Eur J Wildl Res
DOI 10.1007/s10344-009-0280-5

ORIGINAL PAPER

Maintaining the genetic health of putative Barbary lions in captivity: an analysis of Moroccan Royal Lions

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Received: 4 March 2009 / Revised: 30 April 2009 / Accepted: 12 May 2009

Abstract The last representatives of the Barbary lion (*Panthera leo leo*), once numerous in North Africa but exterminated from the wild by the 1940s, are believed to be the captive lions descended from the Moroccan Royal Collection, numbering less than 90 animals in zoos worldwide. The genetic fitness of these captive "Royal Lions" may now be under threat since, although most zoos have avoided hybridisation with animals of other origin, no formal breeding programme currently exists and several institutions have halted breeding activities. This situation has arisen since the distinctiveness of Barbary lions and the representative status of Royal Lions remain inconclusive and definitive molecular studies have yet to be completed. Previously, in the 1970s, morphological and phenotypic traits were used to match Royal Lions and the historic Barbary lion and an *ex situ* breeding programme was initiated involving a number of selected "founder" animals. This paper outlines the status of the descendent population within zoos in Morocco and Europe, including all known pure-bred descendents from the Royal Palace collection. Founder representation is shown to be greater across European collections than the Moroccan collection. Breeding exchanges are recommended between institutions in order to improve genetic diversity and maintain the genetic health of the population and a studbook for European zoo animals has been developed to support this action. This analysis serves as a benchmark for guiding effective maintenance of the captive population, thereby allowing time to clarify the conservation value of Royal Lions and their relevance to North African ecology.

Keywords *Panthera leo* · Inbreeding · Founder representation · Studbook · *Ex situ* conservation · Atlas Mountains

Communicated by W. Lutz

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Introduction

The history of the extinct Barbary lion (*Panthera leo leo*) and the status of the captive Moroccan Royal Lion collection as putative representatives of the subspecies are thoroughly described in the literature (Yamaguchi and Haddane 2002; Hemmer and Burger 2005). The Barbary lion was common in European menageries during the middle ages and more recently in public zoological parks, up to its final extermination from the wild in the Atlas Mountains during the 1940s (IUCN 2008). Since then, several captive lion groups have been proposed as Barbary descendents, but those from the King of Morocco's original collection, and their direct descendents in European zoos, have the strongest circumstantial claim (Yamaguchi and Haddane 2002).

Studies in the early 1970s recognised the potential of lions in the Moroccan Royal collection (hereafter referred

Published online: 10 June 2009

to as "Royal Lions") at Rabat Zoo, as putative representatives of the Barbary lion, and proposed a selective breeding programme involving international zoos (Leyhausen 1975; Hemmer 1978; Frankham et al. 1986; Yamaguchi and Haddane 2002; Hemmer and Burger 2005). The current Moroccan collection has arisen from the individuals recorded in a 1974 census although records are incomplete after 1978. Fortunately, however, all of the European-based individuals can be accurately traced back to the 1974 set of founders.

Within the European group, a number of animals are recorded as descendents from two individuals at Madrid zoo, which were obtained in 1977 from a circus (ISIS 2008) believed to have been originally obtained from the Royal Palace collection prior to 1974. The Madrid female gave birth to cubs in July 1979, suggesting an age on arrival at Madrid of 2 years or more. At that time, private ownership of Royal Lions was common; for example, 28 cubs born at Rabat Zoo were sold to circuses and private collections in Spain, Portugal and France between 1970 and 1973 (Yamaguchi and Haddane 2002).

Over the subsequent decades, Royal Lions and their offspring have been transferred between various institutions, particularly in the US and Europe (Hemmer 1978; Yamaguchi and Haddane 2002; Hemmer and Burger 2005). Several zoos which kept Royal Lions abandoned breeding programmes due to difficulties in obtaining new breeding stock and discouragement arising from the publication of genetic research which appeared to suggest that all lions shared a common ancestor (Yamaguchi 2000; Hemmer and Burger 2005). Nevertheless, in the 1990s, during a revival of interest in Royal Lions, an electronic record was collated from the handwritten breeding log books at Rabat Zoo and data from European and other western zoos known to hold the animals. The intention at that time was to use the data to inform breeding decisions by institutions interested in conserving the animals (Hill and Haynes 1999). However, by 1998, maintenance of this data source had lapsed, as had any clear inter-institutional commitment to preserve Royal Lions. Consequently, there is a risk that the purity and genetic health of the bloodline may be compromised (relative to the original Moroccan Royal Collection) in terms of inbreeding effects and a loss of genetic diversity. These problems would be amplified if future limited transfer of animals between institutions causes isolation of breeding groups. However, several institutions remain committed to maintaining their Royal Lion collections, providing opportunities to manage the population appropriately.

The genetic distinctiveness of the historical Barbary lion has not yet been fully established and the question over whether the Royal Lions are true Barbary lions remains unanswered (Dubach et al. 2005; Yamaguchi 2005; Barnett et al. 2006b; Burger and Hemmer 2006; Antunes et al. 2008). However, the historical Barbary lion is morphologically more distinct than any of the African lion populations (Hemmer 1978), so the guidelines of the precautionary principle (Foster et al. 2000) would suggest that reasonable action to conserve diversity is preferable. This suggests that maintenance of the Moroccan Lions as a separate management unit (albeit in captivity) distinct from other zoo lions is appropriate until better data can clarify their distinctiveness. Furthermore, the continued involvement of committed zoos makes this proposal a perfectly feasible programme of activity.

This study focuses on the European zoo collections (including Hai Kef zoo in Israel), which hold reliable zoo records. In addition, Rabat Zoo holds approximately 25 individuals but breeding records are incomplete and do not appear on the International Species Information System (ISIS 2008). However, reasonable inferences about these animals can be made from historical records of selective breeding concerning the existing Rabat collection.

We review the genetic ancestry of living animals and compile a European studbook in order to identify:

1. Age and gender demographics
2. Reproductive success
3. Founder representation in European collections versus the Rabat group and
4. Implications for a future breeding programme to maintain and improve the genetic health of this captive meta-population

Methods

Data sources and validation

Data to support a demographic assessment of the existing captive population has been drawn from a number of primary and secondary sources, in order to circumvent the lapse of breeding records for Royal Lions since 1998. Sources included data collated from both handwritten breeding records at Rabat Zoo and zoo records in western zoos (hereafter termed the "1998 Studbook"), International Species Information System database entries for "*Panthera leo leo*, North Africa" (ISIS 2008), recent records from zoos (which include paternity/maternity data), official zoo publications (Teichmann 2004; Veselá et al. 2005) and formally published journal articles. Informal sources included zoo websites, newsletters and personal communications between the authors and zoo staff.

A printed version of the 1998 Studbook for Royal Lions was validated against the ISIS species database (ISIS 2008). This information was analysed to identify those founder

animals (born before 1969) recorded in the Hemmer and Leyhausen census of the former Royal Palace collection (Leyhausen 1975), the breeding individuals from that population and subsequent offspring and parentage for all other animals arising between 1970 and 1998. This process was simplified by ignoring any animals which died without breeding (including juvenile deaths). Data for all animals including zoo ID, name (if known), birth location, last location, date of birth, sire and dam was initially collated in Excel (Microsoft Office v2003), then compiled in an Access (Microsoft Office v2003) database.

Data on living lions was collected from the later entries of the 1998 Studbook, together with an ISIS summary of zoos claiming to have holdings of "*Panthera leo leo*, North Africa" (ISIS 2008). Current holdings were cross-referenced against parental histories and individual animal entries for parents, using online ISIS queries, for comparison with the 1998 studbook.

Zoo websites and web-based zoo reports were scrutinised for supporting data on names, dates of birth, gender and parental identifiers. Several zoos claiming to hold "barbary lions" were contacted directly using an email questionnaire. This process revealed that some institutions have either not submitted records to ISIS or have since withdrawn claims for holdings of *Panthera leo leo* from the ISIS records, whilst others actively use the term "barbary lion" in marketing communications to visitors, but do not have ISIS records for these animals. Data validation relied on content validity and consistency of information quoted within a number of different sources.

Information on individual lions within the newly compiled studbook consists of zoo records going back to Hemmer and Leyhausen's 1974 census of pre-1969 founders. It should be noted that the European collections include the known remaining animals from American zoos (Yamaguchi 2005) which participated in the original Hemmer and Leyhausen project (Leyhausen 1975; Hemmer 1978; Yamaguchi and Haddane 2002). Animals in Morocco's Rabat Zoo collection do not have complete zoo records of maternity, paternity, birth date or other demographic information, so an equivalent studbook was not possible.

Each animal was allocated a unique reference number (STUD_ID) within the draft 2008 European Studbook, because many individual animals have multiple zoo ID numbers and, in some instances, multiple names. Only living animals and their parental lines reaching back to the 1969 founder group were included in the studbook. Dead non-breeders or failed breeders (i.e. those with no surviving descendents) were excluded, although all living non-breeding animals have been included since genetic and morphological data for these animals may become important for future analyses.

Analysis of parental lineage and founder gene representation

Initial analysis involved identification of founder representation across the major zoo collections and then for the total known population of Royal Lions (i.e. zoos in Morocco, Europe and outlying regions). Calculation involved mathematical proportioning of founder genes assuming an equal contribution from both sire and dam. The spread of founder gene representation was calculated forwards from the Royal Palace founders (STUD_ID 7, 8, 9, 10, 18, 20, 21, 27, 34, 37) and the two probable founders (STUD_ID 200, 201) from Madrid (Yamaguchi and Haddane 2002; ISIS 2008).

On the basis of these calculations, the proportion of founder genes represented in any one individual was estimated, together with the total proportion across the population. For Rabat Zoo lions, an estimate of founder representation was calculated as an average of the last known proportions of founder representation in 1978. A straightforward Excel (Microsoft Office v2003) spreadsheet was used for calculation of founder representation in animals of known parentage across a maximum of five generations since 1974. This was sufficient to generate the calculations of founder gene representation in each subsequent set of offspring. Excel statistical functions enabled analysis of means across sub-populations, based on the calculated founder representations for individual animals in each sub-population.

Demographic analysis of the Royal Lion population

Data from the draft 2008 European Studbook was used to identify distributions of population age, gender, opportunity for breeding pairs, cub mortality and fecundity and to reveal trends in these variables since the 1974 Royal Palace census.

The population growth rate r was not calculated since large numbers of animals have been removed from breeding programmes in recent years through neutering and contraception (population sizes being limited according to zoo capacity), rendering such calculations uninformative (Balmford et al. 1996).

Juvenile mortality was assessed to identify successful and unsuccessful breeders in the existing Royal Lion population. Details for deaths "at birth" and for cubs less than 1 year old were collected from ISIS (2008). These data were subsequently aligned with records of successful cub survivors in the 2008 European Studbook for both maternal and paternal parents. Percentage cub mortality and survivorship was calculated against the total cubs produced by each breeding male and female. Only births from 1998 were counted, as this included all currently active breeding animals.

Results

Founder representation in the Rabat Zoo collection

This investigation has traced the founding group of 39 animals transferred from the Moroccan Royal Palace collection to Rabat Zoo in 1970 through to the present captive population (Leyhausen 1975; Yamaguchi and Haddane 2002).

Between 1974 and 1979, deaths and transfers of animals, or their surviving offspring, resulted in a marked decline in the number of founders represented in the remaining gene pool at Rabat Zoo. Three of these transferred animals bred for the first time only after leaving Rabat (STUD_ID 30, 34 and 37). By the end of 1974, only three males and three females were breeding in Rabat Zoo. Between 1975 and 1977, ten offspring were born at Rabat Zoo (seven males and three females, all from the same founders) but there is no record of those animals breeding. Seven juveniles died between 1974 and 1978, whilst nine did not breed at Rabat Zoo and were transferred out to European circuses, private collectors and Havana zoo. Due to death of non-breeders, sales and transfers, the initial representation of 39 founders in 1970 was reduced to just ten by 1974. Later deaths, transfers and lack of breeding activity reduced representation to just six founders by 1990 (Table 1). Although a single male was returned to Rabat from Port Lympne in 1996, conversation with Rabat Zoo staff confirm that he has not been involved in breeding activity, and two recent imports from Europe were themselves offspring from Rabat animals previously exported to Germany. Assuming that all animals holding founder genes successfully bred, the remaining genetic diversity of the Rabat group can only be based on a maximum of six founders (Fig. 1). Based on this assumption, a "typical" Rabat Zoo lion would have the representative genome summarised in Table 2.

Founder representation in the European collections of Royal Lions

The population of Royal Lions in European collections includes representation of four founders now certainly lost from the Rabat mix (STUD_ID 21, 27, 34, 37). In addition, 13 animals in European zoos include representation from the two Madrid founders (STUD_ID 200, 201), which are not included in the Rabat calculations as they almost certainly left before the 1974 census (Table 3). The European captive population with these included, is based on 12 founders and this representation is largely unaffected when animals which are neutered, under contraception or now retired from breeding are taken out of the mix. Plans for future breeding should consider the available diversity.

The current captive groups in Spain, Germany, UK, France, Morocco, Czech Republic, Austria and Israel are sourced from four zoo-based family lineages: Rabat, Olomouc (transferred out after 1974), Washington (transferred out in 1976) and Madrid (suspected Moroccan imports from the early 1970s). Lions in the UK are from Washington and Rabat lineages; Central European collections (Czech Republic, Germany and Austria) are derived from Olomouc plus recent Rabat Zoo imports; animals in the main French groups originate from Olomouc, Washington and Rabat lineages; Spanish lions are from Washington and Madrid lineages. Animal exchanges have occurred in recent years between Madrid, Olomouc and France whilst Port Lympne Wildlife Park has sourced two breeding males from Rabat Zoo in the past decade. More recently, animals have arrived in France and Germany from Rabat Zoo, and several have

Table 1 Fate of founders of Rabat Zoo lions

Reason	Founder representation leaving the Rabat gene pool (1974–1978)	Remaining no. of founders
Founders (born before 1969)		39 animals
Died without breeding	1, 2, 3, 4, 11, 12, 13, 14, 16, 22, 25, 28, 31, 38	25
Sale to circuses (non-breeding animals)	35,36, 39	22
Transfer out to other zoos (non-breeders)	6, 19, 33	19
Transfers to Europe/USA (Hemmer & Leyhausen programme)	15, 24, 17, 30 34, 37, 27[a]	13
Death of all surviving offspring	21	12
All surviving offspring transferred	5, 23, 27	9
Survivors to 1990[b] (♀ born before 1969; no record of breeding 1970–1977)	26[b], 29[b], 32[b]	6

[a] Left offspring at Rabat so representation retained

[b] No record of breeding so deleted from founder list

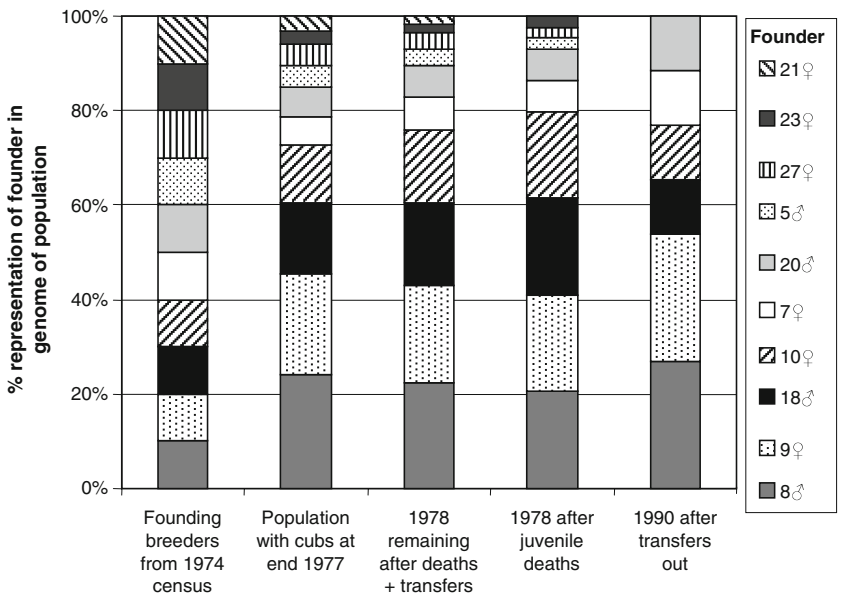

Fig. 1 Reduction in founder representation; Royal Lions at Rabat Zoo, 1977–1990

since achieved breeding successes with their offspring now residing in several German zoos.

The spread of founder genes in each country location is illustrated in Table 4, showing several pronounced differences in representation. An ideal maximum founder spread would see 8.33% representation of each founder, including the Madrid lions (STUD_ID 200, 201), or 10% of each of the ten Rabat-sourced founders from the 1974 census (STUD_ID 07, 08, 09, 20, 21, 24, 34, 37, 200 and 201).

Living animals in European zoos are derived from only two direct female lineages (STUD_ID 09, 37) plus one animal imported from Rabat in recent years. Only three direct male lineages remain; founder 18 (in STUD_ID 216), founder 08 (in STUD_ID 232, 242 and 267), founder 20 (in STUD_ID 249 and 264) plus three males recently imported from Rabat Zoo (STUD_ID 227, 241, 246). The exact paternity for several lions in Spanish zoos is in question (STUD_ID 230, 240, 245, 259, 260, 267) although the sire is certainly one of several Royal Lions (Fig. 5).

Table 2 Estimated founder representation across the Rabat Zoo population

Founder no. (STUDBOOK_ID, Rabat 1969 ID)	Percentage of genome representation across breeding population	Mean representation per individual (proportion of genome)
7♀	11.54	0.083
8♂	26.92	0.292
9♀	26.92	0.292
10♀	11.54	0.125
18♂	11.54	0.125
20♂	11.54	0.083

Reproductive success in Royal Lions (Europe)

Reproductive success can be measured by the number of cubs that survive to maturity. Calculations conducted on data from ISIS (2008) generated statistics for Royal Lions in Europe. Reproductive success of individuals in this group over the past 10 years is as follows:

- Mean no. surviving cubs per breeding female 1998–2008 1.68 (SD±2.25)
- Mean productivity per breeding female (no. of cubs) 3.5 (SD±2.61)
- Mean survival rate per breeding female 42.2%

A mean survival rate of 42% does not fully reflect the skew in female productivity; 12 females successfully produced cubs at a mean survival rate of 78.5%, whilst the remaining ten breeding females produced no survivors (see Fig. 2). Only seven females are currently breeding with a mean survival rate of 72.7% (24 survivors from 33 births). Only three males are actively breeding (STUD_ID 228, 246 and 253, see Fig. 3) with a mean cub survival of 45.5% (ten survivors from 22 births). Breeding males tend to successfully sire multiple litters.

Production of the draft 2008 European Studbook for Royal Lions

This study has collated a draft 2008 European Studbook to replace the incomplete 1998 records. The reliability of European zoo records, plus other findings from our study suggest that this updated studbook would be the most effective start point for initiating any future international breeding programme for Royal Lions. This studbook is the only single source of information that demonstrates known

Table 3 Founder representation across the European population

Founder no. (STUDBOOK_ID, Rabat 1969 ID)	Percentage of genome represented across breeding population	Mean representation per individual (proportion of genome)	Standard deviation per individual
7♀	4.96	0.050	0.0466
8♂	23.34	0.233	0.0807
9♀	23.34	0.233	0.0807
10♀	8.26	0.083	0.0402
18♂	8.26	0.083	0.0402
20♂	13.68	0.137	0.0855
21♀	1.89	0.019	0.0349
27♀	2.76	0.028	0.0393
34♀	4.22	0.042	0.0650
37♀	4.07	0.041	0.0429
200♂ (Madrid)	3.92	0.039	0.0965
201♀ (Madrid)	1.31	0.013	0.0322

parental lineages for lions back to the 1974 Rabat Zoo census [Leyhausen 1975], and will be a valuable repository for additional information such as future genetic profiles and phenotypic data (Fig. 5).

Demographic profile for Royal Lions in European collections

The age profile of European Royal Lions is illustrated in Fig. 4. Male and females become sexually mature at 2 years (Haas et al. 2005), with female reproduction declining at 11 years, stopping at about 15 years. Males are viable breeders up to 16 years. Of the 54 European animals, six of the 35 females are too old to breed, and at least four of the 19 males are neutered. Of the animals born since 2000, 31 (11 males and 20 females) appear to have breeding potential, whilst four have been removed from breeding and one is deceased.

Discussion

Significance of the *ex situ* Moroccan Royal Lion population

On a global scale, *Panthera leo* is rated as vulnerable in the IUCN Red List; however, only the Asiatic subspecies *P. leo persica* is considered separately (IUCN 2008). The Barbary subspecies *P. leo leo*, is rated as extinct in the wild, and no longer categorised separately. However, Red List status masks considerable variation in extinction threat for the remaining wild regional populations. Nearly 90% of wild lions are thought to be found in Eastern and Southern African populations, whilst as few as 1,200–5,200 animals reside in the rest of Africa and Asia combined. Recent analyses suggest that 42% of wild lion populations are in decline and overall the species is estimated to have declined by at least 48% in the past 25 years [IUCN 2008]. Small regional populations are isolated and vulnerable to the

Table 4 Current % representation of founders in Royal Lion captive groups by country

	FOUNDER ID/gender											
	7♀	8♂	9♀	10♀	18♂	20♂	21♀	27♀	34♀	37♀	200♂	201♀
Spain	2.50	21.25	21.25	8.75	8.75	12.50	7.50	0.00	5.00	2.50	7.50	2.50
Czech[a]/Aus	0.00	20.94	20.94	6.88	6.88	8.75	4.38	0.00	9.38	4.38	13.13	4.38
UK	7.15	21.81	21.81	7.50	7.50	20.69	0.00	6.77	0.00	6.77	0.00	0.00
France	2.50	25.21	25.21	4.58	4.58	15.00	0.00	6.25	10.42	6.25	0.00	0.00
Germany	8.33	29.17	29.17	12.50	12.50	8.33	0.00	0.00	0.00	0.00	0.00	0.00
Europe overall	3.43	22.08	22.08	6.97	6.97	14.80	2.46	3.60	5.49	5.30	5.11	1.70
Rabat, Morocco	11.54	26.92	26.92	11.54	11.54	11.54	–	–	–	–	–	–

[a] Including animals now at Hai Kef, Israel

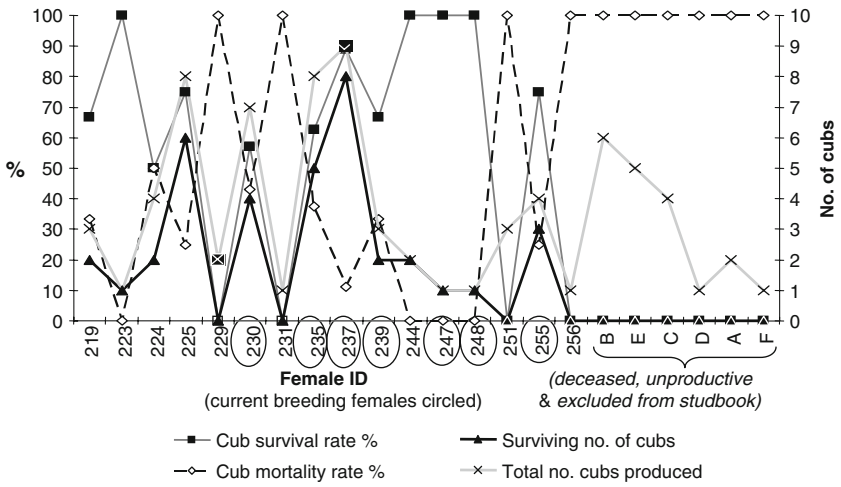

Fig. 2 Female productivity and cub mortality 1998–2008 for Royal Lions (Europe). The ID of current breeding animals is *circled* on the *X*-axis

effects of genetic drift and inbreeding (Bauer and Van Der Merwe 2004; Dubach et al. 2005).

Figure 5 compares population estimates for the small west and central African wild populations, the wild remnant population of Asiatic lion (*P. leo persica*) in India (which receives significant conservation attention), captive Asiatic lions and estimates for the captive population of Royal Lions. Notably, approximately 900 lions are listed in captivity (Gould 2002; ISIS 2008), of which most are hybrids with parents from mixed or unknown regional origins (Barnett et al. 2006a). Although the Moroccan Royal lion population has been dismissed by some as an irrelevant relict group and is certainly only a fraction of the size of the wild populations in India, West and Central Africa, in time, an enlarged captive group of Royal Lions could represent a considerable proportion of genetic diversity within the species. Furthermore, priorities may change if Royal Lions are subsequently identified as genetically distinct from existing wild populations.

Demographic status and recommendations

Only eight juveniles have been produced in the last 2 years, a replacement rate of barely 50% against the ageing population. Given the slow overall rates of productivity, new breeding prides need to be established to produce litters to balance the demographic age distribution. However, current zoo capacities may restrict progress towards this.

The newest offspring locally within each of the European zoos are now related as siblings, cousins or aunt/uncle (Fig. 6). However, across these institutions, a reasonable number of unrelated animals of breeding age are available, some of which are not currently engaged in breeding effort (Fig. 7). This indicates potential for a more proactive international breeding programme.

The current breeding mix at Port Lympne, the largest collection in Europe, is restricted to a single maternal bloodline (STUD_ID 37). However, Port Lympne's representation of founder 27 is not found elsewhere across the

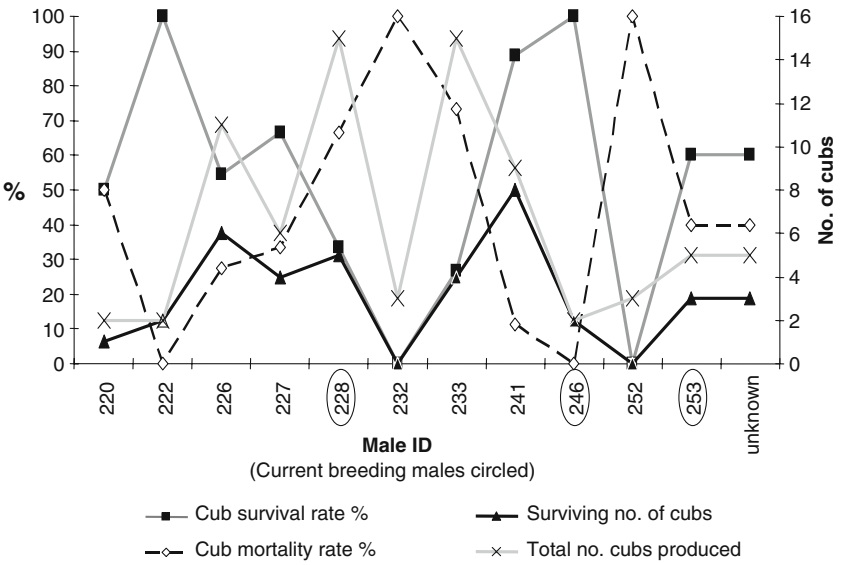

Fig. 3 Male productivity and cub mortality 1998–2008 for Royal Lions (Europe). The ID of current breeding animals is *circled* on the *X*-axis

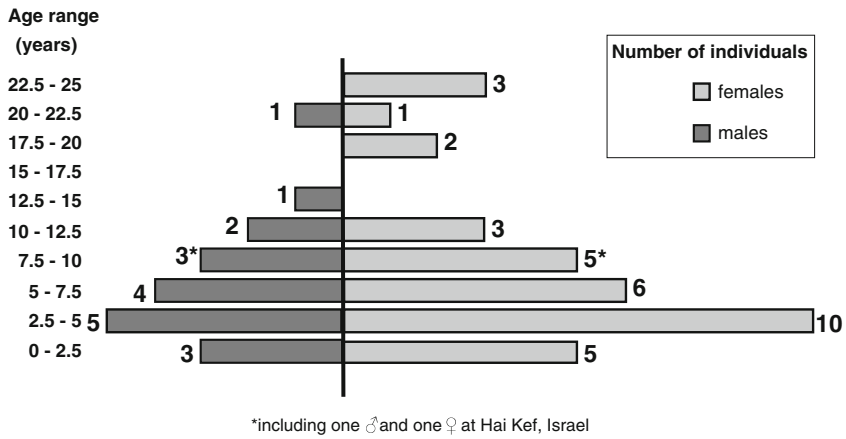

Fig. 4 Age profile for Royal Lions, differentiated by gender, in European Zoos (* includes one male and one female at Hai Kef Zoo, Israel)

population and founders 20 and 37 are presented in much higher proportions than in other groups. Founders 21 and 34, and to some degree founders 07, 10 and 18 are much better represented in Spanish and Czech prides.

In the past, Port Lympne, Olomouc and Neuwied zoos have tended to source new breeding animals from Rabat Zoo, whilst other zoos have usually taken excess stock from other European institutions. This policy needs to be reviewed and a more co-ordinated, purposeful system of breeding exchanges considered in order to: (1) identify good quality breeders using animal health records and cub survival rates, (2) ensure a demographically balanced mix of the gene pool and (3) ensure a recorded studbook. This will enable those zoos previously frustrated with lack of access to new stock, to improve their sourcing options.

The available breeding pool

Depression of reproductive capability is often cited as an outcome of inbreeding effects (Soulé et al. 1986; Frankham et al. 2004). Anecdotal reports of health problems in some Royal Lions (L. Veselá, personal communication) and very poor breeding records for certain individual animals may be indicative of low genetic diversity. Nevertheless, the current breeding animals have achieved fair survival rates in recent years.

The captive population of Royal Lions needs to increase in size and careful management will provide new breeding animals that can maintain genetic diversity. This study suggests that the best source of new bloodlines will be found within European collections; breeding performance is likely to be acceptable and genetic diversity greater. Future

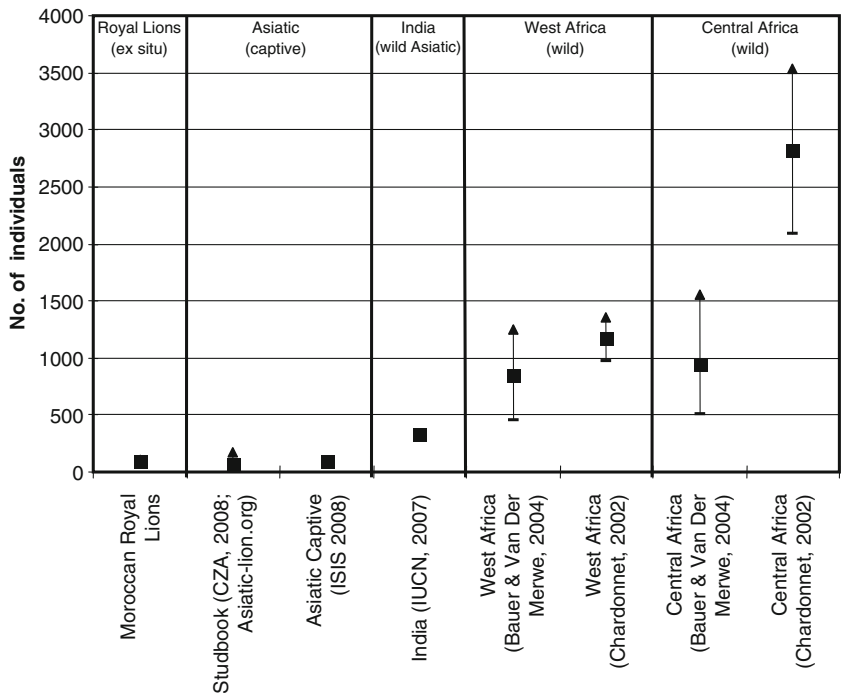

Fig. 5 Comparison of population estimates, for small wild regional groups of *Panthera leo* (IUCN 2008) against the *ex situ* population estimate for Moroccan Royal Lions (ISIS 2008) and Asiatic lions (CZA 2008). Key: ■ mean estimate; ▲ maximum; ▬ minimum

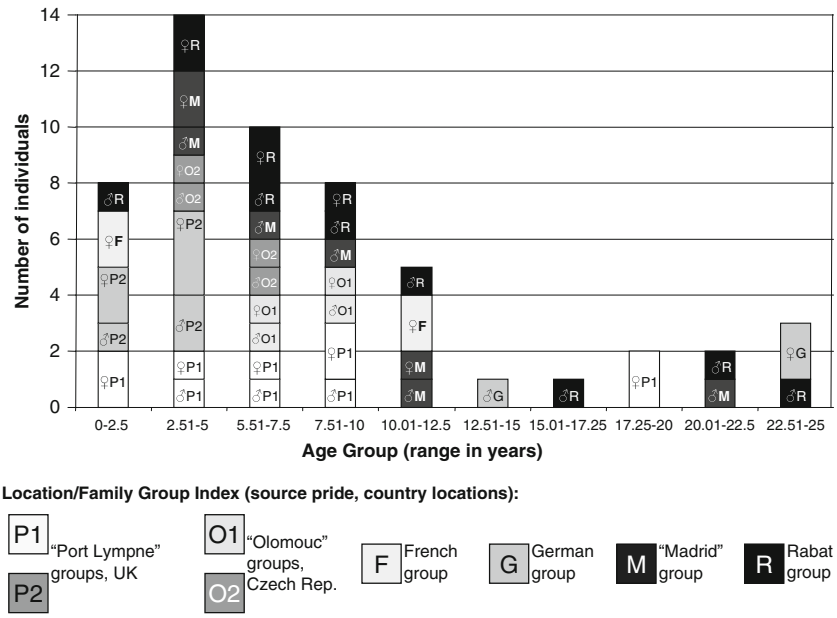

Fig. 6 Demographic spread for Royal Lions in captivity in European zoos. Genders indicated by family lineage. To mitigate inbreeding, intra-family pairs should be avoided, e.g. ♀P1 should not pair with ♂P1 and ideally not with ♂P2, and ♀M should not pair with ♂M

breeding exchanges should seek healthy animals which offer complementary founder gene mixes. However, due to the small overall captive population, future use of good breeding stock from Rabat Zoo will be important. At present, one aim should be for the Rabat Zoo group to regain genetic diversity lost since 1974 for its own viability. This can be achieved by sourcing new stock from Europe. Efforts should also be made to review genetic diversity and establish studbook status for the Rabat Zoo animals.

It may be appropriate to remain cautious about the origins of the Madrid founders (STUD_ID 200 and 201) until genetic work can match their genome to other Royal Lions. However, lions with Madrid lineage may be required to maintain an acceptable level of breeding capacity. A management decision would have to consider whether to

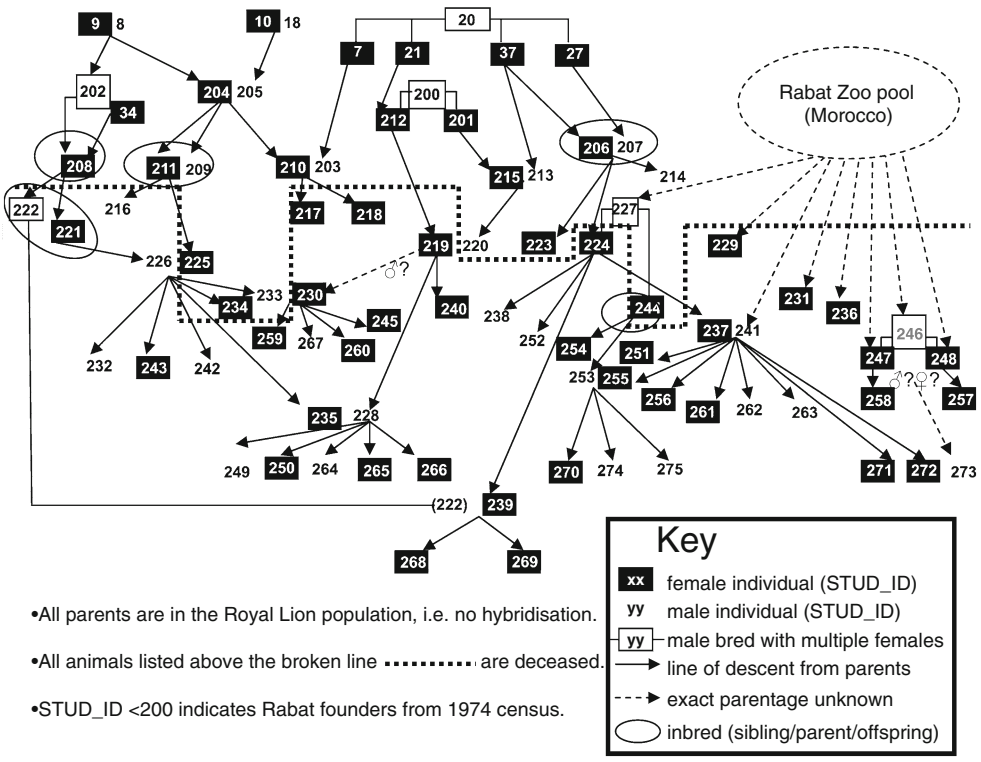

Fig. 7 Family tree for Royal Lions held in captive collections in Europe (1973–2009)

separate animals with Madrid lineage from animals in Olomouc and Port Lympne bloodlines, an approach similar to that taken for European Bison (Frankham et al. 1986; Perzanowski et al. 2004). Alternatively, if Madrid representation is retained, it could be minimised by including only animals in the current Spanish prides (STUD_ID 240, 245, 259, 260, 267).

Future breeding exchanges could establish two new Czech/Port Lympne breeding pairs (by exchanging male animals now at breeding age), plus perhaps one Port Lympne/Spanish breeding pair. Animals from Rabat (including those at Neuwied and Erfurt Zoos) should be considered in the early stages of any breeding programme, based on genetics, health and recent breeding performance. However, a comparison of the genetics of both the German-based animals and earlier Rabat imports with the potentially more diverse bloodlines of longer-established European prides is required. Breeding decisions should be informed by the data from this study, but will need to consider capacity constraints in partner zoos, selection of pride sizes and location of animals for future breeding. The draft European studbook information developed during this research is available from the corresponding author and can be maintained hereafter with the agreement and involvement of participating zoos.

Conclusions

The captive population of Royal Lions is currently held in relatively isolated zoo collections and is vulnerable to the effects of inbreeding depression. Pairing animals from the UK zoos with those from central Europe is a priority to increase diversity and to retain a more even spread of founder genes. The draft 2008 studbook and the founder analysis in this study have identified a number of suitable breeding exchanges. A formal studbook-led breeding programme would enable constructive participation of zoos holding Royal Lions and allow future planning and negotiations. The Hemmer and Leyhausen morphological categorisation has been largely ignored since the 1974 census (Leyhausen 1975; Hill and Haynes 1999; Yamaguchi and Haddane 2002; Tefera 2003) but should be incorporated into the studbook alongside genetic and parental data to enable easier assessment of pedigrees as new genetic knowledge for lions emerges (Dubach et al. 2005; Patterson 2007). Other zoo animals, if genetically proven similar to Royal Lions, could be included in future breeding effort, whilst radical approaches such as *in vitro* procedures might be considered to retain the genes of ageing Royal Lions.

The holding capacity of participating zoos will be a constraint on population growth and institutions will need to be willing to transfer selected animals. However, opportunities exist for mutually beneficial exchanges of animals between zoo institutions that should enable effective maintenance of captive prides in the foreseeable future. The Royal Lion population may yet hold a unique genetic heritage for the global lion population (Patterson et al. 2005; Yamaguchi 2006) and the precautionary principle would suggest that reasonable steps should be taken to preserve Royal Lions until their conservation value has been properly assessed.

Acknowledgements Thanks to the following individuals who have assisted with information and resources to support this research: Nathalie Laurence at Port Lympne Wild Animal Park (UK), Mark Challis and Julie Mansell at Belfast Zoo (UK), Sandrine Silhol at Les Sables d'Olonne Zoo (France), Liba Veselá, Olomouc Zoo (Czech Republic) and Dr. Silvio Pitteri. Thanks are also expressed to the anonymous reviewers for their feedback on an early draft of this paper.

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