

Inbreeding and Offspring Sex Ratio in the Pygmy Hippopotamus (*Cheoropsis liberiensis*) Population Kept in Zoological Gardens

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Accepted December 15, 2014

GRACZYK M., CWIERTNIA P., BOROWSKA A., BARCZAK E., SZWACZKOWSKI T. 2015. Inbreeding and offspring sex ratio in the pygmy hippopotamus (*Cheoropsis liberiensis*) population kept in zoological gardens. *Folia Biologica (Kraków)* **63**: 35-42.

The aim of this study was to estimate the inbreeding level and its impact on offspring sex ratio in the pygmy hippopotamus population kept in zoological gardens. Records of pygmy hippopotamus born between 1873-2013 were extracted from the international studbook. Totally, 1357 individuals originating from 148 breeding units were included (individuals with unknown sex were omitted). The offspring sex ratio is defined as the number of sons to the total number of progeny of each dam and sire. Spearman's rank correlation was employed to examine the relationships between the inbreeding level and offspring sex ratio. Inbreeding coefficients and individual increase in inbreeding coefficients (included as a linear co-variable) were examined as well as the geographic region and birth period using general linear models. The average inbreeding coefficient was 5.39%. The following sex proportion was observed for the inbred population: 57% and 43% for females and males, respectively. A significant relationship between inbreeding level of parents and their offspring sex ratio were estimated for European zoological gardens, whereas in others geographic regions the dependencies were insignificant.

Key words: Captive breeding, pedigree analysis, inbreeding, offspring sex ratio.

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One of the main goals of zoological gardens is preservation of the genetic variability of animal populations, mainly wild species endangered in their natural environments. However, these populations are usually small with undesirable genetic and demographic structures. Moreover, the groups of individuals are relatively small per zoo. In recent decades, breeding conservation programs have been implemented for a number of animal species kept in zoos. They are focused on minimization of mating between relatives and stabilization of genetic variability of a given population. The main factor affecting genetic diversity of populations is inbreeding, which in turn is strongly affected by the number of sires and dams. From a classical perspective introduced by Wright (1921), the inbreeding coefficient describes the probability that two alleles are identical by descent. However, studies performed on various animal populations as well as the history of formation

of some breeds suggest both "bad inbreeding" and "good inbreeding" (thus some traits are consolidated). It should be stressed that the mating of relatives always leads to a reduction of genetic variability and loss of some alleles. As already mentioned, a decrease in fitness and performance due to inbreeding (so-called inbreeding depression) has been documented. A number of reports concern production traits in livestock (GOMEZ *et al.* 2009; KÖNIG & SIMIANER 2006). They indicate that negative inbreeding affects reproduction traits and survival. KELLER and WALLER (2002) and OLECH (2006) hypothesized an inbreeding impact on offspring sex ratio in bison. On the other hand, skewed offspring sex ratio can be the result of a number of other factors. So far, it has been indicated that dams in good condition should tend to produce more sons (TRIVERS & WILLARD 1973). Good condition is a conglomeration of several traits including dam age, nourishment and social

status (GLATSTON 1997; ZSCHOKKE 2002). Furthermore, the progeny sex ratio is also affected by stress (KRUUK *et al.* 1999; RYAN *et al.* 2011) and stress is influenced among others by population density. Because males are perceived as the “more expensive” sex, inbreeding depression may result in more daughters than sons. This may be connected with survivability of the ‘less expensive sex’, i.e. the female. SARAGUSTY *et al.* (2012) showed that offspring sex ratio was similar to sperm sex ratio in collected ejaculates from a pygmy hippopotamus population, which may suggest that sires can ‘control’ spermatogenesis and ‘favor’ sperm with a specific chromosome.

Since the year 2006 the International Union for Conservation of Nature (IUCN) recognized pygmy hippopotamus in natural environments as endangered. Hence, zoological gardens play a key role in the conservation strategy for this species. The European Endangered Species Program (EEP) and Species Survival Plan (SSP) are currently applied. The current population size is 353 animals kept in 133 institutions, whereas the EEP population comprises a total of 126 individuals in 56 institutions (STECK 2014). Considerably more female than male offspring are registered.

The aims of this study were to estimate the inbreeding level and individual increase in inbreeding coefficients as well as its impact on offspring sex ratio in the population of this species kept in zoological gardens.

Material and Methods

Records of pygmy hippopotamus born between 1873-2013 were extracted from the international studbook (STECK 2014). The data structure is as follows: individual number, sex, birth date, death date, sire number, dam number, breeding unit and additional information. The data set contains records of 1357 individuals from 148 zoological gardens: 570 males, 787 females (animals with unknown sex were omitted); 156 animals were founders (wild born).

Pedigree analysis

Prior to the estimation of the inbreeding coefficients, the pedigree completeness was examined using discrete generation equivalents (g_e), according to the formula given by BOICHARD *et al.* (1997):

$$g_e = \sum_{i=1}^{n_j} \left(\frac{1}{2} \right)^{g_{ij}}$$

where: n_j is the number of known ancestors of the j -th individual, and g_{ij} is the number of generations between the i -th ancestor and the j -th animal. An individual inbreeding coefficient (F_i) was ex-

tracted from the additive relationship matrix according to an algorithm described by SARGOLZAEI *et al.* (2005) using the software package CFC – Contribution, Inbreeding (F), Coancestry (SARGOLZAEI *et al.* 2006). It should be noted that the inbred population includes all individuals that had a nonzero inbreeding coefficient. The differences between the average inbreeding coefficients of sires and dams were examined using an unpaired Student’s t -test (KIRK, 2008). Also the individual increase in inbreeding coefficient (ΔF_i) (in consecutive periods) was estimated using the program ENDOG v4.8 (GUTIÉRREZ & GOYACHE 2005) according to the formula described by GUTIÉRREZ & GOYACHE (2010):

$$\Delta F_i = 1 - \sqrt[t]{1 - F_i},$$

where: F_i is the individual coefficient of inbreeding and t is the ‘equivalent complete generations’.

Determination of offspring sex ratio

The offspring sex ratio (OSR) is defined as the number of sons to the total number of progeny per dam vs sire (wild-born animals and parents with one descendant were omitted). The analyses were based on 121 sires and 163 dams as well as 737 offspring (for both parents jointly).

Animals were grouped into four geographical regions (corresponding to the currently implemented breeding programs) on the basis of the location of birth of the parents. The description of the regions is given in Table 1. Moreover, fourteen birth year groups were created. The first one includes individuals born up to 1945, whereas the next groups involve five year birth periods.

Differences between the proportion of sons and daughters were examined with the use of the one sample Z-test for proportions (KIRK 2008).

Relationships between OSR and parental inbreeding coefficients were estimated via Spearman’s rank correlations. The correlation analysis was done within four geographical regions for sires, dams and both parents, respectively. These

Table 1

Structure of geographical regions

Region	Countries
I	Australia, China, Indonesia, Japan, Saudi Arabia, Singapore, Sri Lanka
II	Belgium, Czech Republic, Denmark, France, Germany, Great Britain, Italy, Lithuania, Netherlands, Norway, Poland, Slovakia, Switzerland
III	Canada, USA
IV	Kenya, Republic of South Africa

computations were performed using the CORR (Correlation) procedure of SAS Enterprise package programs (SAS INSTITUTE INC. 2012).

Moreover, dependencies between parental inbreeding and OSR were estimated with the use of the following linear model:

$$y_{ijkl} = \mu + t_i + g_j + b(x_{ijk(l)} - \bar{x}) + e_{ijkl},$$

where: y_{ijkl} – the offspring sex ratio of the $ijkl$ -th parent, μ – the overall mean of sex ratio, t_i – the fixed effect of the i -th birth period, g_j – the fixed effect of the j -th geographical region, b – the partial regression coefficient, $x_{ijk(l)}$ – the inbreeding coefficient of the $ijkl$ -th parent, \bar{x} – the mean parental inbreeding level, e_{ijkl} – the residual effect connected with the $ijkl$ -th observation.

This model was applied for the individual increase in inbreeding coefficients as a covariable. Bonferroni’s correction was made for multiple comparisons. The analysis was done by the GLM (general linear model) and LSD (least significant differences) Student’s t -test procedures of SAS Enterprise package programs (SAS INSTITUTE INC. 2012).

Results

Pedigree analysis

Detailed information on inbreeding structure in the pygmy hippopotamus population is listed in Table 2. Both parents were unknown for 175 individuals in the studied population. Thus, they were

Table 2

Inbreeding level in the population studied

Inbreeding coefficient (%)	Number of animals	Percentage of animals
0	911	67.13
0-5	107	7.89
5-10	67	4.94
10-15	42	3.09
15-20	28	2.06
20-25	106	7.81
25-30	64	4.72
30-35	3	0.22
35-40	29	2.14
Total	1357	100

perceived as founders but only 157 were wild born. The percentage of animals with both known parents was above 86.66% in the whole population. Six individuals had only one known parent (0 with only sire known and 6 with only dam known). The average number of discrete generation equivalents reached 2.10 and the maximum number of discrete generation equivalents reached 5.28. Generally, the number of born males and females increased in consecutive periods (Fig. 1). Moreover, a sex disproportion was registered over time. Totally, 446 individuals were inbred. It should be noted that the pedigree completeness is not satisfactory compared to e.g. livestock popula-

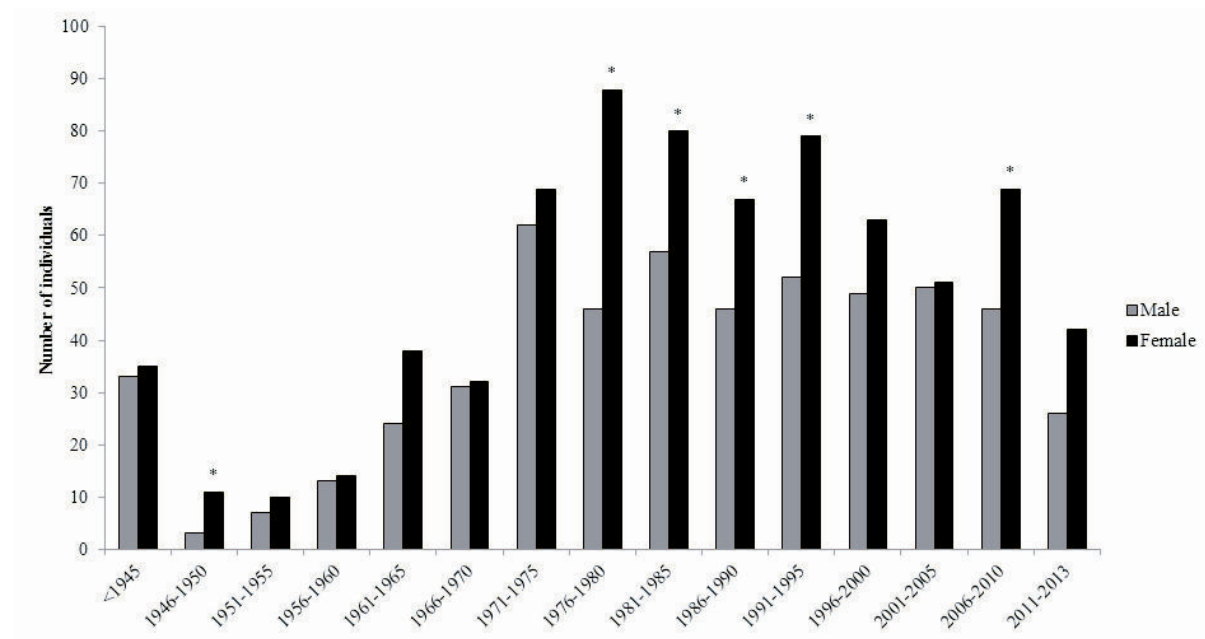


Fig. 1. Number of males and females in the population studied over time. Statistically significant differences ($P < 0.05$) between females and males born in each period are marked by an asterisk.

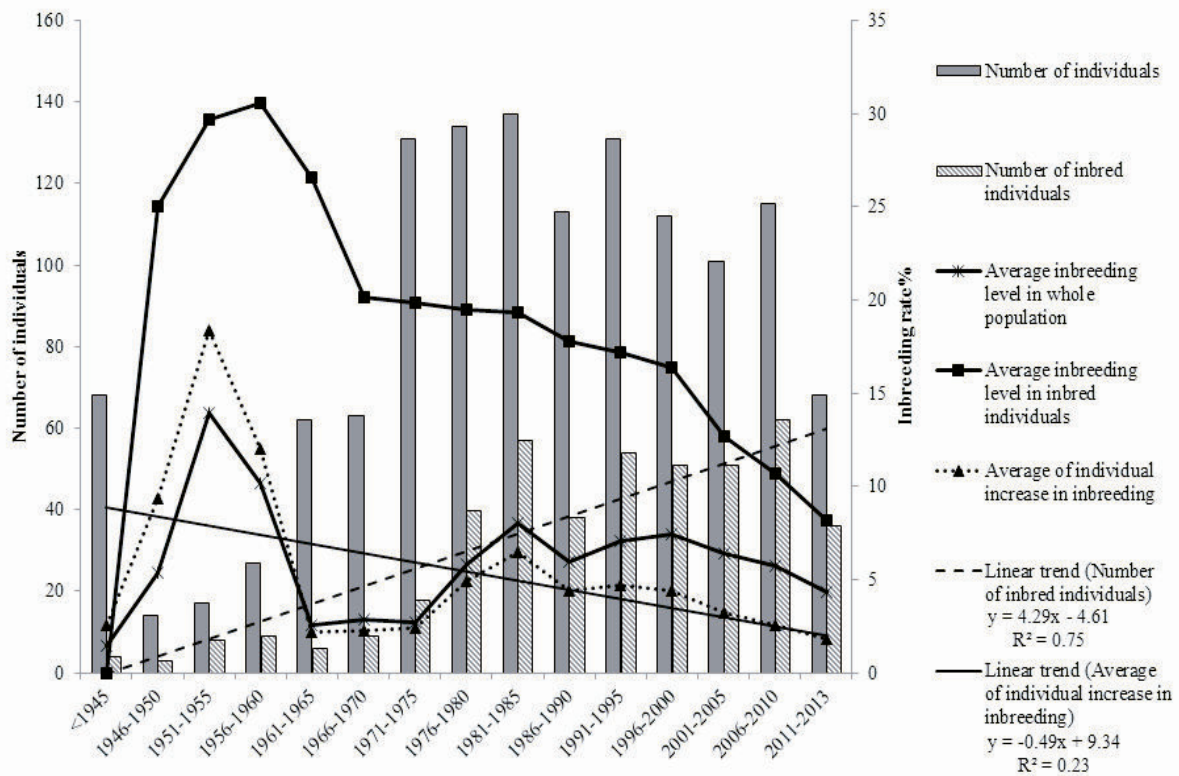


Fig. 2. Number of individuals born in subsequent years and inbreeding level in the population studied.

tions. Hence, the inbreeding coefficients of some individuals might be underestimated. The average inbreeding level was 5.39% and generally, this parameter varied in the studied population (Table 2). A higher inbreeding level was registered for males, 5.46% (ranging from 0.00 to 37.50%), than for females, 5.33% (ranging from 0 to 38.87%), whereas the mean inbreeding level was 16.40% (for inbred individuals). It was mainly affected by inbred females: 16.53% (ranged from 0.24% to 38.87%). The estimated inbreeding level of inbred males was 16.22% (ranged from 0.24% to 37.50%). However, for all males and females and inbred males and females the differences between the averages of inbreeding coefficients were not statistically significant. It should be noted that pedigree information for pygmy hippopotamus (kept in zoos) has been registered since 1873. The number of individuals and inbred individuals over time are presented in Figure 2. The visualization indicates a similar participation of inbred animals in the whole population. However, it clearly shows a decreasing tendency in population size since 1995 with an increase of the participation of inbred animals. It should be noted that 67.13% individuals of the whole population are characterized by a zero inbreeding coefficient (Table 2). The inbreeding level of 202 individuals (14.89% of the population) exceeded 20%. An average individual increase in inbreeding over time is presented in

Figure 2. The tendency decreases in subsequent periods. Since the year 1966 this parameter has been relatively stable and it can be noted from the decreasing tendency, which means that the population size effectively slows down the process of allele loss in the population. The reduction of the mean inbreeding level and individual increase in inbreeding corresponds with a relatively small number of inbred animals in the subsequent periods.

Determination of offspring sex ratio

The results concerning the factors which determine OSR are shown in Tables 3-5. Generally, more females than males were registered in the population: 58.00% and 42.00%, respectively. A relatively similar proportion of sexes was observed for the inbred population (57.00% and 43.00% for females and males, respectively). In both cases the differences are statistically significant ($P < 0.05$).

The estimates of Spearman's rank correlation coefficients between parental (sires, dams, and both) inbreeding and OSR within four geographical regions are listed in Table 3. Generally, both the number of parents and estimated parameters varied across the regions. A lower inbreeding level was estimated for the Africa, where the number of parents was smaller as well. On the other hand, in the case of European zoos (second region) a rela-

Table 3

Spearman's rank correlation between parental inbreeding and offspring sex ratio over four geographical regions

Region	Sires				Dams				Both parents			
	No of sires	Offspring sex proportion (%)	Inbreeding coefficient (%)	Spearman's rank correlation	No of dams	Offspring sex proportion (%)	Inbreeding coefficient (%)	Spearman's rank correlation	No of parents	Offspring sex proportion (%)	Inbreeding coefficient (%)	Spearman's rank correlation
I	12	48.52	2.70	0.162 (P=0.6145)	18	43.47	1.62	-0.182 (P=0.4697)	30	45.492	2.04	-0.029 (P=0.8802)
II	66	41.28	6.12	0.385 (P=0.0014)	92	42.66	5.98	0.144 (P=0.1722)	158	42.087	6.04	0.242 (P=0.0022)
III	37	39.35	3.72	-0.220 (P=0.1905)	44	39.64	3.70	0.206 (P=0.1789)	81	39.505	3.71	0.018 (P=0.8749)
IV	6	57.68	10.43	NE	9	59.31	8.23	-0.347 (P=0.3602)	15	58.669	9.13	-0.280 (P=0.3131)

NE – not estimable.

Table 4

Partial linear regression coefficients of inbred vs individual increase in inbreeding of sires, dams and both parents group for offspring sex proportion

Data set	Inbred			Individual increase in inbreeding		
	Regression coefficient	SE	p-value	Regression coefficient	SE	p-value
Sires	0.6873	0.28727	0.0185	0.4533	0.27193	0.0985
Dams	0.3481	0.24585	0.1589	0.4573	0.25830	0.0787
Both parents	0.4820	0.18297	0.0089	0.4394	0.18268	0.0168

SE – standard error of regression coefficient estimate.

Table 5

Frequency distribution of progeny by sires, dams and both parents

Number of progeny	Number of sires	Number of dams	Number of both parents
2	23	39	62
3	20	27	47
4	11	25	36
5	7	14	21
6	8	13	21
7	14	13	27
8	13	9	22
9	7	5	12
10	3	4	7
11	3	4	7
12	4	1	5
13	1	2	3
14	1	1	2
15	0	1	1
16	1	4	5
17	2	1	3
18	1	0	1
19	2	0	2

tively high level of parental inbreeding was inferred from the largest numbers of studied parents. It seems that this may be connected with the relatively deep pedigrees of the animals. It should be stressed that correlation coefficients were statistically significant for this region only. The dependencies were positive, indicating a higher parental inbreeding level (mainly sires), which leads to an offspring disproportion ratio (with a larger number of sons). As listed in Table 3, the number of parents in other regions (particularly in regions I and IV) is relatively small. Hence, the statistical inference should be cautious. However, the most outlying OSRs (from the theoretical value) were obtained in North American zoological gardens.

Partial linear regression coefficients for parental inbreeding level and individual increase in inbreeding on the offspring sex ratios are given in Table 4. Regression coefficients were statistically significant for sires and both parents. Inbreeding depression was estimated with the use of an individual increase in inbreeding coefficients. These regression coefficients were statistically significant for both parents. The linear partial regression coefficients were positive in both cases. In gen-

eral, this corresponds with the results of correlations between these variables. As already mentioned, changes of OSR over time were also observed (see Fig. 1).

Discussion

It is well known that small populations kept in zoological gardens require more care on the part of their breeding management. Over a number of decades the main problem of captive breeding was mating between relatives as well as the population sex structure. An increase of homozygosity in captive populations is perceived as a serious breeding problem. The average inbreeding level of the population studied was 5.39%. In general, the results correspond to those obtained for pygmy hippopotamus and other species (ZSCHOKKE 2002). WOLC *et al.* (2008) reported that the inbreeding level in captive bred Przewalski horses was 9.4% with considerable fluctuations for five European countries and the USA. However, the population mentioned was rebuilt from a small number of founders. For the last 20 years the individual increase in inbreeding in pygmy hippopotamus has been nearly stable, so the genetic diversity of the population is maintained. It is well known that the inbreeding rate can be underestimated if the completeness of individual pedigrees is not satisfactory. MUCHA (2013) concluded that discrete generation equivalents in fifteen deer populations achieved values 4-5 in the final generations. A similar pedigree completeness (maximum 5.28 whereas the average was only 2.10) was observed in the present study of the pygmy hippopotamus population. By contrast to captive wild animals, the respective parameters for livestock populations are significantly larger. Thus, both inbreeding coefficients and their effects are more precisely estimated.

Over the last decades inbreeding effects have been widely described for captive animals as well. It is well known that an increase of homozygosity leads to a reduction of genetic variability. Moreover, it can also affect inbreeding depression of some traits. Inbreeding has long been known to reduce reproduction and fitness (litter size, gestation length, fertility, body weight, weight (maternal) and offspring survival), and in consequence survival in naturally breeding species (NORBERG & SORENSEN 2007; LEROY 2014). The sex ratio registered in the analyzed pygmy hippopotamus population was 42% of males and was similar to the sex ratio reported by SAGARUSTY *et al.* (2012) – 42.5% and by ZSCHOKKE (2002) – 41%. The sex ratio of species can be modified by many factors including taxonomic order and social organization. In many captive species a bias of the sex ratio

can be observed, e.g.: *Ateles geoffroyi* (female bias), *Lemus catta* and *Oryx dammah* (male bias) (FAUST & THOMPSON 2000). Thus, the sex structure can be modified in the time, leading to a decrease in effective population size and an increase of the probability of homozygosity. Therefore, time fluctuation causes changes in sex ratio in consecutive age classes. The genetic backgrounds of particular sex ratio classes are obviously different. For instance, MARTIN and FESTA-BIANCHET (2011) found positive relationships between mother condition and the number of sons, whereas GRANT and CHAMLEY (2010) indicated negative dependencies between these variables, thus a mother in poor condition had considerably more male offspring. In fact, these investigations were carried out on different animal species. However, a connection between the progeny sex proportion with dam condition suggests putative inbreeding backgrounds, since the increase of homozygosity influences the vigor of animals. Also, some studies hypothesized on the sire's effect on offspring sex ratio. SARAGUSTY *et al.* (2012) noticed that if inbreeding is known to cause a decline in sperm quality, the sex ratio can be influenced. It should be stressed that the above mentioned study was performed on pygmy hippopotamus populations kept in zoos, but this correlation was not found. In the analyzed population a correlation between sex ratio and inbreeding level was found only in region two in sires and both parents. The correlation coefficient in sires and both groups of parents was 0.385 and 0.242, respectively. A positive correlation suggests that we should expect male sex bias but the reverse was observed. FRANKHAM and WILCKEN (2006) found a relationship between inbreeding level and sex ratio in numerous species, e.g.: *Goura cristata*, *Dendrolagus matschiei*, *Canis rufus*, *Oryx leucoryx* (female bias) and *Adax nasomaculatus* (male bias).

A review of many reports on skewed sex ratio clearly showed a number of factors influencing this trait. As already mentioned, interspecific variability in sex ratios has been noted (GLATSTON 1997; ZSCHOKKE 2002). Moreover, these authors reviewed other external factors having an impact on sex ratio, e.g.: parental nutritional status, physical condition, stress, population density, social status, age of mothers and timing of pregnancy. This was confirmed by other authors as well. ZSCHOKKE (2002) indicated that the differences in sex ratio are associated with various methods of feeding and husbandry. SARAGUSTY *et al.* (2009) discussed a more complex determination of the trait including social and biological aspects. However, in captive breeding, the populations are usually small. Hence, some specific factors (typical for a given population) can play a considerable

role in determination of offspring sex ratio. This corresponds with the results obtained in the present study, in which inbreeding effects varied across geographical regions. SARAGUSTY *et al.* (2009) suggested that stillbirths are also a major problem which can be addressed for the pygmy hippopotamus population. As already mentioned, the above factors varied over time and across populations. The results obtained in the present study showed a statistically documented relationship between the inbreeding level of parents and the proportion of male offspring. The four geographical regions analyzed in the present study are subject to different management strategies. Over many decades two of them have been steered by the European Associations of Zoos and Aquaria (region II) and the Association of Zoos and Aquariums (region III). This may involve higher breeding discipline in both regions compared to the other ones. For instance, better pedigree completeness may be expected, which may contribute to a more accurate statistical analysis, including the estimates of inbreeding coefficients. On the other hand, zoological gardens aim to keep their animals in good condition by providing optimal diets, veterinary care, management etc. So, the environmental variability between them has gradually been reduced. However, variability still exists among zoos, as it can be affected among others by the climatic zone.

As presented above, the majority of the estimated rank and linear correlation coefficients between parental inbreeding level and offspring sex ratio are statistically insignificant. Both types of correlations were very similar for respective data subsets. However, the correlations were estimated for a relatively small number of individuals. It can be assumed that a larger sample could allow for a more unambiguous statistical inference.

The obtained results suggest possible interactions between some factors, mainly the inbreeding level and some management conditions which are usually connected with the implemented program. Thus, cumulative interaction effects, such as management conditions, nutrition and animal condition can be larger compared to single effects of particular factors. This can probably be perceived as the main reason for varied results across the geographical regions studied herein.

Acknowledgements

The authors thank Mrs. Beatrice STECK from The International Studbook Keeper from Basel ZOO in Switzerland for providing the studbook. The authors are also grateful to the anonymous reviewer for his/her valuable comments.

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