

HETEROISIS IN THE SECOND AND THIRD GENERATION AFFECTS LITTER SIZE IN A CROSSBREED MINK (*NEOVISON VISON*) POPULATION

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Abstract – Litter sizes in a cross between Brown and Black mink color types were observed through six generations. Litter size was significantly affected by yearly environmental variations. After adjusting for year effects, we found significant increases in litter size in the second and third generations (F₂ and F₃) after crossing. Thereafter, in the following generations, litter size dropped to a level comparable to the mean litter size of the midparent. Increased litter size in F₂ compared to F₁ indicated that maternal effects influenced litter size more than non-maternal effects. The heterosis was mainly caused by an increase in litter size compared to the Black parental line. This indicates that the Black line was affected by inbreeding depression prior to crossing. We also found that two-year old F₁ females had significantly more offspring compared to one-year old F₁ females.

Key words: Inbreeding depression; maternal effect; non-maternal effects; environmental variation; color types.

INTRODUCTION

The positive effect of crossing breeds and lines has been well known for centuries as heterosis (Bruce, 1910; Crow, 1948; Shull, 1948; Chambers and Whatley, 1951; Donald et al., 1977; Crow, 1998), and the effect of heterosis has been explored and exploited in agriculture (Buchanan et al., 1990; Fairfull, 1990; VanRaden and Sanders, 2003) and in wildlife management (Holleley et al., 2011; Pickup, 2012).

Heterosis is described as the reverse of inbreeding, or restoration of inbreeding depression, and is explained as heterozygote superiority by the dominance hypothesis or by the overdominance hypothesis (Springer and Stupar, 2007; Charlesworth and Willis, 2009). In many species, it has been found that inbreeding reduces fecundity (Keller and Waller, 2002). For instance, litter size is reduced in mice (Holt et al., 2005), swine (Farkas et al., 2007) and mink (Berg, 1996; Demontis et al., 2011) due to

inbreeding. Inbreeding depression is a consequence of changes of genotype frequencies, which change toward a higher level of homozygosity in inbred populations compared to non-inbred populations and recessive detrimental alleles will have higher probability of expression in homozygotes. Reduction of the mean phenotypic value of the population is directly proportional to the inbreeding coefficient (Falconer and Macay, 1996) and Demontis et al., (2011) found a strong negative correlation between levels of relatedness between parents and litter size in mink.

Mink is a small carnivore of the Mustelidae family. It is domesticated and bred for the pelt which is used for clothing. Minks mature at one year of age and females are mated in the first 2-3 years of life. They have one litter per year and litter size is approximately 5-6 offspring per litter, however, in rare instances, it can be as high as 12 offspring. Mink breeders on mink farms often experience an increase in litter size after crossing two lines (of the same color) or two color types. Litter size is important for mink production as the number of produced skins has an impact on the economy of the mink breeders. In Denmark, some exchanges of mink between farms exist, but the main reason for the exchange is improvement of body size and fur quality. Hansen et al. (2008) and Nielsen (2006; 2008) previously demonstrated heterosis for litter size in crosses between color types in mink.

The magnitude of heterosis depends on the differences of the allele frequencies in the two populations that are crossed. The effect is greatest when the two populations are fixed for different alleles. The effect of heterosis can be observed in the first two generations (F_1 and F_2 generations), but because of Mendelian segregation the effect in the F_2 is expected to be half the effect in the F_1 (Falconer and Macay, 1996). Unraveling heterosis effect might be complicated by maternal effect (Norberg and Sørensen, 2007). In the first generation after crossing, the increase in the phenotypic value is due to heterosis in the offspring (non-maternal effect), whereas in the second generation, the increase in phenotypic value is due to het-

erosis in both mother and offspring (maternal and non-maternal effect).

The aim of this analysis was to investigate the level of heterosis for litter size in a crossbreed of two mink color types. Based on the theoretical background, we expected to observe heterosis in F_1 and F_2 . If the maternal effect is strong enough, we could expect to observe the greatest effect in F_2 due to both maternal and non-maternal effects. In the following generations, F_3 and onward, we expected to observe stagnation in litter size. For the analysis, two mink color types were crossed and the population was kept at a limited population size for an additional five generations. The two color types were Black short nap mink and Brown mink; these two types were chosen because of phenotypic differences, with the Black type having very small litter sizes while the Brown type having larger litter sizes.

The F_1 females were mated in two succeeding years. This allowed us to compare the number of offspring that was born from one- and two-year-old females. On mink farms, it happens that two-year-old females give birth to more offspring than one-year-olds. This is supported by Ślaska et al. (2009), who found that, at least for some color types, two-year-old females had more offspring in litters than one-year-old females.

MATERIALS AND METHODS

Populations

Brown mink were crossed with Black short nap mink in 2005. Two Brown mink color types (line 74 and line 75) were established and bred at the research farm of Aarhus University in Foulum prior to the experiment. Mink from the Black short nap color type (line 70) were bought from a private mink farmer for the purpose of the experiment. The two color types were chosen based on differences in body size and fur quality. It was assumed that because the two color types were phenotypically different they were also genotypically different. In total, 24 females and 5 males from the Brown color type and 25 females

and 7 males from the Black color type were selected. Brown female mink were mated to Black males and Black females were mated to Brown males. The selected mink for the parental generation were not related: neither sibs and nor mother/offspring were selected. In the following generations, it was attempted to minimize inbreeding, by avoiding mating between close relatives.

A limited number of mink from each generation (F₁-F₅) were mated the following years 2006-2010. F₁ females were mated in two succeeding years: females mated in 2006 were mated to the same males in 2007. As there were no differences in litter size between lines 74 and 75, the two Brown lines were merged into one line; there were also no differences in litter size if Brown females were mated to Black males or if Black females were mated to Brown males. The F₁ and following generations were hereafter characterized as line 71 (see a thorough explanation in Statistical Analysis).

A Brown production line (line 19) was used as a control line. This line was also established and bred at the research farm in Foulum. The population size was large and there was a continuous supply of males from other lines of the Brown color type, which reduced inbreeding in this line. Line 19 represented the overall productivity of the research farm in Foulum. Line 19 had not been involved in other research projects that could affect litter sizes in the years of this project. The number of mink in the two lines and in each generation is listed in Table 1.

Phenotypes

Litter size in the first census (number of offspring observed within the first 48 h after birth) for all females was registered through six generations (2005-2010) in line 71 and in line 19. Litter sizes in line 70 (Black short nap mink), lines 74 and 75 (Brown mink) was the litter size in which the mink was born. Barren females were not registered in these lines and therefore the barren percent for these lines was estimated from the barren percent in 2004 for Black and Brown females on the research farm in Foulum.

Statistical analysis

To analyze whether there was a difference in mean litter size between the two Brown lines (line 74 and line 75) and between mean litter size when mating Brown females (line 74 or line 75) to Black males (line 70) or mating Black females (line 70) to Brown males (line 75), we used a *t*-test.

In the ANOVA analysis, we used a mixed model with mating year and mink line as fixed effects and male identity as random effect. Male identity did not have significant effect (*F* = 0.363, *df* = 1, *p* = 0.55) and the linear model was used for the analysis:

$$Y_{kij} = \mu + l_i + p_j + l_i p_j + \epsilon_{ikj} \tag{1}$$

where *Y_{kij}* is litter size in the *kth* mink female in line *i* (*i* = 19 or 71) in breeding year *j* (*j* = 2004...2010), *μ* is mean value, *l_i* is fixed effect of line *i*, *p_j* is fixed effect of breeding year *j*, *l_ip_j* is interaction between breeding year *j* and line *i* and *ε_{ikj}* is random error. The effects of the parameters in the model were estimated using the R package, doBy version 4.5-3 (Højsgaard and Halekoh, 2012) and were used for estimating least square means of litter size for each year in lines 19 and 71.

Due to the significant effect of year (see results), an adjusted mean litter size was calculated for line 71. The effect of year, calculated from least square means in line 19 was used for adjustment in line 71:

$$\hat{y}_{71j} = 1/n_{71j} \sum_{k=1} (y_{71jk} + [y_{19,2004} - y_{19j}]) \tag{2}$$

where *ŷ_{71j}* is adjusted mean litter size in line 71 in year *j*, *n_{71j}* is number of mink in line 71 in year *j*, *y_{71jk}* is litter size in the *kth* female in line 71 in year *j*, and *y_{19,2004} - y_{19j}* is the effect of year *j* on litter size using line 19 in 2004 as reference.

Percentage of change in litter size using midparent litter size 2004 as reference was calculated as:

$$\text{Change \%} = 100 \cdot ((\hat{y}_{71j} - \hat{y}_{71,2004}) / \hat{y}_{71,2004}) \tag{3}$$

where *ŷ_{71j}* is adjusted mean litter size for line 71 in

Table 1. Least square means of litter size and standard errors (SE) of lines 71 and 19 as well as number of mink in the analyses in the years 2004–2010. Significant deviations of mean litter size from the mean litter size in year 2004 are shown.

Year	Line 71		Line 19	
	Number	Litter size (SE)	Number	Litter size (S.E.)
2004	71	5.90 (0.37)	484	7.23 (0.14)
2005	49	5.18 (0.45)	370	6.24 (0.16)***
2006	97	6.71 (0.32)*	287	6.91 (0.18)
2007	92	6.29 (0.33)**	63	5.60 (0.39)***
2008	18	7.56 (0.74)**	179	6.51 (0.23)**
2009	19	5.37 (0.72)	425	7.14 (0.15)
2010	21	4.52 (0.68)	743	6.14 (0.11)***

Levels of significance: * $p < 0.05$, $p < **0.01$, $p < ***0.001$

Table 2. Three-way ANOVA of the linear model (1). Litter size as response, mating year and line as well as interaction between mating year and line as fixed effects as explanatory variable. Degrees of freedom (df), sum of squares (Sum Sq), mean squares (Mean Sq), F -values and p -values are shown.

	Df	Sum Sq	Mean Sq	F	Pr (>F)
Mating year	6	623	103.81	10.59	1.17e-11***
Line	1	67	67.06	6.84	0.009**
Mating year*line	6	176	29.32	2.99	0.006**
Residuals	2899	28427	9.81		

Levels of significance: * $p < 0.05$, $p < **0.01$, $p < ***0.001$

Table 3. Adjusted litter size and standard errors (SE) in line 71, number of mink, percentage change in litter size with year 2004 as reference, t -values and p -values are shown.

Year / generation	Num.	Adjusted litter size (S.E.)	Change %	t -value	p -value
Brown ^a	31 ^b	6.75 (0.45)			
Black ^a	35 ^b	5.0 (0.36)			
2004 / midparent	61	5.90 (0.37)	0.00		
2005 / F ₁	49	6.18 (0.50)	4.64	-0.50	0.62
2006 / F ₂	97	7.03 (0.29)	19.15	-2.64	0.009**
2007 / F ₂	92	7.92 (0.24)	34.24	-5.28	3.97E-07***
2008 / F ₃	18	8.28 (0.46)	40.34	-3.66	0.0004***
2009 / F ₄	19	5.44 (0.68)	-7.80	0.67	0.54
2010 / F ₅	21	5.61 (0.65)	-4.92	0.43	0.67

^a: parental lines. ^b: Phantom females included.

Levels of significance: * $p < 0.05$, $p < **0.01$, $p < ***0.001$

year j and $\hat{y}_{71,2004}$ is mean liter size for line 71 in year 2004.

To test if there were significant changes from the reference year (2004), t -tests were performed.

To test if two-year-old females had more offspring compared to one-year-old females we used a t -test. F_1

females were mated in two succeeding years. In 2006, the females were one year old and in 2007 the females were two years old. Therefore, adjusted mean litter sizes from years 2006 and 2007 were used for the test. To test if there was a significant change in mean litter size in F_3 compared to F_2 , we used a t -test. Due to the effect of mother's age on litter size, we used one-year-old females in both generations, hence, the adjusted

mean litter size in years 2006 and 2008 were used for the test. The software package Past (Hammer et al., 2001) was used for all *t*-tests.

RESULTS

Barren percentages for Black and Brown females in 2004 were 10.9% and 6.7%, respectively. Three and two phantom females without offspring were therefore added to the parental populations. Mean litter sizes (and standard errors) in Brown and Black were 6.75 (0.45) and 5.0 (0.36) offspring respectively. Mean litter size of the two parental populations (midparent) was 5.9 (0.37) offspring (see Table 1 for least square means of litter size for the two lines). There was no difference between litter size in line 74 and in line 75 ($t = 0.46$, $p = 0.64$). There was no difference in litter sizes regardless of whether Brown females were mated to Black males or Black females were mated to Brown males ($t = 0.060$, $p = 0.55$). Offspring from the crossbreeding (F_1) and following generations (F_2 - F_5) were therefore regarded as the same line (line 71).

Litter size was significantly affected by breeding year, line and interaction between breeding year and line $F_{13, 2904} = 6.98$, $p < 0.001$ (see Table 2 for results of ANOVA). There were significantly fewer offspring in the midparent of line 71 in 2004 (reference year) compared to line 19 in 2004 ($t = 3.33$, $p < 0.001$) where the midparent of line 71 had 18.25% fewer offspring compared to line 19 (the control line). For line 19, there were significant deviations from the reference year in years 2005, 2007, 2008 and 2010, where the litter sizes were significantly smaller compared to the reference year. In line 71, least square means of litter size were significantly increased in the years 2006, 2007 and 2008 compared to the reference year. The least square means of litter size and standard errors (SE) of line 71 and line 19 are listed in Table 1. Significant deviations of mean litter size from the mean litter size in 2004 are shown.

The *t*-test of adjusted mean litter size in line 71 showed significant increase in the years 2006, 2007 and 2008 compared to midparent. Thereafter, the litter size fell to a level comparable to litter size of the

midparent. The improvements in percentage were 19.15, 34.24 and 40.34%, respectively. (See Table 3 for adjusted mean litter size in line 71 and changes in percent using year 2004 as reference). Increase in litter size in 2008 was not significantly different from year 2006 ($t = 1.18$, $p = 0.08$). There were significant differences in litter size between years 2006 and 2007 ($t = 2.36$, $p = 0.02$).

DISCUSSION

Litter size in a crossbreeding between two mink color types was analyzed for heterosis. We found an increase in litter size in F_2 and F_3 generations compared to the mean of the two parental color types, corresponding to midparent. In F_4 and F_5 , the litter size was in the same order of magnitude as the litter size of the midparent.

We found significant effect of line. Line 71, which was our study population, had in general less offspring per litter compared to the control line. The Brown control line (line 19) was a production line with a large population size. Furthermore, there was a continuous supply of males to the line from other lines of the same color type. We therefore assumed that this line did not suffer from an inbreeding depression that could affect litter size. Both the Black and the Brown color type used for our analysis had smaller litter size compared to this control line. In particular, the Black color type had very small litter size. Crossing the two color types resulted in a 23.6% increase in litter size in F_1 compared to the Black color type. This corresponded to 1.18 offspring. Compared to the Brown color type there was a decrease of 8.44% in the F_1 . Based on these results, it is likely that the Black color type suffered from inbreeding depression before crossing, while the Brown color type did not.

We also found large variations in mean litter size between years. The difference between the lowest mean number of offspring (5.6 in 2007) and the highest (7.23 in 2004) in the control line is a 22.5% difference. When we adjusted for this variation between years, we found significant heterosis effect in litter size in the F_2 and F_3 generations after crossing

our studied populations. We did not find significant increase in litter size in F_1 compared to midparent. Heterosis in F_1 is caused by non-maternal effect while the effect in F_2 is caused by both maternal and non-maternal effects. If the maternal and non-maternal effects were in the same order of magnitude, we would have seen effect in F_1 and one and a half of the effect of F_1 in F_2 . As we did not find a significant increase in litter size in F_1 , this might indicate that the non-maternal effect was of less importance and we might therefore assume that, at least for this population, the maternal effect was the most important cause for the observed increase of the mean litter size. The importance of maternal vs. non-maternal effects for litter size is not univocal (e.g. Southwood and Kennedy, 1990; Roehe and Kennedy, 1993), but estimating the effects in mink might be of importance for selection strategies. We expected litter size to stabilize at the level of F_2 in F_3 and in the following generations, but we found a tendency ($p = 0.08$) for increased litter size in F_3 compared to F_2 . This tendency to increased litter size in F_3 could be due to 1) environmental variation that was not grasped by the analysis and we therefore did not take it into account; 2) the very small population size in this generation and therefore large variation in the estimated value; 3) epistatic effect – inbred populations will, if mated at random, reach phenotypic and genotypic equilibrium after one generation. If however there is linkage disequilibrium between loci, an increase in phenotypic value might occur. To our knowledge heterosis effects in F_3 have not been observed in other organisms. Nielsen (2006; 2008) also found an increase in litter size in the third generation after crossing Black and Brown mink color types.

Litter size in F_4 and F_5 fell to a level comparable to midparent. The population sizes in F_3 and the following generations were very small and the population was therefore likely to become inbred. Due to inbreeding or genetic drift, the population might have lost genetic variation and therefore genotypic frequencies might have changed toward higher levels of homozygosity, so the decrease in litter size was probably caused by small population size in F_3 and succeeding generations. The estimated litter sizes

should be interpreted with some caution because of the small population sizes. Variations (SE) in the estimated litter sizes in these generations were very large compared to previous generations and accordingly there might be some uncertainty of the estimates.

The fact that we found significant difference in litter size between F_1 females having offspring as one-year-olds (in 2006) and as two-year-olds (2007), confirms what was previously found for other mink color types by Ślaska et al. (2009), and it is a result that is also often experienced by mink breeders.

We have shown that a cross between color types can increase considerably the mean litter size. Therefore, a breeding strategy focusing on the advantages (due to maternal and non-maternal effects) that can be obtained in the generations following a cross should be considered, especially in those crosses where the other characteristics of the pelt are not excessively altered. Clearly, given the relatively strong contribution of the maternal effects on mean litter size, a long-term breeding strategy should be considered. This breeding strategy should be focused on creating females that harbor the highest possible level of heterozygosity in their genomes. This can be obtained by crossing lines with the highest genetic distance between them, which are not necessarily lines of different colors. If information about the genetic distances between lines is not available, then another option will be to cross lines that show reduced mean litter size. Such a strategy could provide increased economic advantages that outweigh the additional costs associated with the detailed registrations of the numbers of litters born and/or a genetic screening of the lines.

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