

Detecting inbreeding depression is difficult in captive endangered species

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Abstract

During the past two decades, pedigree analysis has documented inbreeding depression in many captive populations. This and subsequent research has led to a recognition that inbreeding depression is a potentially important determinant of small population fitness, in both captivity and the wild. Modern captive-breeding programmes now universally avoid inbreeding. We use simulation to investigate how much traditional pedigree analysis will reveal about the effect of inbreeding in such populations. We find that pedigrees typical of breeding programmes designed to avoid inbreeding have low statistical power to detect inbreeding depression.

INTRODUCTION

In a classic study of conservation biology, Ralls, Brugger & Ballou (1979) demonstrated that inbreeding lowered juvenile viability in populations of captive mammals. In a more detailed analysis, Ralls, Ballou & Templeton (1988) showed that 36 out of 40 zoo populations exhibited a trend towards decreased juvenile viability in inbred individuals. The median estimate of the number of lethal equivalents in a diploid genome was 3.14, but there was much variation among estimates, including populations with non-significant inbreeding depression and increased viability among inbred individuals. Ralls, Ballou *et al.* (1988) also noted that most of their pedigrees were 'small and distributed over only a few levels of inbreeding. The statistical power to detect slopes significantly greater than zero was therefore limited.' In addition, this and subsequent research has shown inbreeding affects different traits to different degrees with variation occurring between species, populations and lineages (e.g. Pray & Goodnight, 1995; Kärkkäinen, Koski & Savolainen, 1996; Lacy, Alaks & Walsh, 1996). These two decades of work have illustrated the complexity of documenting inbreeding depression in both captive and wild populations.

Modern breeding programmes avoid inbreeding by minimizing the mean coefficient of kinship (Ballou & Lacy, 1995), and this has undoubtedly led to widespread improvement in captive-breeding programmes. However, understanding the effect of inbreeding in a particular species may be important, especially when

populations are small or are being intensively managed. In these circumstances, examining the effect of inbreeding upon juvenile viability provides the simplest measure of inbreeding depression. In this paper, we will review how inbreeding depression is detected in pedigrees, propose a criterion for deciding that inbreeding depression is minimal, and explore how characteristics of a pedigree affect the probability of reaching one of these conclusions. As we shall see, breeding programmes designed to avoid inbreeding can make reaching any firm conclusions unlikely.

METHODS

Pedigrees of endangered species in captivity

Although the pedigree of each population will be unique, captive-breeding programmes often cause endangered species to have similar pedigrees. Of the many variables that affect the structure of a pedigree, the number of founders is particularly important. When the number of founders is small, inbreeding occurs early in the breeding programme, often between half siblings ($f = 0.125$, where f is the inbreeding coefficient). Subsequent births will often cluster at a level of inbreeding higher than the first generation of inbred births. When there are more founders, there are more opportunities for non-inbred births and inbreeding can be avoided for longer. In addition, the first inbred births in pedigrees with many founders are generally less inbred than the first inbred births of pedigrees with few founders.

For our analysis, we have defined two idealized pedigrees similar to those of small populations managed to retain genetic variation. The distributions of inbreeding coefficients in these pedigrees, the few-founders

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pedigree and the many-founders pedigree, are shown in Fig. 1 and listed in the Appendix. The few-founders pedigree was modelled after the captive-breeding programme of the Mexican wolf (*Canis lupus baileyi*), which was founded by three wild-caught individuals (Siminski, 1998). In our model pedigree, there are 200 individuals, with eight non-inbred births and 192 inbred births distributed in three levels of inbreeding. The average inbreeding coefficient overall is 0.18, and 0.1875 for inbred births only. Like the Mexican wolf pedigree, 50% of the inbred individuals have an inbreeding coefficient of 0.1875. The many-founders pedigree was modelled after the captive breeding programme of the red wolf (*Canis rufus*), which was founded from 14 individuals (Waddell, 1997). In our model pedigree, there are 600 individuals, with 300 non-inbred births and 300 inbred births distributed among 15 levels of inbreeding. In the red wolf pedigree, most of the first inbred births had $f = 0.0625$ (mostly first-cousin matings) and we incorporated this feature in to our many-founders pedigree. Similar to that of the red wolf pedigree, the average inbreeding coefficient is 0.0313 overall, and 0.0625 among inbred births.

Estimating the effect of inbreeding

In order to estimate the effect of inbreeding upon viability, inbreeding coefficients and viability data (survival to a specified age) are needed for each individual in the analysis. In practice, this usually requires data from a studbook. The impact of inbreeding upon viability can be estimated by fitting the model:

$$S_i = S_0 e^{-Bf_i} \quad (1)$$

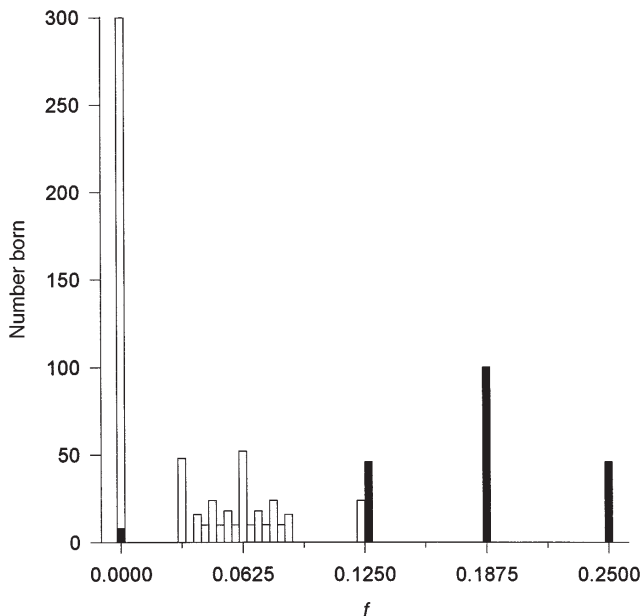


Fig. 1. The distribution of inbreeding coefficients, f , in hypothetical pedigrees with few founders (black) and many founders (white).

to the data, where S_i is the expected viability of an individual with an inbreeding coefficient f_i surviving to a specified age, S_0 is the expected viability of non-inbred individuals, and B is a measure of how fast viability decreases with inbreeding (Morton, Crow & Muller, 1956). In addition, $2B$ is approximately equal to the number of lethal equivalents in a diploid genome. A lethal equivalent is a unit of genetic variation and the number of lethal equivalents in an individual is equal to the sum of the selective disadvantage when that individual is homozygous for all detrimental or lethal alleles (Cavalli-Sforza & Bodmer, 1971).

Deciding whether there is significant inbreeding depression in a pedigree is equivalent to deciding if $2B$ is significantly greater than zero. This can be done by calculating a confidence interval for $2B$ and observing whether the interval includes zero. A confidence interval of $1 - \alpha$ will provide a test of significance of inbreeding depression at the $\alpha/2$ level.

Demonstrating that viability in a pedigree has not been affected by inbreeding is equivalent to showing that $2B = 0$ and not any other value. This is impossible, but we can test whether $2B$ is less than a critical value for which the impact of inbreeding is considered minimal. Figure 2 shows the cost of inbreeding, $1 - S_i/S_0$, expected from the model of Morton *et al.* (1956) as a function of $2B$ and f . As can be interpreted from the figure, $2B = 1$ is the highest number of lethal equivalents that can be considered to have minimal effect upon viability in moderately inbred individuals. For example if $2B = 1$, then matings between half siblings ($f = 0.125$) should have an expected viability of 6% less than non-inbred births and matings between full siblings would have an expected viability of 12% less than non-inbred births. For heuristic purposes, we will consider a pedigree with

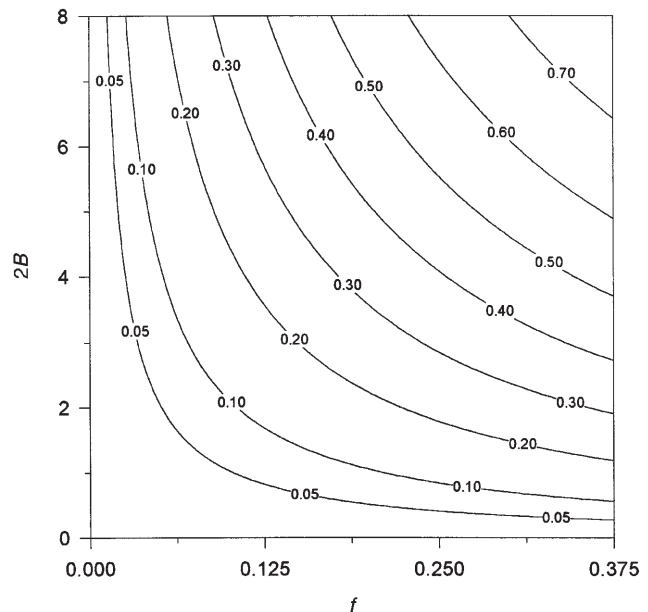


Fig. 2. The expected cost of inbreeding as a function of the inbreeding coefficient, f , and the number of lethal equivalents, $2B$.

an estimate of $2B$ significantly less than 1 to be minimally affected by inbreeding.

Estimating power to describe the effect of inbreeding

We are interested in estimating the probability of a pedigree demonstrating that viability in a population is either affected or not affected by inbreeding, and examining what variables influence this probability. Statistical power is the probability of rejecting a null hypothesis when it is false. In our case, we will be interested in rejecting either of two alternative null hypothesis: $2B = 0$ or $2B > 1$.

We estimated the statistical power of the two idealized pedigrees to detect inbreeding depression (i.e. to show $2B > 0$) by assuming values for S_0 and $2B$, obtaining large numbers of simulated viability data sets from these parameters, estimating $2B$ for each simulated data set, and determining whether the estimate was significantly greater than zero. To be more specific, we varied S_0 from 0.4 to 1.0 in increments of 0.05 and varied $2B$ from 0 to 8 in increments of 1. Simulated data were obtained using eqn (1) to determine expected viability and a random number generator to decide if each simulated birth was viable. We obtained maximum likelihood estimates and 95% confidence intervals (Kalinowski & Hedrick, 1998) for $2B$ in each simulated data set and used this interval to decide if the estimated value of $2B$ was significantly greater than zero. This was repeated 2000 times for each combination of S_0 and $2B$ for the few-founders and many-founders pedigrees, and the

fraction of estimates significantly greater than zero was used as an estimate of the statistical power of the pedigree to detect inbreeding depression. In addition we used this procedure to estimate the power of a pedigree to show that $2B$ is less than 1 when the actual value of $2B$ was zero.

RESULTS

An example

Figure 3 shows one set of simulated viability data for the few-founders pedigree, the maximum likelihood fitted curve ($S_0 = 0.76$, $2B = 3.18$) for the data, and curves with minimum ($S_0 = 0.57$, $2B = 0.00$) and maximum ($S_0 = 0.91$, $2B = 6.10$) values for $2B$. Note that the curve with the steepest decline in viability has the highest non-inbred viability; this allows a curve of that slope to best fit the data. These data were obtained using 3.14 as a value for $2B$ and 0.73 as the value for S_0 . However, because zero is included in the confidence interval for $2B$ in the observed simulated data, we conclude there is no inbreeding depression in these data.

Statistical power as a function of S_0 and $2B$

As Fig. 4 shows, the statistical power of a pedigree to detect inbreeding depression is strongly dependent on the number of lethal equivalents in the population, and on the viability of non-inbred births. Inbreeding depression is more likely to be detected when both S_0 and $2B$ are high. When non-inbred births have a low viability,

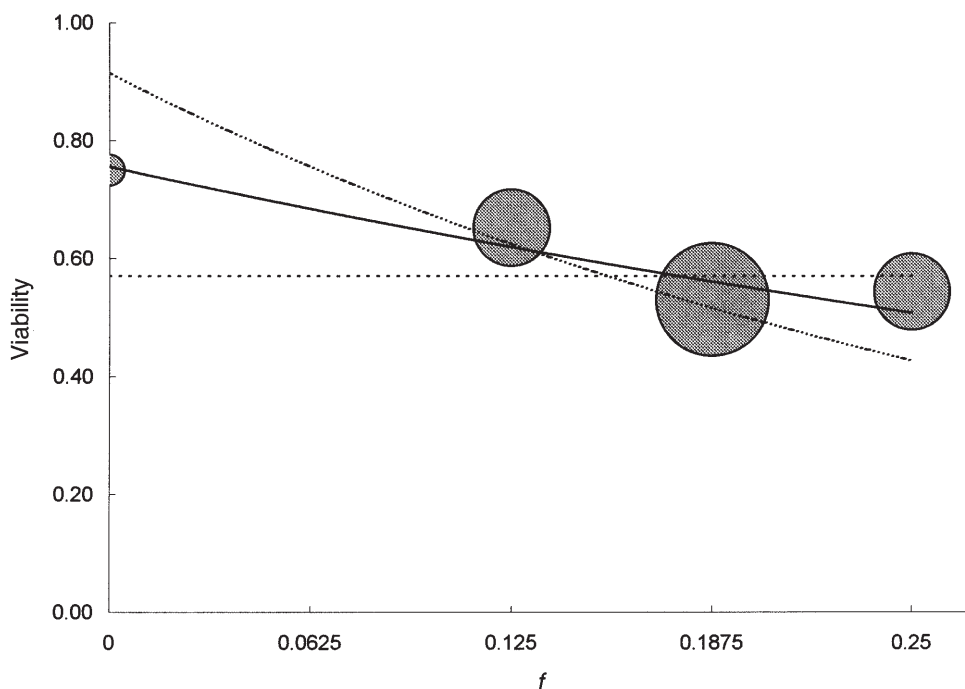


Fig. 3. Simulated viability data as a function of the inbreeding coefficient, f , for the few-founders pedigree (areas of circles are proportional to number of births). Best fit curve for the data (continuous line), and curves with minimum and maximum values for $2B$ for the data (broken lines) are also shown.

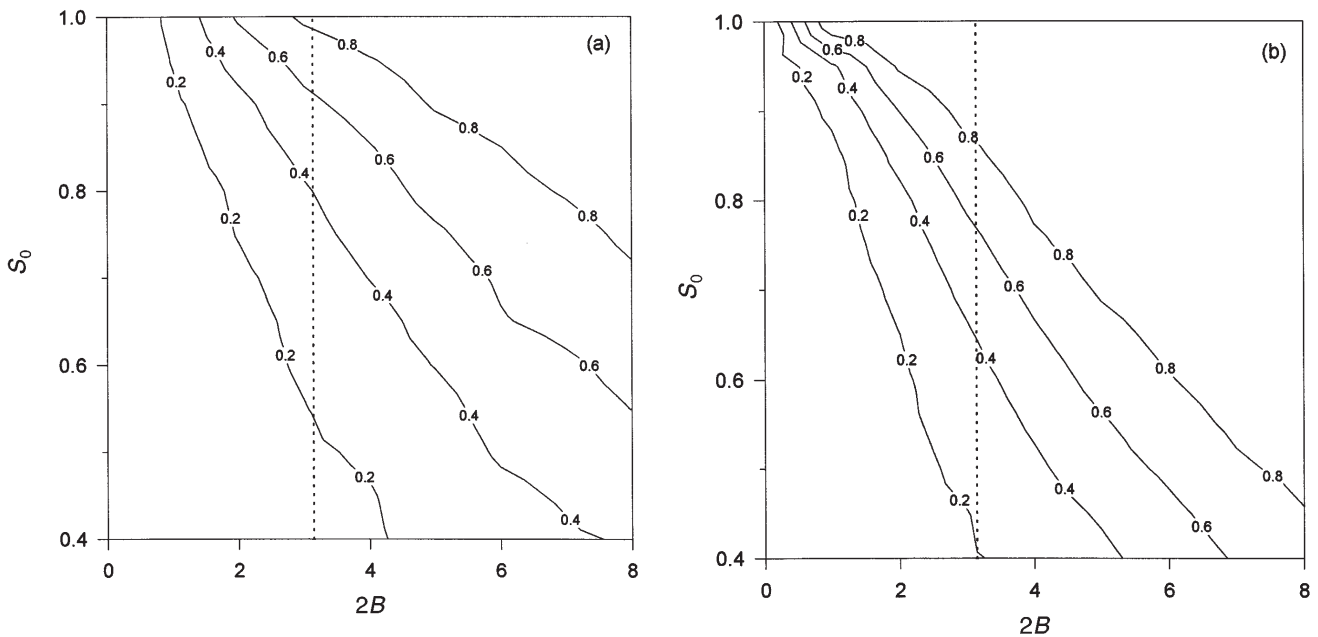


Fig. 4. Statistical power of the (a) few-founders and (b) many-founders pedigrees to detect inbreeding depression as a function of the non-inbred viability, S_0 , and the number of lethal equivalents, $2B$. The broken line indicates the median number of lethal equivalents, 3.14, observed by Ralls, Ballou *et al.* (1988).

detecting a decreased viability among inbred births is more difficult than when non-inbred births have a high viability. The few-founders pedigree (Fig. 4(a)) has less power to detect inbreeding depression than the many-founders pedigree (Fig. 4(b)), even though the average inbreeding coefficient is six times higher in the former. If S_0 and $2B$ equal their median values among mammals in captivity, 0.73 and 3.14 (Ralls, Ballou *et al.*, 1988), respectively, the few-founders pedigree would have an approximately 32% chance of detecting significant inbreeding depression and the many-founders pedigree would have an approximately 53% chance.

Figure 5 shows that demonstrating the effect of inbreeding upon viability to be minimal is unlikely with pedigree structures common to genetically managed endangered species. As can be seen, the probability of a pedigree demonstrating $2B < 1$ is strongly dependent on the viability of non-inbred births. Given 0.73 as a value for S_0 , and no inbreeding depression in the pedigree, there is only a 1% chance of showing $2B$ to be less than 1 with either idealized pedigree. The viability of non-inbred births needs to be approximately 0.95 to have a 50% chance of demonstrating that inbreeding has a minimal effect upon viability. Only one population out of the 40 examined by Ralls, Ballou *et al.* (1988) had a non-inbred viability this high. The steep approach of the two curves to unity when $S_0 > 0.85$ is a consequence of the fact that S_0 cannot be greater than 1.

We note that our criterion for deciding inbreeding has a minimal affect upon viability, less than one lethal equivalent present, was selected for heuristic reasons. Using a more conservative criterion, for example, $2B < 0.5$, would further reduce the probability of a pedigree being able to demonstrate that it was minimally affected by inbreeding.

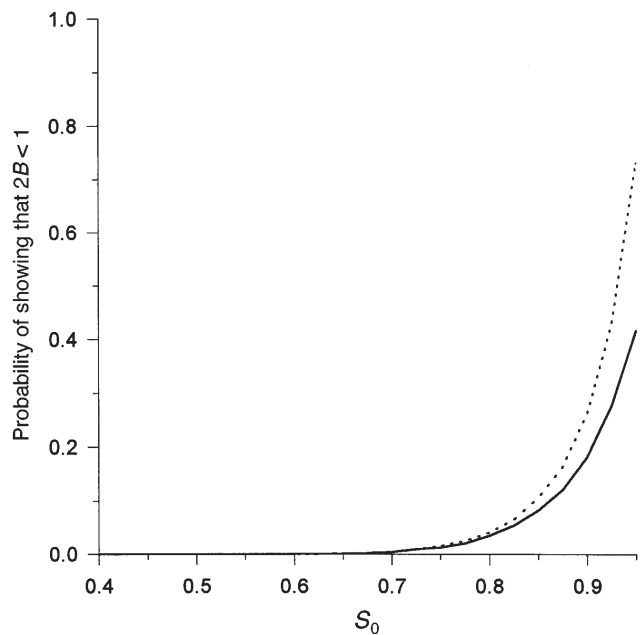


Fig. 5. Probability of the few-founders (broken line) and many-founders (continuous line) pedigrees showing that $2B < 1$ as a function of the non-inbred viability, S_0 , when $2B = 0$.

Statistical power as a function of pedigree structure

We used additional simulations to investigate how the properties of pedigrees affect the probability of demonstrating whether inbreeding depression is present or minimal. To facilitate comparison, we assumed S_0 and $2B$ to have values of 0.73 and 3.14, respectively. First, we examined our pedigree with few founders, which has

Table 1. Changes in the two idealized pedigrees by varying the total number of births, N , and average inbreeding coefficient, \bar{f} , and how this influences the probability of these pedigrees showing $2B > 0$ or $2B < 1$

Pedigree	N	\bar{f}	Probability of showing $2B > 0$ when $S_0 = 0.73$ and $2B = 3.14$	Probability of showing $2B < 1$ when $S_0 = -0.73$ and $2B = 0$
Few-founders				
As described	200	0.1800	0.32	0.01
Even distribution	200	0.1406	0.65	0.07
$2 \times$ individuals	400	0.1800	0.56	0.07
Many-founders				
As described	600	0.0313	0.53	0.01
$0.5 \times$ individuals	300	0.0313	0.30	0.00
$2 \times$ inbreeding	600	0.0625	0.96	0.25

most of its members in a few inbreeding classes of intermediate value. We found that if each inbreeding class had an equal number of individuals, then the pedigree would be twice as likely to detect inbreeding depression (see summary of results in Table 1). Additionally, we found that distributing the individuals equally among the four inbreeding classes resulted in greater statistical power than doubling the number of individuals in the pedigree.

When we examined the pedigree with many founders, we found that detecting inbreeding depression was almost ensured (0.96) if the amount of inbreeding was doubled and unlikely (0.30) when only half as many individuals were examined.

For both pedigrees, the probability of showing that $2B < 1$ when $2B$ was actually zero was quite low, and was less affected by increasing the size of the pedigree or the amount of inbreeding.

DISCUSSION

We have found that the breeding programmes designed to retain genetic variation and avoid inbreeding depression produce pedigrees that make the analysis of the effect of inbreeding upon viability difficult. This difficulty includes demonstrating that inbreeding depression is either present or is minimal and occurs in both of our idealized pedigrees, although it is greatest in the pedigree with few founders.

Departures from the model of inbreeding depression that we have used will affect estimates of inbreeding depression. Such departures are inevitable and may occur in many ways. For example, the actual relationship between inbreeding and viability may be somewhat different from the model of Morton *et al.* (1956), and several alternative models have been proposed. However, current data have been unable to differentiate between models (Makov & Bittles, 1986), largely because many of the models predict very similar relationships. For example, the model we have used is similar to a linear relationship for moderate levels of inbreeding when $2B$ is as high as 8. Perhaps more importantly, variables not accounted for in the model of

Morton *et al.* (1956) may affect viability. Lacy *et al.* (1996) and Ballou (1997) have shown that maternal inbreeding affects viability among offspring. In addition, purging of alleles causing inbreeding depression may decrease inbreeding depression during the course of a breeding programme. Thirdly, and perhaps most important, husbandry conditions may affect viability. For example, Wielebnowski (1996) has documented varying juvenile survival in cheetahs between zoos. In addition, year of birth has been shown to affect viability of non-inbred births (e.g. Wielebnowski, 1996; Ballou, 1997; Kalinowski, Hedrick & Miller, 1999). Furthermore, improving husbandry may reduce observed inbreeding depression without affecting the viability of non-inbred individuals. In addition, viability is only one trait that inbreeding can influence and estimating the number of lethal equivalents is likely to underestimate the total magnitude of inbreeding depression, cumulative over all components of fitness.

Our finding that typical pedigrees of endangered species have low statistical power for detecting inbreeding depression shows that many pedigrees may be consistent with both moderate inbreeding depression and no inbreeding depression. This implies that captive-breeding programmes for endangered species will not reveal the amount of deleterious genetic variation in many species. In fact, breeding programmes designed to maximize gene diversity will produce pedigrees with minimal ability to detect inbreeding depression. In studies of natural populations, low levels of inbreeding and other factors could also limit statistical power to detect inbreeding depression, perhaps even more so than in captive populations. In some circumstances, understanding the effect of inbreeding will be impossible or will require experimental work. For most species, experimental inbreeding will not be appropriate, but this approach has been useful (Sheffer, Hedrick & Velasco, 1999). In the remainder of cases, the conservative approach will be to continue to avoid inbreeding and to accept a fair amount of uncertainty regarding the effect of inbreeding.

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APPENDIX

The number of births with the inbreeding coefficient, f , listed in two hypothetical pedigrees, ‘few-founders’ and ‘many-founders’

f	Number of births	
	Few-founders	Many-founders
0.0000	8	300
0.0313	–	48
0.0391	–	16
0.0430	–	10
0.0469	–	24
0.0508	–	10
0.0547	–	18
0.0586	–	10
0.0625	–	52
0.0664	–	10
0.0703	–	18
0.0742	–	10
0.0781	–	24
0.0820	–	10
0.0859	–	16
0.1250	46	24
0.1875	100	–
0.1250	46	–
Total	200	600