A comment on the use of Nass's contingency test for random association of alleles at different loci

A. L. Mallet* and
E. Zouros†

* Fisheries and Oceans, Marine Ecology Laboratory,
Bedford Institute of Oceanography, P.O. Box 1006,
Dartmouth, N.S., Canada, B2Y 4A2

† Department of Biology, Dalhousie University,
Halifax, N.S. Canada, B3H 4J1

A χ^2 test developed by Nass (1959) and recently used by Ahmad and Hedrick (1985) for the detection of non-random association between alleles of different loci is very sensitive to misclassification of genotypes.

The analysis of electrophoretic data often requires the testing of random associations of alleles among loci to allow inferences about gametic disequilibrium and population subdivisions. The data to be tested involve mutiallelic loci with many rare alleles which lead to contingency tables with very small expectations. To avoid this problem, the usual practice has been either to exclude or to pool frequencies of rare alleles. This practice is however undesirable since rare alleles may provide more information about levels of gene flow among sub-

populations than the more common ones (Slatkin, 1985).

Nass (1959) developed a χ^2 test for small expectations in contingency tables which was used by Ahmad and Hedrick (1985) to test for random associations of alleles between two loci in natural population of the blue mussel, *Mytilus edulis*. They found no evidence for association. We also applied this test in a sample of 2-yr-old mussels, *Mytilus edulis*, for the same purpose (Zouros *et al.*, submitted). 376 mussels were scored for seven

Table 1 Upper diagonal matrix, N = 374; Lower diagonal matrix, N = 376. Three numbers are shown for each entry. The first number is the Chi-square value, then the degree of freedom, and finally, the probability

	AP1	AP2	PG1	PGM	LAP1	LAP2	OcDH
AP1	—	29.0 22.0 0.144	45.6 48.0 0.569	16.7 19.0 0.607	2.1 3.0 0.544	8.3 14.0 0.875	1.8 3.0 0.624
AP2	22.9 12.0 0.003	—	56.4 66.0 0.794	41.8 28.0 0.045	3.1 5.0 0.688	17.5 18.0 0.487	4.5 6.0 0.486
PG1	67.1 43.0 0.001	35.0 37.0 0.562	—	69.8 64.0 0.289	9.5 11.0 0.554	51.1 44.0 0.215	12.3 13.0 0.501
PGM	29.9 18.0 0.004	21.0 16.0 0.179	90.1 55.0 0.002	—	2.1 4.0 0.719	23.5 18.0 0.173	5.6 5.0 0.347
LAP1	2.6 3.0 0.452	1.6 3.0 0.664	9.8 10.0 0.460	2.8 4.0 0.587	—	1.5 3.0 0.680	2.1 1.0 0.143
LAP2	14.4 17.0 0.638	39.5 14.0 0.000	54.4 47.0 0.212	25.5 21.0 0.227	1.9 4.0 0.754	—	5.1 3.0 0.161
OcDH	4.7 3.0 0.197	22.9 3.0 0.000	11.7 11.0 0.384	4.7 4.0 0.322	1.8 1.0 0.175	17.2 4.0 0.002	—

polymorphic loci with number of alleles varying from 4 to 8. We found that the probability for random association was less than 0.05 in six of the 21 pair-wise combinations of loci (table 1, lower diagonal matrix) suggesting that significant levels of association existed among alleles at different loci. A closer examination of the data showed two possible cases of genotype misclassification. The elimination of two animals from the data set made all the previously significant chi-square values non-significant and produced one new significant value (table 1, upper diagonal matrix). This latter result suggests that association did not exist in our sample. Exclusion of rare alleles (frequency <0.01) from our sample also produced no evidence for such association.

In the original application of his test, Nass used cases in which misclassification was impossible. This is however a real possibility in electrophoretic studies. As a result of this, both Type I and Type II error are possible. Type I error will result when common genotypes are misclassified as rare ones

or when one rare genotype is misclassified as another rare genotype. Type II errors will result when rare genotypes are misclassified as common ones. Thus, this test should be applied when genotypes are scored without errors or when the experimenter can subsequently rescored his material.

REFERENCES

- AHMAD, M. AND HEDRICK, P.W. 1985. Electrophoretic variation in common mussel, *Mytilus edulis*: random association of alleles at different loci. *Heredity*, 55, 47-51.
- NASS, C. A. G. 1959. The χ^2 test for small expectations in contingency tables, with special reference to accidents and absenteeism. *Biometrika*, 46, 365-485.
- SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution*, 39, 53-65.
- ZOUROS, E., ROMERO-DOREY, M. AND MALLET, A. L. 1986. Heterozygosity and growth in marine bivalves: further data and the associative overdominance explanation. *Evolution* submitted.