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## **Rates of inbreeding and genetic adaptation for populations managed as herds in zoos with a rotational mating system or with optimized contribution of parents**

Mucha, S; Komen, H

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1 **Rates of inbreeding and genetic adaptation for populations managed as**  
2 **herds in zoos with a rotational mating system or with optimized**  
3 **contribution of parents**

4  
5 Sebastian Mucha<sup>123</sup>, Hans Komen<sup>1§</sup>

6  
7 <sup>1</sup>Animal Breeding and Genomics Centre, Wageningen University

8 P.O. Box 338, 6700 AH, Wageningen, The Netherlands

9  
10 <sup>2</sup>Department of Genetics and Animal Breeding, Poznan University of Life Sciences

11 Wolynska 33, 60-637 Poznan, Poland

12  
13 <sup>3</sup>Scotland's Rural College, Animal & Veterinary Sciences

14 Easter Bush, Midlothian EH25 9RG, United Kingdom

15  
16 <sup>§</sup>Corresponding author

17 Wageningen University

18 P.O. Box 338, 6700 AH, Wageningen, The Netherlands

19 Email: hans.komen@wur.nl

20 Phone: +31317482465

21

## 22 **Summary**

23

24 This study compares two genetic management scenarios for species kept in herds, such as  
25 deer. The simulations were designed so that their results can be extended to a wide range of  
26 zoo populations. In the first scenario the simulated populations of size 3x20, 6x40 or 20x60  
27 (herds x animals in herd) were managed with a rotational mating (RM) scheme in which  
28 10%, 20% or 50% of males were selected for breeding and moved between herds in a circular  
29 fashion. The second scenario was based on optimal contribution theory (OC). OC requires an  
30 accurate pedigree to calculate kinship; males were selected and assigned numbers of  
31 offspring in order to minimize kinship in the next generation. RM was efficient in restriction  
32 of inbreeding and produced results comparable with OC. However, RM can result in genetic  
33 adaptation of the population to the zoo environment, in particular when 20% or less males are  
34 selected for rotation and selection of animals is not random. Lowest rates of inbreeding were  
35 obtained by combining OC with rotation of males as in the RM scheme. RM is easy to  
36 implement in practice and does not require pedigree data. When full pedigree is available, OC  
37 management is preferable.

38

39 Keywords: optimal contribution, rotational mating, breeding circle, zoo populations

40

## 41 **Introduction**

42

43 Maintenance of genetic diversity is particularly important for small populations of wild  
44 animals maintained in zoo gardens. Currently zoos have a rich portfolio of breeding programs

45 that includes more than 500 species survival plan (SSP) programs managed by Association of  
46 Zoos and Aquariums (AZA 2014), and 388 European Endangered species Programs (EEP)  
47 and the European StudBook (ESB) programs managed by European Association of Zoos and  
48 Aquaria (EAZA 2014). The aim of these programs is to make every effort to ensure that  
49 genetic diversity of captive populations is maintained. The key in this case is to maintain  
50 adequate genetic and demographic structure, which will ensure steady growth of the  
51 population over many generations (Lees & Wilcken 2009). Cooperation is indispensable here  
52 as many zoo populations are isolated and too small to conduct a balanced breeding program  
53 (Lees & Wilcken 2009).

54 The aim of every genetic conservation program is to minimize the loss of genetic diversity,  
55 and to control rates of inbreeding as much as possible. However, some reports (Earnhardt et  
56 al. 2001; Zimmermann et al. 2007) suggest that a number of breeding programs in zoos  
57 achieve sub-optimal results. According to Long et al. (2011) the median of gene diversity  
58 retained for 264 SSP populations is 92% and it is predicted to fall to 67% in 100 years. It is  
59 also worth mentioning that 38% of the AZA populations retain less than 90% of genetic  
60 diversity of the founding populations (Long et al. 2011). This points to the need to optimize  
61 breeding programs to better manage available resources. Past methods proposed for  
62 conservation of genetic diversity included tools that affect mating strategies such as  
63 maximum avoidance of inbreeding (Wright 1921), which can be efficient in the short term  
64 but may result in high inbreeding in the long term (Caballero & Toro 2000), and tools to  
65 maintain genetic diversity like population fragmentation and breeding in isolated herds to  
66 conserve genetic diversity on the meta-population level (Leus et al. 2011). However, the  
67 latter strategy is not recommended for small herds as inbreeding will rapidly increase,  
68 resulting in inbreeding depression and accumulation of deleterious alleles (Whiteley et al.  
69 2015). Currently, mating of animals based on mean kinship is the main method used for

70 management of zoo populations (Leus et al. 2011). Mean kinship is the mean of the kinship  
71 coefficients of a given individual with every individual in the population including itself.  
72 Priority is given to matings of animals with low mean kinship as they have few relatives in  
73 the population and are likely to carry alleles that are rare in the population (Ballou & Lacy  
74 1995). Determination of parents and their contributions to the next generation has to be  
75 followed by a decision on how to mate the animals. This can be achieved through a two-step  
76 approach, where first contributions are allocated to the animals and subsequently mating pairs  
77 are optimised (Fernandez & Caballero 2001; Sonesson & Meuwissen 2000), or a single-step  
78 approach that optimises both parameters at the same time (Ballou & Lacy 1995; Fernandez et  
79 al 2001). Fernandez et al. (2001) evaluated performance of both methods using simulations  
80 and concluded that the amount of genetic diversity preserved by the two methods was quite  
81 similar, but the single-step method was more flexible and assured compatibility between the  
82 contributions of males and females. This should therefore lead to overall higher levels of  
83 genetic diversity. A more recent simulation study by Ivy & Lacy (2012) explored the above  
84 mentioned mating strategies with an additional layer of complexity such as variable mortality  
85 rate, reproductive rate, pairing success rate and reproductive success rate. As a result dynamic  
86 mean kinship strategy (two-step approach) was recommended as optimal for populations of  
87 mammals with low fecundity and long lifespans (Ivy & Lacy 2012).

88 Implementation of kinship-based management poses several difficulties that are related to the  
89 limitations of zoo breeding programs. Zoo populations usually have a very limited capacity  
90 that restricts the number of animals which can be kept in the population. Therefore, to  
91 maintain the populations in good condition and avoid overcrowded zoo environment, it is  
92 necessary to either transfer part of the animals to another zoo or to cull them. Transferring of  
93 animals can also pose a significant challenge particularly if the animals that would form  
94 optimal mating pairs are from two distant locations. Therefore it is important to take into

95 account fragmentation of the population and organise the transfer of animals that limits the  
96 costs and effort involved. Additionally, with species such as deer or many other hoofstock  
97 animals, it is important to account for skewed reproduction rates. Because of hierarchical  
98 structure, few dominant males mate with groups of females and have high contributions,  
99 whereas the majority of males have very low or zero contributions to the next generation.

100 The best known strategy to maintain genetic diversity is by optimizing the contributions of  
101 the parents to minimize global kinship in their offspring (optimal contribution theory;  
102 (Meuwissen 1997). In livestock this method is used to optimize genetic gain while  
103 simultaneously restricting the rate of inbreeding. However, efficiency of this method depends  
104 strongly on pedigree completeness (Oliehoek & Bijma 2009). It has been demonstrated that  
105 with pedigrees containing gaps or misidentified parents the method does not perform well  
106 (Oliehoek & Bijma 2009). Low quality of pedigree information in populations of livestock  
107 and zoo animals is a common problem (Earnhardt et al. 2004; Oliehoek & Bijma 2009).  
108 Incorrect entries in pedigrees are usually impossible to detect without the use of molecular  
109 markers. In case of livestock, the level of pedigree errors reported in the literature is around  
110 10% (Oliehoek & Bijma 2009), which can decrease efficiency of the optimal contribution  
111 method.

112 On the other hand there are methods, such as rotational mating, which do not require accurate  
113 pedigree records to control the increase of inbreeding (Nomura & Yonezawa 1996).

114 Rotational mating occurs in several forms, but the basic idea is that females are mated with  
115 males coming from a different herd in a “round robin fashion”. Breeding circle, used  
116 especially in sheep breeding, is one of the most common forms of rotational mating. It is  
117 based on the strategy that each herd never uses its own males for breeding. Exchange of  
118 males between herds is performed in a rotational way. The first herd provides males for the  
119 second herd, the second herd provides males for the third, etc., and the last herd is the source

120 of males for the first herd (Windig & Kaal 2008). Possible errors or missing information in  
121 the pedigrees do not affect performance of the method. Moreover, it can be used even in the  
122 absence of pedigree data under the condition that it is possible to determine the herd of origin  
123 for each animal.

124 Captive breeding programs usually aim to preserve the population so that it can be  
125 reintroduced into the wild in the future (Frankham et al. 2010; Frankham 2015). The success  
126 of such reintroduction widely depends on the level of wild fitness that has been preserved in  
127 captivity. The mechanism of genetic adaptation to the captive environment is driven by  
128 natural selection. Because zoo environments differ from the wild, the genetic variants that  
129 confer the highest fitness in captivity differ from those that are beneficial in natural  
130 environment (Frankham 2008). Genetic adaptation to captivity increases with selection  
131 differential, genetic diversity, effective population size and generations in captivity  
132 (Frankham 2008). Therefore it is important to avoid unintentional selection for characteristics  
133 related to exterior appearance of the animal or personal preference of program coordinators.  
134 This can be easily achieved in genetic management programs that are based on kinship, such  
135 as optimal contribution theory, as it does not involve phenotypic selection, apart from natural  
136 selection. On the other hand, some degree of unintentional phenotypic selection can occur  
137 when only part of the animals are selected.

138 In this paper we use deer species maintained in zoos as an example to compare two strategies  
139 to manage their genetic diversity. Deer populations in zoos are characterized by  
140 fragmentation of the meta-population over several herds, skewed mating ratios ranging from  
141 1 to 5 males per 10 females, and high variance in offspring among males caused by dominant  
142 males in the herds. A recent analysis of studbooks of 15 deer species in European zoos  
143 showed that most studbooks were incomplete, and that 10 out of 15 populations (species) had  
144 low effective population size (between 10 and 20) with significant effect of inbreeding on

145 longevity and survival of offspring (Mucha, unpublished results). This points to the need for  
146 more effective management programs. In theory, breeding circles could offer a simple  
147 alternative to pedigree-based management for deer populations as deer are similar to sheep  
148 populations in a number of ways such as low reproductive rate of females, division in herds  
149 and males with highly variable number of offspring. Therefore, the aim of this paper was to  
150 compare performance of genetic management by rotational mating (breeding circles) with  
151 management based on pedigree and optimal contributions in simulated populations of deer.  
152 The simulations were designed in such a way that implications of the results could be  
153 extended to a wide range of zoo populations of animals with similar live histories and  
154 demographics.

155

## 156 **Materials and Methods**

157

### 158 **Simulated populations**

159 Three populations were simulated that resembled small populations of deer maintained in  
160 zoos in Europe. They were divided into herds resembling the actual number of zoos that bred  
161 each population. The first was a large population (based on *Elaphurus davidianus*) consisting  
162 of 1200 individuals per generation, divided over 20 herds (60 animals per herd). The second  
163 population (based on *Cervus eldi thamin*) consisted of 240 animals, divided in 6 herds (40  
164 animals per herd), and the third (*Axis calamianensis*) of only 60 individuals kept in three  
165 herds (20 animals per herd). In each of the simulated populations half of the animals born  
166 each generation were male and half were female. For simplicity it was assumed that animals  
167 reproduced only once in a lifetime and died afterwards (discrete generations). Furthermore, to  
168 maintain a constant population size in each generation, it was assumed that each female gave



169 birth to two offspring: two males, two females, or one male and one female. The average sex  
 170 ratio of all offspring (population level) in each generation was 1:1. There was no selection in  
 171 females as they all had to participate in the creation of the next generation in order to  
 172 maintain a constant population size. For each population, three mating ratios were simulated,  
 173 where all females were used along with only 50% (1:2 mating ratio), 20% (1:5 mating ratio)  
 174 or 10% (1:10 mating ratio) of males. Using only 10, 20, or 50% of the available males  
 175 implies that (unintentional) selection could take place, which could lead to genetic adaptation.  
 176 We therefore simulated two scenarios: one with random selection of males, and one with  
 177 selection of males on a single (not specified) trait with a heritability of 0.3 and phenotypic  
 178 variance of 1. The effect of inbreeding depression on the simulated trait was not included in  
 179 the simulation.

180 Simulations were performed for 20 generations, and replicated 50 times.

181 All founders had a phenotypic value of 0 and a genetic value calculated as:

$$182 \quad r_{norm} \sqrt{h^2 \sigma_p^2}$$

183 where:  $r_{norm}$  is a random number drawn from a normal distribution with a mean of 0 and  
 184 variance of 1,  $h^2$  is the heritability (0.3), and  $\sigma_p^2$  is the phenotypic variance of the simulated  
 185 trait. In every generation, each offspring was assigned a genetic value  $A$  as follows:

$$186 \quad A = \frac{1}{2} A_s + \frac{1}{2} A_D + MS$$

187 where  $A_s$  and  $A_D$  are genetic values of sire and dam, respectively. Mendelian sampling term  
 188 (MS) was calculated as:

$$189 \quad MS = r_{norm} \sqrt{\frac{1}{2} \left( 1 - \frac{1}{2} (F_s + F_D) \right) h^2 \sigma_p^2}$$

190 where  $F_S$  and  $F_D$  are inbreeding coefficients of sire and dam, respectively. Phenotypic value  
191 of offspring was assigned as:

$$192 \quad P = A + r_{norm} \sqrt{(1 - h^2) \sigma_P^2}$$

193

#### 194 **Rotational mating (RM)**

195 Breeding circles with discrete generations were used. In every generation the first herd  
196 provided male offspring for mating with females from the second herd. The second herd  
197 supplied males to the third herd et cetera. Female offspring remained in the same herd where  
198 they had been born. Two scenarios were considered for selection of males (Table 1). In the  
199 first scenario (1a), males were chosen at random (random selection) and rotated to the  
200 neighbouring herd, where they were used for breeding. The number of selected males  
201 depended on the mating ratio assumed (1:2, 1:5 or 1:10). Selected males were mated at  
202 random with females. Non-selected males did not reproduce. In the second scenario (1b)  
203 selection of males was based on their phenotype for the simulated trait (phenotypic selection).  
204 Males with the highest trait values were selected and rotated to the neighbouring herds, where  
205 they were mated at random with females.

206

#### 207 **Optimal contribution (OC)**

208 The software package Gencont with the option “*minimise ΔF*” (Meuwissen 1997;  
209 Meuwissen, 2002) was used to determine the optimum contributions of animals to the next  
210 generation in order to achieve the lowest possible rate of increase in mean pairwise kinship.  
211 This was achieved by varying the contribution of each animal to the next generation (fraction  
212 of offspring attributable to the parent) according to the equation:

213 
$$\min(\bar{A}_p) = \mathbf{c}'\mathbf{A}\mathbf{c}$$

214 where  $\bar{A}_p$  is the lowest possible average relationship of parents;  $\mathbf{c}$  is a vector of contributions  
215 of length  $n$  ( $n$  = the total number of animals in the population);  $\mathbf{A}$  is a relationship matrix of  
216 selection candidates. The contributions of the selection candidates  $\mathbf{c}$  are optimized by the  
217 Lagrangian multiplier method. The  $\bar{A}_p$  value has to increase over generations due to finite  
218 population size which leads to increase of relationships over time.

219 In order to maintain constant population size, all females ( $N_f$ ) were selected and received  
220 equal contributions of  $1/N_f \cdot 100\%$ . Selection was performed only on the male side and their  
221 contributions differed depending on their relatedness. The process was optimised so that for a  
222 given number of males required for breeding (determined by the mating ratio) a set of males  
223 was selected that would minimise kinship in the next generation, and their contributions were  
224 varied to achieve the optimal result. The population was managed purely on kinship, thus  
225 genetic gain was not expected.

226 With optimal contribution, three alternative management scenarios were considered (Table  
227 1). In the first scenario it was assumed that mating was performed in a meta-population  
228 without any subdivision into herds. Therefore selection and contribution of males based on  
229 the optimal contribution theory was applied on a meta-population scale ( $OC_m$ ). In the second  
230 scenario, selection and mating was performed separately within each herd. There was no  
231 exchange of animals between the herds ( $OC_i$ ). The third scenario was similar to the optimal  
232 contribution performed separately within each herd, but each generation males were  
233 exchanged between herds in a rotational fashion similar to a breeding circle ( $OC_r$ ).

234 Contributions of sires and dams to the next generation were calculated assuming all sires  
235 from the neighbouring herd had been transferred to the herd of destination. Effectively, the  
236 number of sires rotated between the herds was equal to the number of sires with nonzero

237 contributions. In every scenario, three mating ratios (1:2, 1:5 and 1:10) were simulated and  
 238 selected males mated with randomly chosen females. The mating ratio determined the  
 239 number of males with nonzero contributions selected by GENCONT in the optimal  
 240 contribution based scenarios, i.e. a mating ratio of 1:2 in pop3 means that 15 out of 30 sires  
 241 were selected to contribute to the next generation.

242

### 243 **Calculation of parameters**

244 The mean coefficient of inbreeding in each generation ( $\overline{F}_t$ ) was calculated as:

$$245 \quad \overline{F}_t = \frac{\sum_{i=1}^{N_t} F_i}{N_t}$$

246 where:  $F_i$  is the inbreeding coefficient of the  $i$ -th individual from generation  $t$ ,  $N_t$  is the  
 247 number of individuals born in generation  $t$

248 Increase of inbreeding per generation was calculated as:

$$249 \quad \Delta F_t = \frac{\overline{F}_t - \overline{F}_{t-1}}{1 - \overline{F}_{t-1}}$$

250 Where:  $\overline{F}_t$  and  $\overline{F}_{t-1}$  are the mean inbreeding coefficients of the whole population in  
 251 generation  $t$  and  $t-1$ , respectively.

252 Mean increase of inbreeding in generations 5 to 20 was calculated as:

$$253 \quad \Delta F = 1 - \sqrt[15]{\frac{1 - F_{20}}{1 - F_5}}$$

254 To allow for good comparison between rotational mating and optimal contribution selection  
255 schemes, generation 5 was used as starting point assuming that the population had reached  
256 Bulmer equilibrium.

257 Across population mean kinship in each generation was calculated as:

258 
$$\overline{mk}_t = \frac{1}{N_t} \sum_{i=1}^{N_t} mk_i = \frac{1}{N_t^2} \sum_{i=1}^{N_t} \sum_{j=1}^{N_t} f_{ij}$$

259 where:  $mk_i$  is the mean coefficient of kinship of the  $i$ -th individual with the rest of the  
260 population,  $f_{ij}$  is kinship between individuals  $i$  and  $j$  and  $N_t$  represents the total number of  
261 individuals born in the whole population in a given generation. Mean kinship within  
262 individual herds was calculated using the same formula, with  $N_t$  equal to the number of  
263 individuals born in each herd in a given generation.

264 Phenotypic mean was calculated as an arithmetic mean of trait values observed in a given  
265 generation. The mean genetic level was an arithmetic mean of breeding values for the  
266 simulated trait in a given generation.

267

## 268 **Results**

269

### 270 **Rate of Inbreeding ( $\Delta F$ )**

271 *Rate of inbreeding over time:* as expected, in all schemes that used rotational mating (RM and  
272 OCr), inbreeding rates were zero in generations 1 and 2, and at a fairly constant level  
273 thereafter (Figure 1). Inbreeding rates in OCi and OCm schemes increased after generation 1,  
274 and fluctuated across generations, especially in small populations (e.g. 60 animals, see Figure  
275 1).

276 *Effects of population size and mating ratio:* for each scheme tested, rates of inbreeding  
277 increased almost linearly with increasing mating ratio (Table 2). Inbreeding rate was highest  
278 in the smallest population (3x 20 animals) where the mean  $\Delta F$  ranged from 0.71% to 2.20%  
279 (mating ratio 1:2) or from 3.11% to 10.99% per generation (mating ratio 1:10). As the  
280 population size increased the mean  $\Delta F$  decreased (Table 2), irrespective of scheme. Lowest  
281 rates of inbreeding were recorded in the biggest population (20 x 60 animals), ranging from  
282 0.03% to 0.72% at mating ratio 1:2 and from 0.14% to 3.09% at mating ratio 1:10.

283 *Effect of management scheme:* lowest rates of inbreeding were obtained when managing the  
284 meta-population with optimal contribution (OCm). Rates of inbreeding <1% could be  
285 achieved in populations with 240 and 1200 animals, for each mating ratio. In the smallest  
286 population (60 animals), achieving a rate of inbreeding <1% was only possible with a mating  
287 ratio of 1:2 (Table 2). In contrast, the highest rates of inbreeding were realized when  
288 managing the populations with optimal contribution in isolated herds. In the smallest  
289 population (3x20), rate of inbreeding ranged from 2.20% to 10.99% when managed with 1:2  
290 and 1:10 mating ratio, respectively. Mean  $\Delta F$  was smaller in the larger populations, but still  
291 mostly above the 1% threshold (Table 2).

292 Rotational exchange of sires between the herds, managed with (OCr) or without optimal  
293 contribution (RM), produced very similar results. Rates of inbreeding were always below 1%  
294 per generation (Table 2), except for the smallest population, managed with mating ratios of  
295 1:5 or 1:10.

296

## 297 **Kinship**

298 The mean kinship of generation 20, calculated across herds, was very similar for all  
299 management schemes. Mean kinship increased with mating ratio and decreased with

300 population size. Lowest across mean kinship (0.01) was observed in the population with 1200  
301 animals, divided over 20 herds. The highest values (0.30-0.49) were observed in the 3x20  
302 population with a 1:10 mating ratio.

303 Managing isolated herds with optimal contribution, without exchange between herds (OCi),  
304 resulted in individual herds becoming very distinct from each other. Therefore, the ‘across’  
305 mean kinship of the whole population was lower in comparison to the other management  
306 schemes, while the ‘within’ mean kinship was highest, ranging from 0.15 (20x60; mating  
307 ratio 1:2) to 0.91 (3x20; mating ratio 1:10) (Table 2).

308 The mean kinship (within and across) of generation 20 was very similar for RM and OCr  
309 schemes. Due to the exchange of males, herds were more connected than in the previous  
310 scenario (OCi) which led to a higher mean kinship of the whole population.

311

### 312 **Inbreeding rate in RM schemes with and without unintentional selection**

313 Performance of rotational mating schemes was also evaluated in additional simulations where  
314 we assumed unintentional directional selection for a single simulated trait. The results of this  
315 analysis indicate that rotational mating schemes with or without selection produce very  
316 similar mean inbreeding rates in all of the analysed populations, irrespective of mating ratio  
317 (Table 3). However, selection for a moderately heritable trait leads to substantial increase in  
318 genotypic values, up to 2 or more standard deviations from the founder mean, even in small  
319 populations (Table 3). For the larger populations, this is in line with what can be theoretically  
320 expected from phenotypic selection with selected proportions of 10% - 50%, a heritability of  
321 0.3 and a phenotypic standard deviation of 1.

322

## 323 **Discussion**

324

325 Research on effective methods to reduce inbreeding in small zoo populations is particularly  
326 important in the light of recent reports stating that many of the current conservation programs  
327 do not meet the established demographic and genetic assumptions, which may threaten their  
328 survival or adversely affect the health of animals (Lacy 2013; Leus et al. 2011). The main  
329 problem according to Lacy (2013) is that many of the endangered populations have a limited  
330 availability of candidates for selection and low pedigree completeness. Therefore methods  
331 that can mitigate these problems are of particular value for zoo populations. Use of rotational  
332 mating schemes has been evaluated for small breeds of cattle (Colleau & Avon 2008), and  
333 sheep (Windig & Kaal 2008). Optimal contribution has also been studied as a tool to manage  
334 livestock populations (Avendaño et al. 2003; Koenig & Simianer 2006; Sonesson &  
335 Meuwissen 2000). However, to our knowledge the two methods have never been compared.  
336 Particularly, they have not been studied with respect to their performance in populations of  
337 zoo animals managed as herds.

338 Current analysis shows that breeding circles can be a good solution for small populations  
339 maintained in zoos. They can efficiently reduce inbreeding rate with minimum data input. It  
340 only requires information about herd of origin for each animal. This is sufficient to plan  
341 which individuals will be transferred to another herd. Therefore, the quality of pedigrees does  
342 not affect the performance of the breeding program (Windig & Kaal 2008). Moreover, simple  
343 organization of breeding circles makes them easy to set up. It could be relatively easy to  
344 determine which zoos would exchange animals and assign them to the breeding circle.  
345 Assignment of zoos to the breeding circle could be done considering their geographical  
346 locations so that animals are not transported over long distances. In case of populations where



347 the location and costs would prohibit the transfer of animals on a regular basis, the OC  
348 method supplemented with only occasional exchange could be a more feasible option.  
349 Additional research is needed to determine the sensitivity of rotational mating to such factors  
350 as overlapping generations, frequency and complexity of rotation pattern.

351 Rotational mating appears to be a very robust method that can restrict inbreeding regardless  
352 of selection pressure and pedigree completeness. However, caution is required when not all  
353 animals are used for breeding, as is the case for populations where excess males are culled or  
354 removed. Selection criteria in rotational mating schemes are not based on measures related to  
355 genetic diversity harboured by each animal (mean kinship or heterozygosity). It relies entirely  
356 on phenotypic selection which involves a risk of unintentional selection for “the best  
357 animals”; this may lead to genetic adaptation to captivity (Frankham 2008). This risk can be  
358 minimized by selection of males using criteria related to genetic diversity along with careful  
359 management of migration rates.

360 In the rotational mating schemes all of the selected males are assumed to contribute equally  
361 to the next generation. On the other hand, optimal contribution theory allows for optimizing  
362 their contributions and assigning different number of progeny to each male depending on  
363 their relatedness to the population. Therefore optimal contribution requires more control from  
364 species coordinators allowing for a more precise management of the population.

365 Apart from the management scheme used (optimal contribution or rotational mating), the  
366 major factor affecting inbreeding rate was population size. Our results demonstrate that  
367 populations with less than 60 animals cannot be managed without losing genetic diversity at  
368 an unacceptable rate. According to the analyses of programs run by AZA and EAZA more  
369 than a third of the populations are comprised of less than 50 individuals (Leus et al. 2011;  
370 Long et al. 2011). Therefore, in such small populations it is particularly important to

371 maximize the number of males that participate in mating. In the simulated populations, a  
372 mating ratio of 1:2 essentially always resulted in inbreeding rates below 1% per generation.  
373 Higher mating ratios (1:5 and 1:10), where a very limited number of males participated in  
374 mating usually led to a rapid increase of inbreeding regardless of the management scheme.  
375 This was particularly apparent in populations managed as isolated herds, though the problem  
376 exists also in herds with rotational exchange of males. Our results suggest that in a population  
377 with 240 animals spread over 6 herds, the rate of inbreeding can be restricted to <1% per  
378 generation. Therefore six zoos or deer parks could be sufficient to conserve an endangered  
379 population. This points towards the need to increase the size of populations that are  
380 considered as valuable from a conservation stand point.

381 The best results in terms of kinship and rates of inbreeding were obtained when OC was  
382 carried out within the meta-population. In the meta-population there are more parents,  
383 resulting in lower average relatedness. On the other hand, when the population is divided into  
384 isolated herds, a much lower number of parents is available, which translates into a  
385 significant increase of inbreeding. In isolated herds genetic drift reduces genetic diversity  
386 within herds (Frankham et al. 2010; Lacy 1987). When subpopulations become completely  
387 inbred, the total genetic diversity is preserved due to genetic variability between the herds  
388 (Lacy 1987). However, this approach poses a significant risk that some subpopulations will  
389 suffer from inbreeding depression, and may not survive (Leus et al. 2011). Moreover, sub-  
390 populations depart significantly from the founders (Chesser 1983). Therefore it is important  
391 to manage both the exchange of animals between the herds and contributions of animals from  
392 each herd to the next generation (Fernández et al. 2008).

393 Zoo populations usually have a very limited capacity that restricts the number of animals  
394 which can be kept in the population. Therefore, to maintain the populations in good condition  
395 and avoid overcrowded zoo environment, it is necessary to either transfer part of the animals

396 to another zoo or to cull them. In the latter case it is important to remove those animals that  
397 are least valuable to the population. The number of animals that have to be culled (selection  
398 intensity) depends on reproductive capacity of the species. Generally selection intensity will  
399 be higher in males than in females, which is similar to the conditions simulated in the current  
400 study, where mating ratio determined selection intensity. A selective culling strategy can also  
401 increase fitness of the population by eliminating individuals with health and fertility issues.  
402 The main goal of conservation programs is to select animals that are beneficial for genetic  
403 diversity of the population due to their low average relatedness to the population. One must  
404 also bear in mind that in certain cases also some unintentional selection for desired  
405 phenotypic characteristics may take place. This may lead to genetic adaptation to captivity  
406 which is undesirable from a conservation point of view (Frankham 2008). That is why in the  
407 current study, breeding scenarios were evaluated with respect to their potential to restrict  
408 inbreeding under no directional selection pressure and with unintentional selection. Our  
409 results indicate that by using a rotational mating scheme, inbreeding can be restricted with  
410 and without directional selection. However, any form of directional selection will increase  
411 adaptation to captivity and loss of “wild” alleles. Therefore the choice of selection criteria  
412 and breeding scheme is a crucial point in managing populations.

413 It is important to look at the current results in the light of assumptions made during the design  
414 of the simulations. One of the restrictions was that animals could reproduce only once in their  
415 lifetime and were replaced by their offspring thereafter (discrete generations). This  
416 assumption could be valid also in real zoo populations as long as females that had produced  
417 offspring were moved away from males. Otherwise, overlapping generations could be  
418 accounted for in the optimal contribution algorithm, and the average coancestry would be  
419 limited across generations (Meuwissen 1997). Another limitation of the simulated population  
420 was connected with reproductive capacity of females. To keep a constant population size, it

421 was assumed that dams always give birth to two offspring. Removing this restriction will  
422 improve the performance of the OC algorithm. Additionally, to keep a constant population  
423 size, each generation all females had to be used for breeding and selection was possible only  
424 among males. In real populations, where generations overlap, it would be also possible to  
425 perform some selection on the female side. Using the appropriate breeding scheme, this could  
426 have a positive effect on fitness of the population without detrimental effects on the rate of  
427 inbreeding (Meuwissen 2009). Results obtained with the investigated methods could be  
428 further improved by optimisation of mating schemes. In the current simulations it was  
429 assumed that the selected animals were mated at random. Use of a two stage optimisation  
430 methods, where the optimisation of contributions is followed by optimisation of mating pairs,  
431 could have a positive effect on the inbreeding level and the amount of genetic diversity  
432 conserved (Ivy & Lacy 2012). Another alternative could involve a single-step optimisation  
433 process, where both the contributions and mating design would be optimised in one step  
434 (Fernandez et al. 2001).

435

436 Comparison of optimal contributions carried out in a meta-population and in a population  
437 divided into herds, showed that breeding within isolated zoos leads to a significant increase in  
438 inbreeding within individual herds. Optimal contribution method performed better when it  
439 was conducted within a meta-population. However, due to organizational issues global  
440 management of geographically separated herds can be a problem. Therefore, a combination  
441 of the optimal contribution method with rotational mating might be a better alternative. In  
442 this situation, optimal contribution method would be carried out separately within each herd.  
443 Additionally, each generation males would be exchanged between the neighbouring zoos.  
444 Comparison of optimal contribution method with rotational mating demonstrated that they  
445 perform equally well. Simulations showed that rotational mating can efficiently reduce the

446 inbreeding rate. Moreover inbreeding rate in breeding circles with and without selection  
447 pressure was similar. Therefore unintentional selection does not reduce the efficiency of this  
448 scheme with respect to restriction of inbreeding. On the other hand, optimal contributions  
449 require more control over the conservation scheme by varying the contributions of animals to  
450 the next generation. However, optimal contribution requires complete and accurate pedigree  
451 records (Oliehoek & Bijma 2009), which are often unavailable (Mucha et al. unpublished  
452 results). Combination of the two methods (OCr) would result in a robust scheme which slows  
453 down the increase of inbreeding and works irrespective of the pedigree. Accompanied by  
454 optimization of animal contributions within each herd, this scheme would give further benefit  
455 depending on pedigree completeness and would not be sensitive to unintentional selection.

456

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458

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465

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- 545

546 **Table 1.** Selection criteria for males, their contributions to the next generation and exchange  
 547 between herds.

Scenario	Selection	Contributions	Exchange	Abbreviation
Rotational	Random	Equal contributions	Between herds	RM
	Mass			
Optimal contribution	Kinship	Optimal contribution theory	Across meta-population	OC <sub>m</sub>
			Between herds	OC <sub>r</sub>
			Within herds, no exchange	OC <sub>i</sub>

548

549

**Table 2.** Inbreeding rate ( $\Delta F^1$ ), and mean kinship within and across herds after 20 generations of selection.

Population Size <sup>2</sup>	mating ratio	RM			OC <sub>m</sub>		OC <sub>i</sub>			OC <sub>r</sub>		
		$\Delta F$ [%]	Kinship		$\Delta F$ [%]	Kinship	$\Delta F$ [%]	Kinship		$\Delta F$ [%]	Kinship	
			within <sup>3</sup>	across				within <sup>3</sup>	across		within <sup>3</sup>	across
3x20	1:2	0.83	0.19	0.16	0.71	0.14	2.20	0.38	0.13	0.75	0.18	0.15
	1:5	1.89	0.37	0.32	1.53	0.28	4.93	0.65	0.22	1.55	0.32	0.28
	1:10	3.24	0.55	0.49	3.11	0.48	10.99	0.91	0.30	3.14	0.54	0.48
6x40	1:2	0.22	0.08	0.04	0.17	0.04	1.02	0.20	0.03	0.19	0.07	0.04
	1:5	0.52	0.15	0.09	0.37	0.08	2.30	0.39	0.07	0.40	0.12	0.08
	1:10	0.93	0.26	0.16	0.72	0.14	4.74	0.64	0.11	0.76	0.22	0.14
20x60	1:2	0.13	0.05	0.01	0.03	0.01	0.72	0.15	0.01	0.12	0.04	0.01
	1:5	0.31	0.10	0.02	0.07	0.02	1.53	0.28	0.01	0.24	0.09	0.02
	1:10	0.58	0.18	0.04	0.14	0.03	3.09	0.48	0.02	0.45	0.15	0.03

551 <sup>1</sup>Mean increase of inbreeding in generations 5 to 20; <sup>2</sup>nr of herds x nr of animals in each herd; <sup>3</sup>kinship calculated within each herd. OC<sub>m</sub>, OC<sub>i</sub>

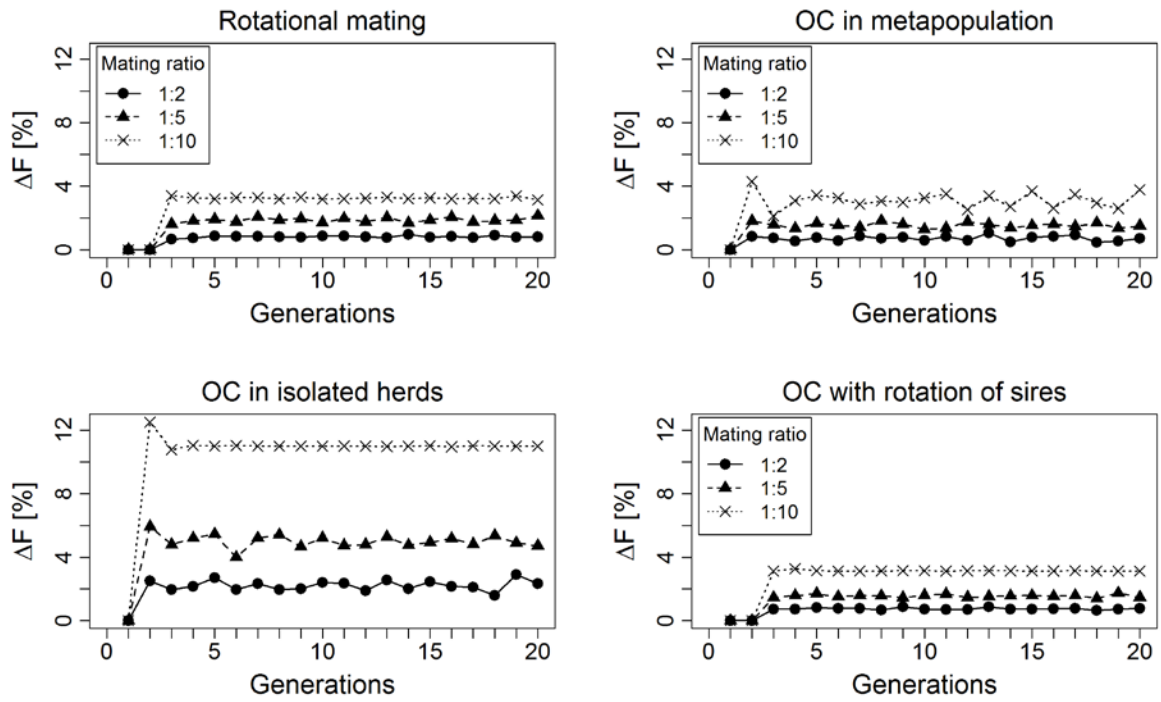
552 OC<sub>r</sub> and RM, see table 1.

**Table 3.** Genetic level, and inbreeding rate in breeding circles with and without random selection of males.

Scenario	mating ratio	Not random		Random	
		$G^1$	$\Delta F^2$	$G^1$	$\Delta F^2$
Three herds with 20 animals per herd	1:2	1.78	0.84	0.04	0.83
	1:5	2.64	1.91	0.05	1.89
	1:10	2.55	3.25	0.03	3.24
Six herds with 40 animals per herd	1:2	1.98	0.23	0.00	0.22
	1:5	3.18	0.53	0.00	0.52
	1:10	3.41	0.94	-0.01	0.93
Twenty herds with 60 animals per herd	1:2	2.01	0.14	-0.01	0.13
	1:5	3.34	0.33	-0.02	0.31
	1:10	3.79	0.61	-0.01	0.58

<sup>1</sup>G – mean genotypic level of the population after 20 generations of selection, expressed in units of standard deviation from the mean of the founder generation.

<sup>2</sup> $\Delta F$  – inbreeding rate in %



**Figure1.** Inbreeding rate in population of 60 animals, divided over 3 herds, managed with different scenarios