



Sveriges lantbruksuniversitet
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Faculty of Veterinary Medicine and Animal Science
Department of Animal Breeding and Genetics

Inbreeding and its effect on fitness traits in captive populations of North Persian leopard and Mhorr gazelle

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Examensarbete / Swedish University of Agricultural Sciences
Department of Animal Breeding and Genetics
463
Uppsala 2009

Master's Thesis, 30 hp
Erasmus Mundus Programme
– European Master in Animal
Breeding and Genetics



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Credits: 30 HEC

Course title: Degree project in Animal Science

Course code: EX0556

Programme: Erasmus Mundus programme

– European Master in Animal Breeding and Genetics

Level: Advanced, A2E

Place of publication: Uppsala

Year of publication: 2009

Name of series: Examensarbete / Swedish University of Agricultural Sciences
Department of Animal Breeding and Genetics, 463

On-line publication: <http://epsilon.slu.se>

Key words: Inbreeding depression, purging, leopard, gazelle, zoo animals



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ABSTRACT

In this study, linear mixed model analyses was conducted to assess inbreeding depression, purging and founder heterogeneity in relation to fitness traits (survival traits and litter size) in captive populations of North Persian leopard and Mhorr gazelle. “*Old*” and “*new*”, ancestral, partial and partial ancestral inbreeding coefficients were included in the models as finer scale measurements in addition to the classical inbreeding coefficient. In North Persian leopard, possible inbreeding depression for survival at days 7 and 30 after birth and weaning age (90 days) is associated with individual/ litter classical inbreeding, further attributed mainly to “*old*” inbreeding. However, a sign of purging can be observed because increased dam inbreeding corresponds with an increased probability for survival of the offspring. Detailed analyses revealed that this effect is significantly associated with the “*new*” inbreeding of the dam. Inbreeding depression is also expressed as litter size reduction. Ancestral inbreeding significantly reduces litter size but has no effect on survival traits. Therefore, no purging could be detected using ancestral inbreeding coefficients. On the other hand, individual classical and “*new*” inbreeding increases the mortality of Mhorr gazelle at weaning (day 180). Sire inbreeding significantly increases mortality at days 7, 30 and 180 which is further associated with “*old*” and “*new*” inbreeding. In both species, there is unbalanced founder contribution of alleles causing inbreeding depression and purging in fitness traits as shown in the results from the analyses including partial and partial ancestral inbreeding coefficients. The study shows that the magnitude of response to inbreeding differs between species and fitness traits.

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1 INTRODUCTION

Captive breeding of endangered or threatened animal populations is becoming more important with the endeavors to maintain genetic variability and avoid inbreeding depression (Hedrick, 1994). Zoo populations may also serve as a reservoir of genetic materials that can be utilized for the reestablishment or reinforcement of wild populations thus, considered essential in the prevention of extinction of a species (Read, 1986; Lacy, 1993). Animals in *ex situ* conservation are also expected to have an improved survival rate as genetic resource when they are reintroduced into the natural population (Ramirez, *et al*, 2006).

However, population sizes in zoos are usually small. Inbreeding is unavoidable, leads to unfavorable consequences such as inbreeding depression. This major risk factor in captive populations of threatened species elevates the risk of extinction in inbred captive populations (Frankham *et al*, 2001). Loss of genetic variability is another consequence which could be due to increase in homozygosity, founding event (founding effect) as subsequent generations emerge or when there is minimum exchange of animals between institutions (Richards, 2000). However, inbreeding also increases the frequency of genotypes being homozygous for deleterious alleles resulting in selection against these alleles, thus, purging the genetic load. Theoretically, purging results in an increase of fitness of a population under random mating with a balance between mutation and selection (Hedrick, 1994). Nevertheless, there are not enough studies on the effect of purging in animals whether in the wild nor in captivity.

This study focuses on the captive populations of the North Persian Leopard (*Panthera pardus saxicolor*) and the Mhorr Gazelle (*Gazella dama mhorr*) which is a subspecies of Dama gazelle (*Gazella dama*). North Persian Leopards are commonly found in the Middle East while Mhorr gazelles habituate the Atlantic Sahara of Africa. The International Union for Conservation of Nature (IUCN) declared the North Persian Leopard as endangered (Khorozyan, 2008) and the Mhorr gazelle as critically endangered (Newby, J. *et al*, 2008).

This study aims to (1) evaluate the genetic variability; (2) examine the significance of various measures of inbreeding to fitness traits; (3) determine the existence and possible effects of purging; and (4) to investigate founder heterogeneity in relation to inbreeding depression and purging in the populations of interest.

2 LITERATURE REVIEW

2.1 *Species biology*

2.1.1 North Persian leopard (*Panthera pardus saxicolor*)

The North Persian leopard is one of the largest among the eight subspecies of leopard in the world. Declared by IUCN as endangered (Khorozyan, 2008), this mammal is a member of the family *Felidae*, subfamily *Pantherinae* which is composed of the “roaring cats” like the lions, tigers, jaguars, snow leopards, clouded leopards and marbled cats. As compared to the spotting pattern of other relatives, clustered spots or “rosettes” of leopards do not contain a spot within (Figure 1).



Figure 1. North Persian leopard. Photo courtesy of Dave Watts

Populations can be found in Iran, Afghanistan, Turkmenistan, Armenia, Azerbaijan, Georgia, Turkey, Russia, North Caucasus and possibly Pakistan, Uzbekistan and Tajikistan. The largest population is found in Iran. IUCN Red List in 2008 declared this species as threatened with decreasing population size. It is also reported that there is no subpopulation that contains more than 100 mature individuals.

They are solitary predators living in exclusive territories and come together only on mating season. Dominant males are called “toms” which occupy larger territories, are typically solitary and mate with several dominant

females. Females have smaller territories than male. In general the leopard's territory depends on the availability of prey and the topography of the inhabited area. Male leopards reach sexual maturity at the age of 2 years while females at 3 years. Females exhibit estrus cycle at an interval of 6 weeks right after puberty. The gestation length is 90 days with litter size ranging from 1- 3. Cubs are weaned at approximately 3 months of age.

The life span is approximately 8 years in the wild and 22 years in captivity (Bies, 2002).

2.1.2 Mhorr gazelle (*Gazella dama mhorr*)

Mhorr gazelle (Figure 2) was declared by CITES as extinct in the wild and is listed as Appendix I species and proclaimed by IUCN as critically endangered. It is reported that there is no living individual in the wild (Newby, J. *et al*, 2008).



Figure 2. Mhorr gazelle. Photo courtesy of <http://www.itsnature.org>

This mammal has the darkest coloration among the Dama gazelle subspecies. The coloration varies with age and season which is typically dark chestnut brown in the upper parts such as the neck. The head is paler white, there is characteristic white coloration surrounding the eyes and the muzzle, with white area just below the throat. All the under parts are white with counter shading. Horns which are S- shape are present in both sexes with

males having thicker and larger than females. Average height ranges from 90- 120 cm (at the shoulder) and weight ranges from 40 – 75 kg.

Data in captivity estimated the gestation period to be 198 days producing a single fawn. Weaning is at 6 months of age. Sexual maturity is reached at the age of 2 years.

Mhorr gazelles have a life span of approximately 12 years in captivity.

2.2 Pedigree analysis

Genetic variability of a population can be evaluated by pedigree analysis using the probability of gene origin. The probability of gene origin can be assessed by determining the founder equivalents or effective number of founders, effective number of ancestors and founder genome equivalent or effective number of founder genes or genomes. These three measures were commonly used in wild populations. Ancestors with unknown parents are considered founders, especially those that are wild- caught (Lacy, 1989). Effective number of founders or founder equivalent is the number of founders that have equal contribution and are expected to produce the same genetic diversity of the population being studied. However, this measure does not take into account effects of bottlenecks. Genetic diversity is maintained and there is equal contribution among founders when the actual number of founders is equal to the number of effective number of founders. However, in real situations, effective number of founders is usually smaller than the actual number of founders (Lacy, 1989; Boichard *et al.*, 1997). Effective number of ancestors is defined as the number of equally contributing ancestors to the genetic diversity of the population under study taking into account a possible bottleneck experienced by the population. In most situations the effective number of ancestors is smaller than the effective number of founders (Boichard *et al.*, 1997). The effective number of founder genes or founder genomes is defined as the number of equally contributing founders with no random loss of founder alleles in the offspring, expected to produce the same diversity as in the population under study. This measure evaluates if the genes from the founders are still present in the population under study. Effective number of genomes is usually smaller than effective number of ancestors since this measure considers gene loss due to unequal founder

contribution, bottlenecks and random genetic drift (Lacy, 1989; Boichard *et al*, 1997).

2.3 Inbreeding depression and purging

Inbreeding is the mating of two animals that are related by descent from a common ancestor (Lacy, 1995). Inbreeding is unavoidable in small populations especially in zoo populations. Consequences of inbreeding include increase in homozygosity of deleterious alleles thus, inbreeding depression and reduction in genetic variability (Wright *et al*, 2008; Read and Harvey, 1986; Crnokrak and Roff, 1999).

Inbreeding depression refers to the reduction of fitness among inbreds compared to the fitness of offspring from randomly mating individuals. It is the major force which affects evolution and viability of small populations (Leberg and Firmin, 2008). Purging is when inbreeding depression is reduced due to selection against deleterious alleles (Ballou, 1997).

The response to inbreeding depression varies between traits wherein traits that involve fitness are the ones critically affected. Fitness traits include survival (number of young that survived), disease resistance, stress resistance and reproduction traits such as fertility, ejaculate volume, mating ability, female fecundity (number of eggs laid, embryogenesis) and litter size (Amos and Balmford, 2001; Crnokrak and Roff, 1999; Falconer and Mackay, 1996; Hedrick, 1994; Lacy *et al*, 1996; Read and Harvey, 1986; Keller and Waller, 2002). Inbreeding depression is accounted in captive, laboratory and wild populations (Ralls *et al*, 1988; Wright *et al*, 2008 ; Crnokrak and Roff, 1999). Inbreeding depression is also recognized as an important factor in determining the fitness of small populations (Kalinowski and Hedrick, 1999).

Two hypotheses were described how fitness is reduced due to inbreeding depression (Amos and Balmford, 2001; Wright *et al*, 2001). The partial dominance hypothesis states that inbreeding depression occurs when deleterious or partially recessive alleles are unmasked as compared when they are in heterozygous state. The overdominance hypothesis states that heterozygotes have superior fitness over the homozygotes and inbreeding depression results from the loss of the favorable heterozygotes. Wright *et al* (2008) pointed out that the partial dominance theory is the major cause of

inbreeding depression while others supported the overdominance theory being the one causing inbreeding depression. Other studies revealed that the two theories work simultaneously (Kristensen and Sorensen, 2005). However, a third hypothesis has been proposed stating that inbreeding depression is due to the separation of epistatic interaction between loci (Templeton and Read, 1994).

There are a number of factors influencing the magnitude, efficiency and detection of inbreeding depression and purging. Population size and structure has an influence on the magnitude of inbreeding depression. Smaller populations promote an increase in the frequency of deleterious alleles and thereby fixation becomes faster (Amos and Balmford, 2001). The domestication selection which enables the species to adapt to captive environment also promotes fixation of deleterious alleles which could be fixed and cannot be purged even with when introduction of new individuals is discontinued (Lynch and O'Hely, 2001). Further, purging was found to be more effective in case of a large population size (Frankham *et al.*, 2001; Boakes, *et al.*, 2006).

Genetic load, alleles involved and allele frequency are also influencing inbreeding depression and efficiency of purging (Bunnell, 1978; Gulisija, 2006; Lacy, 1996; Lynch and O'Hely, 2001; Rodríguez, 1998). The efficiency of purging depends on which alleles exist in the population and which ones are favorable. Purging is effective if there is overdominance of alleles or if the recessive genotype is lethal and/ or the heterozygotes are less viable than the homozygotes of the favorable alleles (Suwanlee *et al.*, 2006, Hedrick, 1994; Lacy *et al.*, 1996; Kristensen and Sørensen (2005; Kalinowski, 2000). Kristensen and Sørensen (2005) affirmed that inbreeding depression is dependent on the allele frequency. Since allele frequency differs between populations, thus, inbreeding depression also varies. Furthermore, if epistasis is absent, inbreeding depression has a linear function with the degree of inbreeding, given that the environment is constant and the trait affected by inbreeding depression is not under selection.

Purging of genetic load in populations is recognized when the level of inbreeding results in the effective selection against recessive or partially recessive detrimental alleles (Barrett and Charlesworth, 1991). With the

removal of the detrimental alleles, mean fitness of the population may return to or exceed the mean fitness of a randomly-mating population (Hedrick, 1994). A population is said to be purged of its genetic load when inbreeding depression is reduced by increasing inbreeding in every generation (Kelly and Tourtellot, 2006). The initial effect of inbreeding is a decrease in fitness due to increased homozygosity, however, if there is effective selection against recessive or partially recessive alleles, then there will be an increase in fitness (Barrett and Charlesworth, 1991).

The rate of inbreeding and the length of time the population has been isolated are also associated with inbreeding depression. Slow inbreeding rate results in less inbreeding depression given that the total inbreeding is the same. With slow inbreeding, more time is given for selection of favourable alleles involving more generations (Frankham *et al* (2001; Bunnell, 1978; Bijlsma *et al*, 2000; Miller and Hedrick, 2001; Hedrick, 1994; Boakes *et al*, 2006).

Environment also plays a role in the manifestation of inbreeding depression and purging. In the wild, environment is more harsh and stressful, therefore inbreeding is more deleterious. Purging was found to be more effective in the wild than in the captive environment (Crnokrak and Roff, 1999). It can be said that efficiency of purging is not the same in all environmental conditions taking also into consideration that certain alleles are expressed only in certain environments (Kristensen *et al.*, 2008). Kristensen and Sorensen (2005)-id is high in harsh environment

Animals in captivity show less inbreeding depression since they are provided with proper husbandry (Kalinowski, 2000). In captive populations, Boakes *et al* (2006) and Ballou (1997) cited reasons for the variation in the detection of purging effects. These include the occurrence of purging in the founder population before they are brought into the zoo, selection intensity between lethal and mildly deleterious recessive alleles, the contribution of the two mechanisms (dominance and overdominance) associated with inbreeding depression; level and rate of inbreeding; population size and number of generations.

Purging has been studied in a number of species. Frankham *et al* (2001) cited the work of Ballou in 1997 who observed small effects of purging

in captive mammals and Visscher *et al* in 2001 who revealed purging in a small feral population of Chilligham cattle in England. The latter however, have no control groups and no specific inbreeding test performed. Table 1 shows a number of studies on inbreeding depression on non-domestic animals.

Table 1. Example of studies on inbreeding depression in non-domestic animals.

Species	Results/ Observations	Environment	Source
Golden lion tamarin	Inbreeding of offspring is not significant predictor on the number of live offspring produced	Wild	Bales <i>et al.</i> , 2007
	Mortality of inbreds are significantly higher than non-inbred offspring	Wild	Dietz <i>et al.</i> ,
Several zoo populations	Inbreeding depression and purging on neonatal survival, survival for neonate to weaning and litter size; significant result on purging in the neonatal survival of 15 out of 17 species studied	Captive	Ballou, 1997
	14 out of 119 zoo populations showed significant purging, however the change in inbreeding depression is so low, <1%	Captive	Boakes <i>et al.</i> , 2006
Mice	New inbreeding has more impact on inbreeding depression than the old inbreeding	Laboratory	Hinrichs <i>et al.</i> , 2007
Dwarf mongoose	No inbreeding depression	Wild	Keane <i>et al.</i> , 1996
Adders (<i>Vipera berus</i>)	Decrease in lower reproductive output and viability due to inbreeding depression	Captive	Madsen <i>et al.</i> , 1996

Oldfield mice	Inbreeding is associated with enhanced manifestation of parental behaviour which contributes to the increase in the survivability of the offspring from inbred parents	Laboratory	Margulis, 1998
Mexican jays	Inbred offspring are less likely to survive	Wild	Brown and Brown, 1998
Ungulates	Mortality is higher in inbred than in the non-inbred juveniles	Captive	Ralls <i>et al.</i> , 1979
Non-human primates	Presented a summary on inbreeding depression in primate species and provided a review on the methods used to detect inbreeding depression	-	Charpentier <i>et al.</i> , 2007
Yellow baboons	Increase in mortality of offspring from inbred parents	Wild	Alberts and Altmann, 1995
African lions	Increases inbreeding results in decreased cub survival	Wild	Packer and Pusey, 1993
Lions	Abnormal sperms and testosterone levels are associated with inbreeding	Wild	Wildt, <i>et al.</i> , 1987
Wild rabbits	Decreased sperm quality is associated with inbreeding	Wild	Keller and Waller, 2002
Black grouse	Decrease in heterozygosity affects mating success and longevity of males	Wild	Höglund, <i>et al.</i> , 2002
Mandrills	Inbreeding is correlated to growth parameters with inbred females being smaller than non-inbred and reach conception at an earlier age	Semi-free-ranging	Charpentier, <i>et al.</i> , 2006
Gazelles	Inbred individuals have higher juvenile survival than non-inbred	Captive	Cassinello, 2005

2.4 Founder Heterogeneity

Variation in the response to inbreeding depression can be traced back to the different numbers of alleles founders have contributed to a population under study. Several studies were conducted which show heterogeneous

founder contributions to the inbreeding depression. Lacy *et al.* (1996) found out that the inbreeding depression exhibited by *Peromyscus polionotus* is due to unequal distribution of deleterious alleles among founders. Rodrigañez, *et al.* 1998 determined that the inbreeding depression on litter size in Large White pigs differs due to alleles coming from specific founder lineages.

2.5 Measures of inbreeding

To investigate the presence of inbreeding depression classical, inbreeding coefficients can be used. The classical inbreeding coefficient (f) is defined as the probability that the two alleles in any homologous locus of an individual are identical by descent originating from a common ancestor of the parents. Therefore f indicates also the relationship between the parents of the individual (Falconer and Mackay, 1996).

To investigate whether purging occurred within a population inbreeding can be split into “old” and “new” inbreeding. “New” inbreeding is described as the inbreeding that occurs in recent generations while “old” inbreeding is the one that precedes the recent inbreeding (Köck *et al.*, 2009). “Old” inbreeding has less influence on inbreeding depression compared to “new” inbreeding. It is brought about by slowly allowing selection over several generations (Kristensen and Sørensen, 2005) while “new” inbreeding refers to the continuing drift of pre-existing deleterious recessive alleles that have not been fixed. “New” inbreeding could also be an indication of emergence of new mutations in the population or natural selection in the loci that display non-additive effects associated with fitness traits (Hinrichs *et al.*, 2007). To measure purging Ballou (1997) came up with the concept of ancestral inbreeding. His basic idea was that inbred individuals with inbred ancestors will show higher fitness compared to inbred individuals with non inbred ancestors if purging exists. The ancestral inbreeding coefficient (f_a) according to Ballou (1997) measures the cumulative proportion of an individual’s genome that has been previously exposed to inbreeding in its ancestors. An individual may have zero classical inbreeding coefficients but may hold an ancestral inbreeding coefficient unequal zero.

Certain inbreeding coefficients can be utilized for the evaluation of founder contributions in relation to inbreeding depression and purging. These

include partial inbreeding and partial ancestral inbreeding coefficients. Founder- specific partial inbreeding coefficient is calculated as the identity-by- descent probability at any given autosomal locus related to a particular founder and allows a more detailed analysis of inbreeding depression on traits (Casellas *et al.*, 2008; Lacy, 1996). Partial inbreeding coefficient (f_i) is defined by Lacy, *et al* (1996) as the probability that an individual is homozygous for an allele that has descended from a specific founder i . The sum of all partial inbreeding coefficients from the founders equals to the total inbreeding coefficient of the individual. This measure analyzes the difference in magnitude and direction of the effects of inbreeding based on the origin of the allele. Partial ancestral inbreeding coefficient (f_{ai}) on the other hand measures the part of the genome which has undergone inbreeding in the past of an individual with regard to alleles originating from a specific founder i . The sum of all partial ancestral inbreeding coefficients equals to the total ancestral inbreeding coefficient of the individual (Baumung, 2009).

3 MATERIALS and METHODS

3.1 Data

The data analyzed were obtained from the studbook records of North Persian leopard and Mhorr gazelle in SPARKS (Single Population Analysis and Records Keeping System) format which were last updated on September, 2008 and March, 2002, respectively. For each species the following information was essential for the analyses: identity number of the individual, sire and dam; sex; birth date; death date or date indicating the last update of the individual in the studbook; parity number; location of birth (zoo) and litter size when appropriate. Table 2 gives an overview for the two populations.

Table 2. Summary of the data and pedigree structure of North Persian leopard and Mhorr gazelle.

	North Persian Leopard	Mhorr Gazelle
No. of animals in the pedigree	639	315
No. of living animals	144 (22.54%)	97 (30.79%)
No. of males	272 (42.47%)	148 (46.98%)
No. of females	275 (43.04%)	167 (53.02%)
No. of sires with offspring	84 (13.14%)	36 (11.42%)
No. of dams with offspring	89 (13.92%)	72 (22.86%)
No. of litters or parities	339	308
Litter size, mean	1.81 (1-5, SD= 0.80)	Not applicable
Parity number, mean & range	3.20 (1-11, SD= 2.20)	4.35 (1-15, SD=3.38)
Pedigree record period	1955– 2008 (53 yrs)	1969– 2000 (31 yrs)
No. of zoos with the species	170	16

3.2 Pedigree analyses for genetic variability

The pedigree records of the two populations were analyzed for genetic variability utilizing the software packages PEDIG (Boichard, 2007) and ENDOG v4.5 (Gutiérrez and Goyache, 2005).

PEDIG was utilized to calculate the effective number of remaining genomes in a defined reference population, while ENDOG was used for estimating the following aspects, effective population size for the whole population, effective population size for a defined reference population (alive or assumed to be alive with known parents), mean maximum generations,

mean complete generations and mean equivalent generations. Both programs were used for the assessment of effective number of founders and ancestors. Maximum generation is the number of generations between an offspring to the farthest ancestor in the pedigree. Complete generation is described as the number of generations that can be traced back with all ancestors known. Equivalent complete generation is the sum of all known ancestors computed as the sum of $(1/2)^n$ where n is the number of generations that can be traced from the offspring to each known ancestor (Maignel *et al.*, 1996; Gutiérrez and Goyache, 2005).

3.3 Inbreeding coefficients

3.3.1 Classical inbreeding coefficient (f)

The classical inbreeding coefficient (f) is the probability that the two alleles in homologous loci of an individual are identical by descent from a common ancestor of the parents. Therefore f indicates the relationship between the parents of the individual. This coefficient is used to examine the general effect of inbreeding on the traits of interest. The individual inbreeding coefficient was calculated using the GRain program in the PEDIG software package (Boichard, 2002).

To investigate further for inbreeding depression and possibly purging, the succeeding inbreeding coefficients were included in the analyses of fitness traits.

3.3.2 “Old” and “new” inbreeding

The effects of inbreeding can be attributed to the inbreeding which happened in the recent past and/ or former generations (Köck *et al.*, 2009). In this analysis, the classical inbreeding coefficients were divided into two parts which are referred to as “old” and “new” inbreeding. The “new” inbreeding coefficients from the three most recent generations were calculated based on Van Raden’s algorithm in the PEDIG program (Boichard, 2002). The “old” inbreeding coefficient was derived by taking the difference between the total inbreeding coefficient and the “new” inbreeding coefficient of each individual.

3.3.3 Ancestral (f_a) inbreeding

Ancestral inbreeding (f_a) is the fraction of an individual's genome that was already exposed to inbreeding in the past (Ballou, 1997). Written below is the formula proposed by Ballou (1997) for the calculation of ancestral inbreeding coefficient. The indices s and d refer to sire and dam.

$$f_a = \frac{[f_{a(s)} + (1 - f_{a(s)})f_s + f_{a(d)} + (1 - f_{a(d)})f_d]}{2}$$

Inbreeding and ancestral inbreeding are not independent to each other, thus, gene dropping is done in a stochastic simulation to solve the problems associated with Ballou's formula (Suwanlee *et al.*, 2007). In the simulation, alleles which are identical by descent (IBD) for the first time are recognized and counted. The ancestral inbreeding coefficient was calculated according to Ballou's definition using GRain in the PEDIG software package (Boichard, 2007) with the following formula:

$$f_{a\text{-gene drop}} = \frac{\text{number of alleles previously IBD}}{2 (\text{number of runs})}$$

Ten thousand gene dropping runs were done in the calculation of ancestral inbreeding coefficient in this study.

3.3.4 Partial inbreeding

The partial inbreeding coefficient is defined as the probability that an individual is homozygous for an allele from a specific founder (Lacy *et al.*, 1996). Again the gene dropping method was used (Suwanlee *et al.*, 2007; Boichard, 2002). In the gene dropping method, unique alleles are assigned to founders. With Mendelian law of segregation, these alleles are passed from parents to offspring. Alleles originating from a certain founder and being IBD are counted. The formula given above is applied resulting in n partial inbreeding coefficients for n founders. Partial inbreeding coefficients were obtained by executing the GRain program in the software package PEDIG (Boichard, 2007). Simulation was done with 10,000 repetitions.

Using SAS procedure CORR, the correlation between the inbreeding coefficients of the important founders in the population was calculated. Founders with partial inbreeding coefficients with a correlation of ≥ 0.60 were considered as a group.

3.3.5 Partial ancestral inbreeding

The partial ancestral inbreeding reveals the part of the genome which has undergone inbreeding due to alleles originating from a specific founder in the past of an individual. The calculation was done analogous to the calculation of partial inbreeding coefficients (see above). Again founders were grouped according to the correlation values of their partial ancestral inbreeding coefficients. Founders with partial ancestral inbreeding coefficient correlation of ≥ 0.60 were considered as one group.

3.4 General linear mixed models

Individual or litter survival as well as litter size were the traits being evaluated. Survival traits include neonatal survival up to 7 and 30 days of age; and survival to weaning age at 90 and 180 days for the North Persian leopard and Mhorr gazelle, respectively. Survival up to 30 days is also analyzed to have additional mortality records.

Each individual was coded as either not surviving (0) or surviving (1) at an age of 7, or 30 days; or at the weaning age of 90 days and 180 days for North Persian leopard and Mhorr gazelle, respectively. A litter was coded as surviving (1) if more than 50% of the individuals within it survived, otherwise coded as not surviving (0). Individuals with missing death dates and no update information were excluded from the analyses.

Parity numbers beyond 10 were clustered to 10. In the case of leopards, birth type of more than 3 was coded as 3 for the analyses of individual/ litter traits due to low number of litters with more than 3 cubs.

Data restrictions were made based on the species and the number of observations per zoo-year combination. Zoo-years with only one observation were excluded from the analyses for all survival traits.

The significance of the different inbreeding coefficients to survival traits of the two populations was analyzed with SAS procedure GLIMMIX (v. 9.2

Statistical Analysis Systems Institute Inc., Cary, NC) while their influence on litter size for the North Persian leopard was analyzed with SAS procedure MIXED (v. 9.2 Statistical Analysis Systems Institute Inc., Cary, NC).

3.4.1 Mortality risk at days 7, 30 and weaning age

Linear mixed model analyses were carried out. The survival traits are considered binary while litter size was regarded as normally distributed. Fixed effects included in the analyses for the survival traits were sex, parity number and birth type (litter size). Dam within zoo-year effect was considered a fixed random effect for all the survival trait analyses since the study is aiming not to compare the performance of zoos with regards to survival of the individuals. Dam is the fixed random effect in the analyses for litter size as the dependent trait.

As a matter of course the fixed effect of sex is excluded from the analyses of litter mortality where no individual sex code is applicable and in addition, fixed effect of birth type is excluded in the analyses for litter size in the North Persian Leopards. Furthermore in Mhorr gazelle analyses, the fixed effect of birth type is excluded considering that only one offspring is normally produced per gestation.

Inbreeding depression and purging were assessed making use of the total, “old”, “new” and ancestral inbreeding

In a basic model, the total inbreeding coefficient of the individual, sire and dam were included in the analyses for survival traits of the North Persian leopard and Mhorr gazelle (Model 1).

$$\mathbf{u} = \mathbf{u}_0 + \beta_f \mathbf{f} + \beta_{f_s} \mathbf{f}_s + \beta_{f_d} \mathbf{f}_d + \beta_{Sex} \mathbf{Sex} + \beta_{Parity\#} \mathbf{Parity\#} + \beta_{BirthType} \mathbf{BirthType} \quad (1)$$

where \mathbf{u} is the logit transformation of a measure of fitness such as mortality, \mathbf{u}_0 is the mean fitness of non-inbred animals, \mathbf{f} is the total inbreeding coefficient of the individual/litter, \mathbf{f}_s is the total inbreeding coefficient of the sire, \mathbf{f}_d is the total inbreeding coefficient of the dam, \mathbf{Sex} is the sex of the individual, $\mathbf{Parity\#}$ is the parity number (1 – 10), $\mathbf{BirthType}$ is the size of the litter to which the individual belongs to (1-3) and $\beta_f, \beta_{f_s}, \beta_{f_d}, \beta_{Sex}, \beta_{Parity\#},$ and $\beta_{BirthType}$

are the regression coefficients associated with f , f_s , f_d , Sex , $Parity\#$, and $BirthType$, respectively.

“Old” and “new” inbreeding coefficients of the individual/litter, sire and dam were included in the analyses of fitness traits using model 2, 3, and 4 respectively.

$$u = u_0 + \beta_{f_old}f_{old} + \beta_{f_new}f_{new} + \beta_{f_s}f_s + \beta_{f_d}f_d + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (2)$$

$$u = u_0 + \beta_f f + \beta_{f_s_old}f_{s_old} + \beta_{f_s_new}f_{s_new} + \beta_{f_d}f_d + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (3)$$

$$u = u_0 + \beta_f f + \beta_{f_s}f_s + \beta_{f_d_old}f_{d_old} + \beta_{f_d_new}f_{d_new} + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (4)$$

where u is again the logit transformation of a measure of mortality, u_0 is the mean fitness of non-inbred animals, f , f_s , f_d , Sex , $Parity\#$, $BirthType$, β_f , β_{f_s} , β_{f_d} , β_{Sex} , $\beta_{Parity\#}$, $\beta_{BirthType}$ are described as defined in Model 1. Indices *old* and *new* refer to the “old” and “new” inbreeding of the individual/ litter, sire and dam.

Model 5 is used in the analyses of the influence of ancestral inbreeding on fitness traits.

$$u = u_0 + \beta_f f + \beta_{f_a}f_a + \beta_{f_s}f_s + \beta_{f_d}f_d + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (5)$$

the parameters are defined as described in Model 1, f_a is the ancestral inbreeding coefficient of the individual/ litter and β_{f_a} is the regression coefficient of the ancestral inbreeding coefficient.

The effects of inbreeding on fitness traits can be due to specific founders or ancestors in the pedigree. Founder lineages vary in their contribution to inbreeding depression (Rodríguez *et al*, 1998). To assess for the founder heterogeneity, the coefficients for partial and partial ancestral

inbreeding were included in the linear mixed model analyses for the fitness traits.

The degree and direction of inbreeding effects depending on the origin of alleles can be analyzed based on partial inbreeding coefficients. The inbreeding coefficient of the individual or litter is divided into parts due to certain founders.

Analyses of the influence of partial inbreeding coefficients of individual/litter, sire and dam, models 6, 7 and 8 were used.

$$u = u_0 + \beta_{f_g1}f_{g1} + \beta_{f_g2}f_{g2} + \beta_{f_g3}f_{g3} + \beta_{f_{f222}}f_{f222} + \beta_{f_s}f_s + \beta_{f_d}f_d + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (6)$$

$$u = u_0 + \beta_{ff} + \beta_{f_s_g1}f_{s_g1} + \beta_{f_s_g2}f_{s_g2} + \beta_{f_s_g3}f_{s_g3} + \beta_{f_s_{f222}}f_{s_{f222}} + \beta_{f_d}f_d + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (7)$$

$$u = u_0 + \beta_{ff} + \beta_{f_s}f_s + \beta_{f_d_g1}f_{d_g1} + \beta_{f_d_g2}f_{d_g2} + \beta_{f_d_g3}f_{d_g3} + \beta_{f_d_{f222}}f_{d_{f222}} + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (8)$$

Parameters are defined as described in Model 1, indices *g1*, *g2*, *g3* and *f222* refer to founder groups 1, 2, 3 and founder number 222, respectively.

To consider if there is heterogeneity of the founders or founder groups in their contribution to inbreeding depression as well as purging, individual and litter fitness traits were analyzed with model 9.

$$u = u_0 + \beta_{f_a_g1}f_{a_g1} + \beta_{f_a_g2}f_{a_g2} + \beta_{f_a_g3}f_{a_g3} + \beta_{f_a_{178}}f_{a_{178}} + \beta_{f_a_{222}}f_{a_{222}} + \beta_{f_s}f_s + \beta_{f_d}f_d + \beta_{Sex}Sex + \beta_{Parity\#}Parity\# + \beta_{BirthType}BirthType \quad (9)$$

where β_{f_a} is the regression coefficient of the partial ancestral inbreeding coefficient with indices *g1*, *g2*, *g3*, *f178* and *f222* referring to the partial ancestral inbreeding of ancestor groups *g1*, *g2*, *g3* and ancestors *178* and *222*, respectively.

The mortality risk of an individual or litter at days 7, 30 and weaning age at a certain level of inbreeding *i.e.*, total, “old”, “new”, partial, ancestral and partial ancestral were calculated based on the formula below (Agresti, 2002):

$$\hat{\pi}(x) = \frac{\exp(\text{intercept} + \text{parameter estimate } x)}{1 + \exp(\text{intercept} + \text{parameter estimate } x)}$$

where $\hat{\pi}(x)$ indicates the probability of mortality of an individual or litter and x the level of inbreeding. Probabilities of mortality with the categorical traits were based on the least square means obtained from the output of SAS procedure GLIMMIX using option *ilink*.

3.4.2 Litter Size

With litter size, the degree of inbreeding effects was calculated based on the least square estimates from the output of SAS procedure MIXED with regression coefficients showing an increase or decrease in number of cubs per 10% increase in inbreeding. The following basic model was used:

$$y_{ijk} = \mu + pn_i + b_{f1}f_1 + b_{fs}f_s + b_{fd}f_d + d_k + \epsilon_{ijk} \quad (10)$$

where y_{ijk} is the litter size of litter i , μ the overall mean, pn_j the parity number j ($j= 1 - 10$), f_1 , f_s , f_d as stated in model 1 are the inbreeding coefficients for litter, sire and dam, respectively, while b refers to the corresponding linear regression coefficients, d_k is the random effect of dam k and ϵ_{ijk} the random residual error.

Variants of this model analogous to those described in chapter 3.4.1. were used to investigate the effect of "old" and "new", ancestral and partial as well as partial ancestral inbreeding coefficients.

4 RESULTS and DISCUSSION

4.1 *North Persian leopard*

4.1.1 Pedigree analysis

The reference population of North Persian leopard is composed of animals that are alive, and with known parents and sex. Animals without death dates are considered alive if they are less than 20 years old (based on their birth dates) which is the approximate life span for this species in captivity.

The results of the pedigree analysis for genetic variability of the reference population are shown in Table 3. The effective number of founders is 7 while the effective number of ancestors is also 7. The analysis also showed that the effective founder genomes in the population is 4. The effective number of founders is lower than the actual number of founders which indicates that there is an imbalance in the expected contribution of each founder in the population. However, the values of the effective number of founders and ancestors are equal, while the effective number of founder genomes is lower, which demonstrates that there is a founder gene loss in the later generations due to random genetic drift (Boichard *et al.*, 1997). The low values of mean maximum generations, mean complete generations and mean equivalent generations shows that there are few generations in the pedigree. The mean maximum generations indicates that on average a maximum of 4.62 generations could be traced back. Mean complete generation show that on the average there are approximately 2.74 generations which separates an individual to its farthest ancestors. Moreover, each individual is separated by 3.35 generations on average (mean equivalent generations) to each of its known ancestors.

Table 3. Measures of genetic variation of North Persian leopards in captivity.

Measures of genetic variation	Value
No. of animals in the reference population (alive)	144 (22.54%)
N_e based on regression of equivalent generations	89
No. of founders	18
Effective number of founders	7
No. of ancestors	13
Effective number of ancestors	7
No. of ancestors explaining 50% of the genetic variation	3
Effective number of founder genomes	4.03 (mean); 0.65 (sd)
Mean maximum generations	4.62
Mean complete generations	2.74
Mean equivalent generations	3.35

4.1.2 Mortality risk up at days 7, 30 and weaning age

To analyze the presence of inbreeding depression as well as purging in the population of North Persian leopards, total, *old* and *new*, and ancestral inbreeding were included in the general linear mixed model analyses for the mortality risk at days 7, 30 and weaning age. To investigate for the contribution of founder inbreeding to inbreeding depression as well as purging, partial and partial ancestral inbreeding coefficients were included in the linear mixed model analyses for the fitness traits.

The mean, standard deviation and range of the individual/ litter, sire and dam total inbreeding coefficients of the North Persian leopard is presented in Table 4. Approximately 70% of the individuals (448 out of 639) and litters (247 out of 353) were inbred. The lowest total inbreeding coefficient of inbred individuals was almost 0.25 (0.2497).

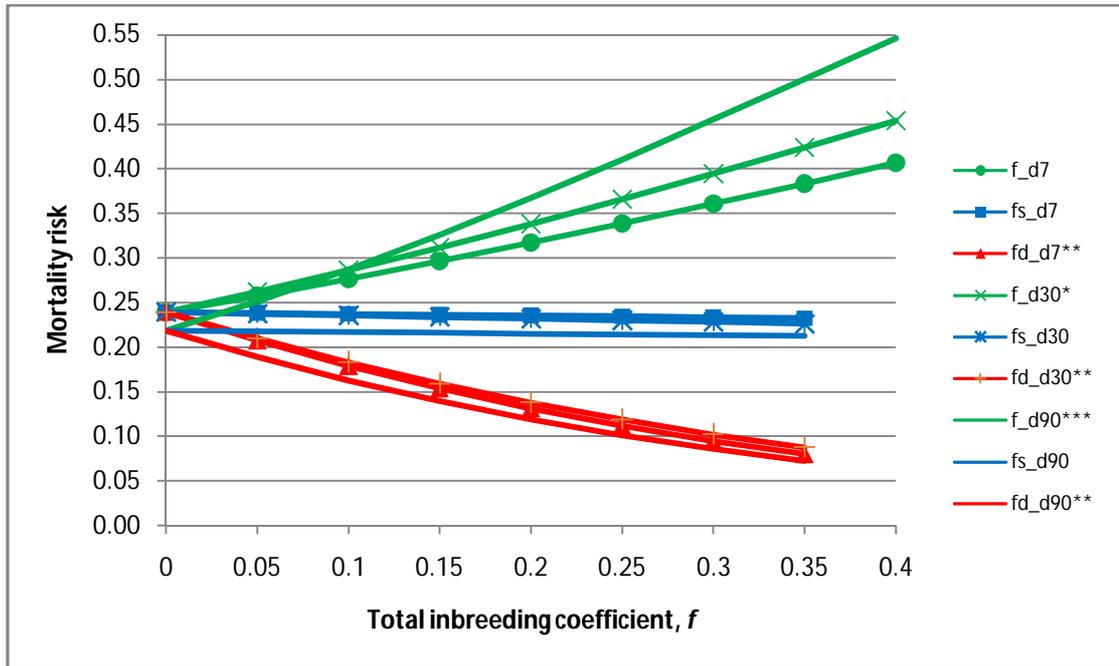
Table 4. Total inbreeding coefficients (f) of individual/ litter, sire and dam.

	Mean	Standard deviation	Maximum
Individual	0.1293	0.1143	0.3975
Litter	0.1260	0.1136	0.3975
Sire	0.0741	0.1063	0.3690
Dam	0.0593	0.0971	0.3012

When the total inbreeding coefficients of the individual, sire and dam were included in the linear mixed model analyses to investigate mortality at days 7, 30 and 90 (weaning age), the total inbreeding of the dam had a significant effect on mortality (α - level 0.10) while total inbreeding of the individual is only significant in survival at days 30 and 90. Total inbreeding coefficient of the sire is not significant in all survivability analyses. The total inbreeding coefficients of the individual and dam have opposite effects on the survival of the individual (Figure 3). (See Appendix 1A.1)

In the litter survival analyses, results showed that only the dam total inbreeding coefficient is significant in mortality at days 7 ($p < 0.05$), 30 ($p < 0.05$) and 90 ($p < 0.10$) (Figure 4, Appendix 1A.2) indicating that as the dam total inbreeding coefficient increases, mortality risk of the litter decreases, which means that the chances of survival of the litter is higher when the dam is inbred. On the other hand, increase of the litter total inbreeding coefficient points into another direction. As litter total inbreeding coefficient increases, mortality risk increases. However, the effect of litter total inbreeding coefficient was not significant in any analyses.

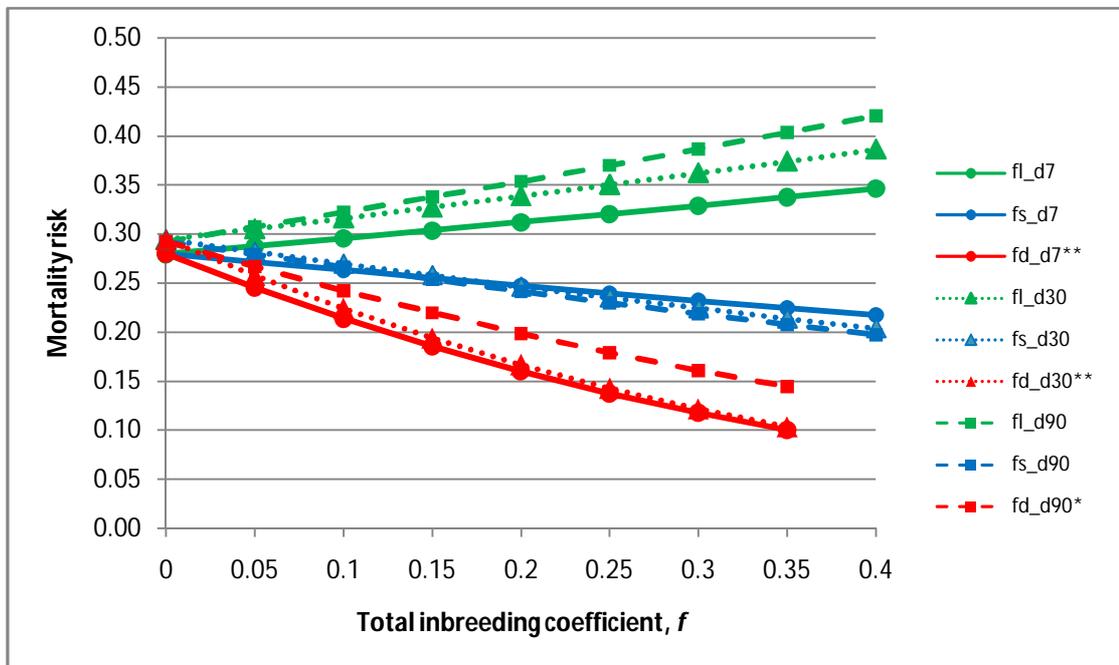
Figure 3. Mortality risk of an individual at days 7, 30 and 90 (weaning age) with total inbreeding coefficients (f) of individual, sire and dam.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f = Individual inbreeding coefficient; f_s = sire inbreeding coefficient; f_d = dam inbreeding coefficient; $d7$ = day 7; $d30$ = day 30; and $d90$ = day 90

Figure 4. Mortality risk of litter at days 7, 30 and 90 (weaning age) with total inbreeding coefficients (f) of litter, sire and dam.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f_l = inbreeding coefficient of the litter; f_s = inbreeding coefficient of the sire; f_d = inbreeding coefficient of the dam; $d7$ = day 7; $d30$ = day 30; and $d90$ = day 90

The results indicate that inbreeding due to individual/ litter, sire or dam has different effects on the individual/ litter survival. The individual inbreeding only influences the survival at 90 days (weaning age).

Inbreeding of the dam has significant effect on all fitness traits which could be an indication of purging, in which, as dam inbreeding increases, the probability of individual and litter mortality also decreases. Ballou in 1997 detected a positive effect of dam inbreeding in European bison, while Lacy *et al.* (1996) and Margulis (1998) observed a positive effect of dam inbreeding in the viability of oldfield mouse (*Peromyscus polionotus*). Margulis (1998) considered that inbred dams have improved maternal behaviour which contributed to the increased survival of the litter, but reproductive success is reduced. Moreover, it was also speculated that inbred females were more likely to experience pseudopregnancy which is due to extended luteal phase and increased progesterone levels (Margulis, 1998). The increase in progesterone levels enhances the manifestation of maternal behaviour which favours survival of the offspring (Dwyer, 2008). Nevertheless, the result in this study is in contrast with the findings of Boakes *et al.* (2006) with the 119 zoo populations where maternal inbreeding has a negative effect on fitness.

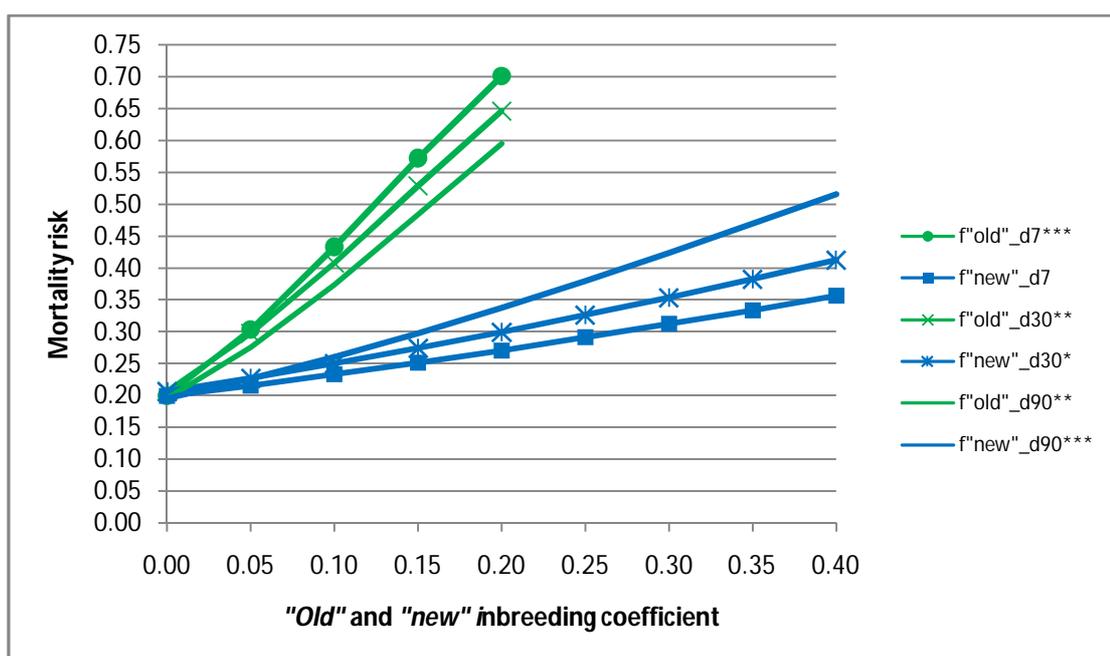
Table 5 shows the mean, standard deviation and maximum values for “old” and “new” inbreeding of the individual/ litter, sire and dam. Figure 5 illustrates the mortality risk of individuals at days 7, 30 and 90 taking individual’s “old” and “new” inbreeding into consideration (see also Appendix 1B.1). “Old” inbreeding is significant for mortality at days 7, 30 and 90, while “new” inbreeding is significant for mortality at days 30 and 90. The mortality risk is higher with “old” inbreeding than with the “new” inbreeding.

Results from the analyses with litter “old” and “new” inbreeding are illustrated in Figure 6 (Appendix 1B.2). The same trend in the probability of non-survival with individual “old” and “new” inbreeding was observed. However, only the effect of “old” inbreeding on all litter survival days is significant.

Table 5. “Old” and “new” inbreeding coefficients of the individual/ litter, sire and dam.

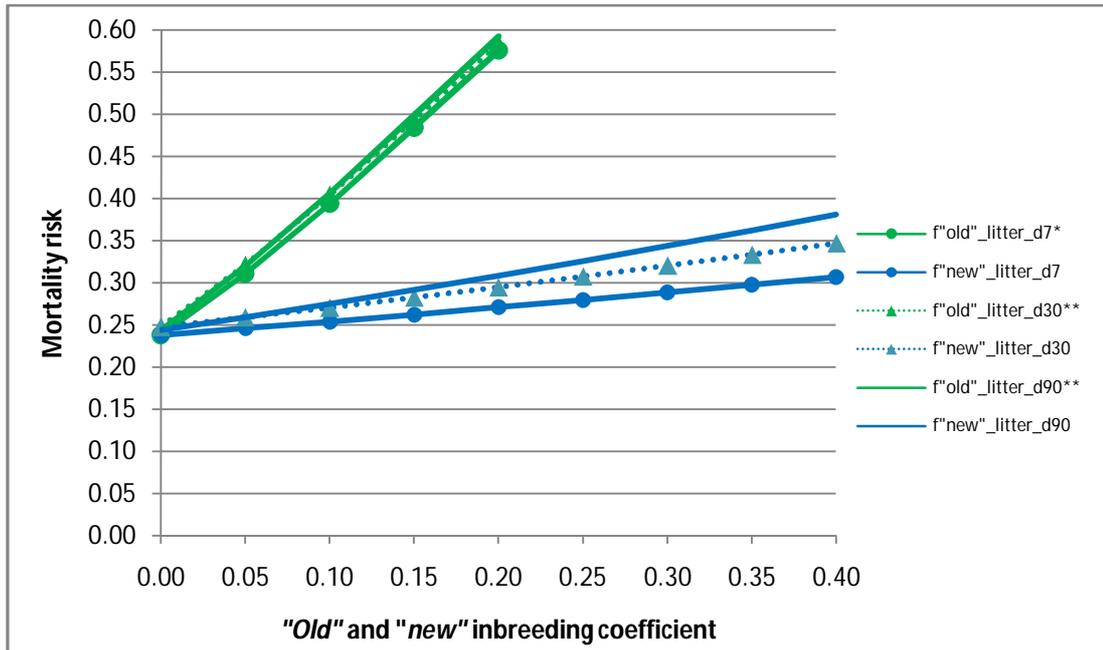
	Mean	Standard deviation	Maximum
Individual			
Old	0.0282	0.0385	0.1533
New	0.0998	0.1188	0.3750
Litter			
Old	0.0300	0.0392	0.1533
New	0.0960	0.1180	0.3750
Sire			
Old	0.0091	0.0257	0.1533
New	0.0673	0.1052	0.3750
Dam			
Old	0.0085	0.0231	0.1015
New	0.0560	0.0993	0.3750

Figure 5. Mortality risk of an individual at days 7, 30 and 90 (weaning age) with “old” and “new” inbreeding coefficients of an individual.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$
f"old" = old inbreeding coefficient of the individual; **f"new"** = new inbreeding coefficient of the individual; **d7**= day 7; **d30**= day 30; and **d90**= day 90

Figure 6. Mortality risk of a litter at days 7, 30 and 90 (weaning age) with “old” and “new” inbreeding coefficients of a litter.



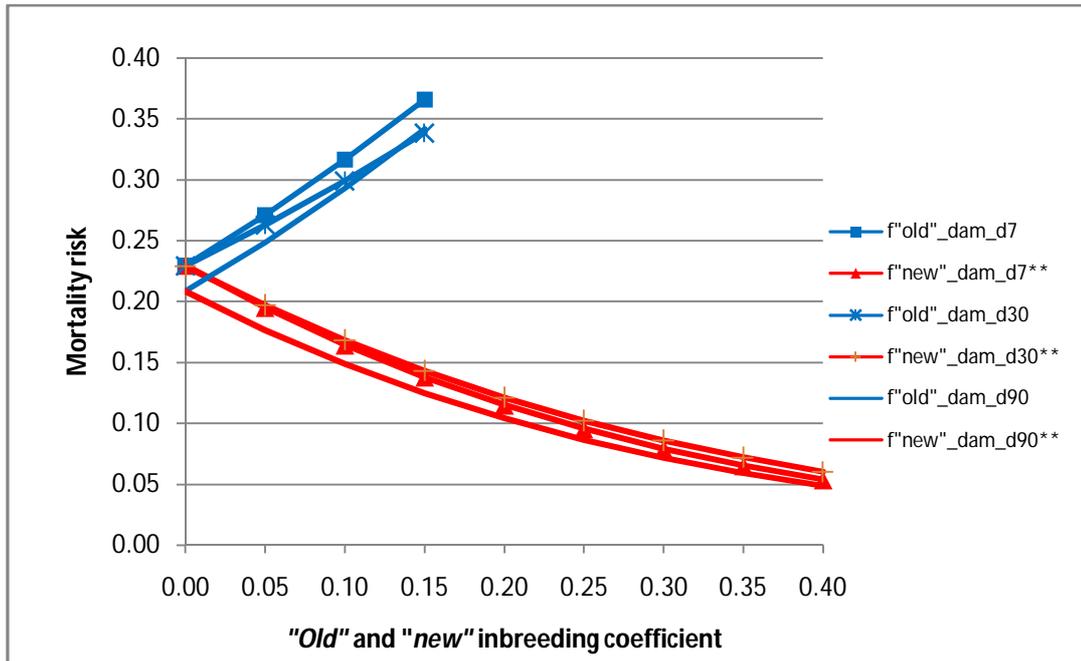
* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_litter = old inbreeding coefficient of the litter; **f"new"_litter** = new inbreeding coefficient of the litter; **d7**= day 7; **d30**= day 30; and **d90**= day 90

“Old” inbreeding of an individual is significant in all survival traits in days while “new” inbreeding is only significant in mortality at days 30 and 90. The impact of “old” inbreeding is higher than the “new” inbreeding. Köck *et al.* (2009) also found a significant effect of both “old” and “new” inbreeding on the survival of Landrace and Large White piglets with “old” inbreeding having a higher impact. This could be an indication that alleles influencing the survival of the individual/litter are of smaller effects, thereby, these alleles are present in the population and continue to segregate for a long period of time (Hinrichs *et al.*, 2007).

The effects of “old” and “new” inbreeding of the dam on individual survival are shown in Figure 7 (Appendix 1B.4). Only the “new” inbreeding of the dam was found to be significant ($p < 0.05$) in all survival analyses. Analyses on litter mortality with “old” and “new” inbreeding of the dam showed that “new” inbreeding has also a significant effect on litter survival (Figure 8, Appendix 1B.5).

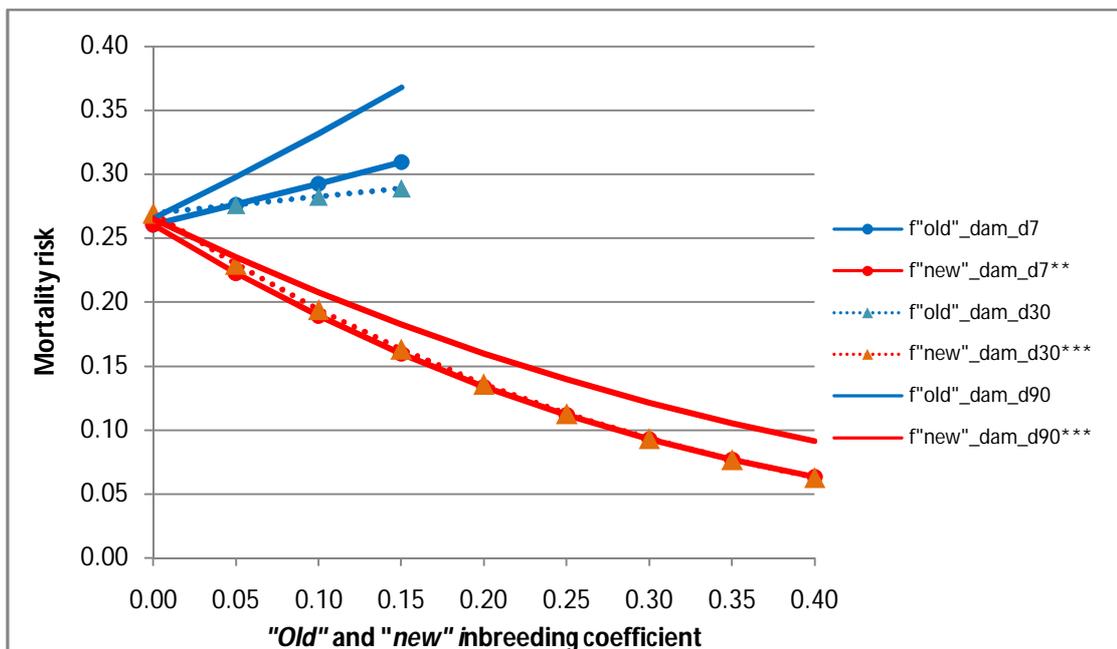
Figure 7. Mortality risk of an individual at days 7, 30 and 90 (weaning age) with “old” and “new” inbreeding coefficients of a dam.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_dam= old inbreeding coefficient of the dam; f"new"_dam = new inbreeding coefficient of the dam; d7= day 7; d30= day 30; and d90= day 90

Figure 8. Mortality risk of a litter at days 7, 30 and 90 (weaning age) with “old” and “new” inbreeding coefficients of a dam.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_dam= old inbreeding coefficient of the dam; f"new"_dam = new inbreeding coefficient of the dam; d7= day 7; d30= day 30; and d90= day 90

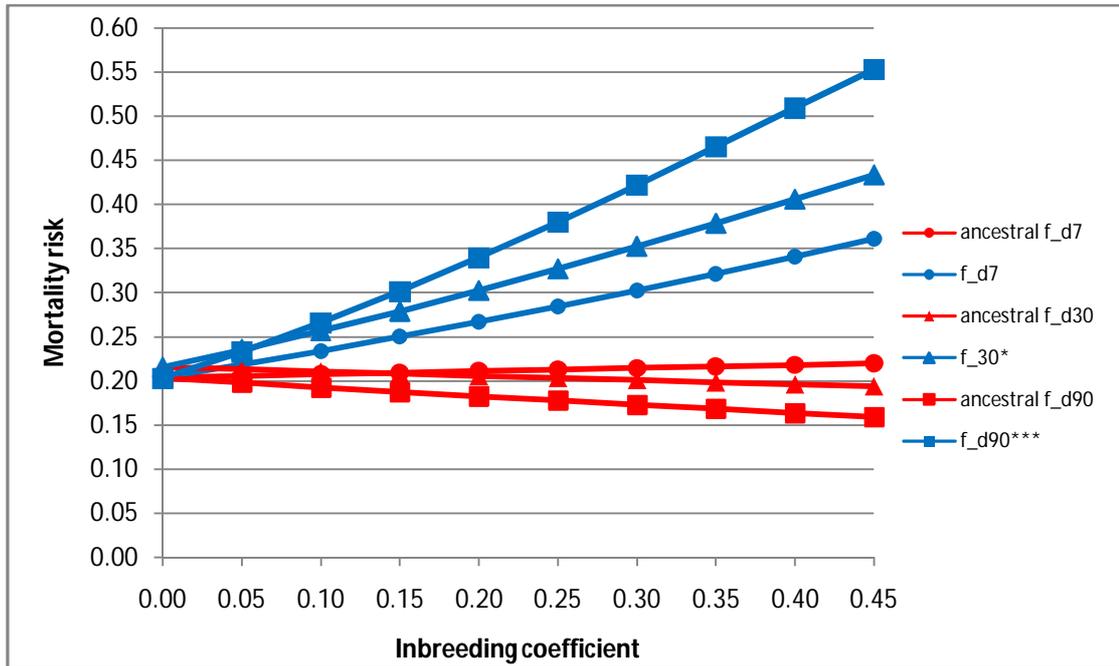
The analyses of survival traits with the “old” and “new” inbreeding of the dam revealed that it is the dam’s “new” inbreeding that is significantly influencing the survival of the offspring/ litter. Interestingly, a positive effect of inbreeding of the dam on survival was found. This could be a possible indication of purging the alleles which have negative effects on achieving maternal success among females. There could also be a possible fixation or epistatic interactions of favourable alleles promoting good mothering ability.

The negative effect of “old” inbreeding is significant in all survival traits. Similar results were obtained by Köck (2009) where the number of Large White and Landrace piglets weaned was reduced by -0.31 and -1.91, respectively, per 10% “old” inbreeding. However, the results on individual/ litter, sire and dam “old” and “new” inbreeding analyses were opposite to the results of Hinrichs *et al.* (2007) with mice, wherein “new” inbreeding was found to cause more inbreeding depression. In addition, the results were also dissimilar to the findings of Hunt (2009) on markhor where “new” inbreeding of the individual, sire and dam decreases the survival of the individual.

The ancestral inbreeding of the population ranges from 0 - 0.41, with a mean of 0.16 (sd= .13). The effects of ancestral inbreeding on the survival traits of the individual and litter are shown in Figures 9 and 10 (see also Appendix 1C.1 and 1C.2).

Ancestral inbreeding increases the chances of survival of individuals, but it has no pronounced effects on litter survival. Moreover, effects of ancestral inbreeding on individual and litter survival were not significant.

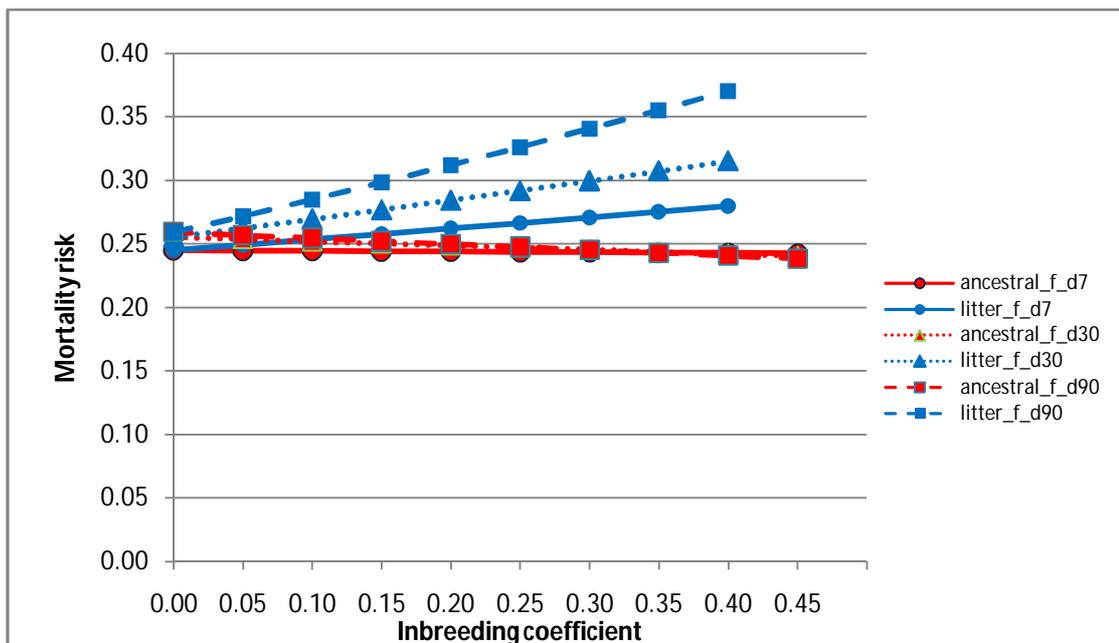
Figure 9. Mortality risk of an individual at days 7, 30 and 90 (weaning age) with ancestral inbreeding coefficient.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

Ancestral f= ancestral inbreeding coefficient; **f**= total inbreeding coefficient; **d7**= day 7; **d30**= day 30; and **d90**= day 90

Figure 10. Mortality risk of a litter at days 7, 30 and 90 (weaning age) with litter ancestral inbreeding coefficient.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

Ancestral f= ancestral inbreeding coefficient of the litter; **litter f**= total inbreeding coefficient of the litter; **d7**= day 7; **d30**= day 30; and **d90**= day 90

In this species, there is an increase in mortality with increasing ancestral inbreeding, thus no purging occurs due to ancestral inbreeding of an individual. Ballou in 1997 obtained a result with 15 out of the 19 taxa showing a reduction in inbreeding depression with increased ancestral inbreeding, however, only one taxon (Sumatran tiger) was found to be significant. Furthermore, he pointed out that ancestral inbreeding may not significantly lessen inbreeding depression due to presence of overdominance (heterozygote advantage) or associative dominance in which case fitness traits are not expected to recover even over prolonged inbreeding. Boakes *et al.* (2006) concluded that purging effects in zoo populations is highly variable thus, unreliable in reducing inbreeding depression.

The analyses of inbreeding depression on survival traits indicate that different traits show different responses to inbreeding depression. The extent of inbreeding depression depends on the genetic load present in the population (recessive or partially recessive, effect of alleles present), allele frequency and characteristics of the loci involved (Hedrick, 1994; Kalinowski *et al.*; 2000; Kristensen and Sørensen, 2005). Furthermore, the presence of inbreeding depression despite the increase of inbreeding as exemplified by increase in mortality risk with increasing inbreeding of individual or litter can be due to the presence of detrimental or deleterious alleles with small effects which are not easily purged from the population, and thereby, they become fixed (Hedrick, 1994; Bijlsma, 1999). The effect of the inbreeding of the dam on the survival of the offspring might be an indication that the inbred dams were purged of the unfavourable alleles associated with maternal success.

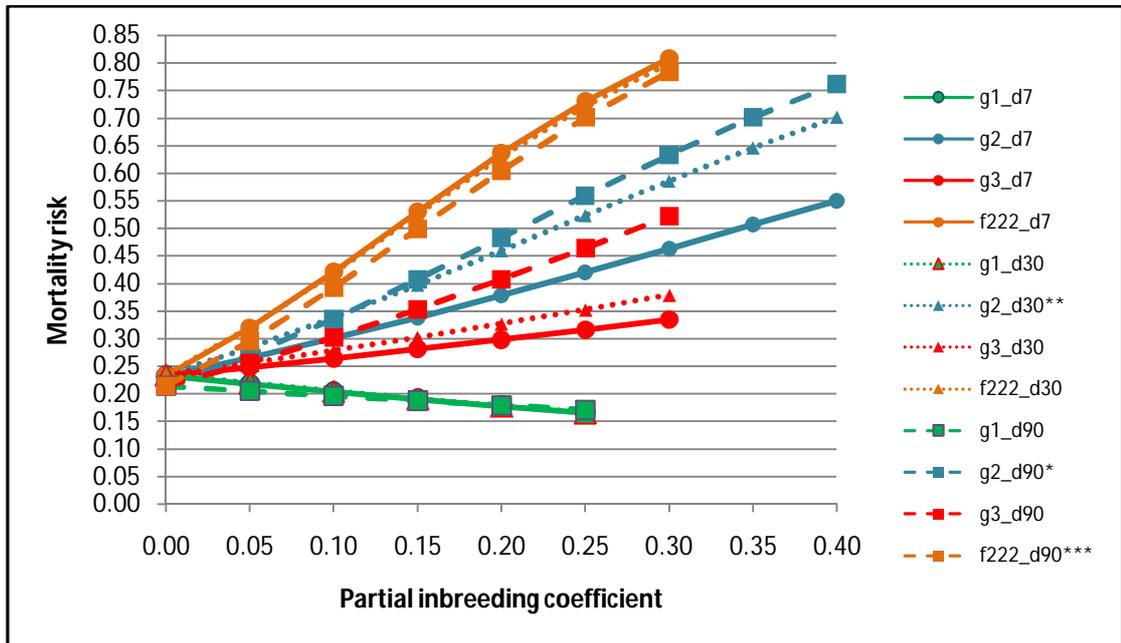
Partial inbreeding coefficients were obtained by using the GRain program. Partial inbreeding coefficients due to the founder or founder groups of individual, sire and dam are shown in Table 6. Based on the correlation of partial inbreeding coefficients of founders, 3 founder groups were formed. One founder (animal 222) was found to have zero correlation with other founders based on partial inbreeding coefficients. The means vary between founder groups which indicate that groups have different contributions to the coefficient of inbreeding of an individual.

Table 6. The partial inbreeding coefficients of founder or founder groups.

Founder/ founder groups	Mean	Standard deviation	Maximum
g1 (f1,f123,f178)	0.0381	0.0608	0.2387
g2 (f13, f333)	0.0207	0.0650	0.3821
g3 (f211,f223)	0.0529	0.0741	0.2564
f222	0.0164	0.0336	0.2576

The magnitude and direction of inbreeding effects between the founder groups on individual and litter mortality are shown in Figures 11-12 (see also Appendix 1D.1 and 1D.2). For the mortality of an individual, only inbreeding due to founder group g1 leads to a decreasing mortality risk which means that it contributes to the increase in survival of an individual. However, founder group g2 is significant for mortality at day 30 ($p < 0.05$) and day 90 ($p < 0.10$), while founder animal 222 is significant at day 90 ($p < 0.05$). The same trend can be observed for the direction of inbreeding effects on litter mortality as contributed by one founder and different founder groups. The decreasing mortality risk associated with founder group g1 means that survival of the litter increases with the contribution of founders belonging to group g1. Significant effects were only found from founder group g2 on mortality at days 30 and 90.

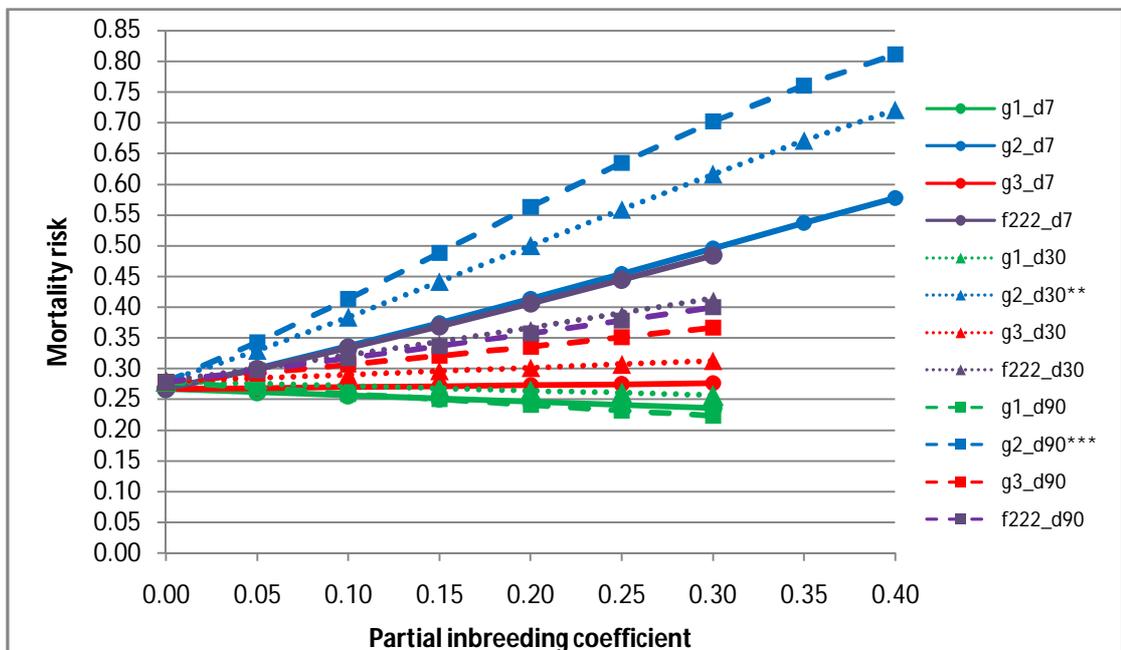
Figure 11. Mortality risk of an individual at days 7, 30 and 90 (weaning age) with partial inbreeding coefficients of founder and founder groups.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

g1= partial inbreeding coefficient of founder group g1; **g2**= partial inbreeding coefficient of founder group g2; **g3**= partial inbreeding coefficient of founder group g3; **f222**= partial inbreeding coefficient of founder animal 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

Figure 12. Mortality risk of a litter at days 7, 30 and 90 (weaning age) with partial inbreeding coefficients of founder and founder groups.

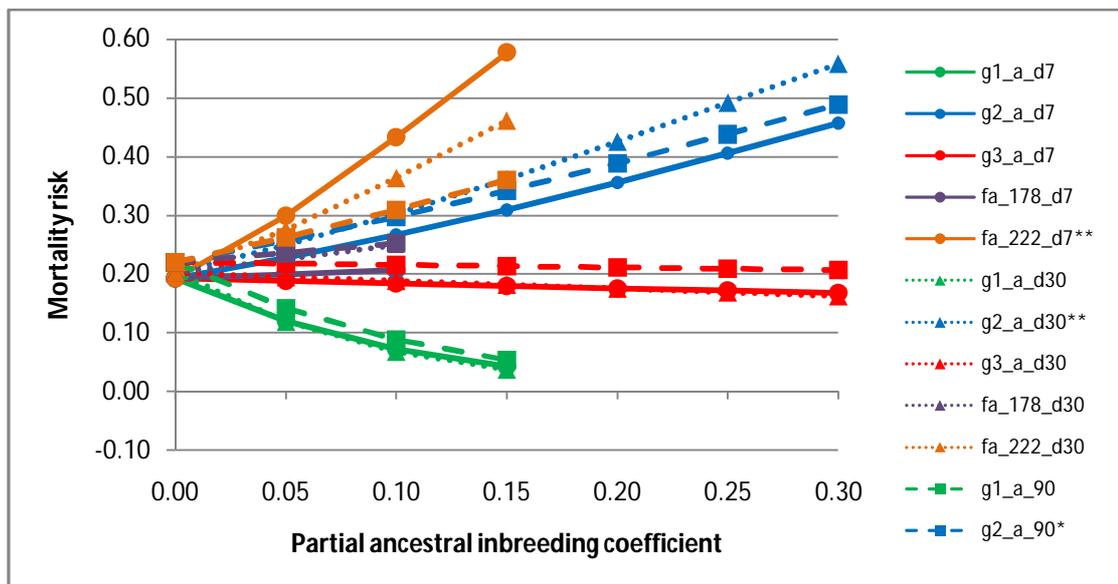


* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

g1= partial inbreeding coefficient of founder group g1; **g2**= partial inbreeding coefficient of founder group g2; **g3**= partial inbreeding coefficient of founder group g3; partial inbreeding coefficient of **f222**= founder animal 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

Figures 13 – 14 (Appendix 1D.3 and 1D.4) show the influence of the partial inbreeding of the different dam founder groups on individual and litter mortality. On mortality of an individual, only one founder group has a positive significant effect on litter survival. Dam_g1 also has a positive effect; however, it was not significant in the analysis. The litter survival analysis also has a similar result with dam founder group g3 (dam_g3) having a significant effect. In addition, dam founder group g2 (dam_g2) has a negative significant effect on survival at day 7 (mortality risk is increases as partial inbreeding coefficient increases).

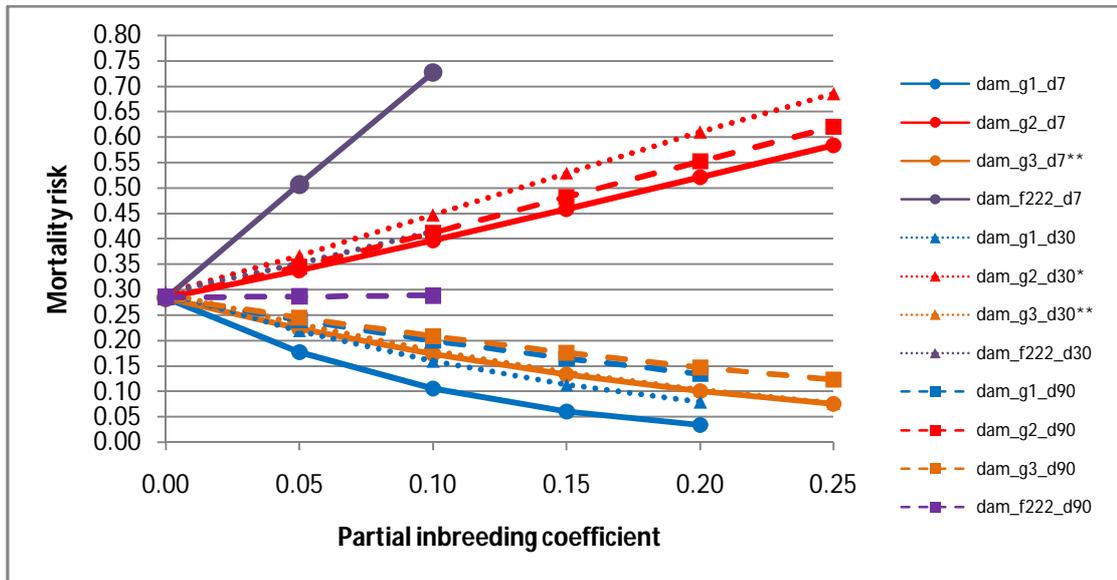
Figure 13. Mortality risk of an individual at days 7, 30 and 90 (weaning age) with partial inbreeding coefficients of dam founder and founder groups.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

dam_g1= partial inbreeding coefficient of dam founder group 1 ; **dam_g2**= partial inbreeding coefficient of dam founder group 2 ; **dam_g3**= partial inbreeding coefficient of dam founder group 3 ; **dam_f222**= partial inbreeding coefficient of dam founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

Figure 14. Mortality risk of a litter at days 7, 30 and 90 (weaning age) with partial inbreeding coefficients of dam founder and founder groups.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

dam_g1= partial inbreeding coefficient of dam founder group 1 ; **dam_g2**= partial inbreeding coefficient of dam founder group 2; **dam_g3**= partial inbreeding coefficient of dam founder group 3; **dam_f222**= partial inbreeding coefficient of dam founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

The results on the analyses of the survival traits with partial inbreeding clearly illustrate that founder groups differ in their contribution to the direction and magnitude of inbreeding depression on survival traits. The increase in survival due to inbreeding of the dam is basically due to alleles coming from founder group g3 and founder group g2 for survival up to day 7. Lacy *et al.* (1996) observed inbreeding depression due to homozygosity of alleles from three founder pairs of mice while the inbreeding depression in viability was traced to four founder lineages. Rodríguez *et al.* (1998) also found differences in survival probabilities in pigs with different founder genes suggesting that there is unequal contribution of the different founders. Köck *et al.* (2009) detected that inbreeding influences the reproductive performance of Landrace and Large White pigs. The influence is said to be in different direction and magnitude which was due to alleles associated to inbreeding that descended from specific ancestors.

Partial ancestral inbreeding determines the partial ancestral inbreeding coefficients from different founders in the pedigree of the individual/ litter. The coefficient reports how much each of the founder or founder groups contribute

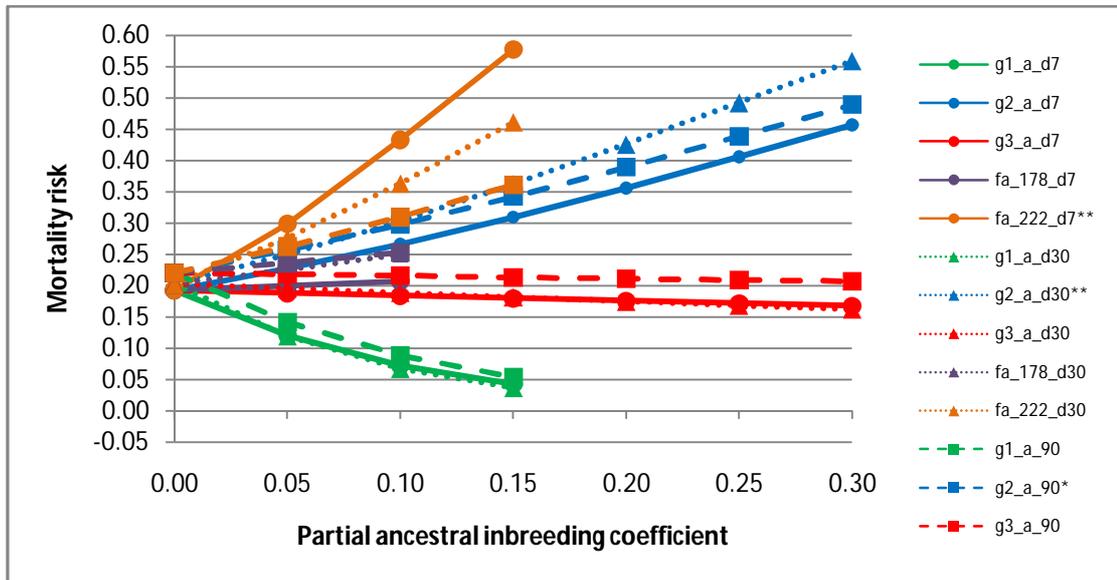
to the inbreeding depression or purging in a population. Partial ancestral inbreeding coefficients of each founder or founder group are shown in Table 7. Founder groups g1 and animal 178 have a negative effect on mortality in days 7, 30 and 90, which means that these founders contribute to the increase in the chances of individual's survival. Founder groups g2 and g3 increase the mortality risk of an individual. Individual number 178 has a different contribution compared to the rest of the groups. It has an effect on mortality at days 7 and 30 but not on day 90. On the other hand, the effects of these founders on mortality were found to be insignificant.

Table 7. The partial ancestral inbreeding coefficients of the founder or founder groups.

Founder groups	Mean	Standard Deviation	Maximum
g1_a (fa_1, fa_123)	0.0135	0.0279	0.1262
g2_a (fa_13, fa_333)	0.0303	0.0551	0.2549
g3_a (fa_211, fa_223)	0.0655	0.0685	0.2796
fa_178	0.0199	0.0251	0.0896
fa_222	0.0253	0.0275	0.1249

Figure 15 shows the contribution of different founders and founder groups to inbreeding depression and possibly purging on litter mortality (see also Appendix 1E.1). Founder group g1 increases the survival of the litter with a decreasing mortality risk as inbreeding increases. However, the effect of founder group g1 was found to be insignificant. Founder group g2 was found to have significant effects on mortality at days 30 and 90, while animal 222 has a significant influence on litter survival up to d7.

Figure 15. Mortality risk of a litter at days 7, 30 and 90 (weaning age) with partial ancestral inbreeding coefficients of founders and founder groups.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

g1_a= partial ancestral inbreeding coefficient of founder group 1 ; **g2_a**= partial ancestral inbreeding coefficient of founder group 2; **g3_a**= partial inbreeding coefficient of founder group 3; **fa_178**= partial ancestral inbreeding coefficient of founder 178; **fa_222**= partial ancestral inbreeding coefficient of founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

The results on the analyzes for the evaluation of founder heterogeneity show further that there is disproportionate contribution of founders and founder groups to the inbreeding depression associated with survival traits and that different founders and founder groups have different influences on survival traits. Furthermore, the results imply that there might be an uneven distribution of the genetic load among founders and the descendants were variably affected by inbreeding (Gulisija *et al.*, 2006; Lacy *et al.*, 1996; Lynch and O’Hely, 2001; Rodrigañez *et al.*, 1998) and the way inbreeding is expressed depends on particular genes that the inbred animal carries and transmits to its offspring (Laikre, 1999).

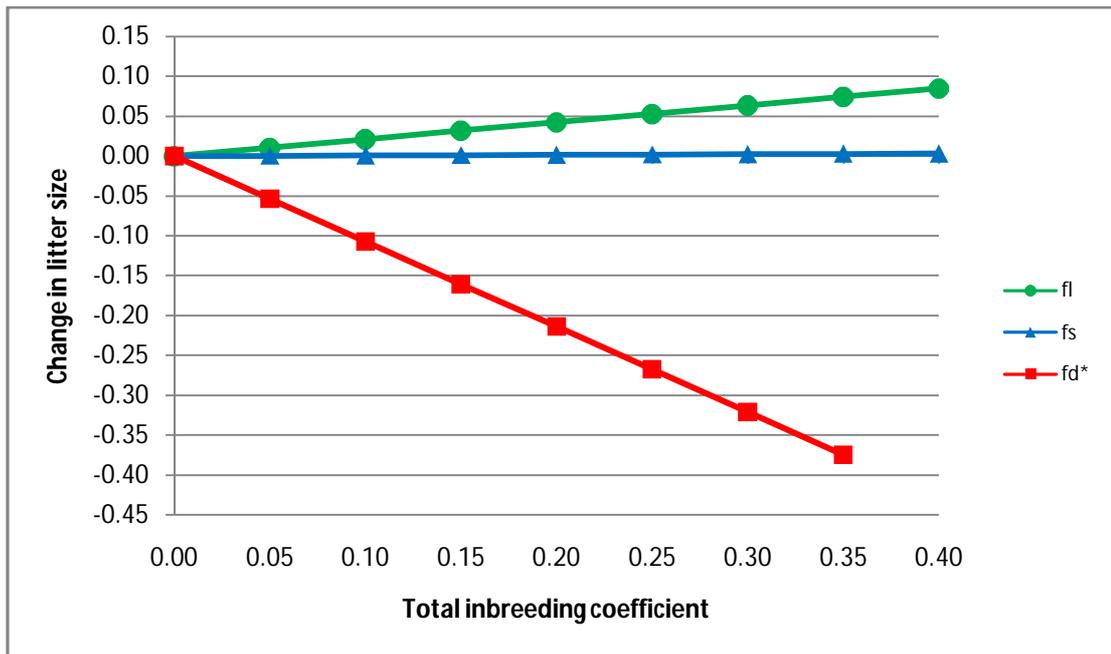
4.1.3 Litter size

The relationship of litter, sire and dam inbreeding on litter size is evaluated with linear mixed model analyses. The following sections discuss the results of the analyses.

The effect of total inbreeding of litter, sire and dam on litter size is illustrated in Figure 16 (see Appendix 1A.3). Increased inbreeding of litter and

sire has a slight positive or no effect on the litter size. However, only the total inbreeding of the dam has a significant negative effect on litter size. Maternal inbreeding had a negative effect on litter size ($p < 0.10$). Studies of Lacy *et al.* (1996) on oldfield mice and Bereskin *et al.* (1968) on pigs also showed inbreeding depression in the form of decreased litter size from inbred dams. The inbreeding depression in the litter size associated with dam inbreeding can be due to the effect of the dam inbreeding on embryo survival in early gestation (Cassell *et al.*, 2003). McCarthy (1967) in his study with mice associated the decrease in litter size with a reduction in the number of eggs ovulated and increased in preimplantation mortality in inbred mothers. Johnson (1990) as cited by Rodríguez *et al.* (1998) also mentioned that litter size is determined by the genotype of the dam and not the litter. Van Arendonk *et al.* (1996) also revealed the presence of maternal genetic influence on piglet survival. Maternal effects on the litter could be considered environmental to the offspring but can have both genetic and environmental components. Furthermore, Peripato *et al.* (2002) in their study identified two QTL that were affecting maternal performance such as nest building, pup grooming, lactation and aggression towards intruders which are essential for the survival of the offspring.

Figure 16. The effect of total inbreeding of litter, sire and dam on litter size.

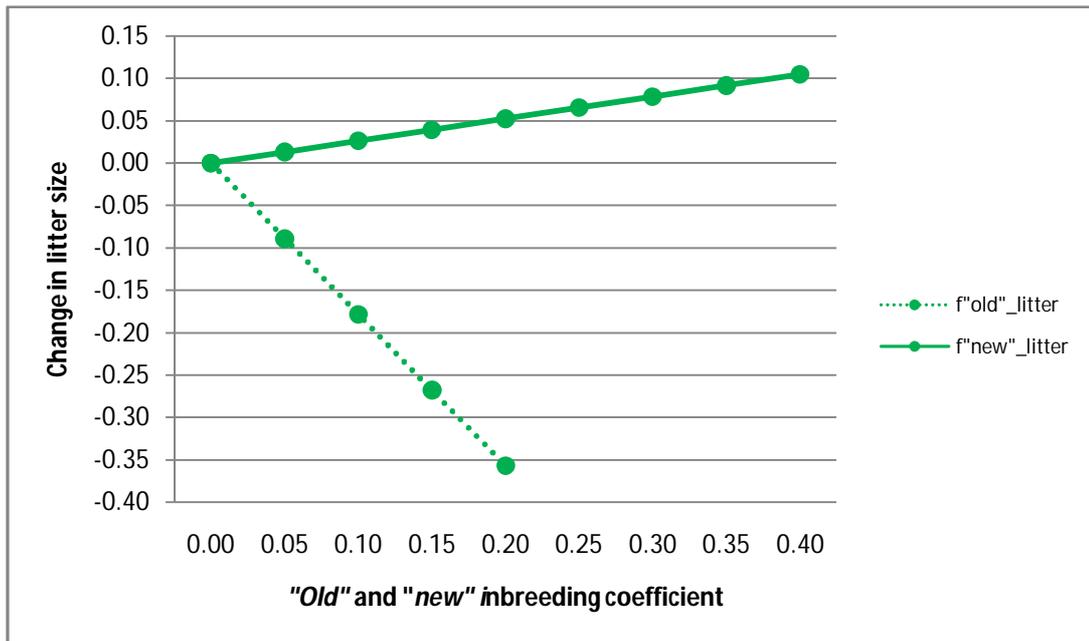


* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f_l = inbreeding coefficient of the litter; f_s = inbreeding coefficient of the sire; f_d = inbreeding coefficient of the dam

The effects of “old” and “new” inbreeding of a litter on litter size were also investigated with the results presented in Figure 17 (Appendix 1B.3). Litter size is affected differently by “old” and “new” inbreeding. With increasing level of “old” inbreeding, litter size is decreasing while it is not influenced by “new” inbreeding.

Figure 17. The effect of “old” and “new” inbreeding of litter on litter size.



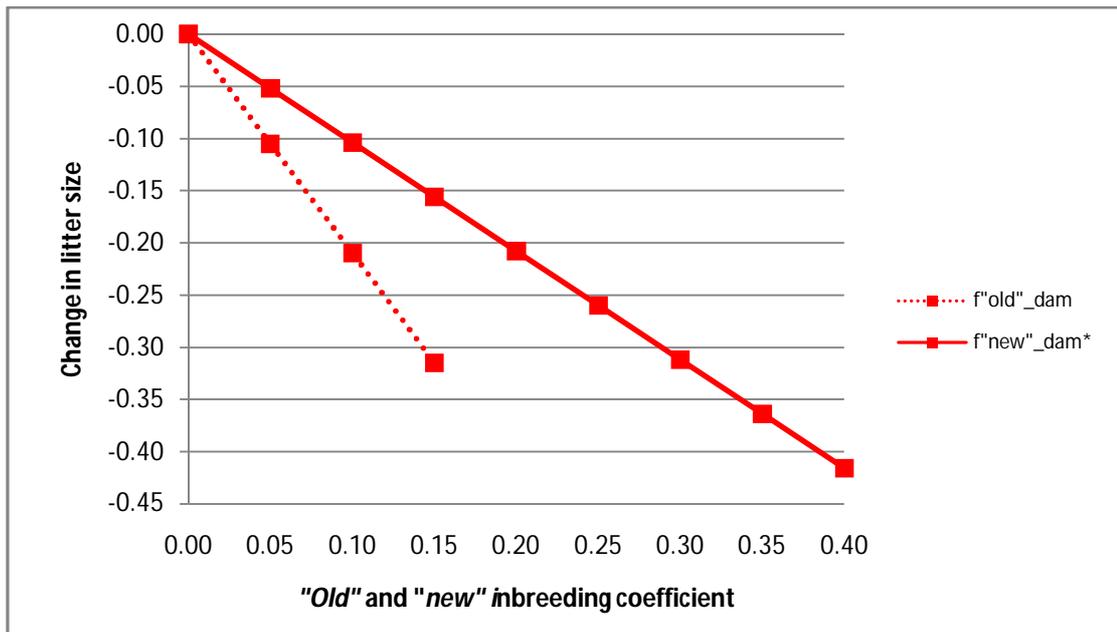
* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_litter = old inbreeding coefficient of the litter; **f"new"_litter** = new inbreeding coefficient of the litter

Both components of inbreeding of the dam (“old” and “new”) clearly have a negative effect on litter size. The effect of “old” inbreeding of the dam is more pronounced (Figure 18) (refer to Appendix 1B.6). Litter size decreases as the level of dam’s “old” and “new” inbreeding increases. However, only the “new” inbreeding of the dam was found to be significantly associated with the decline in litter size. Litter size is decreased by 0.26 if the level of dam “new” inbreeding is at 0.25 ($p < 0.10$).

Figure 19 shows the negative significant effect of sire “old” inbreeding ($p < 0.10$) (Appendix 1B.7). This could be due to a decrease in the fertility, ejaculate volume or semen quality associated with inbreeding (Amos and Balmford, 2001; Crnorkrak and Roff, 1999; Falconer and Mackay, 1996; Lacy *et al.*, 1996; Read and Harvey, 1986).

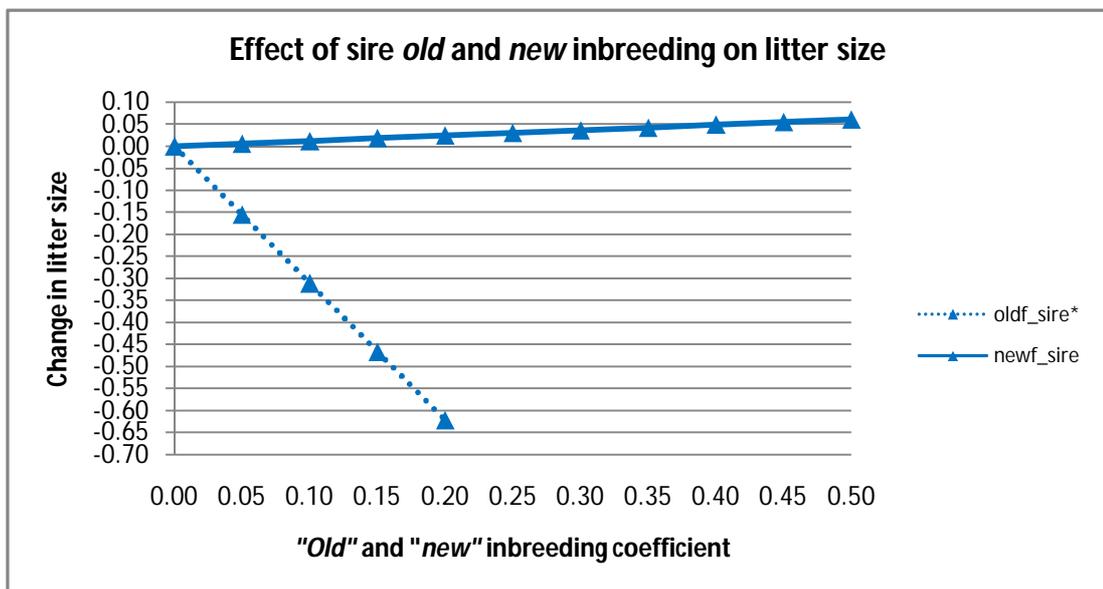
Figure 18. The effect of “old” and “new” inbreeding of dam on litter size.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_dam= old inbreeding coefficient of the dam; **f"new"_dam** = new inbreeding coefficient of the dam

Figure 19. The effect of “old” and “new” inbreeding of sire on litter size.

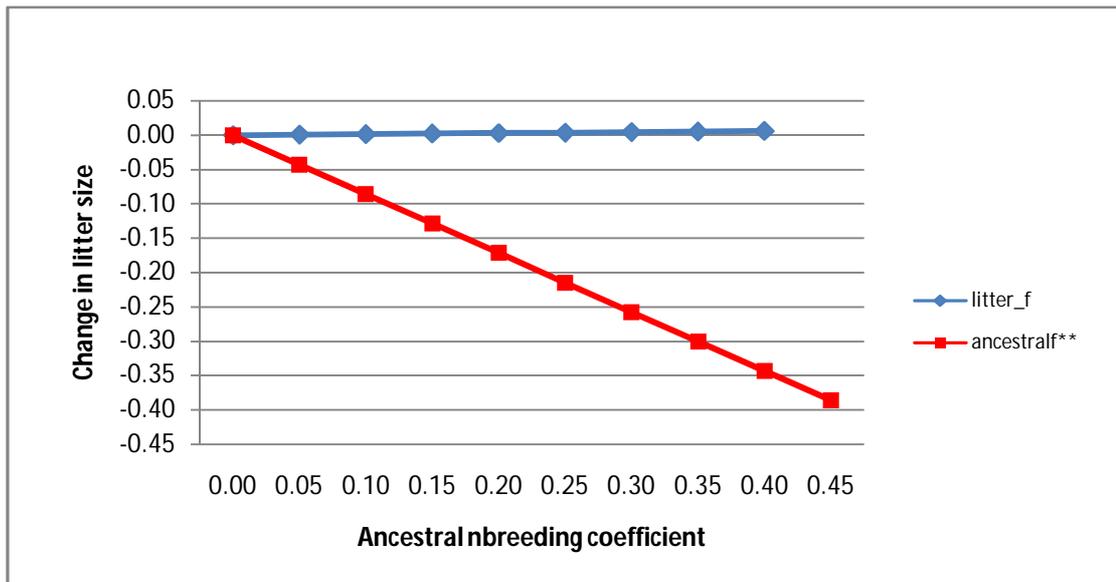


* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_sire= old inbreeding coefficient of the dam; **f"new"_sire** = new inbreeding coefficient of the dam

Figure 20 shows the effect of ancestral inbreeding on litter size (see Appendix 1C.3). It has a significant negative effect on litter size ($p < 0.10$). Litter size decreases as ancestral inbreeding increases. It is only in the analysis of litter size that the ancestral inbreeding of the litter has a negative significant effect as compared to survival traits.

Figure 20. The effect of ancestral inbreeding of litter on litter size.



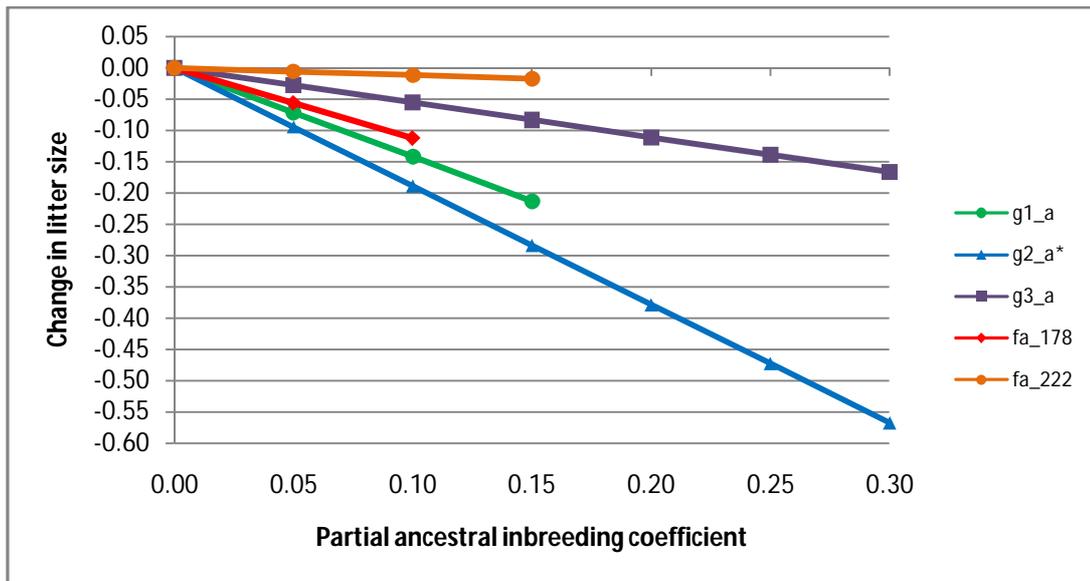
* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

ancestral_f = ancestral inbreeding coefficient of the litter; **litter_f** = total inbreeding coefficient of the litter; **d7** = day 7; **d30** = day 30; and **d90** = day 90

The magnitude and direction of inbreeding depression on litter size influenced by the origin due to specific founders and founder groups is different compared to the effects on survival traits. Analyses with partial inbreeding showed that animal 222 and founder group g1 have positive effects on litter size opposite to founder groups g2 and g3. Nonetheless, the analyses showed that none of the founder or founder groups has a significant effect on litter size. All founders and founder groups of the dam have negative effect on litter size. Nonetheless, all were found to be insignificant.

Figure 21 shows the effect of partial ancestral inbreeding on litter size (see Appendix 1E.2). Founder group g2_a is the one with negative significant effect, decreasing litter size. At 0.25 level of partial ancestral inbreeding, litter size decreases by 0.14.

Figure 21. The effect of partial ancestral inbreeding of litter on litter size.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

g1_a= partial ancestral inbreeding coefficient of founder group 1 ; **g2_a**= partial ancestral inbreeding coefficient of founder group 2; **g3_a**= partial inbreeding coefficient of founder group 3; **fa_178**= partial ancestral inbreeding coefficient of founder 178; **fa_222**= partial ancestral inbreeding coefficient of founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

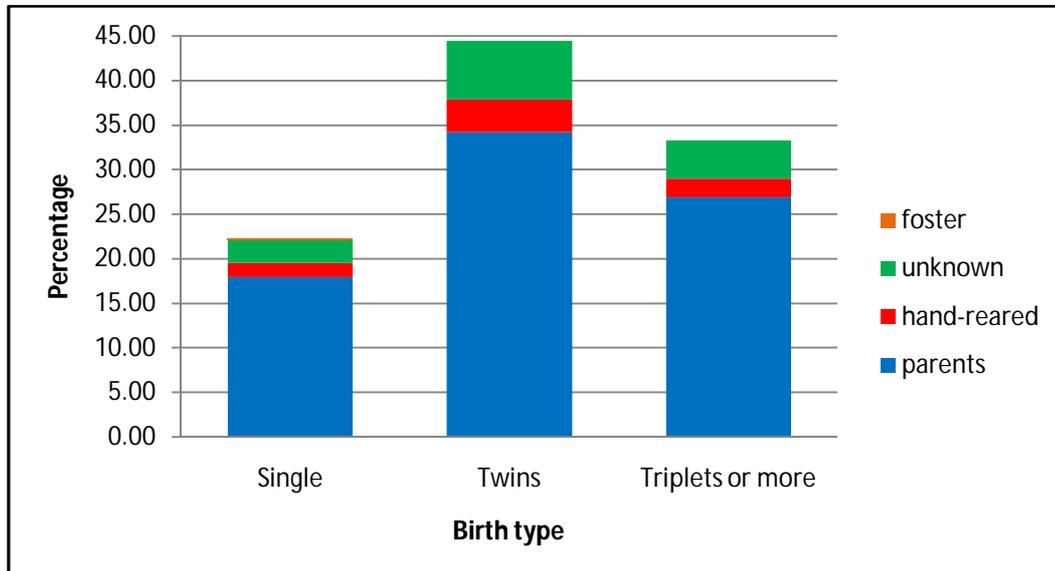
4.1.4 Effects of sex, parity number and birth type

Effects of sex of the individual and parity number were found to be insignificant on the survival of individual or litter in contrast to birth type ($p < 0.10$). Birth type in this study is defined as the size of the litter to which the individual belongs to. The average litter size in North Persian leopard is 2.40 (sd= 0.71). Figure 22 shows the distribution of birth types and the type of rearing provided. Singles have higher a probability (0.51- 0.55) of mortality compared to twins and triplets with probabilities ranging from 0.22 to 0.32 and from 0.25 to 0.27, respectively. However, it was not evaluated if the difference between birth types is significant since it is beyond the scope of the study.

The significance of birth type indicates that litter survival is dependent on litter size. However, a study by Boutin *et al.* (1988) ascertained that survival of juvenile muskrats is not dependent on litter size. He further cited Morris (1987) that individual females may attempt to keep juvenile survival constant

by adjusting litter size. Approximately, 43.85% of the population were born with a twin, 33.75% as triplets or more and 22.40% as singles.

Figure 22. Birth types with type of rearing.



4.2 Mhorr gazelle

4.2.1 Pedigree analysis

Table 8 shows the results of the pedigree analysis of Mhorr gazelle using PEDIG (Boichard, 2007) and ENDOG version 4.5 (Gutiérrez and Goyache, 2005).

The reference population is composed of animals which are alive, with known parents and known sex. If animals are less than 10 years old (based on birth dates up to 2008), they are assumed to be alive. Mhorr gazelle live approximately 12 years in captivity. The following results refer to the reference population.

The effective number of founders is 3.42 when half founder is to be counted) while the effective number of ancestors is 3. The analysis also showed that the effective founder genomes of the population is just 1.44. The effective number of founders and ancestors are lesser than the actual number of founders and ancestors which is an indication that there is an imbalance in the expected contribution of each founder in the population. The values on

the effective number of founders and ancestors are almost equal. This also shows that the animals did not go through a severe bottleneck in captivity. However, effective number of founder genomes is low, which demonstrates that there is gene loss due to drift in the population (Boichard *et al*, 1997). The mean maximum generations indicates that an average a maximum of 6.97 generations could be traced back. Mean complete generation show that on the average there are approximately 4.11 complete generations which separates an individual to its farthest known ancestors. Moreover, each individual is separated by 4.94 generations on average (mean equivalent generations) to each of its known ancestors

Table 8. Measures of genetic variation of Mhorr gazelle population in captivity.

Measures of genetic variation	Value
No. of animals in the reference population (alive)	97 (30.79%)
N_e based on regression of equivalent generations	7
No. of founders	8 (7.5 half founder)
Effective number of founders	3
No. of ancestors	8
Effective number of ancestors	3
No. of ancestors explaining 50% of the genetic variation	2
Effective number of founder genomes	1.44 (mean); 0.41 (sd)
Mean maximum generations	6.97
Mean complete generations	4.11
Mean equivalent generations	4.94

4.2.2 Mortality risk at days 7, 30 and 180 (weaning age)

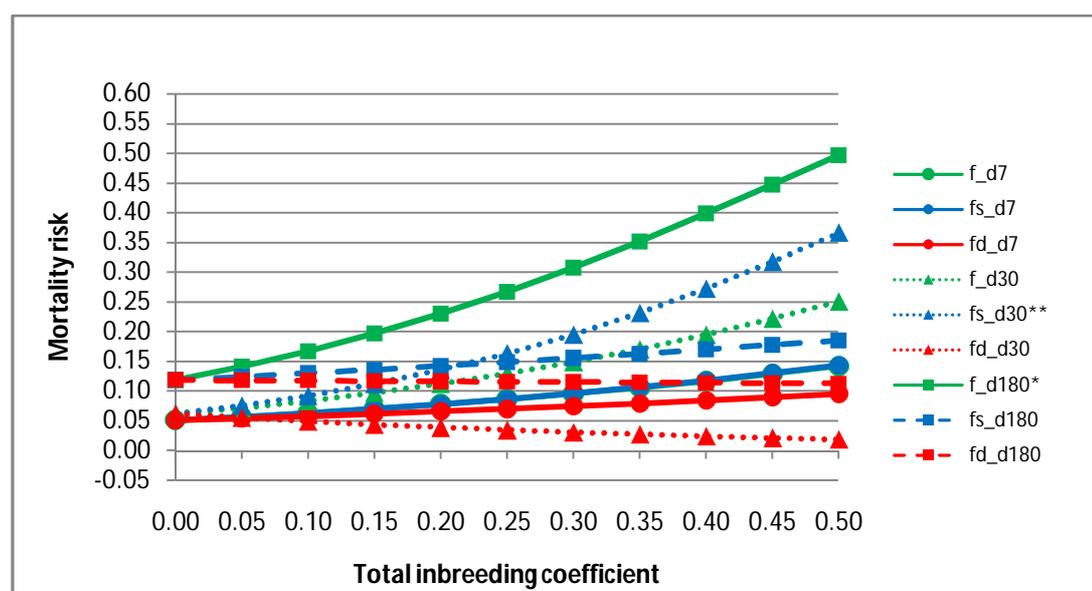
Table 8 shows the mean, standard deviation and maximum value of total inbreeding of the individual, sire and dam (see Appendix 2A). Approximately 70% of the individuals are inbred (219 out of 315). The influence of individual, sire and dam classical inbreeding on mortality at days

7, 30 and 180 (weaning age) is shown in Figure 23. Individual inbreeding has significant effect only in mortality at day 180 or weaning age ($p < 0.10$). Analyses revealed that sire inbreeding is significant in mortality at day 30 ($p < 0.05$). Dam inbreeding has no significant effect in any analyses.

Table 9. Total inbreeding coefficients (f) of the individual, litter, sire and dam.

	Mean	Standard deviation	Maximum
Individual	0.2971	0.1043	0.5247
Sire	0.2300	0.1141	0.4630
Dam	0.2339	0.1070	0.5221

Figure 23. Mortality risk of an individual at days 7, 30 and 180 (weaning age) with total inbreeding coefficients of individual, sire and dam.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f = Individual inbreeding coefficient; f_s = sire inbreeding coefficient; f_d = dam inbreeding coefficient; $d7$ = day 7; $d30$ = day 30; and $d180$ = day 180

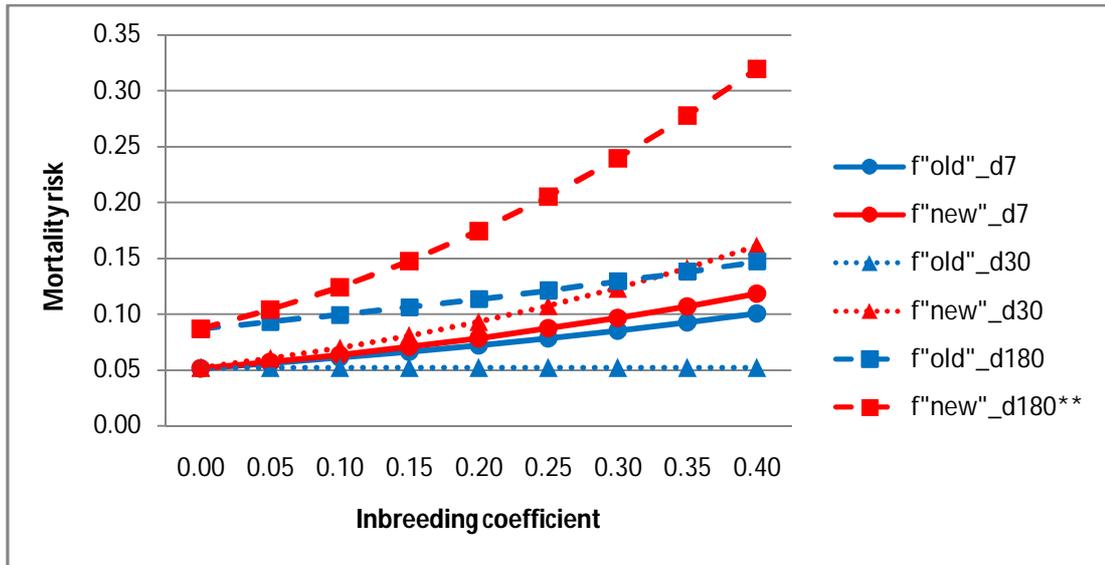
“Old” and “new” inbreeding was considered in the linear mixed model analyses of the survival traits. Table 10 shows the mean, standard deviation and maximum value of individual, sire and dam “old” and “new” inbreeding. Figures 24 and 25 show the impact of individual and sire “old” and “new” inbreeding on mortality at days 7, 30 and 180 (refer to Appendix 2B.1 and 2B.2). Only the “new” inbreeding of the individual was found to have a significant effect on individual mortality at 180 days ($p < 0.05$).

The negative significant effect of individual total inbreeding on mortality at day 180 is an indication that inbreeding of the individual is the one influencing its survival. The same result was found in the study of Cassell *et al.* (2003) on maternal and fetal inbreeding depression in Hosteins and Jerseys. They found out that inbreeding of the calf is the one affecting the survival at later stages of life and not the inbreeding of the sire or dam. Furthermore, the significant effect is highly associated with the “new” inbreeding having a negative effect compared to the “old” inbreeding

Table 10. “Old” and “new” inbreeding coefficients of individual, sire and dam.

	Mean	Standard deviation	Maximum
Individual			
<i>Old</i>	0.1547	0.7999	0.3540
<i>New</i>	0.1412	0.0786	0.3750
Sire			
<i>Old</i>	0.0997	0.0628	0.2765
<i>New</i>	0.1277	0.0797	0.3125
Dam			
<i>Old</i>	0.1075	0.0793	0.2547
<i>New</i>	0.1255	0.0750	0.3750

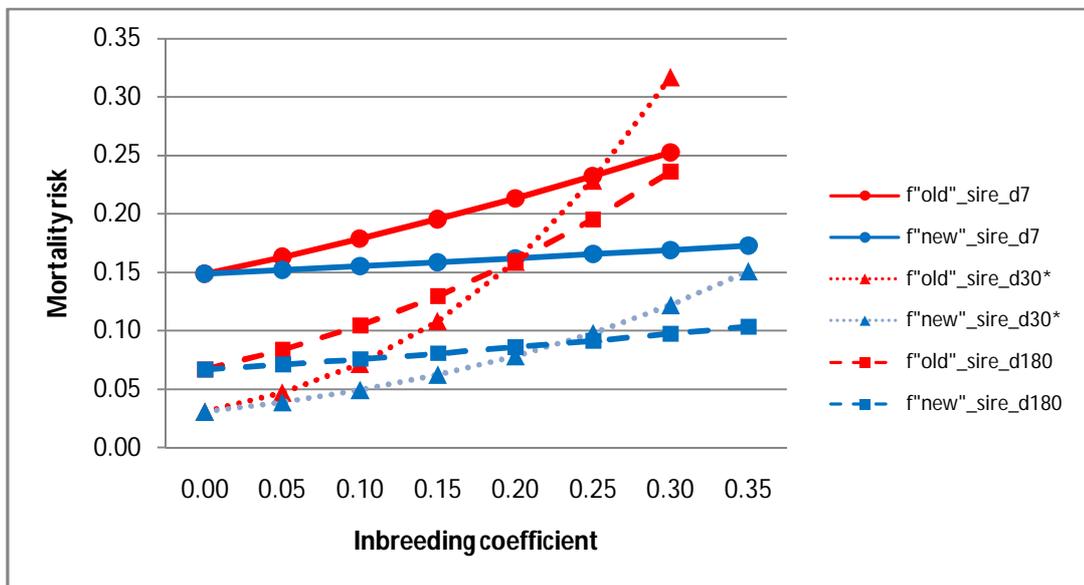
Figure 24. Mortality risk of an individual at days 7, 30 and 180 (weaning age) with “old” and “new” inbreeding coefficients of the individual.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old" = old inbreeding coefficient of the individual; **f"new"** = new inbreeding coefficient of the individual; **d7**= day 7; **d30**= day 30; and **d180**= day 180

Figure 25. Mortality risk of an individual at days 7, 30 and 180 (weaning age) with “old” and “new” inbreeding coefficients of sire.



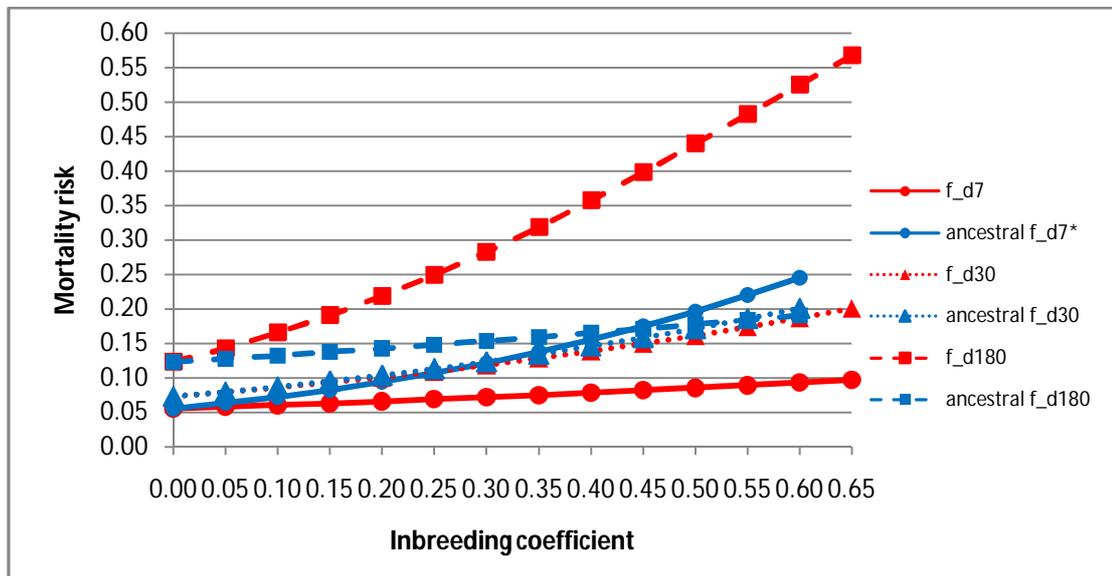
* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_sire= old inbreeding coefficient of the dam; **f"new"_sire** = new inbreeding coefficient of the dam; **d7**= day 7; **d30**= day 30; and **d180**= day 180

Ancestral inbreeding of the Mhorr gazelle population ranges from 0 to 0.69 with a mean of 0.40 (sd = 0.17). Figure 26 shows the effect of ancestral inbreeding on mortality at days 7, 30 and 180 (Appendix 2C). Ancestral inbreeding has a significant effect on mortality at day 7 ($p < 0.10$). The

mortality of an individual at day 7 increases slightly as ancestral inbreeding increases.

Figure 26. Mortality risk of an individual at days 7, 30 and 180 (weaning age) with ancestral inbreeding coefficients of the individual.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

Ancestral f= ancestral inbreeding coefficient; **f**= total inbreeding coefficient; **d7**= day 7; **d30**= day 30; and **d180**= day 180

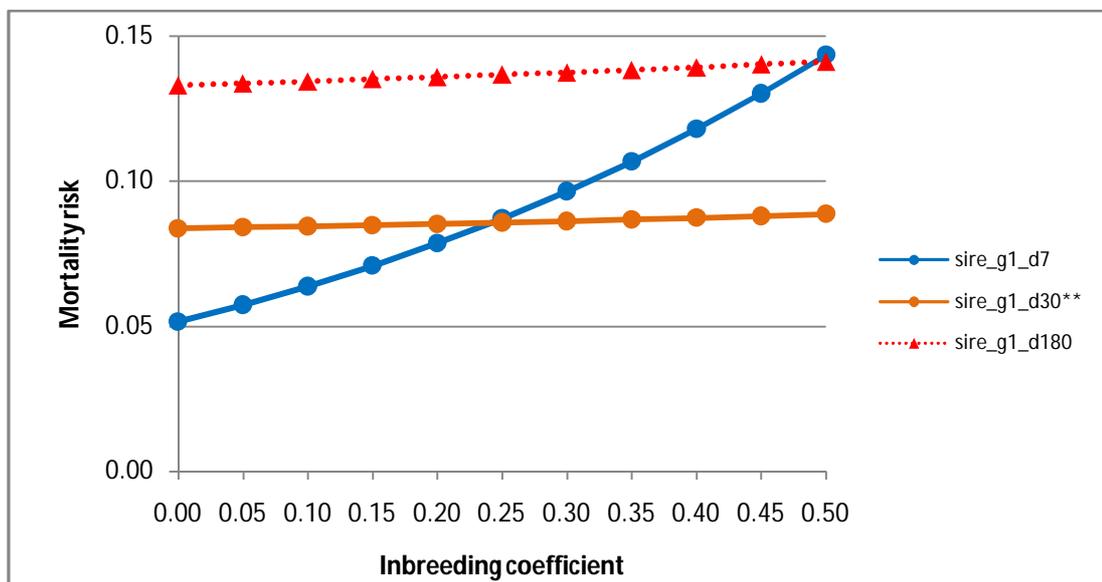
The negative effect of sire inbreeding was also found to be significant in mortality at day 30 (Figure 25, Appendix 2D). Moreover, it is more associated with “new” inbreeding. Gazelles are so called polygynous species, in which males bred with several females. Female gazelles tend to chose males which are more heterozygous. A heterozygous male, which is more attractive to females, was shown to have offspring with increased survival (Byers and Waits, 2006). Byers and Waits (2006) in their study with pronghorns further mentioned, that females use information aside from male ornaments in selecting superior mates. The study of Cassinello (2004) on captive gazelles showed that inbred individuals have declined survival compared to non-inbreds.

The results of the analyses with “old” and “new” inbreeding were similar to the results of Hinrichs *et al.* (2007) with mice, wherein “new” inbreeding was found to cause more inbreeding depression. This could be due to emergence of new mutations in the population or natural selection on the non-

additive loci associated with survival. Epistasis could also be one of the reasons creating new non-additive variation (Hinrichs, 2007).

Analyses of individual partial inbreeding showed that almost no founder or founder group has a significant effect on the mortality of the individual. Founder group g1 which is composed of animals 1, 10, 15, 309 and 311, was found to be the only one contributing to the degree of inbreeding among sires. Its contribution is significant for the mortality of individual at day 30 ($p < 0.05$) (Figure 27).

Figure 27. Mortality risk of an individual at days 7, 30 and 180 (weaning age) with sire founder group 1 inbreeding coefficients.



* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

sire_g1= partial inbreeding coefficient of dam founder group **d7**= day 7; **d30**= day 30; and **d180**= day 180

Analyses on mortality with partial ancestral inbreeding coefficients showed no significant effects. Two founder groups were formed with correlations of their ancestral inbreeding > 0.60 . Founder group 1 (g1_a) is composed of animals 1, 10, 15, 309 and 311) while founder group 2 (g2_a) is composed of animals 9 and 10. Only one group of founders is contributing significantly to the inbreeding depression in the Mhorr gazelle population. This shows that the inbreeding depression in survival traits is associated with sire inbreeding which is due to the alleles coming from the founder group g1_a.

4.2.3 Effects of sex and parity number

The effects of sex and parity number on the survival of the individuals were insignificant.

5 SUMMARY and CONCLUSIONS

5.1 *North Persian leopard*

1. There is considerable founder gene loss due to random genetic drift in the last generations with an effective number of founder genomes lesser than both the effective number of founders and ancestors. However, equal number of effective number of founders and ancestors indicate that the population did not go through severe bottleneck.
2. Inbreeding depression is manifested by an increased mortality risk up to days 7, 30 and 90 after birth as individual/ litter classical inbreeding coefficient increases. Interestingly recent inbreeding has less impact on inbreeding depression compared to "old" inbreeding.
3. A decrease in litter size is significantly associated with increasing litter and dam inbreeding. Sire "*old*" inbreeding is also significantly associated with the decrease in litter size. The effect on litter size by dam inbreeding can be traced to inbreeding of alleles contributed by founder group g2.
4. Purging is apparent when the effect of dam inbreeding on individual/ litter mortality was evaluated. The probability of individual/ litter mortality up to days 7, 30 and 90 decreases as dam inbreeding increases. Further analyses showed that this effect is due to the "*new*" inbreeding of the dam.
5. High ancestral inbreeding has significant negative effects only on litter size. Therefore, no indication for purging was found using ancestral inbreeding coefficients.
6. Certain founder groups are the ones contributing to the increase in mortality of the individual and litter. Inbreeding due to one founder group (g2) and one single founder (animal no. 222) were significantly associated with individual or litter mortality. Founder groups g2 and g3 are the ones contributing to the decrease in litter size, however, the effect is found to be insignificant.
7. The positive effect of increased dam inbreeding on survival of individual or litter can be traced to founder group g2 and g3.

8. Partial inbreeding due to all founders or founder groups decreases litter size. There was no heterogeneity with regard to the founder effects detected.
9. Partial ancestral inbreeding due one founder group g2 and founder number 222 have also a negative significant effect on litter survival, while g2 is also associated with a decrease in litter size.
10. Birth types with 2 or more cubs per litter have decreases probability of mortality compared to singles.

5.2 Mhorr gazelle

1. The effective number of founder genomes is lesser than the effective number of founders and ancestors which indicates that there is substantial gene loss in the last generation due to random genetic drift. The population has not gone through a severe bottleneck as shown by the almost equal values of effective number of founders and ancestors.
2. Inbreeding depression is apparent at weaning age where increasing individual inbreeding corresponds with an increased mortality at day 180. This trend was found to be rather due to “new” inbreeding (during the last 3 generations) of the individual.
3. Sire inbreeding has a negative significant effect on survival of an individual. Both “old” and “new” inbreeding are significantly associated with the decrease in survival.
4. Only one founder group (g1) of the sire is found to be significantly contributing to the mortality of an individual.
5. Ancestral inbreeding significantly increases the mortality of an individual up to day 7.

In conclusion it can be said that different species show different response to inbreeding. Response to inbreeding is also manifested differently among fitness traits investigated. This might be due to the fact that fitness traits are traits which are governed by many alleles and that the two hypotheses (dominance and overdominance) could be acting concurrently on different loci influencing these fitness traits. Moreover, with the unpredictable response of

populations/ species and traits to inbreeding, breeding programs for captive populations should still be designed with the aim to minimize the rate of inbreeding and maximize genetic diversity.

6 LITERATURE CITED

- Agresti, A. 2002. Categorical Data Analysis. 2nd Ed. John Wiley and Sons, Inc., New Jersey. Pp 166- 170.
- Alberts, S. C. and Altmann, J. 1995. Balancing costs and opportunities: dispersal in male baboons. *The American Naturalists* 145: 279- 306.
- Amos, W., and A. Balmford. 2001. When does conservation genetics matter? *Heredity* 87: 257-265.
- Ballou, J. D. 1997. Ancestral inbreeding minimally affects inbreeding depression in mammalian populations. *Heredity* 88: 169- 178.
- Bais, L. 2002. "Panthera pardus" (On-line), Animal Diversity Web. Accessed May 20, 2009 at http://animaldiversity.ummz.umich.edu/site/accounts/information/Panthera_pardus.html.
- Barrett, S. C. H. And Charlesworth, D. 1991. Effects of a change in the level of inbreeding on the genetic load. *Nature* 352: 552- 524.
- Baumung, R. 2009. Personal communication. Vienna, Austria.
- Bies, L. 2002. "Panthera pardus" (On-line), Animal Diversity Web. Accessed May 20, 2009 at http://animaldiversity.ummz.umich.edu/site/accounts/information/Panthera_pardus.html
- Bijlsma, R. Bundgaard, J. and Boerema, A. C. 2000. Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. *J. Evol. Biol.* 13: 502- 511.
- Bereskin, B., Shelby, C. E., Rowe, K. E., Urban, W. E. Jr., Blunn, C. T., Chapman, A. B., Garwood, V. A., Hazel, L. N., Lasley, J. F., Magee, W. T., Mccarty, J. W. and Whatley, J. A., Jr. 1968. Inbreeding and swine productivity traits. *J Anim Sci* 27: 339- 350.
- Boakes, E. H., Wang, J. and Amos, W. 2006. An investigation of inbreeding depression and purging in captive pedigreed populations. *Heredity* 1-11.
- Boichard, D, Maingel, L. and Verrier, E. 1997. The value of using probabilities of gene origin to measure genetic variability in a population. *Genet Sel Evol* 29: 5- 23

- Boichard, D. 2002. Pedig: A fortran package for pedigree analysis suited to large populations. 7th World congress on Genetics Applied to Livestock Production, Montpellier, 19- 23 août 2002, paper 28- 13.
- Boutin, S., Moses, R. A. and Caley, M. J. 1988. The relationship between juvenile survival and litter size in wild muskrats (*Ondatra zibethicus*). *The Journal of Animal Ecology* 57 (2): 455- 462.
- Brown, J.L. and Brown, E. R. 1998. Are inbred offspring less fit? Survival in a natural population of Mexican jays. *Behavioral Ecology* 9 (1): 60- 65.
- Byers, J. A. and Waits, L. 2006. Good genes sexual selection in nature. *PNAS* 103 (44): 16343- 16345.
- Cassell, B. G., Adamec, V. And Pearson, R. E. 2003. Maternal and fetal inbreeding depression for 70-day nonreturn and calving rate in Hosteins and Jerseys. *J. Dairy Sci.* 86: 2977- 2983.
- Casellas, J, Piedrafita, J., Caja, G. and Varona, L. 200. Analysis of founder-specific inbreeding depression on birth weight in Ripollesa lambs. *J Anim Sci* 87: 72- 79.
- Cassinello, J. 2005. Inbreeding depression on reproductive performance and survival in captive gazelles of great conservation value. *Biological Conservation* 122: 453- 464.
- Charpentier, M. J. E., Widdig, A. and Alberts, S. C. 2007. Inbreeding depression in non-human primates: a historical review of methods used and empirical data. *American Journal of Primatology* 69: 1370- 1386.
- Crnokrak, P., and Roff, D.A. 1999. Inbreeding depression in the wild. *Heredity* 83: 260-270.
- Dwyer, C. M. 2008. Genetic and physiological determinants of maternal behaviour and lamb survival: inputs for low- input sheep management. *J. Anim Sci* 86: 246- 258.
- Falconer, D. S. and Mackay, T. F. C. 1996. Introduction to Quantitative Genetics. 4th Edition. Longman Group Ltd., England. p. 247.
- Fitzpatrick, J. L. and Evans, . P. 2009. Reduced heterozygosity impairs sperm quality in endangered mammals. *Biol. Lett.* 5 (3): 320- 323.
- Frankham, R., Gilligan, D. M., Morris, D. and Briscoe, D. A. 2001. Inbreeding and extinction: Effects of purging. *Conservation Genetics* 2: 279-285.

- Gutiérrez, J. P. and Goyache, F. 2005. A note on ENDOG: a computer program for analysing pedigree information. *Journal of Animal Breeding and Genetics* 122: 172- 176.
- Gulisija, D., Gianola, D., Weigel, K. A., and Toro, M. A. 2006. Between-founder heterogeneity in inbreeding depression for production in Jersey cows. *Livestock Science* 104: 244- 253.
- Hedrick, P. W. 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* 73: 363- 372.
- Hinrichs, D., Meuwissen, T. H. E., Ødegard, J., Holt, M. Vangen, O and Woolliams, J.A. Analysis of inbreeding depression in the first litter size of mice in a long-term selection experiment with respect to the age of the inbreeding. *Heredity* 99: 81- 88.
- Höglund, J., Piirtney, S. B., Alatalo, R. V., Lindell, J., Lundberg, A. and Rintamäki, P. T. 2002. Inbreeding depression and male fitness in black grouse. *Proc. R. Soc. Lond.* 269: 711- 715.
- Hunt, V. 2009. Master's thesis. Assessment of conservation breeding programs using pedigree analysis with regard to inbreeding depression. BOKU, Vienna, Austria.
- Keane, B., Creel, S., and Waser, P. M. 1996. No evidence of inbreeding avoidance or inbreeding depression in a social carnivore. *Behavioral Ecology* 4: 480- 489.
- Kalinowski, S. T. and Hedrick, P. W. 1999. Detecting inbreeding depression is difficult in captive endangered species. *Animal Conservation* 2: 131- 136.
- Kalinowski, S. T., Hedrick, P. W. and Miller, P.S. 2000. Inbreeding depression in the Speke's gazelle captive breeding program. *Conservation Biology* 14 (5): 1375- 1384.
- Keller, L. F. and Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17 (5): 230- 241.
- Kelly, J. K. and Tourtellot, M. K. 2006. The genetic analysis of family structured inbreeding depression studies. *Heredity* 97: 345- 364.
- Khorozyan, I. 2008. *Panthera pardus ssp. saxicolor*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 26 April 2009.

- Kristensen, T. N. and Sørensen, A. C. 2005. Inbreeding- lessons from animal breeding, evolutionary biology and conservation genetics. *Animal Science* 80: 121- 133.
- Kristensen, T. N., Loeschcke, V., and Hoffman, A. A. 2008. Linking inbreeding effects in captive population with fitness in the wild: release of replicated *Drosophila melanogaster* lines under different temperatures. *Conservation Biology* 22 (1): 189- 199.
- Lacy, R. C. 1993. Impacts of inbreeding in natural and captive populations of invertebrates: implications for conservation. *Perspect Biol Med* 36: 480-496.
- Lacy, R. C. 1995. Clarification of genetic terms and their use in the management of captive populations. *Zoo Biology*, 14: 565-578.
- Lacy, R. C., Alaks, G and Walsh, A. 1996. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. *Evolution* 50 (6): 2187- 2200.
- Laikre, L. 1999. Conservation genetics of Nordic carnivores: lessons from zoos. *Hereditas* 130: 203- 216.
- Lynch, M., and O'Hely, M. 2001. Captive breeding and the genetic fitness of natural populations. *Conservation Genetics* 2: 363-378.
- Maignel, L., Boichard, D. and Verrier, E. 1996. Genetic variability of French dairy breeds estimated from pedigree information. *Interbull Bull.* 14: 49-54.
- McCarthy, J. C. 1967. The effects of inbreeding on the components of litter size in mice. *Genetical Research* 10: 73- 80.
- Miller, P. S. And Hedrick, P. W. 2001. Purging of inbreeding depression and fitness decline in bottlenecked populations of *Drosophila melanogaster*. *J Evol Biol* 14: 595- 601.
- Newby, J., Wachter, T., Lamarque, Cuzin, F. & de Smet, K. 2008. *Nanger dama*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 26 April 2009.
- Packer, C. and Pusey, A. E. 1993. Dispersal, kinship and inbreeding in African lions, in *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (Thornhill, N.W.) University of Chicago Press. Pp. 375- 391.

- Parland, S. M., Kearney, F., and Berry, D. P. Purging of inbreeding depression within the Irish Holstein- Friesian population. *Genetics Selection Evolution* 41: 16.
- Peripato, A. C., de Brito, R. A., Vaughn, T. T., Pletscher, L. S., Matioli, S. R. and Cheverud, J. M. 2002. Quantitative trait loci for maternal performance for offspring survival in mice. *Genetics* 162: 1341- 1353.
- Ramirez, O., Altet, L., Ensenat, C., Vilá, C., Sanchez, A. and Ruiz, A. 2006. Genetic assessment of the Iberian wolf *Canis lupus signatus* captive breeding program. *Conservation Genetics* 7: 861- 878.
- Read, A. F. and Harvey, P. H. 1986. Genetic management in zoos. *Nature* 322: 408- 410.
- Richards, C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. *Am Nat* 155: 383- 394.
- Rodrigañez, J., Toro, M. A., Rodriguez, C. and Silió. 1998. Effect of founder allele survival and inbreeding depression on litter size in a closed line of Large White pigs. *Animal Science* 67: 573- 582.
- Suwanlee, S. Curik, I., Sölkner, J. and Baumung, R. 2006. Selection Criteria to purge deleterious alleles. In 8th World Congress on Genetics Applied to Livestock Production, August 13- 18, 2006.
- Templeton, A. R. and Read, B. 1994. Inbreeding one word several meanings much confusion. 1996. Estimation of direct and maternal genetic (co)variances for survival within litters of piglets. *Livestock Production Science* 46: 163 – 171.
- Van Arendonk, J. A. M., Van Rosmeulen, C., Janss, L. L. G. and Knol, E. F. 1996. Estimation of direct and maternal genetic co(variances) for survival within litters of piglets. *Livestock Production Science* 46: 163- 171.
- Wright, L. I., Tregenza, T and Hosken, D. J. 2008. Inbreeding, inbreeding depression and extinction. *Conservation Genetics* 9: 833- 843.

APPENDIX 1 – LEOPARD: Inbreeding coefficients, mortality risk and effect on litter size

APPENDIX 1A – TOTAL INBREEDING

1. Mortality risk of an individual at days 7, 30 and 90 with total inbreeding coefficients of individual, sire and dam.

Inbreeding coefficient	ind_ d7	sire_ d7	dam_ d7**	ind_ d30*	sire_ d30	dam_ d30**	ind_ d90***	sire_ d90	dam_ d90**
0.00	0.24	0.24	0.24	0.24	0.24	0.24	0.22	0.22	0.22
0.05	0.26	0.24	0.21	0.26	0.24	0.21	0.25	0.22	0.19
0.10	0.28	0.24	0.18	0.29	0.24	0.18	0.29	0.22	0.16
0.15	0.30	0.24	0.15	0.31	0.23	0.16	0.33	0.21	0.14
0.20	0.32	0.24	0.13	0.34	0.23	0.14	0.37	0.22	0.12
0.25	0.34	0.23	0.11	0.37	0.23	0.12	0.41	0.21	0.10
0.30	0.36	0.23	0.10	0.39	0.23	0.10	0.46	0.21	0.09
0.35	0.38	0.23	0.08	0.42	0.23	0.09	0.50	0.21	0.07
0.40	0.41	0.23	0.07	0.44	0.22	0.08	0.55	0.21	0.06

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f = Individual inbreeding coefficient; f_s = sire inbreeding coefficient; f_d = dam inbreeding coefficient; **d7** = day 7; **d30** = day 30; and **d90** = day 90

2. Mortality risk of a litter at days 7, 30 and 90 with total inbreeding coefficients of litter, sire and dam.

Inbreeding coefficient	f_l _d7	f_s _d7	f_d _d7**	f_l _d30	f_s _d30	f_d _d30**	f_l _d90	f_s _d90	f_d _d90*
0.00	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.29	0.29
0.05	0.29	0.27	0.24	0.31	0.28	0.26	0.31	0.28	0.27
0.10	0.30	0.26	0.21	0.32	0.27	0.22	0.32	0.27	0.24
0.15	0.30	0.26	0.19	0.33	0.26	0.19	0.34	0.25	0.22
0.20	0.31	0.25	0.16	0.34	0.25	0.17	0.35	0.24	0.20
0.25	0.32	0.24	0.14	0.35	0.24	0.14	0.37	0.23	0.18
0.30	0.33	0.23	0.12	0.36	0.22	0.12	0.39	0.22	0.16
0.35	0.34	0.22	0.10	0.37	0.21	0.10	0.40	0.21	0.14
0.40		0.23	0.09		0.20	0.09		0.20	0.13

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f_l = inbreeding coefficient of the litter; f_s = inbreeding coefficient of the sire; f_d = inbreeding coefficient of the dam; **d7** = day 7; **d30** = day 30; and **d90** = day 90

3. The effect of total inbreeding of litter, sire and dam on litter size.

Inbreeding coefficient	f_l	f_s	f_d^*
0.00	0.00	0.00	0.00
0.05	0.01	0.00	-0.05
0.10	0.02	0.00	-0.11
0.15	0.03	0.00	-0.16
0.20	0.04	0.00	-0.21
0.25	0.05	0.00	-0.27
0.30	0.06	0.00	-0.32
0.35		0.00	-0.38

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f_l = inbreeding coefficient of the litter; f_s = inbreeding coefficient of the sire; f_d = inbreeding coefficient of the dam

APPENDIX 1B – “OLD” AND “NEW” INBREEDING

1. Mortality risk of an individual at days 7, 30 and 90 with “old” and “new” inbreeding coefficients of individual, sire and dam.

Inbreeding coefficient	<i>f</i>''old'' _d7***	<i>f</i>''new'' _d7	<i>f</i>''old'' _d30**	<i>f</i>''new'' _d30*	<i>f</i>''old'' _d90**	<i>f</i>''new'' _d90***
0.00	0.20	0.20	0.21	0.21	0.20	0.20
0.05	0.30	0.22	0.30	0.23	0.28	0.23
0.10	0.43	0.23	0.41	0.25	0.37	0.26
0.15	0.57	0.25	0.53	0.27	0.48	0.30
0.20	0.70	0.27	0.65	0.30	0.59	0.34
0.25		0.29		0.33		0.38
0.30		0.31		0.35		0.42
0.35		0.33		0.38		0.47
0.40		0.36		0.41		0.52

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f''old'' = old inbreeding coefficient of the individual; *f*''new'' = new inbreeding coefficient of the individual; **d7**= day 7; **d30**= day 30; and **d90**= day 90

2. Mortality risk of a litter at days 7, 30 and 90 with “old” and “new” inbreeding coefficients of litter.

Inbreeding coefficient	<i>f</i>''old'' _litter _d7*	<i>f</i>''new'' _litter _d7	<i>f</i>''old'' _litter _d30**	<i>f</i>''new'' _litter _d30	<i>f</i>''old'' _litter _d90**	<i>f</i>''new'' _litter _d90
0.00	0.24	0.24	0.25	0.25	0.24	0.24
0.05	0.31	0.25	0.32	0.26	0.32	0.26
0.10	0.39	0.25	0.41	0.27	0.41	0.28
0.15	0.48	0.26	0.49	0.28	0.50	0.29
0.20	0.58	0.27	0.58	0.30	0.59	0.31
0.25		0.28		0.31		0.33
0.30		0.29		0.32		0.34
0.35		0.30		0.33		0.36
0.40		0.31		0.35		0.38

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f''old''_litter = old inbreeding coefficient of the litter; *f*''new''_litter = new inbreeding coefficient of the litter; **d7**= day 7; **d30**= day 30; and **d90**= day 90

3. The effect of “old” and “new” inbreeding of litter on litter size.

Inbreeding coefficient	f”old”_litter	f”new”_litter
0.00	0.00	0.00
0.05	-0.09	0.01
0.10	-0.18	0.03
0.15	-0.27	0.04
0.20	-0.36	0.05
0.25		0.07
0.30		0.08
0.35		0.09
0.40		0.10

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$; **f”old”_litter** = old inbreeding coefficient of the litter; **f”new”_litter** = new inbreeding coefficient of the litter

4. Mortality risk of an individual at days 7, 30 and 90 with “old” and “new” inbreeding coefficients of the dam.

Inbreeding coefficient	f”old”_dam_d7	f”new”_dam_d7**	f”old”_dam_d30	f”new”_dam_d30**	f”old”_dam_d90	f”new”_dam_d90**
0.00	0.23	0.23	0.23	0.23	0.21	0.21
0.05	0.27	0.19	0.26	0.20	0.25	0.18
0.10	0.32	0.16	0.30	0.17	0.29	0.15
0.15	0.37	0.14	0.34	0.14	0.34	0.12
0.20		0.11		0.12		0.10
0.25		0.10		0.10		0.09
0.30		0.08		0.09		0.07
0.35		0.07		0.07		0.06
0.40		0.05		0.06		0.05

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$; **f”old”_dam** = old inbreeding coefficient of the dam; **f”new”_dam** = new inbreeding coefficient of the dam; **d7**= day 7; **d30**= day 30; and **d90**= day 90

5. Mortality risk of a litter at days 7, 30 and 90 with “old” and “new” inbreeding coefficients of the dam.

Inbreeding coefficient	f”old”_dam_d7	f”new”_dam_d7**	f”old”_dam_d30	f”new”_dam_d30***	f”old”_dam_d90	f”new”_dam_d90***
0.00	0.26	0.26	0.27	0.27	0.26	0.26
0.05	0.28	0.22	0.28	0.23	0.30	0.23
0.10	0.29	0.19	0.28	0.19	0.33	0.21
0.15	0.31	0.16	0.29	0.16	0.37	0.18
0.20		0.13		0.14		0.16
0.25		0.11		0.11		0.14
0.30		0.09		0.09		0.12
0.35		0.08		0.08		0.11
0.40		0.06		0.06		0.09

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$; **f”old”_dam** = old inbreeding coefficient of the dam; **f”new”_dam** = new inbreeding coefficient of the dam; **d7**= day 7; **d30**= day 30; and **d90**= day 90

6. The effect of “old” and “new” inbreeding of dam on litter size.

Inbreeding coefficient	f”old”_dam	f”old”_dam*
0.00	0.00	0.00
0.05	-0.11	-0.05
0.10	-0.21	-0.10
0.15	-0.31	-0.16
0.20	-0.42	-0.21
0.25		-0.26
0.30		-0.31
0.35		-0.36
0.40		-0.42

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f”old”_dam= old inbreeding coefficient of the dam; **f”new”_dam** = new inbreeding coefficient of the dam

7. The effect of “old” and “new” inbreeding of sire on litter size.

Inbreeding coefficient	f”old”_sire	f”new”_sire
0.00	0.00	0.00
0.05	-0.16	0.01
0.10	-0.31	0.01
0.15	-0.47	0.02
0.20	-0.62	0.02
0.25		0.03
0.30		0.04
0.35		0.04
0.40		0.05

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f”old”_sire= old inbreeding coefficient of the dam; **f”new”_sire** = new inbreeding coefficient of the dam

APPENDIX 1C- ANCESTRAL INBREEDING

1. Mortality risk of an individual at days 7, 30 and 90 with ancestral inbreeding coefficients.

Inbreeding coefficient	ancestral f_d7	f_d7	ancestral f_d30	f_30*	ancestral f_d90	f_d90***
0.00	0.20	0.20	0.22	0.22	0.20	0.20
0.05	0.21	0.22	0.21	0.24	0.20	0.23
0.10	0.21	0.23	0.21	0.26	0.19	0.27
0.15	0.21	0.25	0.21	0.28	0.19	0.30
0.20	0.21	0.27	0.21	0.30	0.18	0.34
0.25	0.21	0.28	0.20	0.33	0.18	0.38
0.30	0.21	0.30	0.20	0.35	0.17	0.42
0.35	0.22	0.32	0.20	0.38	0.17	0.47
0.40	0.22	0.34	0.20	0.41	0.16	0.51
0.45	0.22		0.19		0.16	

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

ancestral f= ancestral inbreeding coefficient; **f**= total inbreeding coefficient; **d7**= day 7; **d30**= day 30; and **d90**= day 90

2. Mortality risk of a litter at days 7, 30 and 90 with litter ancestral inbreeding coefficients.

Inbreeding coefficient	ancestral_f_d7	litter_f_d7	ancestral_f_d30	litter_f_d30	ancestral_f_d90	litter_f_d90
00.00	0.25	0.25	0.26	0.26	0.26	0.26
0.05	0.24	0.25	0.25	0.26	0.26	0.27
0.10	0.24	0.25	0.25	0.27	0.25	0.29
0.15	0.24	0.26	0.25	0.28	0.25	0.30
0.20	0.24	0.26	0.25	0.28	0.25	0.31
0.25	0.24	0.27	0.25	0.29	0.25	0.33
0.30	0.24	0.27	0.25	0.30	0.25	0.34
0.35	0.24	0.28	0.24	0.31	0.24	0.36
0.40	0.24	0.28	0.24	0.32	0.24	0.37
0.45	0.24		0.24		0.24	

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

ancestral_f= ancestral inbreeding coefficient of the litter; **litter_f**= total inbreeding coefficient of the litter; **d7**= day 7; **d30**= day 30; and **d90**= day 90

3. The effect of ancestral inbreeding of litter on litter size.

Inbreeding coefficient	litter_f	ancestral_f**
0.00	0.0000	0.00
0.05	0.0008	-0.04
0.10	0.0015	-0.09
0.15	0.0023	-0.13
0.20	0.0030	-0.17
0.25	0.0038	-0.21
0.30	0.0046	-0.26
0.35	0.0053	-0.30
0.40	0.0061	
0.45	0.0068	

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

ancestral_f= ancestral inbreeding coefficient of the litter; **litter_f**= total inbreeding coefficient of the litter; **d7**= day 7; **d30**= day 30; and **d90**= day 90

APPENDIX 1D- PARTIAL INBREEDING

1. Mortality risk of an individual at days 7, 30 and 90 with partial inbreeding coefficients.

Inbreeding coefficient	g1_ d7	g2_ d7	g3_ d7	f222_ d7	g1_ d30	g2_ d30**	g3_ d30	f222_ d30	g1_ d90	g2_ d90*	g3_ d90	f222_ d90***
00.00	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.21	0.21	0.21
0.05	0.22	0.27	0.25	0.32	0.22	0.28	0.26	0.32	0.21	0.27	0.26	0.30
0.10	0.20	0.30	0.26	0.42	0.20	0.34	0.28	0.42	0.20	0.34	0.30	0.39
0.15	0.19	0.34	0.28	0.53	0.19	0.40	0.30	0.53	0.19	0.41	0.35	0.50
0.20	0.18	0.38	0.30	0.64	0.18	0.46	0.33	0.63	0.18	0.48	0.41	0.60
0.25	0.17	0.42	0.32	0.73	0.17	0.52	0.35	0.72	0.17	0.56	0.46	0.70
0.30		0.46	0.33	0.81		0.59	0.38	0.80		0.63	0.52	0.78
0.35		0.51				0.65				0.70		
0.40		0.55				0.70				0.76		

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

g1= partial inbreeding coefficient of founder group g1; **g2**= partial inbreeding coefficient of founder group g2; **g3**= partial inbreeding coefficient of founder group g3; **f222**= partial inbreeding coefficient of founder animal 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

2. Mortality risk of a litter at days 7, 30 and 90 with litter partial inbreeding coefficients.

Inbreeding coefficient	g1_ d7	g2_ d7	g3_ d7	f222_ d7	g1_ d30	g2_ d30**	g3_ d30	f222_ d30	g1_ d90	g2_ d90***	g3_ d90	f222_ d90
0.00	0.27	0.27	0.27	0.27	0.28	0.30	0.28	0.28	0.28	0.28	0.28	0.28
0.05	0.26	0.30	0.27	0.30	0.28	0.33	0.28	0.30	0.27	0.34	0.29	0.30
0.10	0.26	0.34	0.27	0.33	0.27	0.38	0.29	0.32	0.26	0.41	0.31	0.32
0.15	0.25	0.37	0.27	0.37	0.27	0.44	0.30	0.34	0.25	0.49	0.32	0.34
0.20	0.25	0.41	0.27	0.41	0.26	0.50	0.30	0.37	0.24	0.56	0.34	0.36
0.25	0.24	0.45	0.27	0.44	0.26	0.56	0.31		0.23	0.64	0.35	
0.30		0.50	0.28	0.48		0.62	0.31			0.70	0.37	
0.35		0.54				0.67				0.76		
0.40		0.58				0.72				0.81		

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

g1= partial inbreeding coefficient of founder group g1; **g2**= partial inbreeding coefficient of founder group g2; **g3**= partial inbreeding coefficient of founder group g3; partial inbreeding coefficient of **f222**= founder animal 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

3. Mortality risk of an individual at days 7, 30 and 90 with partial inbreeding coefficients of the dam.

Inbreeding coefficient	dam_g1_d7	dam_g2_d7	dam_g3_d7**	dam_f222_d7	dam_g1_d30	dam_g2_d30	dam_g3_d30*	dam_f222_d30	dam_g1_d90	dam_g2_d90	dam_g3_d90*	dam_f222_d90
0.00	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.22
0.05	0.11	0.26	0.20	0.67	0.13	0.26	0.21	0.53	0.15	0.23	0.19	0.33
0.10	0.05	0.27	0.17	0.93	0.07	0.29	0.18	0.80	0.10	0.23	0.16	0.46
0.15	0.02	0.28	0.14		0.03	0.31	0.15		0.06	0.24	0.13	
0.20	0.01	0.30	0.11		0.02	0.34	0.12		0.04	0.24	0.11	
0.25	0.00	0.31	0.09		0.01	0.36	0.10		0.02	0.25	0.09	
0.30		0.33	0.08			0.39	0.09			0.25	0.07	

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

dam_g1= partial inbreeding coefficient of dam founder group 1 ; **dam_g2**= partial inbreeding coefficient of dam founder group 2; **dam_g3**= partial inbreeding coefficient of dam founder group 3; **dam_f222**= partial inbreeding coefficient of dam founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

4. Mortality risk of a litter at days 7, 30 and 90 with partial inbreeding coefficients of the dam.

Inbreeding coefficient	dam_g1_d7	dam_g2_d7	dam_g3_d7**	dam_f222_d7	dam_g1_d30	dam_g2_d30*	dam_g3_d30**	dam_f222_d30	dam_g1_d90	dam_g2_d90	dam_g3_d90	dam_f222_d90
0.00	0.28	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
0.05	0.18	0.34	0.22	0.51	0.22	0.37	0.23	0.35	0.24	0.35	0.24	0.29
0.10	0.11	0.40	0.17	0.73	0.16	0.45	0.18	0.41	0.20	0.41	0.21	0.29
0.15	0.06	0.46	0.13		0.11	0.53	0.14		0.16	0.48	0.18	
0.20	0.03	0.52	0.10		0.08	0.61	0.10		0.13	0.55	0.15	
0.25		0.58	0.08			0.69	0.08			0.62	0.12	

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

dam_g1= partial inbreeding coefficient of dam founder group 1 ; **dam_g2**= partial inbreeding coefficient of dam founder group 2; **dam_g3**= partial inbreeding coefficient of dam founder group 3; **dam_f222**= partial inbreeding coefficient of dam founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

APPENDIX 1E- PARTIAL ANCESTRAL INBREEDING

1. Mortality risk of a litter at days 7, 30 and 90 with partial ancestral inbreeding coefficients.

Inbreeding coefficient	g1_a_d7	g2_a_d7	g3_a_d7	fa_178_d7	fa_222_d7**	g1_a_d30	g2_a_d30**	g3_a_d30	fa_178_d30	fa_222_d30	g1_a_d90	g2_a_d90*	g3_a_d90	fa_178_d90	fa_222_d90
0.00	0.19	0.19	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.22	0.22	0.22	0.22	0.22
0.05	0.12	0.23	0.19	0.20	0.30	0.12	0.25	0.20	0.23	0.28	0.14	0.26	0.22	0.24	0.26
0.10	0.07	0.27	0.18	0.21	0.43	0.07	0.30	0.19	0.25	0.36	0.09	0.30	0.22	0.25	0.31
0.15	0.04	0.31	0.18		0.58	0.04	0.36	0.18		0.46	0.05	0.34	0.21		0.36
0.20		0.36	0.18				0.43	0.17				0.39	0.21		
0.25		0.41	0.17				0.49	0.17				0.44	0.21		
0.30		0.46	0.17				0.56	0.16				0.49	0.21		

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$; **g1_a**= partial ancestral inbreeding coefficient of founder group 1 ; **g2_a**= partial ancestral inbreeding coefficient of founder group 2; **g3_a**= partial inbreeding coefficient of founder group 3; **fa_178**= partial ancestral inbreeding coefficient of founder 178; **fa_222**= partial ancestral inbreeding coefficient of founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

2. Effects of different levels of partial ancestral inbreeding on litter size

Inbreeding coefficient	g1_a	g2_a*	g3_a	fa_178	fa_222
0.00	0.00	0.00	0.00	0.00	0.00
0.05	-0.07	-0.09	-0.03	-0.06	-0.01
0.10	-0.14	-0.19	-0.06	-0.11	-0.01
0.15	-0.21	-0.28	-0.08		-0.02
0.20		-0.38	-0.11		
0.25		-0.47	-0.14		
0.30		-0.57	-0.17		

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$ **g1_a**= partial ancestral inbreeding coefficient of founder group 1; **g2_a**= partial ancestral inbreeding coefficient of founder group 2; **g3_a**= partial inbreeding coefficient of founder group 3; **fa_178**= partial ancestral inbreeding coefficient of founder 178; **fa_222**= partial ancestral inbreeding coefficient of founder 222; **d7**= day 7; **d30**= day 30; and **d90**= day 90

APPENDIX 2 - MHORR: Inbreeding coefficients, mortality risk and effect on litter size

APPENDIX 2A – TOTAL INBREEDING

Mortality risk of an individual at days 7, 30 and 180 with total inbreeding coefficients of individual, sire and dam.

Inbreeding coefficient	f_d7	f_s_d7	f_d_d7	f_d30	f_s_d30**	f_d_d30	f_d180*	f_s_d180	f_d_d180
0.00	0.05	0.05	0.05	0.06	0.06	0.06	0.12	0.12	0.12
0.05	0.06	0.06	0.05	0.07	0.08	0.06	0.14	0.12	0.12
0.10	0.06	0.06	0.06	0.08	0.09	0.05	0.17	0.13	0.12
0.15	0.07	0.07	0.06	0.10	0.11	0.04	0.20	0.14	0.12
0.20	0.08	0.08	0.07	0.11	0.14	0.04	0.23	0.14	0.12
0.25	0.09	0.09	0.07	0.13	0.16	0.03	0.27	0.15	0.12
0.30	0.10	0.10	0.07	0.15	0.19	0.03	0.31	0.16	0.12
0.35	0.11	0.11	0.08	0.17	0.23	0.03	0.35	0.16	0.11
0.40	0.12	0.12	0.08	0.19	0.27	0.02	0.40	0.17	0.11
0.45	0.13	0.13	0.09	0.22	0.32	0.02	0.45	0.18	0.11
0.50	0.14	0.14	0.10	0.25	0.37	0.02	0.50	0.19	0.11

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f = Individual inbreeding coefficient; **f_s** = sire inbreeding coefficient; **f_d** = dam inbreeding coefficient; **d7** = day 7; **d30** = day 30; and **d180** = day 180

APPENDIX 2B – ‘OLD’ AND ‘NEW’ INBREEDING

1. Mortality risk of an individual at days 7, 30 and 180 with “old” and “new” inbreeding coefficient of the individual.

Inbreeding coefficient	f"old" _d7	f"new" _d7	f"old" _d30	f"new" _d30	f"old" _d180	f"new" _d180**
0.00	0.05	0.05	0.05	0.05	0.09	0.09
0.05	0.06	0.06	0.05	0.06	0.09	0.10
0.10	0.06	0.06	0.05	0.07	0.10	0.12
0.15	0.07	0.07	0.05	0.08	0.11	0.15
0.20	0.07	0.08	0.05	0.09	0.11	0.17
0.25	0.08	0.09	0.05	0.11	0.12	0.21
0.30	0.09	0.10	0.05	0.12	0.13	0.24
0.35	0.09	0.11	0.05	0.14	0.14	0.28
0.40	0.10	0.12	0.05	0.16	0.15	0.32

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old" = old inbreeding coefficient of the individual; f"new" = new inbreeding coefficient of the individual; d7= day 7; d30= day 30; and d180= day 180

2. Mortality risk of an individual at days 7, 30 and 180 with “old” and “new” inbreeding coefficient the sire.

Inbreeding coefficient	f"old" _sire_d7	f"new" _sire_d7	f"old" _sire_d30*	f"new" _sire_d30*	f"old" _sire_d180	f"new" _sire_d180
0.00	0.15	0.15	0.03	0.03	0.07	0.07
0.05	0.16	0.15	0.05	0.04	0.08	0.07
0.10	0.18	0.16	0.07	0.05	0.10	0.08
0.15	0.20	0.16	0.11	0.06	0.13	0.08
0.20	0.21	0.16	0.16	0.08	0.16	0.09
0.25	0.23	0.17	0.23	0.10	0.20	0.09
0.30	0.25	0.17	0.32	0.12	0.24	0.10
0.35		0.17		0.15		0.10

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

f"old"_sire= old inbreeding coefficient of the dam; f"new"_sire = new inbreeding coefficient of the dam; d7= day 7; d30= day 30; and d180= day 180

APPENDIX 2C – ANCESTRAL INBREEDING

Mortality risk of an individual at days 7, 30 and 180 with ancestral inbreeding coefficients of the individual.

Inbreeding coefficient	f_d7	ancestral f_d7*	f_d30	ancestral f_d30	f_d180	ancestral f_d180
0.00	0.06	0.06	0.07	0.07	0.12	0.12
0.05	0.06	0.06	0.08	0.08	0.14	0.13
0.10	0.06	0.07	0.09	0.09	0.17	0.13
0.15	0.06	0.08	0.09	0.10	0.19	0.14
0.20	0.07	0.09	0.10	0.10	0.22	0.14
0.25	0.07	0.11	0.11	0.11	0.25	0.15
0.30	0.07	0.12	0.12	0.12	0.28	0.15
0.35	0.08	0.14	0.13	0.13	0.32	0.16
0.40	0.08	0.16	0.14	0.15	0.36	0.17
0.45	0.08	0.18	0.15	0.16	0.40	0.17
0.50	0.09	0.20	0.16	0.17	0.44	0.18
0.55	0.09	0.22	0.17	0.19	0.48	0.18
0.60	0.09	0.25	0.19	0.20	0.53	0.19
0.65	0.10		0.20		0.57	

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

Ancestral f= ancestral inbreeding coefficient; **f**= total inbreeding coefficient; **d7**= day 7; **d30**= day 30; and **d180**= day 180

APPENDIX 2D – PARTIAL INBREEDING

Mortality risk of an individual at days 7, 30 and 180 with partial inbreeding coefficient of sire founder group 1.

Inbreeding coefficient	sire_g1_d7	sire_g1_d30**	sire_g1_d180
0.00	0.05	0.08	0.13
0.05	0.06	0.08	0.13
0.10	0.06	0.08	0.13
0.15	0.07	0.08	0.14
0.20	0.08	0.09	0.14
0.25	0.09	0.09	0.14
0.30	0.10	0.09	0.14
0.35	0.11	0.09	0.14
0.40	0.12	0.09	0.14
0.45	0.13	0.09	0.14
0.50	0.14	0.09	0.14

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

sire_g1= partial inbreeding coefficient of dam founder group **d7**= day 7; **d30**= day 30; and **d180**= day 180