Simultaneous inbreeding and outbreeding depression in reintroduced Arabian oryx

T. C. Marshall[1] and J. A. Spalton[2]

[1]Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK
[2]Office of the Adviser for Conservation of the Environment, Diwan of Royal Court, P.O. Box 246, Muscat 113, Sultanate of Oman

Abstract

In most species the offspring of closely related parents have reduced fitness compared with the offspring of unrelated parents, a phenomenon known as inbreeding depression. However if parents are very distantly related, their offspring may also have reduced fitness. This pattern, outbreeding depression, has been most commonly observed in plants and only rarely in animals. Here we examine the consequences of inbreeding and outbreeding on juvenile survival of reintroduced Arabian oryx (*Oryx leucoryx*) in Oman, a population with a small number of founders drawn from a number of sources. Using microsatellite-based measures of inbreeding and outbreeding, there was no apparent relationship between inbreeding or outbreeding and survival when inbreeding and outbreeding were tested in separate statistical models. However when inbreeding and outbreeding were tested in the same statistical model, we found simultaneous inbreeding depression and outbreeding depression acting on juvenile survival. Outbreeding depression may be more common in vertebrates than previously supposed, and conservation strategies that seek to maximize the genetic diversity of managed populations may risk mixing lineages that are sufficiently differentiated to cause outbreeding depression among descendants.

INTRODUCTION

Animal and plant breeders have known for a long time that inbreeding generates offspring with lower fitness than their non-inbred counterparts (Darwin, 1868; Falconer & Mackay, 1996), an observation termed inbreeding depression. Inbreeding depression is thought to arise from the accumulation of deleterious recessive mutations or the existence of loci with heterozygote advantage (Charlesworth & Charlesworth, 1987). More recently a number of studies have demonstrated inbreeding depression in non-domestic animals both in captivity (for reviews, see Ralls, Brugger & Ballou, 1979; Ballou & Ralls, 1982; Lacy, Petric & Warneke, 1993) and in the wild (Greenwood, Harvey & Perrins, 1978; Van Noordwijk & Scharloo, 1981; Ralls, Harvey & Lyles, 1986; Stockley et al., 1993; Bensch, Hasselquist & Von Schantz, 1994; Jiménez et al., 1994; Keller et al., 1994; Coltman, Bowen & Wright, 1998; Coulson et al., 1998; Keller, 1998; Coltman et al., 1999; Slate et al., 2000), although some studies of wild populations (Gibbs & Grant, 1989; Hoogland, 1992; Grant & Grant, 1995) failed

to find inbreeding depression (see also reviews by Frankham, 1995a; Crnokrak & Roff, 1999). Inbreeding depression may be sufficiently intense to cause local population extinction if population sizes are small (Frankham, 1995b; Saccheri et al., 1998; see also Frankham, 1998).

However, offspring fitness may not be maximized by breeding with the most distantly related mate (Templeton et al., 1986). Low fitness of offspring under outbreeding, known as outbreeding depression, occurs either by the disruption of complexes of interacting genes that have jointly evolved under natural selection or by mixing of genomes adapted to different environments (Templeton, 1986; Waser, 1993). Outbreeding depression is thought to be less common than inbreeding depression. It is most frequently observed in plants (for a review, see Waser, 1993) but has also been detected in marine invertebrates (for a review, see Knowlton & Jackson, 1993) and in captive callimico (*Callimico goeldii*; Lacy et al., 1993). Most recently, Coulson et al. (1998, 1999) found that different components of fitness in free-living red deer (*Cervus elaphus*) may be negatively or positively associated with outbreeding.

Here we examine the impact of inbreeding and outbreeding on juvenile survival of reintroduced Arabian

All correspondence to : Dr Tristan Marshall. Tel: +44 7932 698 445; Fax: +44 131 650 6564; E-mail: tristan.marshall@ed.ac.uk.

oryx (*Oryx leucoryx*), a species regarded as a paradigm of conservation by captive breeding and reintroduction (Stanley Price, 1989). The captive populations that provided oryx for the reintroduction in Oman had a small number of founding animals. The reintroduced population may therefore be vulnerable to inbreeding depression. However the captive populations are themselves thought to have been founded from several geographical areas, and it is therefore possible that hybrids between lineages may be vulnerable to outbreeding depression. In this study we use microsatellite-based measures of inbreeding and outbreeding to show that reintroduced Arabian oryx in Oman suffer, simultaneously, from both inbreeding depression and outbreeding depression.

MATERIALS AND METHODS

Study population

The Arabian oryx was formerly distributed across most of the Arabian peninsula, but hunting pressure, particularly in the 1950s and 1960s, led to a dramatic decline in its range and eventually to extinction in the wild in 1972 (Henderson, 1974). Captive breeding was initiated at Phoenix Zoo, Arizona in 1963 using three oryx captured in eastern Yemen in 1962, one captured in central Oman in 1960, four captive oryx from Saudi Arabia and one non-breeding captive animal from Kuwait. A second breeding group was founded at Los Angeles Zoo in 1967 with a pair from the same Saudi Arabian group that contributed to the Phoenix breeding programme. Between 1963 and 1977, the captive population in the USA increased at 17.2% per year and, in 1978, the first oryx were sent back to the Middle East (Stanley Price, 1989).

From 1980 onwards Arabian oryx were gradually reintroduced to the Jiddat-al-Harasis, a stony desert plateau in central Oman that is now a World Heritage Site known as the Arabian Oryx Sanctuary (AOS). Five releases took place in the AOS in the 1980s and early 1990s involving 34 captive-bred animals, mostly from the USA but also some animals of Qatari ancestry (Fig. 1). A three year drought and the presence of a sterile dominant male slowed the rate of increase of the
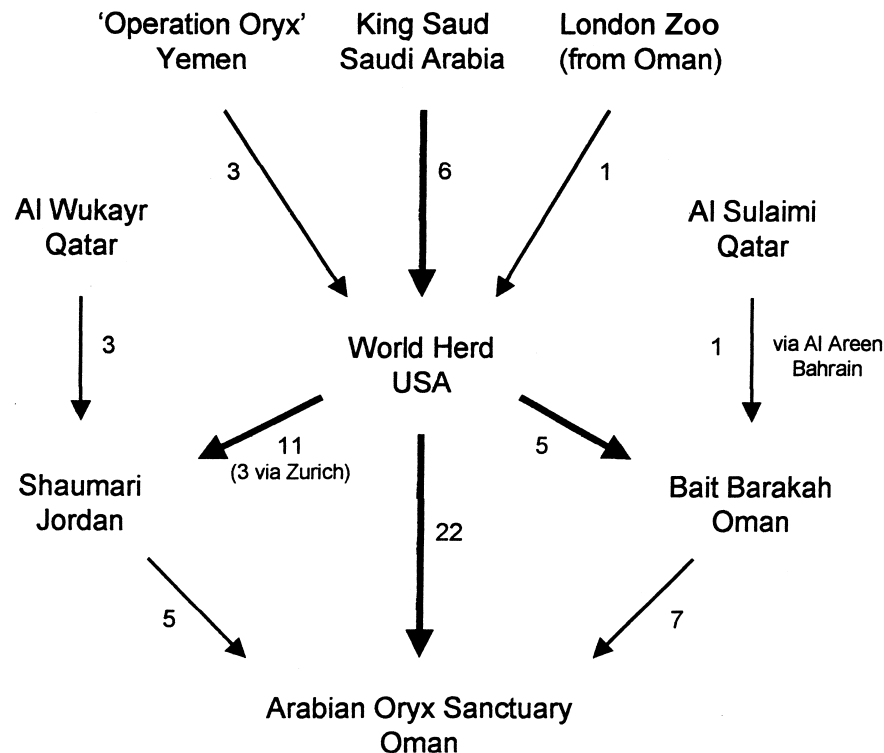

Fig. 1. The sources of Arabian oryx reintroduced to the Arabian Oryx Sanctuary, Oman up to December 1993. 'Operation Oryx' was a capture expedition mounted in the Eastern Aden Protectorate, now Yemen (Grimwood, 1962), and the animal from London Zoo was captured in central Oman (Woolley, 1962). HM King Saud of Saudi Arabia provided two pairs of oryx for the initial breeding herd in the USA at Phoenix Zoo, and it is thought that a third pair, later sent from Riyadh to Los Angeles Zoo, was from the same source. King Saud's oryx are thought to have originated from one or more capture expeditions. Al Wukayr and Al Sulaimi were two separate private collections in Qatar thought to have been founded independently using wild-caught oryx. A non-breeding female sent from Kuwait to Phoenix Zoo has been omitted for clarity. The Shaumari Wildlife Reserve in eastern Jordan and the Bait Barakah Breeding Centre in Muscat, Oman are two additional locations at which oryx reintroduced to the Arabian Oryx Sanctuary were bred. The number of oryx transferred is indicated in each case, and a bold arrow is used to indicate the lineage that provided the largest number of oryx to found each group. Further details of the history of Arabian oryx in the Middle East may be found in Stanley Price (1989), Marshall (1998) and Marshall *et al.* (1999).
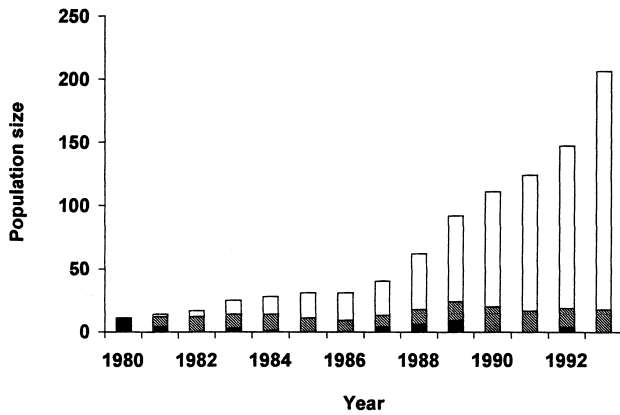
Fig. 2. Number of Arabian oryx in the reintroduced population on the Jiddat-al-Harasis, Oman on 31st December each year from 1980–1993, including animals in the pre-release enclosure. Note that only 34 of the 41 captive-bred oryx brought to the Jiddat-al-Harasis were actually released during this period. [☐], wild-born oryx; [▨], surviving captive-bred oryx; [■], new captive-bred oryx.

Fig. 3. Number of Arabian oryx calves born on the Jiddat-al-Harasis, Oman from 1987–1993. For each year calves that died during their first year of life (■) are shown separately from calves that survived (☐) this period. The data for 1993 include only calves born before 8th May.

reintroduced population during the early years. Following rainfall in 1986, when supplementary feeding was discontinued, the population increased at an average rate of 31% per year between 1987 and 1993 (Fig. 2).

Between January 1987 and May 1993, J.A.S. collected detailed data to assess the factors influencing mortality of the individually-monitored Arabian oryx population in the Sanctuary (Spalton, 1995). A total of 173 calves were born during this period. Mortality of calves during their first year of life accounted for 53% of all recorded deaths and was concentrated in the drought of 1991 (Fig. 3; Spalton, 1993). Blood samples were obtained from live oryx whenever possible, and skin or horn samples were collected post mortem. Oryx that died as juveniles were significantly more likely to have been sampled than oryx that survived (16 out of 27 versus 41 out of 146; $\chi^2 = 8.66$, d.f. = 1, $P = 0.003$), but we believe that among oryx that died and oryx that survived, probability of sampling was random with respect to inbreeding and outbreeding, and therefore that statistical models relating juvenile survival to inbreeding and outbreeding should not be affected by the bias in sampling in favour of oryx that died.

In a previous analysis, crude protein content of *Stipagrostis* spp., the preferred food of Arabian oryx, was calculated for each oryx on each day. Information on the decline in crude protein content following last rainfall (Spalton, 1999), obtained from a detailed vegetation survey carried out in 1989, was combined with long-term spatial rainfall records and daily data on the location of each oryx. Percentage crude protein content of *Stipagrostis* grasses available to the calf's mother in the 30 days before and 60 days after parturition was the major factor influencing juvenile survival of the 173 Arabian oryx born between January 1987 and May 1993. Higher levels of crude protein, corresponding to periods immediately following rainfall, led to higher juvenile survival (Spalton, 1995).
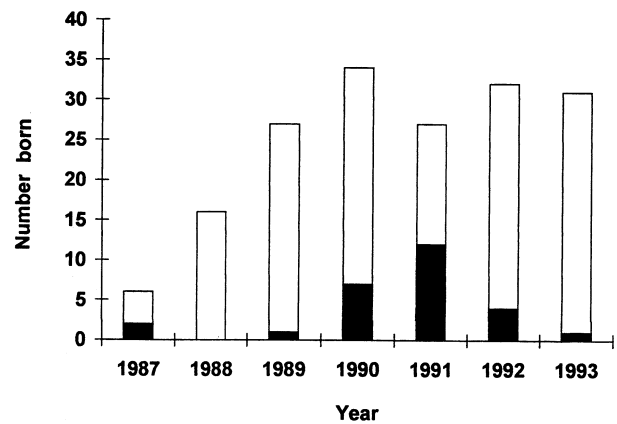
Measures of inbreeding and outbreeding

DNA samples were obtained from 57 oryx born between 1987 and 1993 (33% of 173). We typed all 57 oryx for six microsatellite loci (Marshall *et al.*, 1999). Using these data we calculated individual heterozygosity (a measure of inbreeding) and the microsatellite-based measure mean d^2 (a measure of outbreeding) across the six loci for each oryx (Table 1).

Heterozygosity at each locus is a binary variable, with the value 1 if alleles differ and the value 0 if they do not. Individual heterozygosity is the mean across loci of these heterozygosity values. More inbred animals have lower individual heterozygosity because there is a higher probability of both alleles at each locus having been descended from a single common ancestor. Although in our analysis individual heterozygosity could only take five discrete values (the 57 oryx were heterozygous for between zero and four loci out of six), we treated it as a continuous variable to give optimum statistical power, noting that the generalized linear models used in our analysis (see Statistical analysis, below) are tolerant of explanatory variables that deviate from a normal distribution.

Table 1. Microsatellite loci used in analysis of inbreeding and outbreeding in Arabian oryx

Locus	Number of alleles	Average heterozygosity	Maximum d^2	Average d^2
RPB3	2	0.088	1	0.088
OarFCB304	2	0.456	1	0.456
MAF50	4	0.456	4	0.956
MAF46	4	0.421	25	4.281
OarCP26	5	0.491	25	2.018
OarHH64	5	0.596	64	5.737

Primer references and typing protocols may be found in Marshall *et al.* (1999). The figures given for the number of alleles, average observed heterozygosity, maximum d^2 and average d^2 score refer to the 57 oryx that were sampled in this study.

Mean d^2 (Coulson *et al.*, 1998) is related to individual heterozygosity but incorporates allele length information. The value of d^2 at each locus is the squared difference in allele lengths, measured in repeat units (one repeat was 2 base pairs (bp) for all loci in this study). Mean d^2 is the mean across loci of these d^2 values. More outbred individuals have higher mean d^2 scores because on average at each locus a longer time has elapsed since the two alleles at each locus shared a common ancestor, and hence there has been more opportunity for the allele lengths to diverge via stepwise mutation (Pemberton *et al.*, 1999). Mean d^2 was treated as a continuous variable. Although the maximum d^2 varies considerably between loci (Table 1), it is not appropriate to scale d^2 values by maximum d^2 at each locus prior to taking the mean (this procedure would constrain scaled d^2 values to vary between zero and one), because those loci with high maximum d^2 carry much more information about outbreeding than loci with low maximum d^2.

Following Coulson *et al.* (1999) we also calculated mean $d^2_{outbreeding}$ by taking the mean of d^2 values for each animal across heterozygous loci only. Because three oryx were homozygous at all six loci, mean $d^2_{outbreeding}$ could be calculated for only 54 out of the 57 sampled oryx. The distributions of mean d^2 and mean $d^2_{outbreeding}$ scores were right-skewed, and, following Coltman *et al.* (1998), scores were transformed as $\log_e (x + 1)$ prior to statistical analysis.

Individual heterozygosity was positively correlated with mean d^2 ($r = 0.522$, d.f. = 55, $P < 0.001$) but not significantly correlated with mean $d^2_{outbreeding}$ ($r = 0.210$, d.f. = 52, $P = 0.131$). Mean d^2 and mean $d^2_{outbreeding}$ were highly positively correlated ($r = 0.956$, d.f. = 52, $P < 0.001$).

Statistical analysis

Logistic regression models (McCullagh & Nelder, 1989) were developed using GENSTAT 5 (NAG, Oxford), with a binomial response of 1 representing survival to one year of age and 0 representing failure to survive to one year. In all models we fitted a term representing the percentage crude protein content of *Stipagrostis* spp. (Spalton, 1995, 1999) prior to testing the genetic terms individual heterozygosity, mean d^2 and mean $d^2_{outbreeding}$. The significance of terms not included in the final model was assessed by the change in deviance, distributed approximately as χ^2, when they were added to the final model, while the significance of terms included in the final model was assessed by the change in deviance when they were dropped from the final model.

RESULTS

We assessed the impact of inbreeding and outbreeding on survival of 57 Arabian oryx calves for which individual heterozygosity and mean d^2 were available. Controlling for crude protein content of *Stipagrostis* spp. ($X^2 = 11.09$, d.f. = 1, $P = 0.001$), we found that fitted singly, neither individual heterozygosity ($X^2 = 2.29$, d.f. = 1, $P = 0.130$) nor mean d^2 ($X^2 = 1.94$, d.f. = 1, $P = 0.164$) were associated with juvenile survival. However when individual heterozygosity and mean d^2 were fitted to the model jointly, both were significant (individual heterozygosity: $X^2 = 7.14$, d.f. = 1, $P = 0.008$; mean d^2: $X^2 = 6.79$, d.f. = 1, $P = 0.009$; both terms together: $X^2 = 9.09$, d.f. = 2, $P = 0.011$). Crude protein and individual heterozygosity were positively associated with juvenile survival and mean d^2 was negatively associated with juvenile survival (Fig. 4). Crude protein, individual heterozygosity and mean d^2 together explained 27% of the total deviance in the model. The robustness of the model was supported by a balanced bootstrap analysis (1000 replicates): 95% confidence intervals for crude protein, individual heterozygosity and mean d^2 coefficients in the logistic model did not include zero. Furthermore restricting the analysis to those animals born in less favourable environmental conditions (when crude protein content of *Stipagrostis* spp. was less than 6.5%: see Fig. 4(a)) made no difference to the results (data not shown).

We examined the model further by replacing mean d^2 with mean $d^2_{outbreeding}$, a derivative of mean d^2 that is not correlated with individual heterozygosity because mean $d^2_{outbreeding}$ is calculated only across heterozygous loci (see Materials and Methods, above). Controlling for crude protein content of *Stipagrostis* spp. ($X^2 = 10.30$, d.f. = 1, $P = 0.001$), we found that fitted singly, individual heterozygosity was not associated with juvenile survival ($X^2 = 2.43$, d.f. = 1, $P = 0.119$) while mean $d^2_{outbreeding}$ was negatively associated with juvenile survival ($X^2 = 4.46$, d.f. = 1, $P = 0.035$). When individual heterozygosity and mean $d^2_{outbreeding}$ were fitted to the model jointly, both were significant (individual heterozygosity: $X^2 = 4.60$, d.f. = 1, $P = 0.032$; mean $d^2_{outbreeding}$: $X^2 = 6.63$, d.f. = 1, $P = 0.010$; both terms together: $X^2 = 9.05$, d.f. = 2, $P = 0.011$). Again crude protein and individual heterozygosity were positively associated with juvenile survival and mean $d^2_{outbreeding}$ was negatively associated with juvenile survival. This model illustrates clearly that the association between mean d^2 and survival is independent of the association between individual heterozygosity and survival.

Following the methodology described by Coulson *et al.* (1999) and Pemberton *et al.* (1999), we also tested the model for dependence on particular loci by testing each locus individually and by dropping each locus in turn from the calculation of both individual heterozygosity and mean d^2 (Table 2). Of the individual loci tested, only d^2 at locus OarCP26 showed a significant association with survival ($X^2 = 4.52$, d.f. = 1, $P = 0.038$). However the influence of this locus alone cannot explain the strength of the association found between overall mean d^2 and survival ($X^2 = 6.79$, d.f. = 1, $P = 0.009$), implying that other loci must have contributed to this association.

When each locus was dropped in turn from the analysis, the model changed in two cases. First, individual heterozygosity became non-significant when the locus MAF50 was excluded ($X^2 = 3.24$, d.f. = 1, $P = 0.071$), and second, mean d^2 became non-significant when the

locus OarCP26 was excluded (X^2 = 2.98, d.f. = 1, P = 0.084). However in both cases there remained a trend ($P < 0.1$) in the predicted direction. We conclude that the effects of individual heterozygosity and mean d^2 across all six loci cannot be completely explained by the influence of one locus alone.

DISCUSSION

Individual heterozygosity measures recent inbreeding, and mean d^2, while incorporating a component of heterozygosity, is thought to be most sensitive to outbreeding, particularly between populations that have
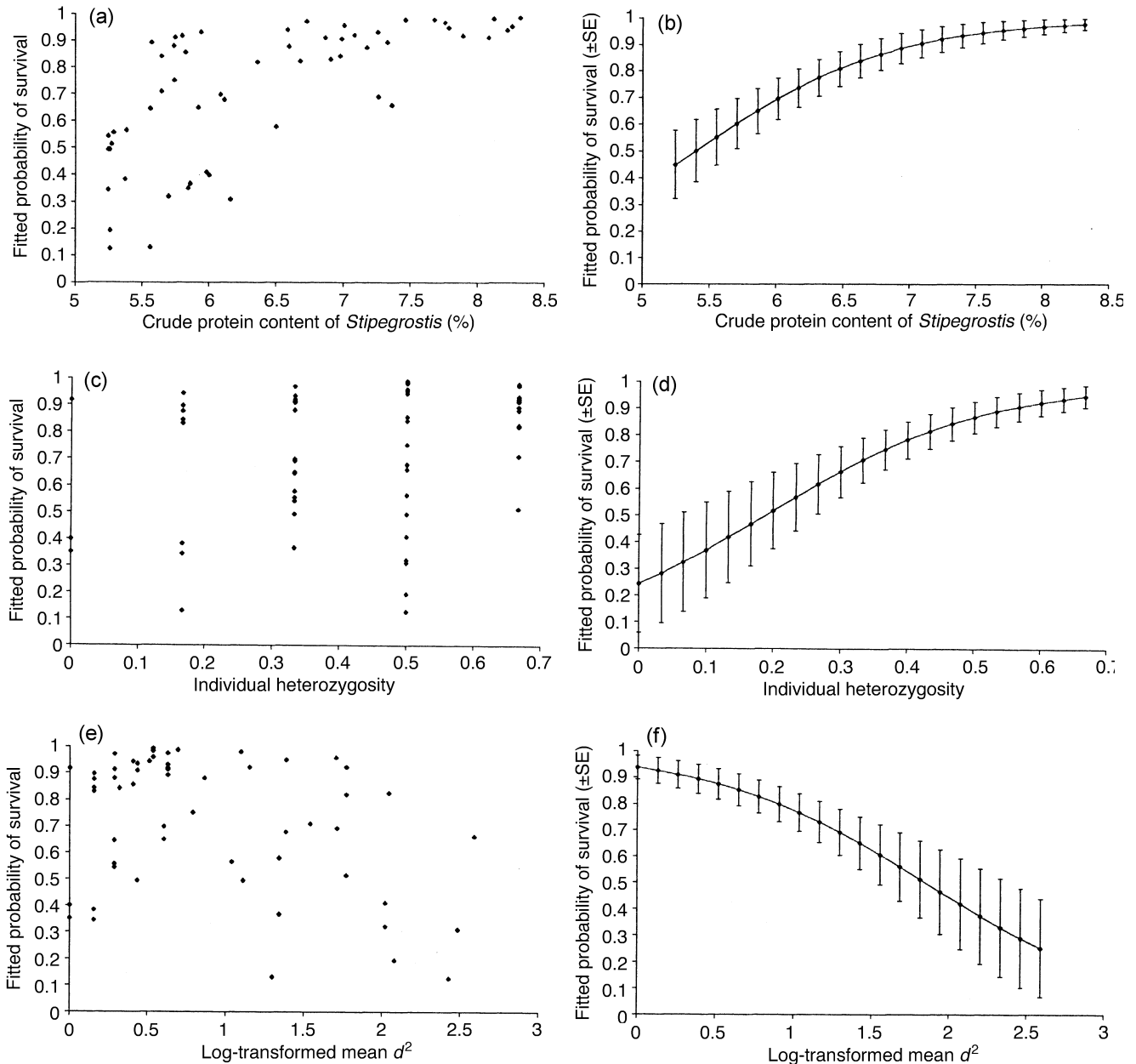

Fig. 4. Association of crude protein, individual heterozygosity and mean d^2 with juvenile survival. (a) and (b) relationship between crude protein and probability of survival. (c) and (d) relationship between individual heterozygosity and probability of survival. (e) and (f) relationship between mean d^2 and probability of survival. Plots (a), (c) and (e) show the fitted survival probability from the full model for each animal plotted against the explanatory variable, while plots (b), (d) and (f) show the predicted survival probability for 21 selected values and approximate standard errors of the predictions (error bars), along with a logistic curve illustrating the predicted relationship between survival probability and the explanatory variable. Predicted survival probabilities were obtained by setting each of the two explanatory variables not under consideration to its mean value across all individuals.

Table 2. An evaluation of the influence of individual loci on the model of the relationship between individual heterozygosity, mean d^2 and juvenile survival

Locus	Effect of locus fitted individually		Effect of removing locus	
	Heterozygosity	d^2	Heterozygosity	Mean d^2
RPB3	N/A	N/A	**0.011**	**0.014**
OarFCB304	N/A	N/A	**0.045**	**0.025**
MAF50	0.692	0.216	0.077	**0.019**
MAF46	0.613	0.346	**0.007**	**0.016**
OarCP26	0.110	**0.038**	**0.017**	0.090
OarHH64	0.386	0.319	**0.009**	**0.015**

In each cell the P-value for a model testing the influence of heterozygosity or d^2/mean d^2 at the given locus is shown. P-values shown in bold are significant ($P < 0.05$). Crude protein was fitted in all models; in models testing heterozygosity, d^2 or mean d^2 was fitted, while in models testing d^2 or mean d^2, heterozygosity was fitted. The effect of RPB3 and OarFCB304 as individual loci could not be assessed since these two loci were biallelic, and therefore heterozygosity and d^2 terms were completely correlated.

been separated sufficiently long for microsatellite allele lengths to diverge (Pemberton *et al.*, 1999). We therefore interpret the positive association between individual heterozygosity and juvenile survival as inbreeding depression, and the negative association between mean d^2 and juvenile survival as outbreeding depression. The fact that both very inbred and very outbred calves had low survival explains why neither measure showed an association with survival when fitted singly.

Only six loci were used in this study, and hence estimates of inbreeding provided by heterozygosity and of outbreeding provided by mean d^2 are relatively crude. However in common with several previous studies using individual heterozygosity and mean d^2 as measures of inbreeding and outbreeding (Coltman *et al.*, 1999; Coulson *et al.*, 1999; Pemberton *et al.*, 1999), we have tested the influence of individual loci, and believe that the absence of strong influences of individual loci on fitness favours the idea that individual heterozygosity and mean d^2 are measures of genome-wide inbreeding and outbreeding.

Given the modest number of individuals and loci typed, it is surprising that we were able to demonstrate inbreeding depression and outbreeding depression, and implies that these are strong influences on juvenile survival. Although deviances are not strictly additive in a logistic model, the change in deviance when individual heterozygosity and mean d^2 were both included in the model of survival ($X^2 = 9.09$) is of similar magnitude to the change in deviance when crude protein was included in the model ($X^2 = 11.09$), suggesting that inbreeding depression and outbreeding depression are together almost as important an influence on juvenile survival as the quality of grazing available to the oryx.

Inbreeding depression may be concealed by outbreeding depression

Inbreeding depression is an almost routine observation in many higher taxa including ruminants (Ralls *et al.*, 1979; Ballou & Ralls, 1982), and most studies use pedigree-based analysis of inbreeding coefficients to look for evidence of inbreeding depression (Crnokrak & Roff, 1999). A pedigree-based analysis in this same population of Arabian oryx did not detect a significant association between inbreeding coefficient and juvenile survival among 122 calves, although there was a non-significant trend ($P < 0.1$) for lower survival among more inbred calves (T. C. M., unpublished results). The absence of an association between inbreeding coefficient and fitness was surprising given that 24% of Arabian oryx in this population are closely ($F = 0.25$) or moderately ($F = 0.125$) inbred (T. C. M., unpublished results), giving a substantial sample of inbred matings in which to detect inbreeding depression.

To our knowledge this is the first study to document inbreeding depression concealed by simultaneous outbreeding depression. We suggest that studies finding no association between inbreeding and fitness should be wary of concluding that inbreeding depression is absent, and that it should become standard practice to test for outbreeding depression as well as inbreeding depression.

Causes of outbreeding depression in Arabian oryx

Hybridization is a term typically used to indicate mating between individuals belonging to distinct species. Hybridization commonly results in hybrid progeny with low fitness, if viable hybrids can be formed at all (Harrison, 1993; see also Greig, 1979). In contrast, the term outbreeding is most often used to refer to mating of distantly related individuals within a given species. Outbreeding depression therefore generally refers to the reduced fitness of progeny of distantly related individuals within a species. The implication is that the two individuals are so distantly related that their genes are no longer adapted to work with each other or with the environment in which they find themselves (Templeton, 1986; Waser, 1993).

The founders of the Arabian oryx population studied here are from at least five independent capture expeditions (Fig. 1). While documentation on all except two of these founder groups is extremely limited, it is unlikely that all five populations were founded with animals from the same part of the Arabian oryx's former range. The existence of outbreeding depression suggests that two or more of the founder animals or groups came from genetically differentiated populations.

Surprisingly allozyme and microsatellite data suggest relatively low levels of genetic differentiation between contemporary Arabian oryx populations (Vassart, Granjon & Greth, 1991*a*; Marshall *et al.*, 1999). This may be because these populations are mixtures of lineages, and samples representing the lineages prior to mixing are not available. However previous karyotyp-

ing work on Arabian oryx (in which the standard chromosomal complement is $2n = 58$) detected a Robertsonian translocation (a fusion between chromosomes 17 and 19) segregating in several populations in the Middle East (Vassart *et al.*, 1991*b*). In a limited survey we found that two males, introduced to the AOS population from Shaumari in Jordan, were heterozygous ($2n = 57$) for this same translocation (J. A. S., unpublished results). The pattern of occurrence in all herds is consistent with a single source of the translocation in the Al Wukayr population in Qatar, and this suggests some degree of genetic differentiation between the Al Wukayr lineage and other lineages (Fig. 1).

Implications for conservation

Our findings are important for two reasons. First, the existence of outbreeding depression implies that prior to extinction in the wild, there were genetically distinct but phenotypically cryptic races of Arabian oryx. Founders of the reintroduced population came from a vast span of the Middle East, and in each geographical area, genes may have become adapted to work with each other or with the local environment (Templeton, 1986). Our study suggests that intra-specific genetic differentiation sufficient to cause outbreeding depression may be a more widespread phenomenon in vertebrates than was previously thought.

Second, well-managed captive-breeding programmes aim to minimize inbreeding. However, in seeking to avoid inbreeding depression and ensuring future adaptability by maximizing the number of founders, populations managed for conservation may be vulnerable to outbreeding depression if founders are taken from genetically differentiated populations. In captive callimico, in which simultaneous inbreeding and outbreeding depression has also been found, inbreeding was an order of magnitude more powerful an influence on fitness than outbreeding (Lacy *et al.*, 1993). However in AOS Arabian oryx, outbreeding depression is a much more important influence on fitness: outbreeding explains as much variation in juvenile survival as inbreeding.

In Omani Arabian oryx, the high rate of intrinsic population growth (Fig. 2) suggests that the simultaneous inbreeding and outbreeding depression acting on the population is not currently a major threat to population viability. We note that the level of inbreeding in the AOS population of Arabian oryx (average $F = 0.10$) is considerably lower than the level at which population viability is likely to be threatened by inbreeding (Frankham, 1995*b*). Furthermore, subsequent experience has shown us that human factors, particularly illegal hunting, represent much greater threats to reintroduced Arabian oryx (Spalton, Lawrence & Brend, 1999). However, in other less fecund species, the joint action of inbreeding depression and outbreeding depression may in itself represent a serious obstacle to successful captive breeding and reintroduction.

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