

Inbreeding depression in captive bighorn sheep

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Abstract

We estimated the extent of inbreeding depression for juvenile survival in 589 captive-born bighorn sheep, and, unlike an earlier report, found no evidence of significant inbreeding depression. There did not appear to be any overall effect of year of birth, place of birth, subspecies, sex or ancestral inbreeding upon the viability of inbred animals as compared to non-inbred animals. However, for the first few years of data, there was lower survival for inbred than non-inbred offspring. After this period, there was an increase in the viability of inbred offspring, probably reflecting a general improvement in husbandry conditions in the zoos. It should be emphasized that the lack of significant inbreeding depression does not necessarily imply that there may not be inbreeding depression for other traits or in a less benign or more natural environment for juvenile viability.

INTRODUCTION

Inbreeding depression has been found in a large proportion of species examined (Ralls, Ballou & Templeton, 1988; Lacy, 1997), including the cheetah (Wielebnowski, 1996) and highly selfed plants (Husband & Schemske, 1996), although the statistical power to detect an effect is limited in many species and generally only part of fitness has been evaluated in a given species (Hedrick & Kalinowski, 2000). As a result, captive-breeding programmes have been designed so that the reduction of fitness resulting from inbreeding depression can be avoided. However, in some species with relatively large sample numbers and reasonable statistical power, no inbreeding depression has been detected (Kalinowski, Hedrick & Miller, 1999). It is not clear how often low amounts of inbreeding depression occur but there does not appear to be significant inbreeding depression for all traits in all environments.

Bighorn sheep, *Ovis canadensis*, have greatly declined in numbers over the twentieth century. As a result, the peninsular bighorn sheep in southern California has been listed as a federal endangered species and the desert bighorn in New Mexico has been listed as endangered in that state. A number of factors have been implicated in this decline, particularly disease transmission and competition from livestock, destruction and fragmentation of habitat, and hunting. However, the decline in fitness in some populations has been related by several

authors (e.g. DeForge *et al.*, 1979; Hass, 1989) to inbreeding depression, and Berger (1990) suggested that smaller populations of bighorn sheep are more likely to go extinct, partly because of genetic factors. As suggested by Gilpin & Soulé (1986), inbreeding depression and other factors reducing population numbers may interact and result in a greater decline than predicted from these factors individually.

Sausman (1984) compared 6-month survival of 172 non-inbred and inbred bighorn sheep, captive-born in the 1970s and early 1980s. She found that survival of the non-inbred animals was 78% while that of the inbred animals was only 46%, suggesting that inbreeding depression was quite important in this species. In addition, she found that the survival of inbred female sheep was significantly lower than inbred male sheep. Since Sausman's study, a large number of additional bighorn sheep have been born in captivity. To determine whether the higher mortality she found in inbred offspring in the early sample is present in these more recently born sheep, we examined the survival of these additional animals. Overall, the inbreeding depression that she observed does not appear to be present in this more recent sample. We examine factors that may explain the basis for the difference in these results and discuss the implications of our findings.

MATERIALS AND METHODS

We used studbook data submitted to the International Species Information System (ISIS) by North American zoos to examine the effect of inbreeding upon the

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juvenile viability of captive-born individuals. This studbook does not have a designated keeper, so cautious use of these data is necessary in order to prevent missing or incorrect data from affecting the analysis (see Discussion). With this in mind, we used the Single Population Analysis Records Keeping System (SPARKS, 1994) batch edit function to identify obvious errors in the data. For example, individuals born before their parents, animals that had sire or dam identification that was not consistent with the naming convention of the database, and some other errors, were not included in our analysis. We defined juvenile viability as survival to 180 days. Inbreeding coefficients were calculated assuming that unknown parents were founders of the captive population. This assumption can potentially influence results (see Discussion), so we did not include individuals with unknown parentage in our viability analysis, although we did include their descendants. The studbook contained individuals meeting the above criteria born from 1964 to 1995, although few individuals were born before 1976. Births occurring before 1976 accounted for only 1.7% of the data, and most years in this period had zero or one births. In order to increase the homogeneity of the data, we included only individuals born in 1976 or later. After these considerations, data from 589 births remained.

We used the maximum likelihood approach outlined by Kalinowski & Hedrick (1998) to determine the effect of inbreeding on survival based on the model of Morton, Crow & Muller (1956). Using this approach, $2B$ is approximately equal to the average number of lethal equivalents for a diploid individual. If $2B = 0$, then survival does not decline with increased inbreeding. As shown by Kalinowski & Hedrick (1998), the maximum likelihood approach for estimation of $2B$ is median unbiased and gives confidence intervals with the stated degree of accuracy, unlike the linear regression approach which has been previously used (Templeton & Read, 1983; Ralls *et al.*, 1988).

We then examined the effect of time of birth, place of birth, subspecies, sex and history of inbreeding on survival for non-inbred and inbred sheep. In all these cases, we used the G test (Sokal & Rohlf, 1995) to test for significant differences between categories.

RESULTS

Figure 1 gives a plot of the 6-month survival for all the animals, categorized by their different inbreeding coefficients. The area of the circle is proportional to the number of individuals born with each inbreeding coefficient. For example, there are 416 animals with an inbreeding coefficient of 0.0. The maximum likelihood estimate of B is 0.23 with a 95% confidence interval of [0.00, 0.79]. If the estimate of B is 0.23, then the estimate of $2B$ is twice that, i.e., 0.46, and the confidence interval is [0.00, 1.58). In other words, there is no evidence of a statistically significant decline in survival with increasing inbreeding coefficient. In fact, the upper confidence limit here for $2B$ (1.58) is lower than the

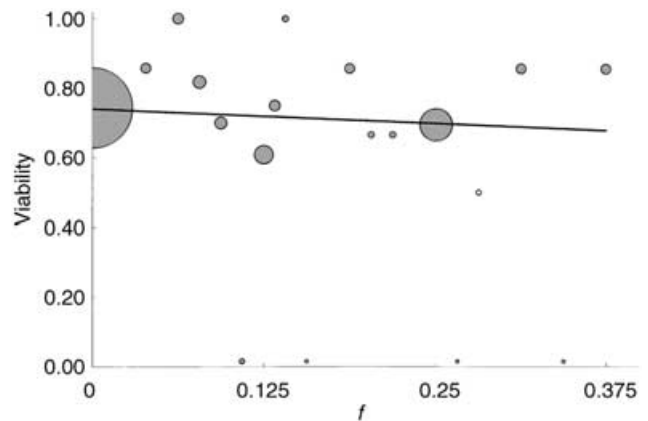


Fig. 1. Six-month survival as a function of the inbreeding coefficient where the sizes of the circles indicate sample sizes for the different inbreeding classes and the line is the maximum likelihood fit.

median (3.14) observed over a number of species by Ralls *et al.* (1988), suggesting that inbreeding has a smaller impact on juvenile survival in bighorn sheep than in many other species.

We then categorized animals as non-inbred ($f = 0$) or inbred ($f > 0$) to determine whether other factors may make obvious contributions to survival. First, Fig. 2 gives the survival as a function of year of birth. For the first 4 years, 1976–79, the survival of inbred sheep was much reduced, and significantly different, from that for non-inbred sheep. In this period, 28 out of 42 non-inbred sheep survived (67%) and 9 out of 24 inbred sheep survived (38%). On the other hand, in the remainder of the data set (1980–95), the viability of inbred sheep was slightly higher, but not statistically significant, than non-inbred sheep (77% versus 73%, respectively).

To examine the effect of different facilities on survival, we examined survival of non-inbred and inbred animals at the nine facilities with more than 20 births since the study of Sausman (173 of the 181 births she

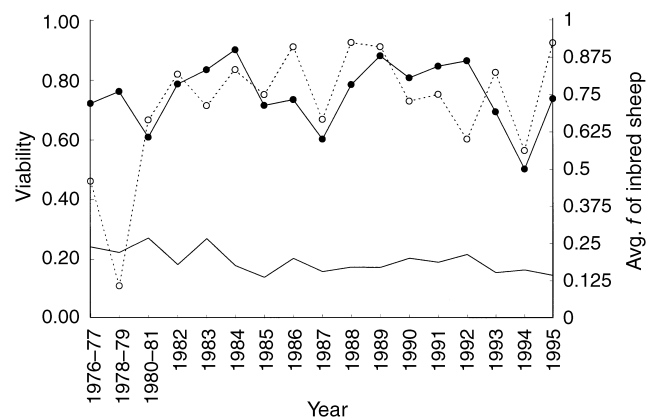


Fig. 2. Six-month survival presented by year of birth for inbred animals (broken line and open circles) and non-inbred animals (solid line and closed circles). Also given is the average inbreeding coefficient of the inbred sheep for different years (lower solid line).

examined were listed in the ISIS group we examined and are given in the first line of Table 1 as old records). Of the eight facilities that had both non-inbred and inbred offspring, four had higher survival for non-inbred offspring, three had higher survival for inbred offspring, and one had equal survival of non-inbred and inbred offspring (Table 1). None of the observed differences is significant at the 0.05 level although the comparisons for the Denver Zoo ($P = 0.08$) and the Los Angeles Zoo ($P = 0.06$) were near statistical significance (the Los Angeles Zoo for an apparent increase in viability).

Of the sheep, 538 were categorized to subspecies and Table 2 gives the 6-month survival for these sheep. There were no significant differences between non-inbred and inbred sheep for any of the subspecies. Although the overall survival varied with subspecies, with *Ovis canadensis cremnobates* having the lowest survival, this difference was not statistically significant. Data analysis also showed that there were not significant two-way interactions for any of the comparisons. In addition, there were 15 births of crosses between subspecies. Of the five non-inbred offspring, four survived

(80%), while for the ten inbred offspring, only three survived (30%). Although this sample size is very small, the large difference merits further investigation.

Of the sheep, 572 were of known sex, 289 females and 283 males (Table 3). The survival for female and male sheep, both non-inbred and inbred, was nearly identical, i.e., there was no significant sex-specific effect of sex on inbred survival.

Templeton & Read (1983) suggested that in the captive population of Speke's gazelle the survival of inbred animals that had inbred parents was higher than that of inbred animals with no history of inbreeding. The inbred bighorn sheep were classified into those whose parents were non-inbred and those that were inbred (Table 4). The survivorship for the two categories was nearly identical, indicating that survivorship in this sample was not influenced by previous inbreeding.

DISCUSSION

We examined the relationship between juvenile viability and inbreeding for captive bighorn sheep born from

Table 1. The number of bighorn sheep born (N) in the facilities with more than 20 births, the number of survivors (N_S), and the survivorship (S) for both the non-inbred and the inbred individuals. The average inbreeding coefficient f for the inbred births is also given. The old records are the animals examined by Sausman (1984) that we included in our overall data

Zoo	Total	Non-inbred			Inbred			
		N	N_S	S	N	N_S	S	f
Old records	173	124	90	0.73	53	30	0.57	0.225
Buffalo	88	57	52	0.91	31	26	0.84	0.147
Calgary	117	117	90	0.77	—	—	—	—
Denver	61	38	30	0.79	23	12	0.52	0.212
London RP	25	9	9	1.00	16	14	0.88	0.151
Los Angeles	37	27	16	0.59	10	9	0.90	0.153
Palm Desert	47	20	14	0.70	27	15	0.52	0.240
Phoenix	35	21	18	0.86	14	12	0.86	0.268
St Louis	23	17	11	0.65	6	5	0.83	0.344
Edmonton	27	23	15	0.65	4	3	0.75	0.250
18 other zoos	129	93	54	0.58	42	29	0.69	0.122
Total	589	416	309	0.74	173	124	0.72	0.191

Table 2. The number of non-inbred and inbred sheep born (N) surviving for 6 months (N_S) and the survivorship (S) for the five different subspecies

Subspecies	Total	Non-inbred			Inbred		
		N	N_S	S	N	N_S	S
<i>O. c. californiana</i>	38	27	20	0.74	11	9	0.82
<i>O. c. canadensis</i>	383	292	221	0.76	91	65	0.71
<i>O. c. cremnobates</i>	27	12	7	0.58	15	9	0.60
<i>O. c. mexicana</i>	43	28	23	0.82	15	13	0.87
<i>O. c. nelsoni</i>	47	35	22	0.63	12	11	0.92
Total	538	394	293	0.74	144	107	0.74

Table 3. The number of non-inbred and inbred, female and male, sheep born (N), surviving for 6 months (N_S), and the survivorship (S)

Sex	Total	Non-inbred			Inbred		
		N	N_S	S	N	N_S	S
Female	289	209	161	0.77	80	59	0.74
Male	283	195	145	0.74	88	65	0.74
Total	572	404	306	0.76	168	124	0.74

Table 4. The number born (N), surviving for 6 months (N_S) and the survivorship (S) of inbred lambs with non-inbred and inbred parents

	Total	N	N_S	S
Inbred offspring with non-inbred parents	204	119	85	0.71
Inbred offspring with inbred parents	93	54	39	0.72
Total	297	173	124	0.72

1976 to 1995 and found no evidence for inbreeding depression. In addition, we examined the effect of year of birth, place of birth, subspecies, sex and ancestral inbreeding upon viability. None of these variables had a strong or directional effect, or a statistically significant effect, upon viability, except that inbred sheep born in the early years of the study had significantly higher mortality than non-inbred ones.

In general, interpreting evidence for a lack of inbreeding depression must be done cautiously, for there are no good examples of a mammalian species being unaffected by inbreeding (Lacy, 1997). Although inbreeding depression appears to be almost universal, its effects vary across species, populations and traits, and it is difficult to measure (for reviews, see Lynch & Walsh, 1998; Hedrick & Kalinowski, 2000). In fact, Lacy, Alak & Walsh (1996) found that different lineages of the field mouse, *Peromyscus polionotus*, founded from the same population had different levels of inbreeding depression.

First, the structure of some pedigrees can hinder detection of inbreeding (Kalinowski & Hedrick, 1999), particularly if most of the individuals have a similar inbreeding coefficient. However, the bighorn sheep pedigree does not seem to suffer from this problem, because the confidence limits for the number of lethal equivalents are fairly narrow. Second, although we have found no evidence for inbreeding affecting juvenile survival, it is probably appropriate to assume that other traits will be affected by inbreeding. A third possibility for why we observed no inbreeding depression is that the captive environment compensated for the effects of deleterious genetic variation (see below).

Fourth, another possible explanation for the observed lack of inbreeding depression is that missing or inaccurate data influenced our results. In particular, Earnhardt, Thompson & Willis (1995) have suggested that the ISIS database may have a number of problems that may make it unsuitable for planning, management or evaluation of captive populations. However, Flesness *et al.* (1995), in a re-evaluation of the data examined by Earnhardt *et al.* (1995), found that the 'ISIS census data are reasonably accurate for the North American zoo populations'. In fact, Flesness *et al.* (1995) found that most of the census differences between the ISIS database and the records of the AZA (American Zoological Association) occurred because of non-zoo animals. When non-zoo animals were removed from the census counts, the overall correlation between the data sets was very high at 0.974. In our bighorn sheep data set, virtually all animals were from zoos, suggesting that many of the problems pointed out by Earnhardt *et al.* (1995) are not common in our data set.

In analysis of inbreeding levels, uncertain parentage in a pedigree can bias estimates of inbreeding depression downward. We assumed all unknown individuals to be unrelated founders, and in fact a large number of bighorn sheep have been brought into captivity from natural populations in which their relationship to other sheep was unknown. If some of these individuals were actually related, then we would have classified inbred individual progeny as non-inbred. If inbred individuals had a lower viability than non-inbred individuals, then this misclassification could then lower the estimated viability of non-inbred individuals. We partially addressed this issue in our analysis by not including individuals with unknown parents in our analysis (although we included their offspring).

At least two other approaches are possible. For example, descendants of unknown parents can be excluded from analysis. This approach can be overly conservative because it ignores what we do know about the recent pedigree of individuals with one or more unknown ancestors. For example, removing an individual with one unknown great-grandparent seems excessive. A potentially more refined approach is to calculate unbiased estimates of inbreeding coefficients (Ballou & Lacy, 1995), based on the proportion of each individual's genome that can be traced to wild-caught individuals. We applied both of these approaches to the bighorn sheep data and obtained essentially the same results as we have presented.

For example, of the 589 sheep in our data set, we were able to determine the complete ancestry of 123 sheep back to wild founders, so that their genomes are completely pedigreed. The maximum-likelihood estimate of $2B$ for this group is 1.20 with a 95% confidence interval of [0.00, 4.90], somewhat higher than for the total sample but still not significantly different from zero. For 180 sheep, we have 75% or more of the genome pedigreed. Based on an unbiased estimate of f calculated from the fraction of the genome that is known, the maximum-likelihood estimate of $2B$ for these sheep is 0.00 with a fairly narrow 95% confidence interval of [0.00, 1.54]. In other words, using the subset of data known with the greatest accuracy, our conclusions are still that there is no statistical support for inbreeding depression for juvenile survival.

Another test of this possibility can be made by comparing previous analysis of inbreeding depression in bighorn sheep by Sausman (1984) to our results. Sausman compared the viability of captive-born inbred bighorn sheep to non-inbred sheep and found a uniformly strong effect of inbreeding upon survival. She analyzed records from individual zoos and presumably had a better opportunity to prevent missing data from biasing results. Our larger and more recent data set does not show this trend. Sausman also observed a higher mortality among inbred females than males. We did not observe this in our data. Our data have only limited overlap with the data of Sausman, but a few interesting comparisons are possible.

We can compare our estimates of non-inbred viabil-

ity in the old records to those of Sausman to see if uncertain parentage and inbreeding has lowered our estimates of the viability of non-inbred individuals. In the three zoos with more than five non-inbred births (Buffalo, Calgary and Denver), our estimates of non-inbred viability (0.91, 0.77 and 0.79 respectively) are very similar to the previous estimates of Sausman (0.92, 0.76 and 0.75 respectively). This leads us to conclude that statistical artefacts are not responsible for the observation of no inbreeding depression.

One explanation for why we observed less inbreeding depression than Sausman (1984) is that changing zoo environments may have improved the viability of inbred births since Sausman collected her data. This hypothesis, unfortunately, is difficult to test, but available evidence seems to support it. Perhaps, most significantly, the lowest inbred viability in our data occurred before 1980, in the few years that our data overlap with the data of Sausman. As an example, for Palm Desert the survivals, after the early animals are removed, for non-inbred and inbred progeny are not significantly different (0.67 and 0.71 respectively). The apparent increase in viability after this period may reflect a permanent improvement in husbandry conditions among zoos. For example, the prophylactic use of anti-parasite medication became widespread for captive zoo ungulates during this period (D. Wharton, pers. comm.) and may have provided a significant change in husbandry. We can also compare our viability data for inbred individuals to that of Sausman in three zoos. In the Los Angeles Zoo, the viability of inbred sheep seems to have increased from Sausman's estimate of 0.54 to our estimate of 0.90 ($P = 0.06$), but this could be explained by a decrease in inbreeding among the inbred individuals. In the Denver and Palm Desert zoos, our estimates of inbred viability (0.52 and 0.52 respectively) are similar to those of Sausman (0.50 and 0.38 respectively).

Our suggestion that the viability of inbred individuals may increase with improving environmental conditions is not unusual (e.g., Ralls *et al.*, 1988; Kalinowski *et al.*, 1999). Empirical evidence for increased inbreeding depression in stressful environments is accumulating (Hedrick & Kalinowski, 2000). However, our suggestion that this increase in viability may occur undetected without an increase in non-inbred viability is more novel. There is little evidence of this phenomenon, but the Speke's gazelle captive-breeding programme (Templeton & Read, 1983) may be an example. This breeding programme has popularly been discussed as an example of selection reducing the genetic load of a captive population, but reanalysis of the data has shown that the viability of first-generation inbred gazelles increased during the course of the captive-breeding programme without affecting the viability of non-inbred births (Kalinowski *et al.*, 2000). This appears to explain the increase in viability that has previously been described as occurring after the first generation of inbreeding.

We have found no evidence for inbreeding depression in the captive bighorn sheep data that we have reviewed,

and we suggest that improving zoo conditions could explain the difference from the earlier results of Sausman (1984). We can interpret these results as a caution that inbreeding can be difficult to measure, even in captive environments. Further, this suggests that using captive populations to estimate the effect of inbreeding in wild environments will also be difficult.

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