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An Improved Method for Estimating Inbreeding Depression in Pedigrees

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Fitness is expected to decrease with inbreeding in proportion to the amount of deleterious genetic variation present in a population. The effect of inbreeding on survivorship is usually modeled as a negative exponential relationship, and this model has been widely used to estimate the amount of deleterious genetic variation in populations. Linear regression has traditionally been used to estimate the parameters of the model, including the number of lethal equivalents. This article describes an alternative method for estimating parameters and their confidence limits: the maximum likelihood approach. The accuracy of regression and maximum likelihood estimates of the number of lethal equivalents is compared through simulation. The maximum likelihood approach is found to be both median unbiased and capable of estimating confidence limits with nearly the stated degree of accuracy, while the linear regression approach is found to be median biased. The significance of this on previous estimates of inbreeding depression is discussed. Zoo Biol 17:481–497, 1998. © 1998 Wiley-Liss, Inc.

Key words: endangered species; fitness; lethal equivalents; maximum likelihood

INTRODUCTION

The deleterious effects of inbreeding have long been recognized in domesticated species [Darwin, 1868, 1876], and more recently were documented in both captive [Ralls et al., 1979, 1988] and wild [Jimenez et al., 1994; Keller et al., 1994] populations. Most evidence suggests that a large proportion of inbreeding depression is caused by deleterious recessive alleles [for review, see Charlesworth and Charlesworth, 1987]. Inbreeding is expected to affect many aspects of fitness, but the impact of inbreeding on viability is often the most straightforward to examine.

To quantify the number and impact of deleterious alleles on survivorship, Morton et al. [1956] defined a lethal equivalent as an unit of deleterious genetic variation and developed a model to estimate the genetic load present in a genome. One lethal equivalent is defined as a set of alleles of such number that, if dispersed in different individuals, would, on average, be lethal in one individual of the group. For ex-

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ample, if an allele or set of alleles causes death 50% of the time, this is equal to one-half of a lethal equivalent. Morton et al. chose the number of lethal equivalents in a doubled haploid genome as a statistic to quantify the deleterious alleles present in a genome. This statistic originally was called the total mutational damage of an individual, a term no longer used. In this article, we continue the imprecise, but common and convenient, practice of using the term *lethal equivalent* as an abbreviation for the number of lethal equivalents in a doubled genome (either haploid or diploid). Morton et al. [1956] showed that survivorship is expected to decline in inbred individuals in proportion to the number of lethal equivalents within the population and used this relationship to estimate the average number of lethal equivalents per gamete from pedigrees with known levels of inbreeding. The number of lethal equivalents within a genome, therefore, also serves as a measure of the magnitude of inbreeding depression.

The model of Morton et al. [1956] has been used to estimate the number of lethal equivalents in many populations, and such research has influenced conservation priorities and actions. For example, in a classic study, Ralls et al. [1988] examined 40 mammalian zoo pedigrees and found decreased survivorship among inbred individuals in 36. The median estimate for the number of lethal equivalents per gamete in these 40 pedigrees was 1.57 and the mean was 2.33. These results and others have made inbreeding depression a concern for small population conservation, both in captivity and the wild [Bleich et al., 1990; Hedrick and Miller, 1992; Ballou et al., 1995]. In addition, recognition of the detrimental consequences of inbreeding has led to increased interest in the possibility of removing deleterious genetic variation from captive populations [Templeton and Read, 1983; Hedrick, 1994; Ballou, 1997; Lacy and Ballou, 1998].

Unfortunately, the linear regression method normally used to estimate the number of lethal equivalents within pedigrees [Templeton and Read, 1983] has at least one limitation that may lead to flawed estimates. Templeton and Read circumvented this problem with a data transformation that has become common [Ralls et al., 1988] and controversial. Willis and Wiese [1997], however, argued that the statistical technique is flawed, and Lacy [1997] has maintained it produces inappropriately low estimates. In response, Templeton and Read [1998] admitted that their method will produce biased estimates but argue that the bias is small and the method sound. This discussion, however, lacked a thorough quantitative analysis of how well the linear regression method works, and the issue remains unresolved. We begin this article by describing how the traditional linear regression method estimates the number of lethal equivalents in a pedigree. Next, we describe an alternative mathematical technique, maximum likelihood, that avoids the shortcomings of linear regression estimation in this application. Last, we test how well both of these techniques estimate the number of lethal equivalents in simulated data when the true value is known a priori.

ESTIMATING THE NUMBER OF LETHAL EQUIVALENTS

Modeling the Impact of Inbreeding on Viability

Estimating the number of lethal equivalents in individuals of a population is usually done from captive populations with a known pedigree. Each individual in the pedigree is characterized by two values: its inbreeding coefficient and its viability (survival or not to a specified age). To summarize these data, let us define an inbreeding class as all the individuals in a pedigree sharing the same inbreeding coefficient. Let N_i represent the number of individuals born with the inbreeding coefficient f_i and let $N_{s(i)}$ equal the number of the individuals in the *i*th inbreeding class that survived to a specified age. The observed viability $S_{obs(i)}$ in the *i*th inbreeding class equals

$$S_{obs(i)} = \frac{N_{s(i)}}{N_i}.$$

If *m* represents the number of inbreeding classes in the pedigree, then the total number of births in the pedigree *N* equals

$$N = \sum_{i=1}^{m} N_i.$$

Morton et al. [1956] developed a model to describe the relationship between survivorship and inbreeding. This model assumes all individuals with the same inbreeding coefficient have the same probability of surviving, independent of other factors such as location or year of birth. If loci affecting survivorship have independent, deleterious, and multiplicative effects, then survivorship is expected to decline approximately exponentially as a function of the inbreeding coefficient. This can be expressed as

$$S_i = S_0 e^{-Bfi} \tag{1a}$$

where S_i is the probability of an individual with an inbreeding coefficient of f_i surviving to a specified age, S_0 is the survivorship of non-inbred individuals, and *B* is a constant describing the rate of decline of survivorship with inbreeding for the population. In addition, *B* is approximately equal to the number of lethal equivalents in a haploid genome [Morton et al., 1956]. This model has the advantages of making few assumptions and having biologically meaningful parameters. Consequently, many studies used this model to estimate the number of lethal equivalents, 2*B*, in the diploid genomes of various species by fitting the model to survivorship data obtained from pedigrees. Because other traits besides viability may be affected by inbreeding, the magnitude of inbreeding depression quantified by *B* may be an underestimate of the true cost of inbreeding.

Estimating *B* with Linear Regression

Two methods commonly used to estimate model parameters are least-squares linear regression and maximum likelihood. Both methods have advantages and potentially significant drawbacks. We first consider linear regression, as it has traditionally been used to estimate S_0 and *B* [Templeton and Read, 1983; Ralls et al., 1988].

B can be estimated by expressing Equation (1a) in a linear form,

$$\ln Si = \ln S_0 - Bf_i \tag{1b}$$

and fitting this relationship to the data with weighted least-squares regression. The intercept of the fitted line will be an estimate of $\ln S_0$, and the slope of the fitted line will be an estimate of *B*. Specifically, estimates of S_0 and *B*, $\hat{S}_0$ and $\hat{B}$, can be calculated

$$\hat{B} = \frac{\Sigma f \ln(S_{obs})}{\Sigma f^2} \tag{2a}$$

$$\hat{S}_0 = avg(\ln S_{obs}) - \hat{B}[avg](f), \tag{2b}$$

where

$$\Sigma f \ln(S_{obs}) = \sum_{i=1}^{m} N_i [f_i - avg(f)] \left[\ln S_{obs(i)} - avg(\ln S_{obs})\right] \tag{2c}$$

$$avg(f) = \sum_{i=1}^{m} \frac{N_i f_i}{N} \tag{2d}$$

$$avg(\ln S_{obs}) = \sum_{i=1}^{m} \frac{N_i \ln S_{obs(i)}}{N} \tag{2e}$$

[Sokal and Rohlf, 1995, Box 14.4]. However, in practice, this method will not work when there have been no survivors in an inbreeding class (i.e., a $S_{\text{obs}(i)} = 0$), for the logarithm of zero is undefined. Templeton and Read [1983] advocate dealing with this problem by adjusting observed viabilities with a "small sample size correction." Specifically, they replaced $S_{\text{obs(i)}}$ with $S_{\text{adj}(i)}$

$$S_{adj(i)} = \frac{N_{s(i)} + 1}{N_i + 2}$$

in Equations (2a), (2b), (2c), and (2e). In this article, we call estimates of B obtained in this way "adjusted linear regression" estimates.

Confidence intervals of width $1 - \alpha$ for B are typically obtained [Sokal and Rohlf 1995, Box 14.5]

$$\hat{B} \pm t_{\alpha,m-2} s_B$$

where $t_{\alpha,m-2}$ is the t statistic with $m - 2$ degrees of freedom, and the standard error for B, s_B, is

$$s_B = \sqrt{\frac{s^2}{\sum_{i=1}^{m} x^2}}$$

where

$$s^2 = \frac{\sum_{i=1}^{m} N_i (S_{adj(i)} - \hat{B} f_i - \hat{S}_0)^2}{m-2}$$

$$\sum_{i=1}^{m} x^2 = \sum N_i \left(f_i - avg(f)\right)^2.$$

Estimating *B* with Maximum Likelihood

Maximum likelihood theory offers a general and commonly used method for estimating parameters in models. The first step in obtaining maximum likelihood estimates for a parameter is to model how the data were obtained and define the probability of obtaining the observed data from the model. This probability is the likelihood of the parameters in the model, given the data. The model of Morton et al. [1956] implies the likelihood L, of S_0 and B, given pedigree data, to be

$$L = \prod_{i=1}^{m} C_i (\hat{S}_i)^{N_{s(i)}} (1 - \hat{S}_i)^{N_i - N_{s(i)}} \tag{4a}$$

where $\hat{S}_\mathrm{i} = S_0{}^{e\text{-}Bfi}$ and C_i is the appropriate binomial coefficient. Note that L is a function of S_0 and B. The maximum likelihood estimates of S_0 and B are those that maximize L. Likelihood equations are typically log transformed as

$$\ln L = \sum_{i=1}^{m} \left[N_{s(i)} \ln \hat{S}_i + (N_i - N_{s(i)} \ln(1 - \hat{S}_i) \right] + \sum_{i=1}^{m} \ln C_i \tag{4b}$$

to ease computation, maximizing the log-likelihood is equivalent to maximizing the likelihood. Maximum likelihood estimates for S_0 and B may be obtained analytically or with maximization algorithms, such as those in widely available in software packages [e.g., Microsoft Excel 97].

We now construct maximum likelihood confidence intervals for B based on a region of acceptance obtained with likelihood ratio tests, as this method is preferred when dealing with small samples [Kendall and Stuart, 1979]. A confidence interval for B contains all values of B that the data cannot reject as significantly different from the maximum likelihood estimate of B. Likelihood ratio tests begin by defining two hypotheses, H_1 and H_2. In our case, let us define $H_1{:}B \geq 0$, $0 \leq S_0 \leq 1$, and $H_2{:}B = B_\mathrm{Alt}$, $0 \leq \mathrm{S}_0 \leq 1$. Let us define, L_1 and L_2 as the highest likelihood possible under H_1 and H_2, respectively. L_1 is obtained with the maximum likelihood estimates of S_0 and B, and L_2 is obtained by selecting the value of S_0 that maximizes the likelihood given B_Alt. A 95% confidence interval for B can be obtained [Rice, 1995] by finding the highest and lowest values of B_Alt that conform to the inequality

$$2\ln\left(\frac{L_1}{L_2}\right) \leq \chi^2_{0.05,1}.$$

An Example

As an example, consider the survivorship data of the black and white ruffled lemur *Varecia variegata variegata* [summarized by Berg, 1997], presented in Appendix I. Table 1 presents the adjusted linear regression and maximum likelihood estimates of B along with 95% confidence intervals for B for this example. Appendix I details how a spreadsheet can be used to obtain a maximum likelihood estimate of B. Figure 1 shows the observed viability of each inbreeding class and the maximum likelihood curve. On this graph, B represents the rate at which expected viability declines.

TABLE 1. Adjusted linear regression and maximum likelihood estimates of S_0 and *B*, with 95% confidence interval minima and maxima for *B*, calculated for 10 pedigree data sets

				Adj. linear regression		Maximum likelihood	
	N	m	$\bar{f}$	$\hat{S}_0$	$\hat{B}(B_{min}, B_{max})$	$\hat{S}_0$	$\hat{B}(B_{min}, B_{max})$
Amur leopard[a]	340	32	0.15	0.64	–0.12 (–0.69, 0.46)	0.70	0.00 (0.00, 0.30)
Ceylon leopard[a]	142	7	0.05	0.54	–0.91 (–2.09, 0.27)	0.59	0.00 (0.00, 0.51)
Chinese leopard[c]	531	30	0.21	0.49	0.15 (–0.79, 1.08)	0.52	0.09 (0.00, 0.67)
Black and white lemur[a]	85	12	0.10	0.88	2.84 (1.99, 3.69)	0.92	2.72 (1.37, 4.48)
Black lemur[a]	132	5	0.06	0.53	1.94 (–0.93, 4.80)	0.53	2.14 (0.05, 4.77)
Speke's gazelle[b]	64	5	0.11	0.80	3.09 (1.39, 4.79)	0.81	2.75 (0.96, 4.97)
Speke's gazelle[c]	46	11	0.18	0.81	1.35 (–0.12, 2.83)	0.86	1.18 (0.00, 3.10)
Mohr gazelle[a]	150	29	0.18	0.82	1.36 (0.50, 2.22)	0.97	1.76 (0.63, 2.54)
Human[d]	1744	4	0.01	0.91	1.59 (–0.58, 3.76)	0.91	1.48 (0.70, 2.36)
Human[e]	5273	3	0.01	0.88	0.99 (–6.51, 8.49)	0.88	1.03 (0.45, 1.67)

N, the number of individuals in each pedigree.
m, the number of inbreeding classes.
f, the average inbreeding coefficient.
[a] Data summarized by Berg [1997].
[b] Data of Templeton and Read [1983], offspring of non-inbred parents.
[c] Data of Templeton and Read [1983], offspring of inbred parents.
[d] Data of Sutter and Tabah from Loir et Cher including all deaths, see Morton et la. [1956] for data and reference.
[e] Data of Arner, see Morton et al. [1956] for data and reference.

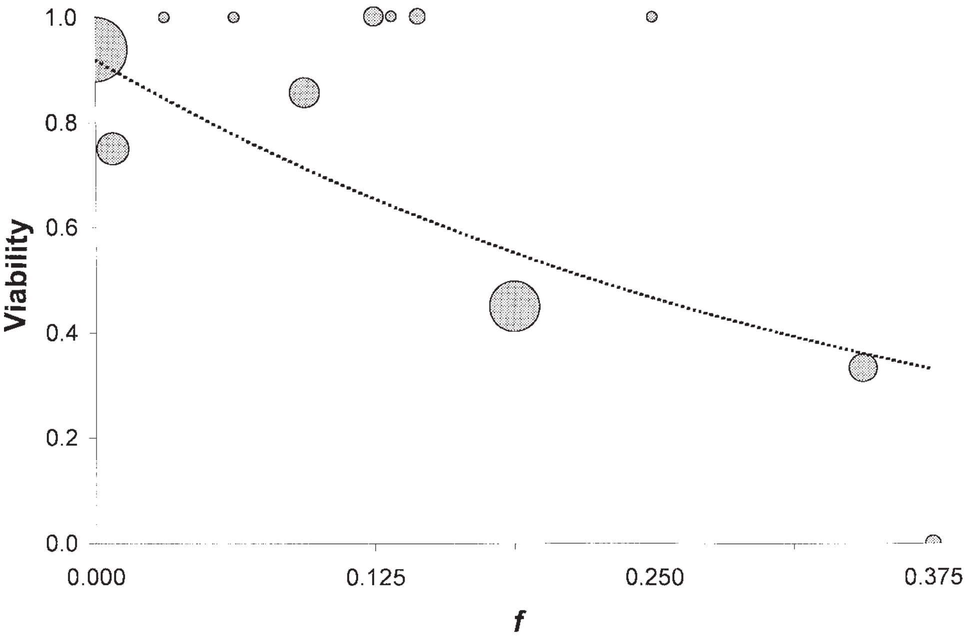

Fig. 1. Observed viability of the black and white ruffled lemur data of Appendix I (circles) and maximum likelihood fit of the model of Morton et al. [1956] to the data. The area of circles is proportional to the number of individuals born.

METHODS

We first compare adjusted linear regression and maximum likelihood estimates of S_0 and B obtained from 10 real pedigree data sets. These pedigrees include the amur leopard (*Panthera pardus orientalis*), Ceylon leopard (*Panthera pardus kotiya*), Chinese leopard (*Panthera pardus japonensis*), black and white ruffled lemur (*Varecia variegata variegata*), black lemur (*Elemur fulvus mayottensis*), mohr gazelle (*Gazella dama mohr*), two Speke's gazelle (G*azella spekei*) data sets, and two human data sets. Regression calculations used the small sample size correction of Templeton and Read [1983]. Maximum likelihood values for S_0 and B were calculated as described above, with the restriction that $B \geq 0$.

Next we sought to evaluate how well adjusted linear regression and maximum likelihood methods estimate B. To do this, we generated large numbers of simulated pedigree data sets from hypothetical populations with a defined value of B and then compared how well each statistical method estimated the known value of B. In each simulated pedigree, the number of individuals born in each inbreeding class was defined. Viability was simulated using the model of Morton et al. [1956] to determine expected viability and a random number generator to decide whether each simulated birth was a survivor or non-survivor.

We examined three variables that might affect estimates of B: the total number of births in the pedigree, the actual magnitude of B, and the structure of the pedigree (i.e., the distribution of inbreeding coefficients in the pedigree). In our simulations, we used 40, 80, 160, and 1,000 for values of N, and 0.0, 1.0, 2.0, and 4.0 for parametric values of B. In all cases S_0 equaled 0.75. From the infinite diversity of possible pedigree structures, we defined four hypothetical pedigrees: even, outbred, endangered, and complex (Table 2). The even pedigree has the same proportion of births in each of five inbreeding classes. The outbred pedigree consists of 75% non-inbred births and 25% inbred births distributed in three inbreeding classes. The endangered pedigree is modeled to represent the distribution of births in a small captive population managed to minimize inbreeding. Last, the complex pedigree contains 13 inbreeding classes with most classes containing a small percentage of the births.

TABLE 2. The percentages of births in four hypothetical pedigrees (even, outbred, complex, and endangered) having the inbreeding coefficient f_i

	Pedigree structure			
f_i	Even	Outbred	Complex	Endangered
0.00000	0.200	0.750	0.400	0.050
0.03125	—	—	0.025	—
0.06250	—	—	0.050	—
0.09375	—	—	0.025	—
0.12500	0.200	0.100	0.150	0.200
0.15625	—	—	0.025	—
0.18750	0.200	0.050	0.050	0.500
0.21875	—	—	0.025	—
0.25000	0.200	0.100	0.100	0.200
0.28125	—	—	0.025	—
0.31250	—	—	0.050	—
0.34375	—	—	0.025	—
0.37500	0.200	—	0.050	0.050

Fifty thousand data sets were generated from each of the 64 combinations of these three variables, and adjusted linear regression and maximum likelihood estimates of B were calculated. Maximum likelihood estimates of B were constrained to be positive. Figure 2 shows the distribution of adjusted linear regression and maximum likelihood estimates of B for the complex pedigree with 80 members when B equaled one and when B equaled four. The two distributions of adjusted linear regression estimates of B both appear symmetric. In contrast, the distribution of maximum likelihood estimates was close to symmetric only when B equaled four. When B equaled one, many of the maximum likelihood estimates were zero.

Our first evaluation of these two methods of estimating B was to examine how well estimates of B clustered around the parametric value of B used to generate the data. Traditionally, this is done by calculating (or estimating) the bias of a statistic

$$\text{estimated bias} = B - \text{mean}(\hat{B}). \tag{5a}$$

However, the asymmetry of maximum likelihood estimates of B created by the restriction to non-negative values makes the median a more appropriate measure of central tendency. Therefore, we estimated median bias

$$\text{estimated median bias} = B - \text{median}(\hat{B}). \tag{5b}$$

to evaluate how well estimates of B clustered around the parametric value.

Our second evaluation of the two methods of estimating B was to determine how well confidence intervals for B captured the actual value of B. We calculated adjusted linear regression and maximum likelihood 95% confidence intervals for each estimated value of B and recorded the proportion of times these confidence intervals contained the value of B used to generate the data.

RESULTS

Table 1 shows that the adjusted linear regression and maximum likelihood estimates of B in 10 real pedigrees were fairly similar. Estimates of B ranged from near zero to approximately three. The largest difference between adjusted linear regression and maximum likelihood estimates of B occurred in the mohr gazelle pedigree, with an adjusted linear regression estimate of 1.36 and a maximum likelihood estimate of 1.76. Estimates of the non-inbred viability S_0 were also fairly similar, with the largest difference occurring, again, in the pedigree of the mohr gazelle. Confidence intervals for B were similar, expect for the Human 2 data, for which the adjusted linear regression method produced an exceptionally wide interval [–6.51, 8.49].

Simulation showed that the magnitude of median bias for least-squares estimates of B ranged from very small to substantial, depending on characteristics of the pedigree. As can be seen in Table 3, all three variables that we examined affected the amount of median bias of adjusted linear regressions estimates of B. Two trends are apparent. First, increasing the size of the pedigrees led to decreased median bias. Second, the median bias of estimates of B was dependent on B. For example, adjusted linear regression estimates of B were too high for the complex pedigree when B equaled zero but were too low when B was greater than ~1.5. Last, we found that the pedigree structure affected the magnitude of direction of median bias. As ex-

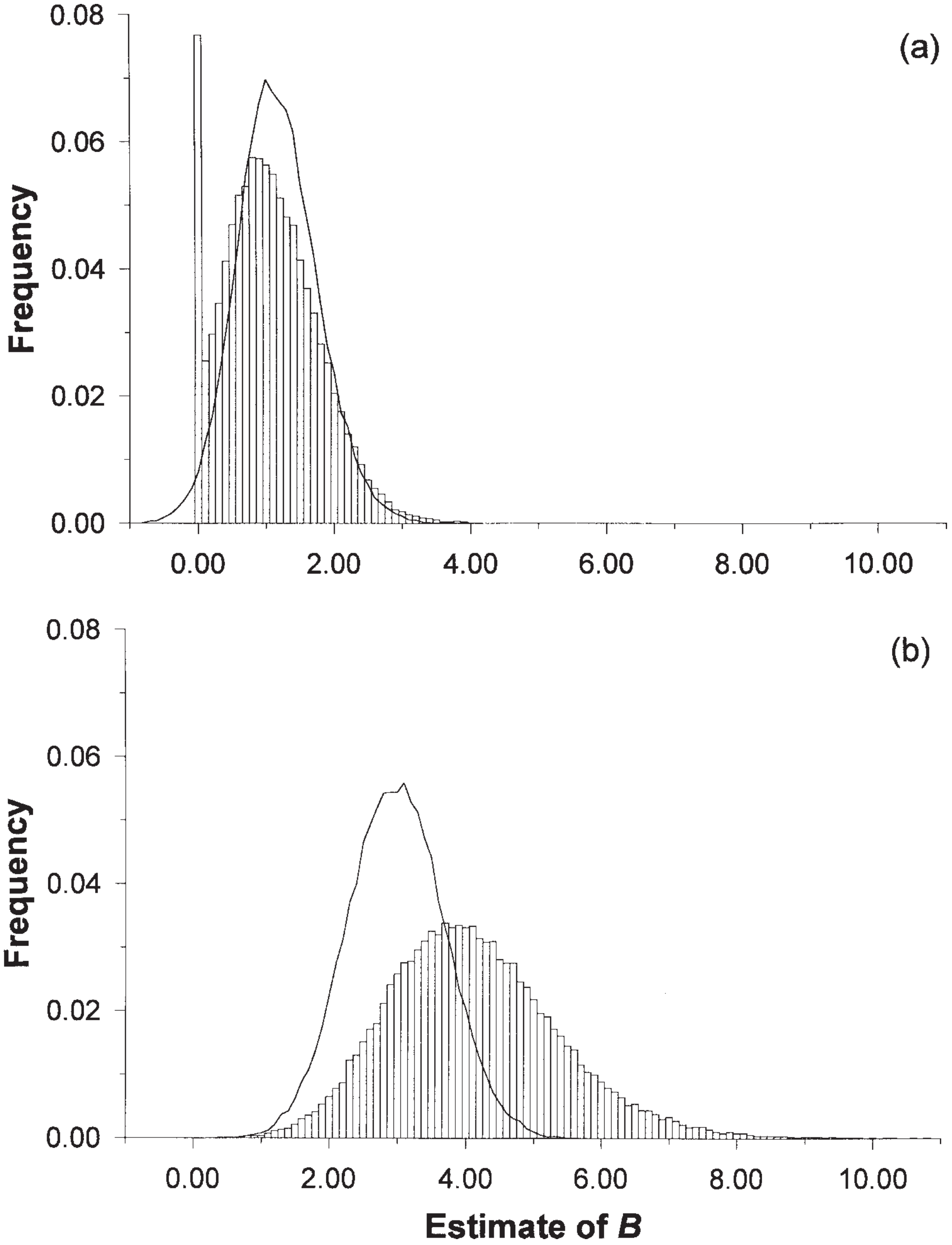

Fig. 2. The distribution of 50,000 maximum likelihood (histogram) and adjusted linear regression (line) estimates of B when S_0 equaled 0.75 and B equaled 1.0 (**a**) and 4.0 (**b**).

pected, the pedigree with the least number of individuals per inbreeding class (complex) produced the most median biased estimates.

As Table 3 also shows, maximum likelihood estimates of B were much less median biased than adjusted linear regression estimates, with a mean absolute value of only 0.024. Two minor trends are apparent. First, increasing the size of the pedi-

TABLE 3. Estimated median bias, *B* – median ($\hat{B}$), for 50,000 estimates of *B* obtained from simulated pedigrees data sets

		Linear regression				Maximum likelihood			
N	*B*	Even	Out	Com	End	Even	Out	Com	End
40	0	0.00	–0.59	–0.52	0.00	0.00	0.00	0.00	0.00
80	0	0.00	–0.36	–0.36	0.00	0.00	0.00	0.00	0.00
160	0	0.00	–0.20	–0.23	0.00	0.00	0.00	0.00	0.00
1,000	0	0.00	–0.04	–0.05	0.00	0.00	0.00	0.00	0.00
40	1	0.16	–0.34	–0.14	0.25	–0.02	0.02	0.00	–0.20
80	1	0.08	–0.21	–0.14	0.21	–0.01	0.01	0.01	–0.06
160	1	0.05	–0.13	–0.10	0.11	0.00	0.00	0.00	–0.03
1,000	1	0.01	–0.02	–0.02	0.02	0.00	0.00	0.00	0.00
40	2	0.34	–0.03	0.33	0.70	–0.03	0.03	–0.01	–0.29
80	2	0.18	–0.07	0.15	0.46	–0.01	–0.01	–0.01	–0.06
160	2	0.10	–0.04	0.06	0.25	–0.01	0.00	–0.01	–0.02
1,000	2	0.02	–0.01	0.00	0.05	0.00	0.00	0.00	0.00
40	4	0.92	0.71	1.57	1.87	–0.06	–0.10	–0.05	–0.42
80	4	0.53	0.41	1.02	1.14	–0.02	–0.04	–0.03	–0.13
160	4	0.28	0.19	0.59	0.69	–0.01	–0.03	0.00	–0.05
1,000	4	0.05	0.03	0.09	0.13	0.00	–0.01	0.00	0.00

Simulated pedigrees varied by structure (even, outbred, (out) complex (Com) and Endangered (End), size (*N* = 40, 80, 160, and 1,000), and the value of *B* used to generate the data (*B* = 0, 1, 2, and 4).

gree appears to have decreased the median bias, and second, the endangered pedigrees appear to have slightly more median bias than the other three pedigree structures we examined.

Table 4 shows that adjusted linear regression 95% confidence intervals for *B*

TABLE 4. The proportion of 50,000 95% confidence intervals for *B* that did not contain the value of *B* used to generate simulated data

		Linear regression				Maximum likelihood			
N	*B*	Even	Out	Com	End	Even	Out	Com	End
40	0	0.04	0.04	0.17	0.01	0.04	0.03	0.03	0.01
80	0	0.05	0.04	0.13	0.03	0.03	0.03	0.03	0.03
160	0	0.05	0.05	0.09	0.04	0.03	0.03	0.02	0.03
1,000	0	0.05	0.05	0.06	0.05	0.03	0.02	0.02	0.03
40	1	0.05	0.06	0.09	0.03	0.05	0.03	0.04	0.01
80	1	0.05	0.06	0.07	0.03	0.05	0.04	0.05	0.04
160	1	0.05	0.05	0.06	0.04	0.05	0.05	0.05	0.05
1,000	1	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05
40	2	0.05	0.04	0.12	0.04	0.06	0.05	0.06	0.02
80	2	0.05	0.05	0.08	0.04	0.06	0.06	0.05	0.05
160	2	0.05	0.05	0.07	0.04	0.05	0.05	0.05	0.07
1,000	2	0.05	0.05	0.06	0.04	0.05	0.05	0.05	0.05
40	4	0.08	0.11	0.56	0.06	0.06	0.06	0.06	0.03
80	4	0.06	0.07	0.28	0.05	0.05	0.05	0.05	0.06
160	4	0.05	0.06	0.16	0.05	0.05	0.05	0.05	0.07
1,000	4	0.05	0.05	0.08	0.03	0.05	0.05	0.05	0.05

Pedigrees varied by structure (even, outbred, (out) complex (Com) and Endangered (End), size (*N* = 40, 80, 160,and 1,000), and the value of *B* used to generate the data (*B* = 0, 1, 2, and 4).

often had a type I error rate of 5% as desired. However, when samples were small or when B was large, the type I error rate was high. Again, the complex pedigree performed the most poorly.

In contrast, we found that maximum likelihood 95% confidence intervals contained the value of B used to generate the simulated data ~95% of the time. In other words, the type I error rate was quite close to its desired value of 0.05. Specifically, the error rate ranged from 0.012 to 0.071, as shown in Table 4. A few trends are apparent. First, the error rate for samples drawn from a species with $B = 0$ was <0.05 and closer to 0.025, which is the expected value when $B = 0$ as discussed below. Second, the structure of the pedigree influenced the error. When N was small and $B > 0$, the endangered pedigree had an error rate that was both <0.05 and lower than the error rates of the other three pedigree structures. Last, the reliability of the maximum likelihood confidence intervals appears to approach 0.05 with increasing sample sizes when $B > 0$.

DISCUSSION

To evaluate how biased previous estimates of B might be, we now focus on identifying and understanding circumstances in which the adjusted linear regression method performs poorly. To do this, we produced a detailed description of median bias of adjusted linear regression estimates of B using simulations similar to those described above. Figure 3a and b depict the estimated median bias of estimates of B in the complex, and endangered pedigrees with N varying from 40 to 240, B varying from 0 to 5, and S_0 equaling 0.75. From these figures, we see that median bias of adjusted linear regression estimate of B can be quite substantial but also see that a broad region exists where the median bias of adjusted linear regression estimates of B is small. These figures suggest that estimates of B for species with an average number of lethal equivalents ($B \approx 1.5$–2.5) [Ralls et al., 1988] may have little bias. The similarity of estimates of B in the 10 real data sets presented above seems to corroborate this.

Figure 4 directly illustrates the relationship between the parametric value of B and adjusted linear regression estimates of B for the complex, outbred, and endangered pedigrees. The figure shows that each pedigree type can closely estimate B for some ranges of B. This implies that general conclusions regarding the effect of inbreeding on viability are unlikely to be affected by bias.

Along with B, S_0 affects bias. Simulations showed that raising S_0 from 0.75 to 0.90 affected the bias of estimates of B. Compare Fig. 3c, which shows the bias in the complex pedigree when S_0 equaled 0.90, to Fig. 3a, where S_0 equaled 0.75.

Templeton and Read [1983, 1984, 1998] describe their small sample size adjustment as a correction that “smoothes over the irregularities” in the data [1983] to produce “conservative” [1984] estimates of viability. Despite these seemingly positive attributes, the small sample adjustment only improves estimates of viability in the sense that it forces them to conform to the assumptions of linear regression. Three lines of reasoning show that the consequence of this is to produce the bias that we have measured in our simulations. First, linear regression should produce unbiased estimates of B, without making assumptions not implicit in the model of Morton et al. [1956] [Guttman, 1982, p. 14]. That this expectation was not met in our simulations suggests that the median bias that we observed was created by the

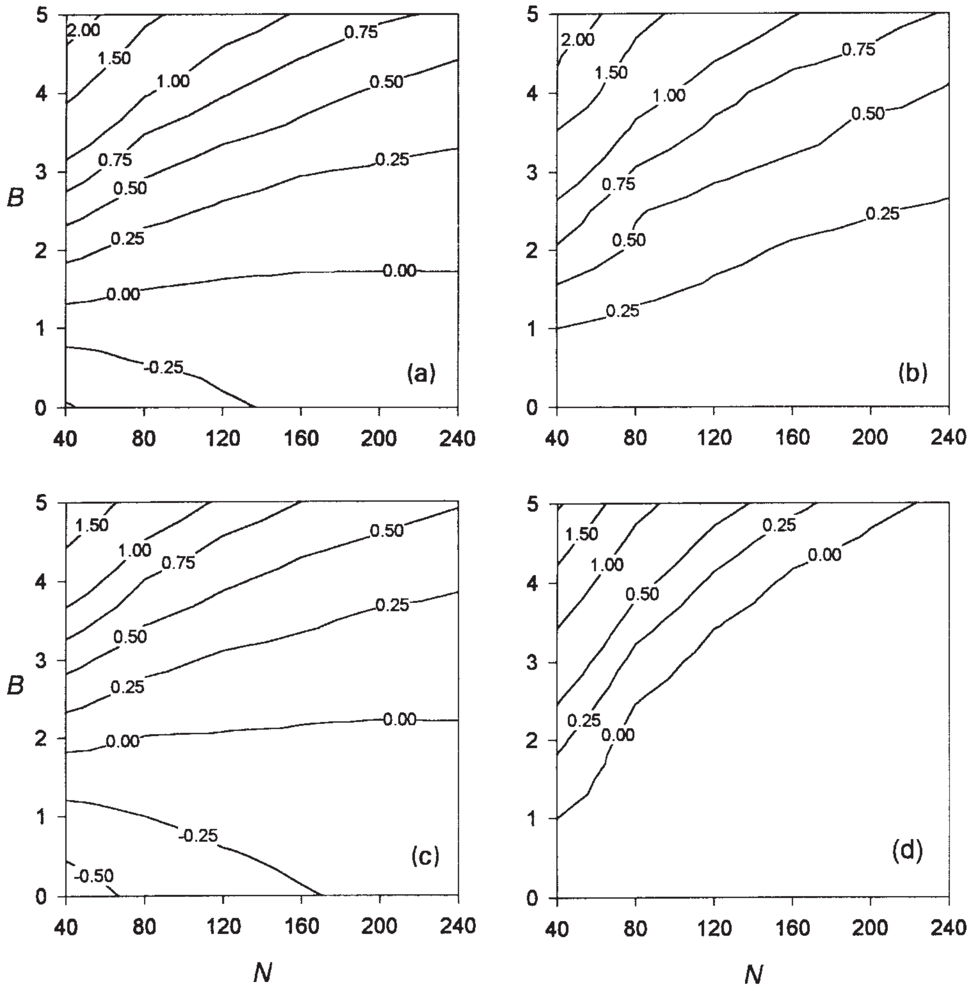

Fig. 3. Estimated median bias of adjusted linear regression estimates of *B*. **a:** Complex pedigree with S_0 equal to 0.75, small sample size adjustment of Templeton and Read. **b:** Endangered pedigree with S_0 equal to 0.75, small sample size adjustment of Templeton and Read. **c:** Complex pedigree with S_0 equal to 0.90, small sample size adjustment of Templeton and Read. **d:** Complex pedigree with S_0 equal to 0.75, the small sample size adjustment of Templeton and Read used only in inbreeding classes with no survivors.

small sample size adjustment of Templeton and Read. The plausibility of this explanation is supported by the apparently arbitrary nature of the small sample size adjustment of Templeton and Read. This small sample size adjustment was used in general surveys of inbreeding depression [Ralls et al., 1988], but no justification for its use has been provided. Instead, Templeton and Read argued that their small sample size adjustment is intuitively reasonable [1984] and that its bias is low in one pedigree [1998].

Empirically, we can see the small sample size adjustment causes bias by using a less influential small sample size adjustment and observing that bias declines. We applied the small sample size correction of Templeton and Read only when there were no survivors in an inbreeding class and found much less median

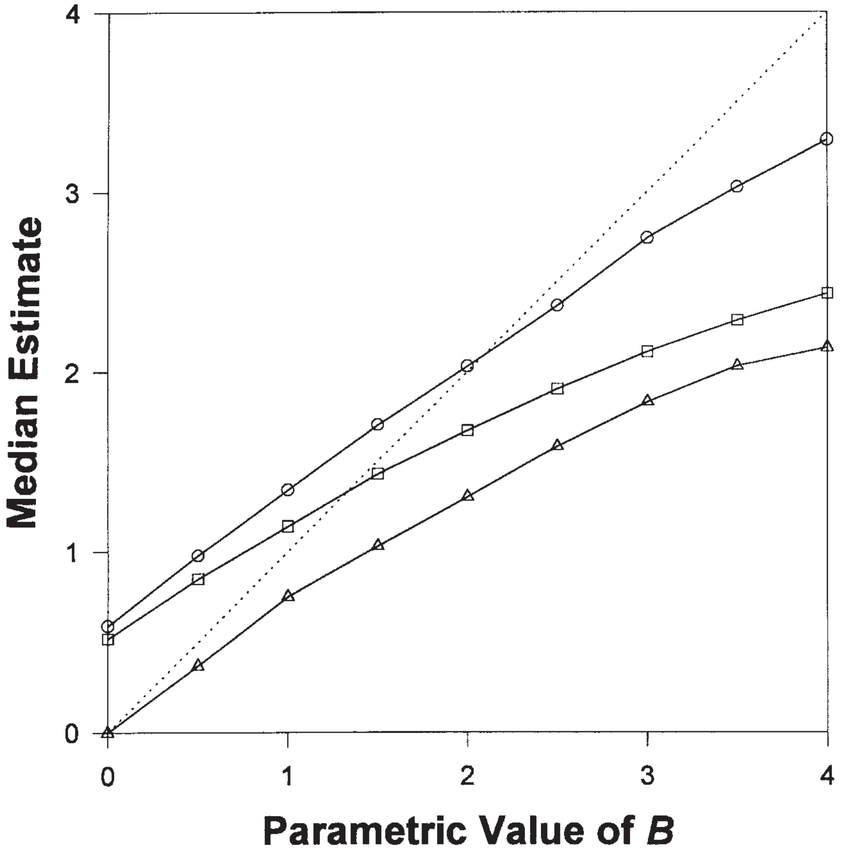

Fig. 4. Medians of 50,000 adjusted linear regression estimates of *B* compared to the parametric value of *B* in the outbred (circles), complex (squares), and endangered (triangles) pedigrees.

bias with this alternative method. Compare the median bias in Fig. 3a with the decreased median bias in Fig. 3d. Many other small sample size adjustments could be proposed and tested, but we see no compelling reason not to use maximum likelihood estimates of *B*.

Last, we can see that the small sample size adjustment of Templeton and Read causes bias by observing how it changes estimates of viability. Pedigree structure, parametric values of S_0 and *B*, and the small sample adjustment all interact to cause bias in ways that may be difficult to predict, but simple examples demonstrate the dynamics of these variables. Consider the idealized effects of the small sample adjustment on estimates of viability in two symbolic pedigrees illustrated in Fig. 5. When $B = 0$, $S_0 > 0.50$, and individuals are distributed evenly across inbreeding classes (Fig. 5a), the small sample correction reduces estimates of viability equally in all inbreeding classes. This will change the intercept of a fitted line but will not affect the slope. When $B = 0$ and there are more non-inbred than inbred individuals (Fig. 5b), the small sample size adjustment will lead to inappropriately high estimates of *B*. When *B* and S_0 are high (Fig. 5c and d), the small sample size adjustment decreases estimates of *B*. The results of our simulations are consistent with these illustrations.

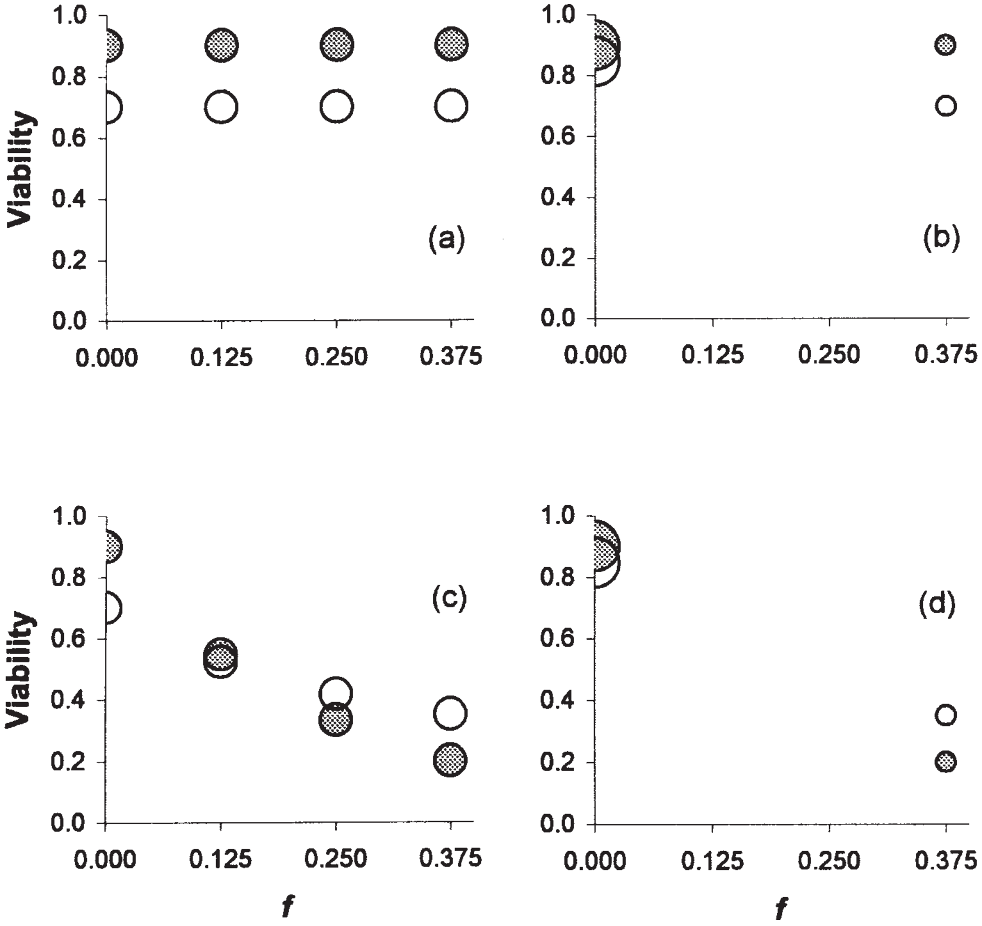

Fig. 5. Observed (shaded circles) and adjusted (open circles) viabilities in two idealized pedigrees when *B* equaled zero (**a,b**) and four (**c,d**).

Unlike least-squares estimates, maximum likelihood estimates of parameters are generally only asymptotically unbiased [Wetherill, 1981, p. 79], which means that their bias decreases to zero as sample sizes increase to infinity. Our results suggest that the magnitude of this bias is small enough to be inconsequential.

Our use of median bias instead of bias is unusual but reasonable and appropriate. Kendall and Stewart [1977, p. 4] noted "there is nothing except convenience to exalt the arithmetic mean above other measures of location as a criterion of bias. We might equally well have chosen the median of the distribution'' or its mode. The mean value is used, as always, for its mathematical convenience." In any case, our conclusions regarding the bias of adjusted linear regression estimates are not affected by the measure of central tendency that we used. We calculated the mean of all distributions, as well as the median, and found the mean and median adjusted linear regression estimates to be very similar. Maximum likelihood estimates of median bias become similar to estimates of bias when *B* and *N* are large.

A noteworthy characteristic of the maximum likelihood approach to estimating

B presented here is that *B* is restricted to be non-negative. In other words, the expected viability of an inbreeding class is not permitted to rise with increasing levels of inbreeding. This restriction is imposed to prevent viabilities from becoming greater than one. Besides being biologically impossible, viabilities greater than one would make the log likelihood undefined. The restriction of *B* to non-negative values appears to have caused the low error rate for pedigree data sets drawn from a population with $B = 0$. When pedigrees are generated from a population with $B = 0$, half of the pedigrees produced are expected to have increased survivorship in inbred individuals. All these samples will be assigned a maximum likelihood value of 0.0 for *B*, and their confidence intervals will include 0.0 as a lower bound. Since 0.0 is the value of *B* used to generate the samples, the confidence intervals will be correct each time there is an apparent trend toward increasing survivorship, which means that the expected error rate for the maximum likelihood technique is 2.5%. This expectation is approached in pedigrees with large sample sizes.

We conclude by mentioning that the small sample size correction of Templeton and Read is intuitive and reasonable when viewed as pragmatic solution to a statistical obstacle. In many pedigrees, including perhaps the majority of typical ones, adjusted linear regression produces estimates of *B* with little bias. However, because the bias can be fairly large and because estimating the impact of inbreeding can be an important part of population viability analysis, we see no reason to continue using this method of estimating *B*. Until a new data transformation is demonstrated to produce estimates of *B* with less median bias than the maximum likelihood method or can produce estimates of *B* with an equal median bias and less variance, we recommend using the maximum likelihood method to estimate *B*.

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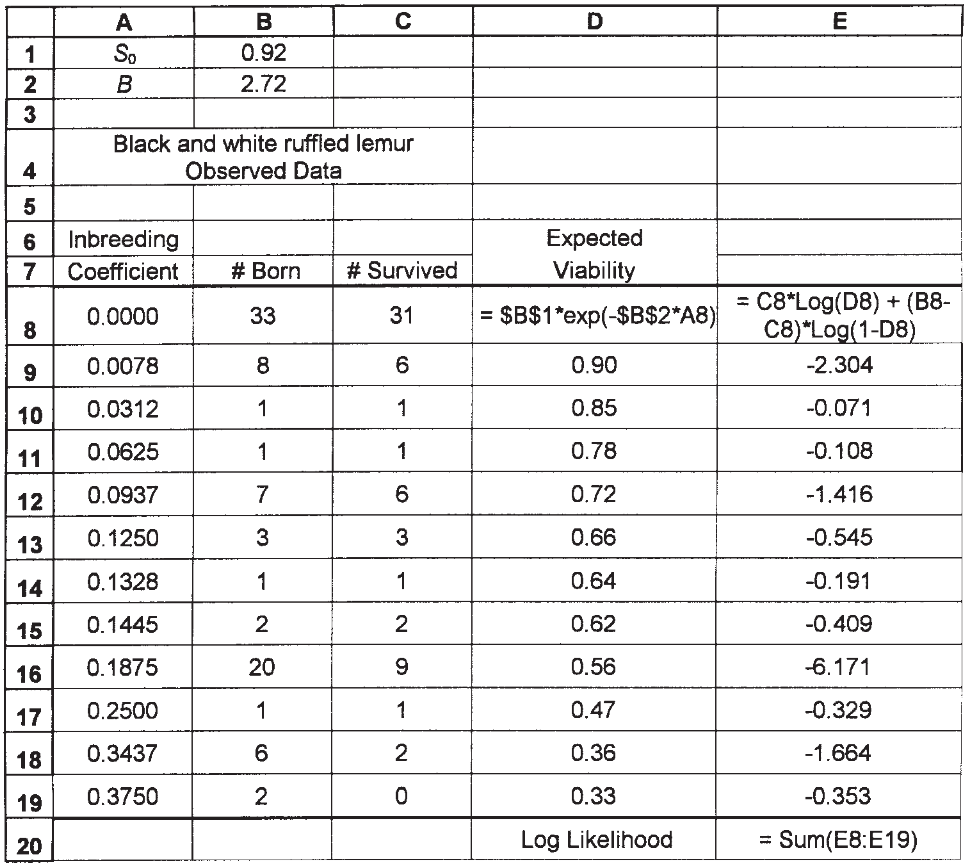
APPENDIX I. Using Microsoft Excel 97 to find maximum likelihood estimates of S_0 and B. The spreadsheet below can be used to obtain maximum likelihood estimates of S_0 and B for the black and white ruffled lemur data [summarized by Berg, 1997] below. Once the spreadsheet has been set up with the data and formulas as shown below, the Solver macro found in the Tools menu can be used to find values for S_0 and B that maximize the likelihood of the data. To do this, set the target cell, E20, to be maximized, by changing cells B1 and B2 subject to the constraints B1 > 0, B1 < 1, and B2 > 0. Then press Solve. Note that the binomial coefficients log C_i have been omitted because they do not affect the relative likelihoods of different values of S_0 and B.



	A	B	C	D	E
1	*S_0*	0.92			
2	*B*	2.72			
3					
4	Black and white ruffled lemur Observed Data				
5					
6	Inbreeding			Expected	
7	Coefficient	# Born	# Survived	Viability	
8	0.0000	33	31	= \$B\$1*exp(-\$B\$2*A8)	= C8*Log(D8) + (B8-C8)*Log(1-D8)
9	0.0078	8	6	0.90	-2.304
10	0.0312	1	1	0.85	-0.071
11	0.0625	1	1	0.78	-0.108
12	0.0937	7	6	0.72	-1.416
13	0.1250	3	3	0.66	-0.545
14	0.1328	1	1	0.64	-0.191
15	0.1445	2	2	0.62	-0.409
16	0.1875	20	9	0.56	-6.171
17	0.2500	1	1	0.47	-0.329
18	0.3437	6	2	0.36	-1.664
19	0.3750	2	0	0.33	-0.353
20				Log Likelihood	= Sum(E8:E19)