A BIAS IN ESTIMATION OF VIABILITIES FROM COMPETITION EXPERIMENTS

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SUMMARY

Some current estimators of the relative viabilities of genotypes used in competition experiments, are shown to be biased, the bias being a function of the sample size and the gene frequency. From their use, an erroneous conclusion of a frequency-dependent phenomenon can be drawn. Almost unbiased estimators are advocated. The calculation of the exact distribution of these estimators for each experimental situation is the only correct way to test the significance of an observed value.

1. INTRODUCTION

In this paper, we consider the bias, due to sample size, of some estimators of the relative viabilities of different types in a population. The existence of this kind of bias has long ago been noted. Its approximate value has been given by Haldane (1956) in a particular case, and by Manly (1972) in the context of the mark-release-recapture method. But, as far as we know, there has been no systematic study of its importance and characteristics. The methods of estimation in general use do not take it into account, probably because the investigators believed that they were dealing with a "large sample". Insufficient attention has been given to the question: what is a "large sample"? There is no general answer; the sample size to be considered as "large" depends on the algebraic form of the estimator and on the frequencies of the types in the population.

In many studies, particularly of enzyme polymorphism the size of the sample is very rarely more than 100, and we shall show that such a sample size does not allow one to neglect the bias. Several methods of estimating viability, as part of the total fitness are in current use in studies of *Drosophila* (Spiess, 1958, 1979; Anxolabéhère, 1976).

The experimental approach is the following. Two random samples A and B of eggs are taken in a population and grown with or without competition respectively. For an autosomal diallelic locus, the number of adults of each of the three possible genotypes A_1A_1 , A_1A_2 , A_2A_2 is obtained in each condition. The symbols are as follows:

Adults obtained	Genotypes			Total
	A_1A_1	A_1A_2	A_2A_2	
From sample A: (without competition)	n_{11}	n_{12}	n_{13}	N_1
From sample B: (with competition)	n_{21}	n_{22}	n_{23}	N_2

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Let P_1, P_2, P_3 , be the proportions of the three genotypes A_1A_1, A_1A_2, A_2A_2 in the egg population, v_1, v_2, v_3 their survival probabilities without competition up to the adult stage, v'_1, v'_2, v'_3 the same probabilities with competition. The adult probabilities of the three genotypes are then:

	A_1A_1	A_1A_2	A_2A_2
without competition	$P_1 v_1 / \bar{v}$	$P_2 v_2 / \bar{v}$	$P_3 v_3 / \bar{v}$
with competition	$P_1 v'_1 / \bar{v}'$	$P_2 v'_2 / \bar{v}'$	$P_3 v'_3 / \bar{v}'$

with

$$\bar{v} = P_1 v_1 + P_2 v_2 + P_3 v_3 \quad \bar{v}' = P_1 v'_1 + P_2 v'_2 + P_3 v'_3$$

Comparing the two conditions, we can look at the presence of differential selection by testing the homogeneity of proportions. When differential selection is present, several quantitative measures of it can be used.

In the first method (Bundgaard and Christiansen, 1972; Anxolabéhère, 1976; Johnson, 1976), the estimators of the differential selection

$$W_1 = \frac{n_{21}}{n_{22}} \cdot \frac{n_{12}}{n_{11}} \quad (1)$$

$$W_3 = \frac{n_{23}}{n_{22}} \cdot \frac{n_{12}}{n_{13}}$$

are the ratios of estimators of the relative viabilities of the homozygotes against the heterozygotes. For instance, n_{21}/n_{22} estimates the relative viability of the A_1A_1 genotype against the A_1A_2 genotype. Then, W_1 and W_3 estimate respectively $\sigma_1 = v'_1/v'_2 \cdot v_2/v_1$ and $\sigma_3 = v'_3/v'_2 \cdot v_2/v_3$, the differential selection indices of the homozygous genotypes. It is important to note that these estimators depend as much on relative viabilities without competition as on relative viabilities with competition. Particular attention must then be given to their biological significance.

It is often implicitly considered that there is no selection in the condition without competition, and the estimators of differential selection are then taken as measures of the selection in the condition with competition: W_1 and W_3 are so used to estimate v'_1/v'_2 and v'_3/v'_2 . If for instance, selection on viabilities exists in the A sample, but not in the B sample, the estimations of W_1 and W_3 could give the erroneous impression of selection in B.

It is not unrealistic to think of a situation where selection would exist at a given locus in an uncrowded condition, this selection disappearing with high density. Then, the W_1 and W_3 estimators, used in this context, could lead erroneously to the conclusion of a selection in the B sample.

In the second method (Polivanov, 1964; Kojima and Tobari, 1969; Yarbrough and Kojima, 1967; Johnson, 1976; Spiess, 1979), Hardy-Weinberg proportions in the sample without competition are assumed. The ratio of the number of homozygotes to heterozygotes in the sample with competition is then divided by the ratio of the panmictic expectation of number

of homozygotes to heterozygotes in the sample without competition. The allelic frequencies are estimated from this sample. The estimators are:

$$\begin{aligned}
 A_1A_1 \quad X_1 &= \frac{n_{21}}{n_{22}} \cdot \frac{2(2n_{11}+n_{12})(n_{12}+2n_{13})}{(2n_{11}+n_{12})^2} \\
 &= 2 \cdot \frac{n_{21}}{n_{22}} \cdot \frac{(n_{12}+2n_{13})}{(2n_{11}+n_{12})} \\
 A_2A_2 \quad X_2 &= \frac{n_{23}}{n_{22}} \cdot \frac{2(2n_{11}+n_{12})(n_{12}+2n_{13})}{(2n_{13}+n_{12})^2} \\
 &= 2 \cdot \frac{n_{23}}{n_{22}} \cdot \frac{(2n_{11}+n_{12})}{(2n_{13}+n_{12})},
 \end{aligned} \tag{2}$$

noting that $(2n_{11}+n_{12})/2N_1$ and $(n_{12}+2n_{13})/2N_2$ are the estimators of the allelic frequencies p and q in the sample without competition. X_1 and X_3 are, in fact, the maximum likelihood estimators of the v'_1/v'_2 and v'_3/v'_2 ratios when random mating is assumed, and as such reliable for large samples only.

In a previous paper (Goux and Anxolabéhère, 1980), we showed that indices of sexual isolation and selection in current use are so strongly biased in small samples, and their sampling distribution present such peculiarities, as to need caution in their practical use by investigators. Since that time, our attention has been drawn to the formal similarities in these parameters and the differential selection indices; we consider here the properties of small samples.

2. BIASES OF THE CURRENT ESTIMATORS OF DIFFERENTIAL SELECTION

By using the Taylor's series expanded to the second order at the point $E(a), E(b), E(c), E(d)$, it is easy to show (Goux and Anxolabéhère, 1980) that:

$$\begin{aligned}
 E\left(\frac{ad}{bc}\right) &= \frac{E(a) \cdot E(d)}{E(b) \cdot E(c)} \left[1 + \frac{\text{var } b}{E^2(b)} + \frac{\text{var } c}{E^2(c)} - \frac{\text{cov}(a, b)}{E(a) \cdot E(b)} - \frac{\text{cov}(a, c)}{E(a) \cdot E(c)} \right. \\
 &\quad \left. + \frac{\text{cov}(a, d)}{E(a) \cdot E(d)} + \frac{\text{cov}(b, c)}{E(b) \cdot E(c)} - \frac{\text{cov}(b, d)}{E(b) \cdot E(d)} - \frac{\text{cov}(c, d)}{E(c) \cdot E(d)} \right] + \varepsilon.
 \end{aligned}$$

This formula will be used to establish the small sample properties of the estimators.

(i) The estimators W_1 and W_3

(a) Expectation of W_1 and W_3 under the null hypothesis of no selection

If P_1, P_2, P_3 are the theoretical frequencies of the three genotypes in the egg population, we have for the adults, under the null hypothesis of

no selection,

$$\begin{aligned} E(n_{11}) &= N_1 P_1, & E(n_{21}) &= N_2 P_1, & E(n_{12}) &= N_1 P_2, \\ E(n_{22}) &= N_2 P_2, & \text{var}(n_{11}) &= N_1 P_1(1 - P_1), & \text{var}(n_{21}) &= N_2 P_1(1 - P_1), \\ & & \text{var}(n_{12}) &= N_1 P_2(1 - P_2), & \text{var}(n_{22}) &= N_2 P_2(1 - P_2), \\ \text{cov}(n_{11}, n_{12}) &= -N_1 P_1 P_2, & \text{cov}(n_{21}, n_{22}) &= -N_2 P_1 P_2, \\ \text{cov}(n_{11}, n_{21}) &= \text{cov}(n_{11}, n_{22}) = \text{cov}(n_{12}, n_{21}) = \text{cov}(n_{12}, n_{22}) = 0. \end{aligned}$$

From these values, we obtain directly:

$$E(W_1) = E\left(\frac{n_{21}}{n_{22}} \cdot \frac{n_{12}}{n_{11}}\right) = 1 + \frac{1}{N_1 P_1} + \frac{1}{N_2 P_2} + \varepsilon$$

and by substitution

(3)

$$E(W_3) = E\left(\frac{n_{23}}{n_{22}} \cdot \frac{n_{12}}{n_{13}}\right) = 1 + \frac{1}{N_1 P_3} + \frac{1}{N_2 P_2} + \varepsilon.$$

The estimators W_1 and W_3 are biased and their bias depends on the size of the samples and the genetic composition of the population. They are then frequency-dependent.

The investigators, basing their assumptions on the properties of large samples, compare the observed values of the estimators to unity, 1 being taken as their expectation under the null hypothesis of no differential selection. This is incorrect if the samples are not very large. In the particular case where $N_1 = N_2 = N$, as is often the case in electrophoretic experiments, and $P_1 = p^2$, $P_2 = 2pq$, $P_3 = q^2$, (random mating with gene frequencies p and q in eggs).

$$E(W_1) = 1 + \frac{1}{N} \left(\frac{1}{p^2} + \frac{1}{2pq} \right) + \varepsilon$$

with an analogous formula for $E(W_3)$.

Fig. 1 shows the variation of these expectations with sample size and allelic frequency. A simulation of the distribution of W_1 under the null hypothesis of no selection either in A sample or B sample, shows a strong departure of $\bar{W}_1$ from one and the skewness of the distribution (fig. 2).

(b) *Expectation of W_1 and W_3 , with selection.*

If v'_1, v'_2, v'_3 , are the viabilities of the three genotypes in the condition with competition, the expectation of adults numbers are:

$$E(n_{21}) = \frac{N_2 P_1 v'_1}{\bar{v}'}, \quad E(n_{22}) = \frac{N_2 P_2 v'_2}{\bar{v}'}, \quad E(n_{23}) = \frac{N_2 P_3 v'_3}{\bar{v}'}$$

and their variances:

$$\begin{aligned} \text{var}(n_{21}) &= \frac{N_2 P_1 v'_1}{\bar{v}'} \left(1 - \frac{P_1 v'_1}{\bar{v}'} \right) & \text{var}(n_{22}) &= \frac{N_2 P_2 v'_2}{\bar{v}'} \left(1 - \frac{P_2 v'_2}{\bar{v}'} \right) \\ \text{var}(n_{23}) &= \frac{N_2 P_3 v'_3}{\bar{v}'} \left(1 - \frac{P_3 v'_3}{\bar{v}'} \right) & \text{cov}(n_{21}, n_{22}) &= -\frac{N_2 P_1 P_2 v'_1 v'_2}{\bar{v}'^2}. \end{aligned}$$

FIG. 1.—Expectation of the differential selection index W_1 under the null hypothesis of no selection, for different sample sizes N and different values of the allelic frequency (with Hardy-Weinberg proportions among the zygotes).

At the second order of Taylor's series:

$$E(W_1) = \frac{v'_1 v_2}{v'_2 v_1} \left[1 + \frac{\bar{v}}{N_1 P_1 v_1} + \frac{\bar{v}'}{N_2 P_2 v'_2} \right] + \varepsilon$$

$$E(W_1) = \sigma_1 \left[1 + \frac{\bar{v}}{N_1 P_1 v_1} + \frac{\bar{v}}{N_2 P_2 v'_2} \right] + \varepsilon. \quad (4)$$

FIG. 2.—Distribution of the differential selection index from 250 drawings, simulated under the null hypothesis of no selection ($\sigma_1 = \sigma_2 = \sigma_3 = 1$) at the allelic frequency $p(A_1) = 0.2$, with zygotes in Hardy-Weinberg proportions and $N_1 = N_2 = N = 60$.

The similarity with formula (3) is evident. An analogous formula for $E(W_3)$ is obtained directly, by replacing P_1 by P_3 , σ_1 by σ_3 and v_1 by v_3 . It can be shown that $E(W_1)$ and $E(W_3)$ are correlated, the bias of $E(W_1)$ depending on σ_3 and conversely the bias of $E(W_3)$ depending on σ_1 .

In the particular case of no selection in the condition without competition, we have:

$$\sigma_1 = \frac{v'_1}{v'_2}, \quad \sigma_2 = 1, \quad \sigma_3 = \frac{v'_3}{v'_2},$$

and the formula (4) becomes:

$$E(W_1) = \sigma_1 \left[1 + \frac{1}{N} \left(\frac{1}{P_1} + \frac{\bar{\sigma}}{P_2} \right) \right] + \varepsilon$$

if $N_1 = N_2 = N$.

The graph of $E(W_1)$ on allelic frequency in the Hardy-Weinberg conditions is given in fig. 3.

We have tabulated a particular case, where the true values of the viabilities are respectively linearly frequency-dependent for A_1A_1 and constant for A_2A_2 (fig. 4). The values of $E(W_1)$ in this case are not very

FIG. 3.—Expectations of the differential selection index W_1 under a particular hypothesis of selection (with constant coefficients, $\sigma_1 = 0.8$, $\sigma_2 = 1$, $\sigma_3 = 1.2$) for different sample sizes N and different values of the allelic frequency (with Hardy-Weinberg proportions among the zygotes).

FIG. 4.—Expectations of the differential selection indices W_1 and W_3 with a sample size $N_1 = N_2 = 60$, under a particular frequency-dependent differential selection hypothesis ($\sigma_1 = -1.71p + 1.91$, $\sigma_2 = 1$, $\sigma_3 = 0.71$) for different values of the allelic frequency (with Hardy-Weinberg proportions among the zygotes), in the absence of selection where there is no competition.

different, for small p , from the values obtained either under the null hypothesis of no selection (fig. 1) or the constant viability selection case (fig. 3). Such a situation can then be misleading.

(ii) *The estimators X_1 and X_3*

If there is no selection either in the A or in the B sample,

$$E(X_1) = 2 \frac{P_1 P_2 + 2P_3}{P_2 (2P_1 + P_2)} \left[1 + \frac{1}{N_2 P_2} + \frac{2}{N_1} \frac{4P_1 P_3 + P_2(1 - P_2)}{(P_2 + 2P_3)(2P_1 + P_2)^2} \right] + \varepsilon \quad (5)$$

and as the X_i estimators are constructed under the Hardy-Weinberg hypothesis, we can write by substitution of P_1 , P_2 , P_3 by p^2 , $2pq$, q^2 , and with $N_1 = N_2 = N$

$$E(X_1) = 1 + \frac{1}{N} \frac{1+q}{2pq} + \varepsilon$$

this expectation being one for $N = \infty$ (fig. 5).

FIG. 5.—Expectations of the differential selection index X_1 for different sample sizes N and different values of the allelic frequency (with Hardy-Weinberg proportions among the zygotes) (a) under the null hypothesis of no selection, (b) under a particular hypothesis of a differential selection with constant coefficients ($\sigma_1 = 0.8$, $\sigma_2 = 1$, $\sigma_3 = 1.2$), in the absence of selection where there is no competition.

If there is selection, we have, with $\sigma_1 = v'_1/v'_2$,

$$E(X_1) = 2\sigma_1 \frac{P_1 P_2 + 2P_3}{P_2 2P_1 + P_2} \left[1 + \frac{\bar{v}'}{N_2 P_2 v'_2} + \frac{2[4P_1 P_3 + P_2(1 - P_2)]}{(P_2 + 2P_3)(2P_1 + P_2)^2} \right] + \varepsilon \quad (6)$$

admitting no selection in the condition without competition. In Hardy-Weinberg terms,

$$E(X_1) = \sigma_1 \left[1 + \frac{1}{N} \left(\frac{\bar{v}'}{2pqv'_2} + \frac{1}{2p} \right) \right] + \varepsilon$$

with $\sigma_1 = v'_1/v'_2$ and $N_1 = N_2 = N$.

If $N \rightarrow \infty$, $E(X_1) \rightarrow \sigma_1$.

The same formulae hold for $E(X_3)$ with P_1 replaced by P_3 and σ_1 by σ_3 . As for the W_i , we find a frequency-dependence of the expectations for finite N , and a strong bias depending on N , for small values of allelic frequencies (fig. 5).

It is easy to show that the X_i are less biased than the W_i , the ratio of the biases of $E(W_1)$ and $E(X_1)$ being $2/p$. Nevertheless, the W_i are not submitted to the limitation of the Hardy-Weinberg conditions.

3. A NEW SET OF ESTIMATORS

Following Haldane (1956) who suggests adding one to the denominator in estimating viabilities from ratios to give better approximation, we can consider the estimator:

$$H_1 = \frac{n_{21} \cdot n_{12}}{(n_{22} + 1)(n_{11} + 1)}. \quad (7)$$

If we look at each sample as a trinomial drawing, the theoretical proportions of the three genotypes in eggs being P_1, P_2, P_3 respectively and the two samples being independently drawn, the probability of a particular experimental result, under the null hypothesis of no selection in both conditions, is:

$$\begin{aligned} P_0 &= P(n_{11}, n_{12}, n_{13}/N_1) \times P(n_{21}, n_{22}, n_{23}/N_2) \\ &= \frac{(N_1)!(N_2)!}{n_{11}!n_{12}!n_{13}!n_{21}!n_{22}!n_{23}!} P_1^{(n_{11}+n_{21})} P_2^{(n_{12}+n_{22})} P_3^{(n_{13}+n_{23})} \end{aligned} \quad (8)$$

If selection is present, the probability becomes:

$$\begin{aligned} P_s &= P(n_{11}, \dots, n_{23}/N_1, N_2) \\ &= P_0 \cdot (v_1)^{n_{11}}(v_2)^{n_{12}}(v_3)^{n_{13}}(\bar{v})^{-N_1} \cdot (v'_1)^{n_{21}}(v'_2)^{n_{22}}(v'_3)^{n_{23}}(\bar{v}')^{-N_2}. \end{aligned} \quad (9)$$

If there is no selection in the condition without competition, this formula reduces to:

$$P_s = P_0 \cdot \sigma_1^{n_{21}} \cdot \sigma_3^{n_{23}} \cdot (\bar{\sigma})^{-N_2}$$

with

$$\sigma_1 = \frac{v'_1}{v'_2}, \quad \sigma_2 = 1, \quad \sigma_3 = \frac{v'_3}{v'_2}.$$

We can then consider the bias in H_1 .

(1) *Under the null hypothesis of no selection*, the expectation of $n_{21}/(n_{22} + 1)$ is the sum of all possible products

$$P(n_{21}, n_{22}, n_{23}/N_2) \times \frac{n_{21}}{n_{22} + 1} = \frac{N_2!}{(n_{21} - 1)!(n_{22} + 1)!n_{23}!} P_1^{n_{21}} P_2^{n_{22}} P_3^{n_{23}}$$

and

$$E\left(\frac{n_{21}}{n_{22} + 1}\right) = \frac{P_1}{P_2} \sum \frac{N_2!}{(n_{21} - 1)!(n_{22} - 1)!n_{23}!} P_1^{n_{21}-1} P_2^{n_{22}+1} P_3^{n_{23}}$$

the sum containing all the terms of $(P_1 + P_2 + P_3)^{N_2}$, except the terms with $n_{22} = 0$.

Then, we have:

$$E\left(\frac{n_{21}}{n_{22} + 1}\right) = \frac{P_1}{P_2} [1 - (P_1 + P_3)^{N_2}].$$

In the same way,

$$E\left(\frac{n_{12}}{n_{11} + 1}\right) = \frac{P_2}{P_1} [1 - (P_2 + P_3)^{N_1}].$$

Finally,

$$E(H_1) = E\left(\frac{n_{21} \cdot n_{12}}{(n_{22}+1)(n_{11}+1)}\right) = [1 - (P_1 + P_3)^{N_2}][1 - (P_2 + P_3)^{N_1}] \quad (10)$$

(2) When selection is present, the expectations become

$$E\left(\frac{n_{21}}{n_{22}+1}\right) = \frac{P_1 v'_1}{P_2 v'_2} \left[1 - \left(\frac{P_1 v'_1 + P_3 v'_3}{\bar{v}'}\right)^{N_2}\right]$$

$$E\left(\frac{n_{12}}{n_{11}+1}\right) = \frac{P_2 v_2}{P_1 v_1} \left[1 - \left(\frac{P_2 v_2 + P_3 v_3}{\bar{v}}\right)^{N_1}\right]$$

with

$$\sigma_1 = \frac{v'_1}{v'_2} \cdot \frac{v_2}{v_1},$$

$$E(H_1) = E\left(\frac{n_{21} \cdot n_{12}}{(n_{22}+1)(n_{11}+1)}\right)$$

$$= \sigma_1 \left[1 - \left(\frac{P_1 v'_1 + P_3 v'_3}{\bar{v}'}\right)^{N_2}\right] \left[1 - \left(\frac{P_2 v_2 + P_3 v_3}{\bar{v}}\right)^{N_1}\right]. \quad (11)$$

Analogous formulae can be obtained for

$$H_3 = \frac{n_{23} \cdot n_{12}}{(n_{22}+1)(n_{13}+1)}.$$

(3) *Either without or with selection*, it is clear that $E(H_1)$ tends very quickly to 1 or σ_1 , when N_1 and N_2 increase. Using Haldane's terms, we can then say that the H_1 estimator is almost unbiased. In the same way, $E(H_3)$ tends to 1 or σ_3 .

4. VARIANCES OF THE H_1 ESTIMATORS

The use of the classical formulae on variances of ratios and products shows that, under the null hypothesis of no selection,

$$\text{var}(H_1) = \text{var} \frac{n_{21} \cdot n_{12}}{(n_{22}+1)(n_{11}+1)}$$

$$= \frac{P_1^2}{P_2^2} [1 - (P_1 + P_3)^{N_2}]^2 \left[\frac{N_1 P_2}{N_1 P_1 + 1} \right]^2 \cdot B$$

$$+ \frac{P_2^2}{P_1^2} [1 - (P_2 + P_3)^{N_1}]^2 \left[\frac{N_2 P_1}{N_2 P_2 + 1} \right]^2 \cdot A$$

$$+ \left[\frac{N_2 P_1}{N_2 P_2 + 1} \right]^2 \left[\frac{N_1 P_2}{N_1 P_1 + 1} \right]^2 \cdot A \cdot B \quad (12)$$

with

$$A = \frac{N_2 P_1 (1 - P_1)}{(N_2 P_1)^2} + \frac{N_2 P_2 (1 - P_2)}{(N_2 P_2 + 1)^2} + \frac{2 N_2 P_1 P_2}{N_2 P_1 (N_2 P_2 + 1)}$$

$$B = \frac{N_1 P_2 (1 - P_2)}{(N_1 P_2)^2} + \frac{N_1 P_1 (1 - P_1)}{(N_1 P_1 + 1)^2} + \frac{2 N_1 P_1 P_2}{N_1 P_2 (N_1 P_1 + 1)}$$

annroximativ.

But owing to the intrinsic asymmetry of the distribution of H_1 , the use of classical symmetric confidence intervals, restricted to situations where normality can be accepted, is possible only with large samples. If it is not the case, the true distribution of H_i must be obtained by tabulation of (8).

In the case where $(P_1 + P_3)^{N_2} \sim 0$, $(P_2 + P_3)^{N_1} \sim 0$, and if it is possible to admit that $N_2 P_2 + 1 \sim N_2 P_2$ and $N_1 P_1 + 1 \sim N_1 P_1$, we can write, with $N_1 = N_2 = N$:

$$\text{var}(H_1) = \frac{2}{N} \left[\frac{1}{P_1} + \frac{1}{P_2} \right] + \frac{1}{N^2} \left[\frac{1}{P_1} + \frac{1}{P_2} \right]^2. \quad (13)$$

A numerical example, corresponding to a common situation in electrophoretic investigations, is $N = 60$, $P_1 = 0.25$, $P_2 = 0.50$, which gives $E(H_1) \sim 1$ and $\sqrt{\text{var}(H_1)} \sim 0.447$ under the null hypothesis.

5. CONCLUSION

In selection experiments, the estimators of relative viabilities in common use are biased when the sample sizes are not very large, because they are based on ratios of numbers. Their bias depends on sample size and genotypic frequencies, and then is frequency dependent. We present new almost unbiased H_i estimators, free of the Hardy-Weinberg condition, deducing from their distribution approximate formulae for their variances. Nevertheless, the intrinsic asymmetry of their distribution often prevents the use of classical symmetric confidence intervals and the true distribution must be tabulated to obtain correct intervals.

Our study does not review all methods of estimating viabilities in current use. In particular, investigators (Anxolabéhère, 1971; Kojima and Huang, 1972; Morgan, 1976; Birley and Beardmore, 1977; Snyder and Ayala, 1979) look at the viabilities of two different types by putting known numbers R_1 and $N_1 - R_1$ of each of two types, on the medium where competition will proceed. The relative viability is then measured by the ratio A'/B' of the survivors of each of the two types, after selection. The underlying probability distribution is hypergeometric and it is easy to show that, under the null hypothesis of no selection:

$$E\left(\frac{A'}{B'}\right) = \frac{R_1}{N_1 - R_1} \left[1 + \frac{1}{N} \left(\frac{N_1 - N}{N_1 - R_1} \frac{N_1}{N_1 - 1} \right) \right] + \varepsilon$$

where $N = A' + B'$.

The estimator A'/B' is then biased, the bias depending on N and on the relative frequencies of the input types. The importance of this bias will be considered elsewhere, in the context of a critique of the regression methods used in experiments advocating the existence of a frequency-dependent selection.

In the experimental system studied in this paper the samples are not constitutively small, as was the case in our previous work on isolation and sexual selection indices (Goux and Anxolabéhère, 1980). Nevertheless, owing to experimental constraints, the sample size is not very large ($N = 60$ is widely used). This is the reason why the maximum likelihood estimators can lead to erroneous conclusions. If not taken into account, the bias can easily lead to the erroneous conclusion of a true frequency-dependent effect on selective values.

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