genotype frequencies: $s.e.(p_{ij}) = p_{ij}(1-p_{ij})/N$, 95% c.i. = $p_{ij} \pm 1.96 s.e.(p_{ij})$; (b) for the items labelled as expected, the same procedure is applied but the standard errors are calculated after $s.e.[P(A_iA_j)] = s.e.(p_{ij}) = \sqrt{[4p_i^3(1-p_i)/2N]}$ if $i=j$, $s.e.[P(A_iA_j)] = s.e.(p_{ij}) = \sqrt{[4p_i p_j (p_i + p_j - 4p_i p_j)/2N]}$ otherwise (Weir, 1999).

The following is a standard program text output obtained by running the program with the genotype data $D = N(AA) = 119$, $H = N(Aa) = 42$ and $R = N(aa) = 39$ and selecting all tests provided:

HARDY-WEINBERG TESTING

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	AA	Aa	aa	N
obs. abs. frequencies	119	42	39	200
exp. abs .freq. (without correction)	98.000	84.000	18.000	200

exp. abs. freq. (Hogben/Levene)	97.895	84.211	17.895	200
exp. abs. freq. (Cannings & Edwards)	97.948	84.105	17.948	200

obs. rel. frequencies	0.595	0.210	0.195	-
exp. rel. freq. (without correction)	0.490	0.420	0.090	-
exp. rel. freq. (Hogben/Levene)	0.489	0.421	0.089	-
exp. rel. freq. (Cannings & Edwards)	0.490	0.421	0.090	-

p = P(A) = 0.7000

q = P(a) = 0.3000

H0:{d,h,r} s.e.(p) = s.e.(q) = 0.0281

Ha:{p²,2pq,q²} s.e.(p) = s.e.(q) = 0.0229

P(chi-squared test without correction) < 10⁻⁶

P(chi-sq. test with Yates' correction) < 10⁻⁶

P(c.s. test w/ Hogben/Levene correct.) < 10⁻⁶

P(c.s. test w/ Cannings & Edwards corr.) < 10⁻⁶

P(G or log-likelihood test without cor.) < 10⁻⁶

P(log-likelih. test w/ continuity corr.) < 10⁻⁶

P(Fisher's exact test) < 10⁻⁶

P(Haldane's exact test) < 10⁻⁶

'EXACT' BOOTSTRAP (1000 SIMULATIONS) ESTIMATES

OBS. SAMPLE p = P(A) = (2D+H)/2N = 0.7000

BOOTSTRAP EST. p = 0.7014

EXACT PROBABILITY (1000 SIMUL.) < 10⁻⁶

genotype	normal approximation		bootstrap (1000 simul.)		
	calc.P	95% C.I.	meanP	med.P	95% C.I.
AA	obs. 0.595	{0.527,0.663}	0.597	0.595	{0.525,0.670}
	exp. 0.490	{0.427,0.553}	0.492	0.490	{0.420,0.565}
Aa	obs. 0.210	{0.154,0.266}	0.210	0.210	{0.155,0.270}
	exp. 0.420	{0.384,0.456}	0.419	0.420	{0.350,0.490}
aa	obs. 0.195	{0.140,0.250}	0.193	0.190	{0.140,0.250}
	exp. 0.090	{0.063,0.117}	0.089	0.090	{0.055,0.125}

Besides generating 1,000 populations in expected HW proportions {P(AA) = p², P(Aa) = 2pq, P(aa) = q²}, the program simulates also 1,000 populations with frequencies {d = D/N, h = H/N, r = R/N}, where D, H and R are the observed numbers of sampled genotypes AA, Aa and aa respectively. These population points are then plotted on an isosceles ternary diagram (Otto and Benedetti, 2000) that shows the HW parabola {p², 2pq, q²} and its 95% chi-squared confidence intervals corresponding to the population of size N, represented by curves {p² + pqF_{LL}, 2pq(1-F_{LL}), q² + pqF_{LL}} and {p² + pqF_{UL}, 2pq(1-F_{UL}), q² + pqF_{UL}} with lower and upper limits F_{LL} = + √(3.841/N) and F_{UL} = - √(3.841/N) (Bautzer Santos, 2006; Graffelman & Camarena, 2008).

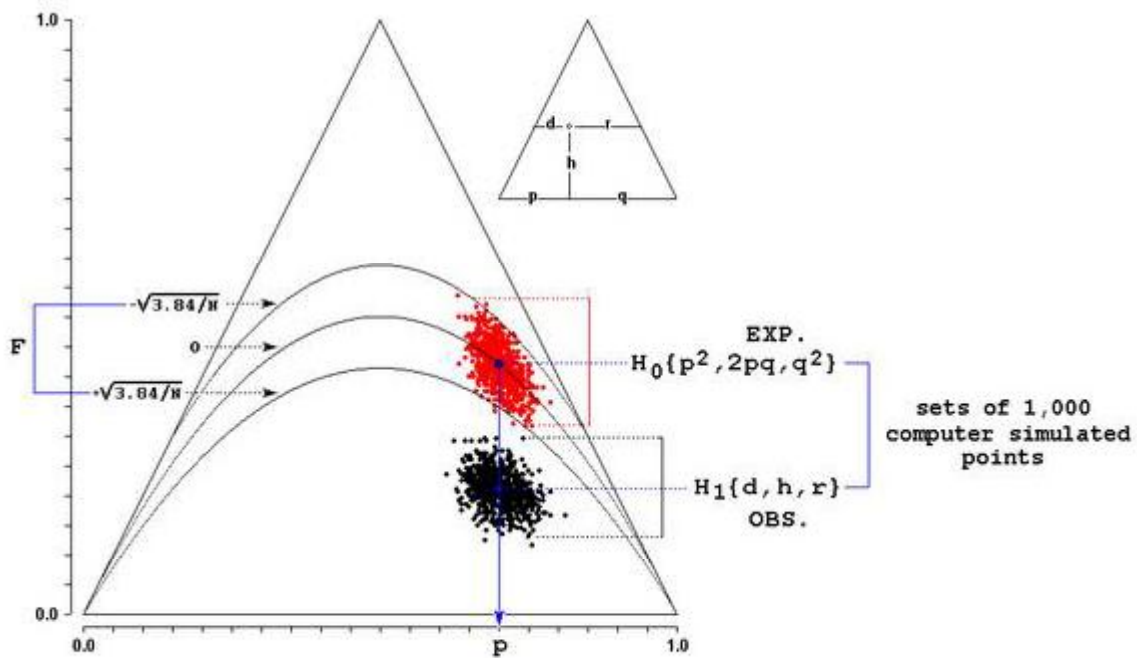


Figure 3 - Trilinear diagram showing the results obtained with the observed sample $N(AA) = 119$, $N(Aa) = 42$, and $N(aa) = 39$. Please consult the preceding text for explanations.

(2) THE GENERALIZED CASE OF k ALLELES

If a number larger than 2 is entered (k -allele case) into the initial input window (Figure 1), the user is prompted with the window shown in Figure 4.

Hardy Weinberg Equilibrium Test

File About

HARDY WEINBERG EQUILIBRIUM TEST

Statistical Tests

☒ Chi-square without correction ☒ Exact probability by simulation

Number of Genotyped Individuals

	A	B	C	D
A	2	4	6	8
B		10	12	14
C			16	18
D				20

Run

Figure 4 - Prompting window for choosing options and entering genotype data in the generalized k-allele case. The window shown above corresponds to the case of four alleles (**A, B, C, D**), in which the exact test option has been checked and the following genotype numbers were entered: **N(AA) = 2, N(AB) = 4, N(AC) = 6, N(AD) = 8, N(BB) = 10, N(BC) = 12, N(BD) = 14, N(CC) = 16, N(CD) = 18, N(DD) = 20.**

As in the two-allele case, in order to avoid running problems, the user should not use small total sample sizes (v.g. of the order of **10k** or less, where **k** is the number of alleles). Many null entries should also be avoided; in the common case when some of these occur, and especially when the total sample size is relatively small, the user is advised to properly agglutinate some value classes, thus reducing the number of alleles, and improving the power of the test and the performance of the program. Just like in the two-allele case, there is no sense at all, either, in testing sample sizes of the order of about **10k** or less, for the reasons already explicated. And for any sample size, obtained test probability values of the order of less than 10^{-6} should be considered cautiously because they could result exclusively from genotyping errors with a large probability.

WHAT THE PROGRAM DOES IN THE k-ALLELE CASE

In the k-allele case the program performs the chi-squared test and the 'exact' test based on computer bootstrap simulations.

(1) Chi-squared HW test without continuity correction

As in the two-allele case, in the n-allele case Hardy-Weinberg (HW) equilibrium is usually a null hypothesis $\{H_0: P(AA) = p_1^2, P(AB) = 2p_1p_2, P(AC) = 2p_1p_3, \dots\}$ tested by Pearson's non-parametric chi-squared statistics $\chi^2 = \sum(o_{ij} - e_{ij})^2 / e_{ij} = \sum(o_{ij}^2 / e_{ij}) - N$, where o_{ij} is the genotype observed absolute frequency, e_{ij} its corresponding expected figure based on HW proportions, **N** the total sample size, the summation taking place from $i = j = 1$ to $i = j = k$. As there are **k** different alleles and the $k(k+1)/2$ expected genotype absolute frequencies are calculated conditional to the sample size **N** and to the fixed value of **k-1** different allele frequencies extracted from the same sample, the number of degrees of freedom of the HW chi-squared test is calculated after $k(k+1)/2 - k = k(k-1)/2$. No continuity correction is applied to the test, since this procedure is appropriate only for the two-allele case, when the number of degrees of freedom is 1.

(2) Exact tests based on computer simulations

Just like in the two-allele case, the program calculates the probability of occurrence of the observed sample **N(AA), N(AB), N(AC), ...,** under the hypothesis of panmixia $\{N(AA) = Np_1^2, N(AB) = 2Np_1p_2, \dots\}$ and conditional to allele absolute frequencies $\{N(A) = 2Np_1, N(B) = 2Np_2, \dots\}$ estimated from the sample. The formula for this probability (**P₀**) is similar to the one used in the two-allele case, taking into account the complications associated to the multinomial case, that is, that now there exist **k** alleles, $k(k+1)/2$ different genotypes and **k** different types of

heterozygous individuals. The rest of the program works exactly as in the two-allele case, with the difference that instead of creating a single normalized random number and verifying its correspondence to p_1^2 , $2p_1p_2$, $2p_1p_3$, ..., p_k^2 , the program generates two random numbers and verifies the correspondence of each of them with each of the k different possible allele frequencies $p_1 = P(A)$, $p_2 = P(B)$, ...; each pair of two consecutive random numbers, on its turn, thus automatically generates a genotype occurring under 'perfect' panmictic conditions.

The program calculates also approximate and 'exact' confidence intervals for genotype frequencies, the latter based on algorithms that use random numbers to simulate genetic populations, exactly as described in the two-allele case, in which the formulas used are already shown in their generalized form for the k -allele case.

The following is a standard program text output obtained by running the program with the genotype data described on the legend of Figure 4 and selecting the exact test option.

HARDY-WEINBERG TESTING

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	genotype absolute frequencies		genotype relative frequencies	
	observed	expected	observed	expected
AA	2	1.100	0.018	0.010
AB	4	5.000	0.036	0.045
AC	6	6.800	0.055	0.062
AD	8	8.000	0.073	0.073
BB	10	5.682	0.091	0.052
BC	12	15.455	0.109	0.140
BD	14	18.182	0.127	0.165
CC	16	10.509	0.145	0.096
CD	18	24.727	0.164	0.225
DD	20	14.545	0.182	0.132
N	110			

	allele freq. (pi)	s.e. (pi) H0:{d,h,r}	s.e. (pi) H1:{p^2,2pq,q^2}
A	0.100	0.021	0.020
B	0.227	0.031	0.028
C	0.309	0.035	0.031
D	0.364	0.036	0.032

P(chi-squared test without correction) = 0.0465

genotype	normal approximation			bootstrap (1000 simul.)		
	calc.P	95% C.I.		meanP	med.P	95% C.I.
AA	obs. 0.018	{0.000,0.043}		0.018	0.018	{0.000,0.045}
	exp. 0.010	{0.002,0.018}		0.011	0.010	{0.004,0.021}
AB	obs. 0.036	{0.001,0.071}		0.037	0.036	{0.009,0.073}
	exp. 0.045	{0.026,0.065}		0.046	0.046	{0.026,0.068}
AC	obs. 0.055	{0.012,0.097}		0.054	0.055	{0.018,0.100}
	exp. 0.062	{0.037,0.087}		0.063	0.062	{0.039,0.087}
AD	obs. 0.073	{0.024,0.121}		0.074	0.073	{0.027,0.127}
	exp. 0.073	{0.044,0.101}		0.073	0.073	{0.046,0.104}
BB	obs. 0.091	{0.037,0.145}		0.092	0.091	{0.045,0.145}
	exp. 0.052	{0.026,0.077}		0.053	0.052	{0.030,0.079}
BC	obs. 0.109	{0.051,0.167}		0.110	0.109	{0.055,0.173}
	exp. 0.140	{0.105,0.176}		0.142	0.142	{0.109,0.179}
BD	obs. 0.127	{0.065,0.190}		0.129	0.127	{0.073,0.191}
	exp. 0.165	{0.127,0.204}		0.167	0.167	{0.128,0.207}
CC	obs. 0.145	{0.080,0.211}		0.148	0.145	{0.082,0.218}
	exp. 0.096	{0.058,0.133}		0.098	0.096	{0.065,0.142}
CD	obs. 0.164	{0.095,0.233}		0.162	0.164	{0.100,0.236}
	exp. 0.225	{0.183,0.267}		0.226	0.225	{0.184,0.268}
DD	obs. 0.182	{0.110,0.254}		0.185	0.182	{0.118,0.264}
	exp. 0.132	{0.086,0.178}		0.135	0.136	{0.093,0.183}

EXACT PROBABILITY (1000 SIMUL.) = 0.0380

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