The correlated effects of genetic selection for growth on reproductive fitness

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Declaration

I declare that this thesis was composed by myself and that the work contained therein is my own, except where explicitly stated otherwise in the text.

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Abstract

In many experiments of genetics of growth a correlated effect on reproductive fitness, mostly on litter size, has been observed. As selection experiments are usually done with restricted population size, a certain increase of inbreeding is inevitable. Inbreeding has in general a deleterious effect on the fitness of an individual and tends to depress the reproduction from the expected level.

This study addressed the question of effects of growth selection and inbreeding on reproductive fitness in the first parity of mice. A range of components was analysed in a unique set of inbred mouse lines derived from seven genetically high growth lines and four low growth lines, with the average weight at mating of high and low line females respectively 48g and 15g. The collection of 'replicated' growth lines allowed more general conclusions to be drawn about the effects that were investigated.

A correlated response of growth selection was observed on litter size. Surprisingly the litter size of the growth lines did not greatly decrease over the period of full-sib matings, but some other traits such as the number of infertile matings were suspected to be showing inbreeding depression. Therefore, an experiment was designed to estimate the correlated effects of growth selection on components of reproductive fitness. The effects of inbreeding were removed at a foetus level by crossing inbred lines and at parental level by producing two-way crosses.

Growth selection had a strong effect on the components of reproductive fitness, for example the ovulation rate was on average 17 ova in high and 9 ova in low lines. The regression of ovulation rate on body weight was consistent in different size of females and was not greatly affected by the inbreeding. However, the litter size did not have such a strong relationship with body weight due to positive regression between embryonic mortality and body weight. The heavier the females were, the lower was the survival rate from ovulated egg to foetus. Also lower pregnancy rates were observed among the heavy females, which would further reduce the expected litter size of heavy females.

The effects of growth selection on foetuses were confounded by the mating partner effect, i.e. mating pairs with very different size (up to six fold size differ-

ence). The mating partner effect had a negative impact on the ovulation rate of females, on the survival of foetuses and on the pregnancy rates which were up to 40% lower compared with matings between same size pairs.

Both the inbreeding of the foetus and of the dams had a negative effect on the reproductive performance, but the latter was more important. Inbreeding on foetuses mainly affected the losses after implantation and the inbreeding of mothers the losses before implantation and the pregnancy rate.

The reproductive fitness was highest among outbred animals. The medium size outbred females had higher offspring production than the average of extremes.

Chapter 1

General introduction

Growth is an economically important trait in farm animal production and has been widely studied for a long time. There is a particularly large number of mouse studies on the genetics of growth (Goodale, 1938; Roberts, 1965; Eisen, 1974; Barria and Bradford, 1981a; McCarthy and Roberts, 1989; Bünger et al., 2001b). It has a similar pattern across mammalian species (Eisen, 1974), which allows the results from mouse experiments to be applied in breeding of other mammals. Mice have often been used as model animals for studies of quantitative genetics, because they are relatively easy and cheap to maintain and because the generation interval is shorter than in large mammals making them suitable model animals. Body weight is a trait often under selection in mouse studies of growth and has been succesfully selected over very long periods of time (Bünger et al., 2001b). It is easily changed by selection due to its high heritability, usually around 40%, and it has rather predictable response to selection under standard environmental conditions.

In most of the growth selection studies a positive correlated response in litter size has been observed (Eisen, 1974; McCarthy, 1982). This is an important observation, since in farm animal production the reproduction should be maintained on a high level otherwise not much genetical improvement can be achieved. Litter size on its own has not been studied as widely as the aspects of growth rate. It is a trait with a low heritability, estimates ranging from 8% to 23% (Roberts, 1981), which is typical for traits related with fertility or viability. Some long-term selection experiments dedicated just to selection on litter size in mice have been reported (Eisen, 1978; Falconer, 1971), one of them lasting over one hundred generations and covering a range of issues related to litter size (Vangen, 1999).

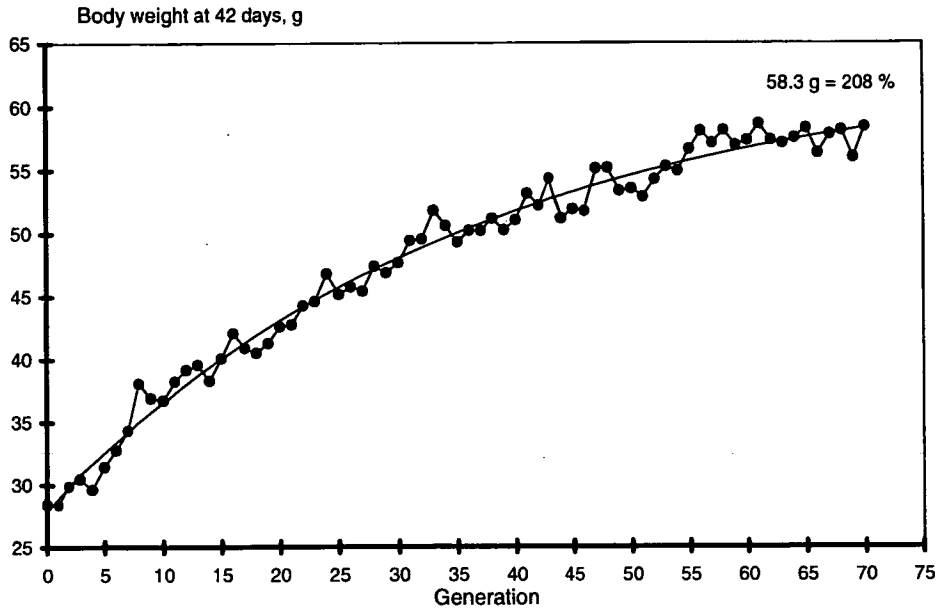
High positive genetic correlations have been observed between litter size and growth traits, for example 0.6 for six week body weight (Eisen, 1978), 0.3 for lean mass (Beniwal et al., 1992) and 0.9 for body weight gain (Rahnefeld et al., 1966). Hence, in general, large animals have large litters and small animals small litters. However, extremes in either direction tend to be less fit and therefore have a reduced fertility and also litters of extreme size tend to be less fit (Roberts, 1981). Genes influencing both the growth and reproductive traits has been found, for example gene for obesity (*ob*) and abnormal growth (*dwarf*) which both reduce the fertility (Austin and Short, 1985). However, animals homozygous for mutant gene *lit*, can be as light as 8g, but still be fertile (Bünger et al., 1998).

There is a positive genetic correlation between growth and litter size, however selection for growth can result in a response over many generations (figure 1.1), whereas the correlated effect on litter size usually levels off after few generations (figure 1.2). This indicates that the genetic correlation decreases during the selection experiment, i.e. the response to direct selection continues but the correlated response on litter size ceases. In a Dummerstorf mouse experiment the time to reach half of the maximum theoretical selection response (half life) for body weight was estimated to be 25 generations and for litter size just 3 generations (Bünger et al., 1993). To understand what could be behind these observations, the components of litter size and the whole reproductive performance must be considered first.

1.1 Reproductive fitness

Reproductive fitness can be defined as a total contribution of an individual to the gene pool (Falconer and Mackay, 1996). It can be divided into two main parts, the total production of the offspring and the quality of the offspring weaned (table 1.1). Because studies covering the life-time aspect would be very long, even in mice from two to three years, studies mainly concentrate on the first parity results. Thus also this study had to be restricted on the first parity results and particularly on the components of litter size. First a brief description of maternal performance and the total number of litters will be given and then a more detailed description of each of the components of litter size and the relationship between

Figure 1.1: The body weight in grams at 42 days of DUH-6 line over 70 generations (Bünger et al., 1993)

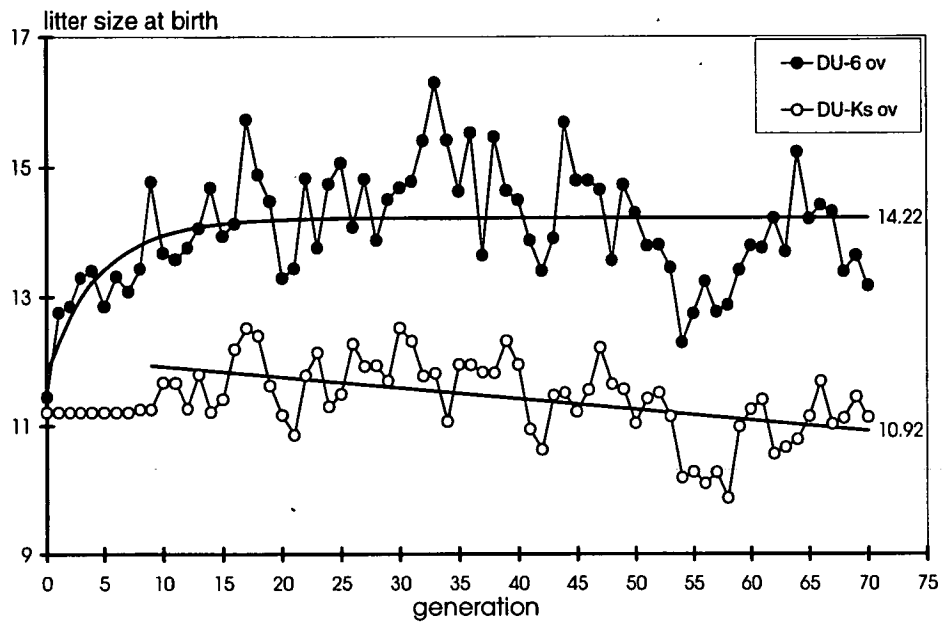


the components.

Apart from the total number of litters and litter sizes there are many external and internal factors affecting possibly all components of reproductive fitness (table 1.1). The most interesting factors for this study are growth, body composition and inbreeding, which will be discussed in more detailed below. Other factors like impaired health clearly have an effect on both growth and reproduction as well as stress (Nalbandov, 1976; Silver, 1995). Underfeeding has been shown to slow down the sexual and physiological development (Eisen, 1975a), to reduce ovulation rate and to increase the time to mating (Meyer and Bradford, 1974). There are several hormonal factors affecting the reproductive fitness at different stages (e.g. Snell, 1941).

Parental care: Maternal performance describes the ability of taking care of the pups and it affects the survival of the offspring until their own sexually active life. The milk yield of the mother increases with the number of pups born, but the increase is not proportional and when litters are very large the mother is not able

Figure 1.2: The litter size at birth in DUH-6 line and in DU-Ks control line over 70 generations with fitted lines



to provide enough milk for optimal growth and development for all pups (Silver, 1995). Animals from smaller litters grow faster than those from large litters, because of the better pre-weaning environment. Thus, there are two opposing factors, firstly the positive genetic regression between mothers and daughters in terms of litter size and secondly the negative environmental correlation (Falconer, 1960). Standardisation of the litter size after birth to a certain level has been shown to be an important factor for both body weight and consequently for future reproduction of the offspring (Eisen, 1978; Bünger et al., 1992). A daughter-dam regression for litter size that is close to zero, though negative, has been observed from litters which were not standardised after birth (Falconer, 1960). This shows that the genetical potential of the offspring to give birth to large litters has been suppressed by the maternal environment.

Total number of litters: The total number of offspring produced by a female over its life-time depends on components such as the number of litters and the litter sizes. The number of litters a female produces during its life-time depends

Table 1.1: The components of reproductive fitness (Bünger, 1987; Falconer and Mackay, 1996)

Total number of offspring born (fertility)	Total number of litters	Litter sizes
	sexual maturity	ovulation rate
	interval between litters	fertilisation rate
	length of reproductive life span	implantation rate
	frequency of infertile matings	embryonic mortality
	Other inner and environmental factors	
	growth, body composition	
	inbreeding, health status, stress	
	food, light, temperature	
Quality of offspring weaned	Offspring survival	
	parental care	
	milk yield	
	stress susceptibility	

on the age when she becomes sexually mature and the length of the reproductive life. Most female mice reach sexual maturity at an age of 7 weeks and males on average one week earlier (Silver, 1995) and the reproductive life of the female lasts on average until an age of 40 weeks (Roberts, 1961). In laboratory conditions the mice can survive up to three years, but the litter size tends to decrease already after five litters (Grüneberg, 1943; Silver, 1995). Therefore the parity seems to have a larger influence on the litter size than the maternal age itself.

The total output of the fertile life-time is affected by the interval between the litters, i.e. how fast the female recovers from the birth and is ready for a new pregnancy. According to Silver (1995) the time from being born to giving birth is on average ten weeks for mice. The usual cycle length in mice is from 4 to 6 days and the gestation length is 19 days, but less regular cycles and longer gestation periods in some selected mouse lines has been reported (Bradford, 1971). In mice the ovulation takes place independently of the copulation, spontaneously, and the mating occurs during a heat period which takes place a few hours after oestrus (Grüneberg, 1943; Snell, 1941). In outbred female mice the conception

rates are rather high, over 90% in general (Bradford, 1969). However, infertile matings have a large impact on an individual's reproductive fitness, e.g. Vangen (1999) reported a loss of lines after four parities due to large percentage of infertile females. A sterile copulation could induce a pseudopregnancy, which last about half of the normal pregnancy and thus delays the new pregnancy by extending the metaestrus phase in which the corpora lutea develops (Silver, 1995). In the mouse the copulation happens only once in a heat period, since after copulation a vaginal plug from coagulating sperm is formed in the female vagina. Vaginal plug prevents any further copulations, but it also a necessary mechanism for the early stages of pregnancy (Snell, 1941).

Litter sizes: Litter size is a product of many factors and furthermore complicated by the fact that it is partly affected by the parental generation and partly by the offspring generation. The main factors affecting litter size are the ovulation rate, fertilisation rate, implantation rate and embryo survival (table 1.1). A high genetic correlation has been found between litter size and ovulation rate, but selection on ovulation rate has not always produced correlated response in litter size, while the selection for embryo survival had an increasing effect on litter size (Bradford, 1969). The most effective way to improve litter size might be achieved from increasing the ovulation rate and simultaneously maintaining a low level of embryonic mortality. To further examine the factors affecting the changes in litter size some aspects of each one of the components will be discussed below.

Number of eggs released in ovulation can be counted by counting the corpora lutea in ovaries. Ovulation rate can be divided in two components as explained by Land and Falconer (1969), namely the activity of follicle stimulating hormone (FSH) and the sensitivity of ovary to the hormone. Selection on ovulation rate revealed a different pattern of these components, so that increase in ovulation rate increased the FSH activity and decrease in ovulation rate decreased the ovarian sensitivity. Land and Falconer (1969) did not find changes in sensitivity in the high ovulation rate line, but an increase in ovary weight and consequently in ovarian sensitivity has been reported in lines selected for high litter size (Durrant et al., 1980).

Ovulation rate can be said to set the limit to litter size, since in mice monozygotic twinning is very rare (Wan et al., 1982; McLaren et al., 1995). Litter size

and ovulation rate measure nearly the same trait, but at a different stage of the gestation. The estimates of the heritability for ovulation rate are similar but mostly higher to those for litter size ranging from 0.1 to 0.3 (Bradford, 1969; Land and Falconer, 1969; Nielsen et al., 1996). In some of the experiments the standard errors were large so that the estimates only hinted that ovulation rate could be a improved by selection. This has been shown in selection experiments in which ovulation rate clearly responded (Land and Falconer, 1969; Bradford, 1969; Land, 1970). Selection response in litter size has been mainly attributable to the changes in ovulation rate in species such as mice, pigs and rabbits (Perez-Enciso and Bidanel, 1997). However, ovulation rate selection has not consistently resulted in major changes in litter size (Land and Falconer, 1969; Perez-Enciso and Bidanel, 1997), which suggests a negative correlation between ovulation rate and pre-natal survival.

As pointed out above the ovulation rate equals the maximum potential litter size, but the other factors affecting the litter size actually determine the final outcome, i.e. the litter size. The eggs released from the ovaries have to meet the sperm at the fertilisation site at the upper end of the oviduct and be succesfully fertilised, in which process many eggs are lost. Except the ability of the sperm to travel to the fertilisation site, the process might be affected by the sperm quality, timing of mating or capacitation, which is the time sperm is required to be exposed to vaginal secretions before it is able to fertilise eggs (Nalbandov, 1976; Bateman, 1966). The success of penetration and conjugation could be due to the sperm or as well to the maturity of the egg. Fertilisation rate might not be related to the ovulation rate, thus it is not necessarily a limiting factor of the litter size in mice (Bowman and Roberts, 1958). This agrees with the results from livestock (cattle, sheep and pigs) in which the fertilisation rates are usually high, around 90 to 95% (Bazer et al., 1990).

Once the egg is fertilised it must implant to the uterus before it starts developing to an embryo. Around five days after fertilisation the embryos will float free in the reproduction tract, thus this pre-implantation period can be seriously affected by an external event (Silver, 1995; de A. Ribeiro et al., 1996). Such things in the environment of the mother like erratic lightning, extreme temperature or humidity, food and water supply and quality, noise or stress might cause a failure

in implantation of the embryos. The size of the female might have an impact on the pre-implantation losses, e.g. large mice tend to be more phlegmatic, i.e. more calm than small mice (Vangen, 1999). Thus, failure in implantation includes embryos with reduced viability in addition to the uterine environment provided by the mother.

Ribeiro et. al. (1996) found a positive phenotypic correlation between ovulation rate and the number of implantation sites. The distance between implantation sites in uterus is usually equal (Nalbandov, 1976) and in crowded uterus the space between embryos will become small which might affect the survival of the embryos. In species like pigs the fertilised eggs can migrate to the other uterine horn which might have more space available, but in mice migration from one horn to the other does not occur (Ribeiro et al., 1997).

A low implantation rate might already at this early stage reflect the uterine capacity. However, it has been shown that in mice both ovulation rate and body mass have a positive genetic correlation with uterine capacity (Nielsen et al., 1996), which would be expected since large animals tend to have larger internal organs. Thus, a large female is expected to have high ovulation rate, consequently a high number of implantation sites and survival of the embryos not limited by the uterine capacity. In mice the natural ovulation rate has been estimated to be lower than the uterine capacity and it would be possible to test the capacity only by using superovulation treatments (Ribeiro et al., 1997).

Some of the eggs attached to the uterine wall will not survive during the pregnancy, which further reduces the number of live foetuses at the end of the pregnancy. In livestock the losses decrease the offspring production on average by 25 to 50% (Bazer et al., 1990) and they could be a result of either zygotic factors or maternal factors, e.g. overcrowded uterus, or both of them. Losses happening before implantation or after implantation appear to change independently from each other, e.g. they are not physiologically linked (Bateman, 1966). A review of estimates of genetic correlations between ovulation rate and pre-natal survival in mice showed a range from -0.1 to -0.9, with heritability estimates for pre-natal survival being rather small, below 0.05 (Perez-Enciso and Bidanel, 1997). Also, Bradford (1969) observed that the estimates of genetic correlations were not consistent between lines selected for high ovulation rate and for high embryonic

survival. In terms of correlated response a similar pattern can be seen, since selection on litter size did not increase the embryonic mortality (Perez-Enciso and Bidanel, 1997), while increase in embryonic mortality was apparent in high ovulation rate lines (Bradford, 1969). Conclusions from the experimental results is that both ovulation rate and pre-natal survival are genetically controlled, but might still be genetically independent.

After the birth the litter size might be futher reduced, because female mice tend to destroy some of the pups which does not seem to be fit enough or maybe the litter size seems too large to maintain and the mother reduces the litter size size by killing some of the offspring (Silver, 1995). These losses are difficult to study, since mice give birth during the night and when the litter size is observed in the following morning there might not be any traces left of the destroyed pups. However, Falconer and Roberts (1960) observed only a small difference between number of live foetuses at end of pregnancy and number of pups born.

Male effect: The influence of the male fertility has not been considered so far. The importance of the male seems to vary from population to population and also greatly depends which aspect of the reproductive fitness has been studied. For example the ovulation rate can be seen more as a trait of the female alone, but fertilisation failure might be due to the male sperm or libido. However, Bateman (1966) did not report any male influence on losses before or after implantation, but Fowler and Edwards (1960) reported a reduced fertility in large mice due to the low libido or even sterility of the males.

The growth and development of the embryos or foetuses are affected by several hormonal factors and these might be influenced by the genotype of sire, but also dam or foetus itself (Bazer et al., 1990). Eisen (1977) observed that the male accounted for 6% to 13% of the variance in litter size. It can be concluded that the male clearly has an effect on the reproduction, but compared to the effect of the female or the offspring it is neglible.

1.1.1 The effects of growth and body composition

In general a positive correlation has been found between the body weight and litter size as discussed earlier. Because of this general observation, with very few exceptions (e.g. Bradford, 1971), a pleiotropic gene action has been suggested

to act between litter size and body weight. In a large line the increase in litter size was observed to be due to increased ovulation rate and the decrease of litter size in the low line due to increased losses either before or after implantation of eggs (Falconer, 1960; Falconer, 1971). The relationship between components of the reproductive fitness and growth selection will be considered more in detailed here.

Sexual maturity: Eisen (1975a) suggested that the time of the onset of puberty (vaginal opening) is close to the inflection point of the growth curve and pairing of sexually immature females with mature males produced an accelerated growth rate. Body weight selection might not affected greatly the age of puberty (Crane et al., 1972), but selection for slower gain has delayed the reproductive onset (Ernst et al., 1999). From a line selected for extremely slow gain between 28 and 56 days only 42% of the females actually reached oestrus, while the litter size of the fertile ones was not seriously affected (Ernst et al., 1999). The lines with greatest 56 day weight reached 80% of their mature weight before onset of puberty, but in slow gain females 91% of mature weight was required. However, the body weight at six weeks (42d) has a positive correlation with the body weight at the vaginal opening but negative correlation with the age (Eisen, 1978). The age at vaginal opening was similarly correlated with the litter size than with the body weight, which again suggested an accelerated development rate due to earlier vaginal opening. Mice selected for heavy body weight are expected to be heavier than control mice at the time of puberty and tend to produce large litters. Thus, the body weight at puberty seems more important than the age at puberty for the reproductive performance of the female.

Length of reproductive life span: Roberts (1961) compared the life time production of large and small mice and found that the small mice weaned nearly double the number of pups than did the large females. A line of large mice was reported to have a very low incidence (below 25%) of second litters and hardly any litters after the second litter (Barria and Bradford, 1981b). The high growth rate and high lifetime reproduction seem to be opposing each other. The first parity results are positively correlated with the mothers body size, but in later parities large size is not advantageous.

Frequency of infertile matings: Large mice tend to have larger litters than

the small mice, but large size is connected with reproductive difficulties (Roberts, 1981; Siedwert et al., 2000). As low pregnancy percentage as 42% has been observed in mice selected for rapid gain compared with the high pregnancy rate of the control line (Bradford, 1971). The poor fitness in the heavy line was observed to be mainly due to the extreme size rather than accumulated inbreeding (Barria and Bradford, 1981b). On the other hand, small mice have been observed to have impaired oestrus cycle and therefore lower mating performance (Fowler and Edwards, 1960). Also Falconer (1960) observed that small mice had somewhat higher percentage of failures to produce litters. The reason for the reduced fertility in small mice could have been affected by changes in hormonal function, which would cause an ovulation failure, longer oestrus cycle and possibly have an effect on the implantation rate.

Ovulation rate: Ovulation rate shows more consistent genetic relationship with body weight than does litter size. In studies where the correlated response in litter size moved in the same direction than body weight, the reason has been found to be due to the changes in ovulation rate (Fowler and Edwards, 1960; Land and Falconer, 1969). If a gene contributes to a large body size, it is likely that also the internal organs get larger, e.g. ovaries (Roberts, 1978). Also more hormones are likely to be produced and together with large ovaries, the result could be a larger ovulation rate. This is supported by results of positive genetic correlation between body weight and the two components, FSH activity and ovarian sensitivity, of ovulation rate (Land, 1970). The estimates of genetic correlations between body weight and ovulation rate vary from 0.4 (Land, 1970) to 0.8 (Nielsen et al., 1996).

The regression of corpora lutea on body weight was estimated to be 0.24 ova/g (Falconer and Roberts, 1960), which stresses the fact that in studies of ovulation rate the body weight differences should be taken into account. For other mammals, e.g. pigs, body weight has not been found to have as large correlation with ovulation rate as in mice (King and Young, 1957).

Embryonic mortality: The losses at later stage of pregnancy are due to the genotype and phenotype of the mother as well as the offspring. In large mice the foetuses are significantly heavier from day 10 onwards than in small mice, and in crosses between large and small mice the foetuses of large mothers are

larger (Güneren et al., 1996). However, the maternal genotype accounted for only one third of the difference in foetal body weight between the reciprocal crosses. In some cases the large mice have had problems to carry the pregnancy to full term, so that the large ones had the most losses occurring after implantation (Fowler and Edwards, 1960), which might reflect the harmful combination of large size of the foetuses and limited uterine capacity.

Body composition: Large mice can eat more than small mice, but if the food is not used efficiently it can lead to excess of fat. Most of the large mice have been reported to increase their fatness at older age when no energy is needed for the growth (Roberts, 1978; Roberts, 1981; McCarthy and Roberts, 1989).

In many of the growth selection experiments lines have been completely lost due to infertility and in some cases the difficulties in reproduction might be due to the fatness of the animals instead of the inbreeding effects (Roberts, 1981). Roberts reported studies of a line which was saved, because matings were done before the animals became fat. The infertility problem is also seen in other extremely fat mice, like the homozygotes for obesity (*ob*) gene (Holness et al., 1999). The testis size of the male has been used a a measure of fertility and was observed to be smaller in fat males, which could be due to the negative relationship between male hormones (testosterone) and fat content (Hastings et al., 1991).

No difference in litter size was found in lines divergently selected for fatness, the lines did not differ in the fat free body weight, but difference in absolute fat was large (Brien et al., 1984; Martinez et al., 2000). Since the body weights remained the same, it would also be expected that the ovulation rate was the same between high and low fat lines. The lines selected for high fat showed lower reproductive rate than the low fat lines, possibly due to lower pregnancy rate. Hastings et. al. (1991) found no difference in the ovulation rates between the high or low females. But suprisingly the fat line was associated with lowest number of post-implantation losses and thus had the higher pre-natal survival rate (Hastings et al., 1991). Possibly some excess fat could be utilised as an extra energy resource required during the pregnancy and hence increases the prenatal survival of the fatter ones.

1.1.2 The effect of inbreeding

Selection experiments are often done using a small population size, in which case a depression in traits might arise from the effect of accumulating inbreeding. In general inbreeding has a harmful effect on traits related with fitness, but not so much on those related with growth (Falconer and Mackay, 1996). Crossing of lines produces offspring with better mean value for those traits most affected by inbreeding, i.e. the traits show heterosis which is like reversed inbreeding depression (Falconer and Mackay, 1996). A large positive heterosis for litter size of mice (90%) and small positive heterosis for body weight (5%) has been observed (Mohamed et al., 2001). Some of the positive heterosis could be due to the changes in pre-natal survival (Hastings et al., 1991). The fertility traits like embryonic mortality might be controlled by rare recessive genes and the heterozygotes are mainly the most fit, while the growth traits and also ovulation rate have expressed additive gene action (Falconer, 1960; Falconer and Mackay, 1996). Thus, when selection on growth is practised, the fitness, e.g. survival of embryos, could be expected to change (Roberts, 1978; Latter and Robertson, 1962).

It has been estimated that the litter size of mice is reduced by 0.5 pups for 10% increase in inbreeding coefficient (Falconer, 1960). The reduction was mainly attributable to the fertility of the mother (60%), but the viability of the litter has also a effect (40%). The litters tend to be more inbred than the parents in experiments with increasing levels of inbreeding. However, the inbreeding of mother has such an influence that heterozygosity of the offspring is not enough to increase the litter size (Roberts, 1960). In Roberts (1960) experiment an increase of inbreeding coefficient of the offspring reduced the litter size by 1.4 pups and when inbreeding was introduced to the mothers further decrease of a pup was observed. But only the removal of the effects of inbreeding from the mother recovered the litter size to the original level. The inbreeding of the male has very little influence on the litter size (Falconer, 1971; Falconer, 1960) or on its components (Falconer and Roberts, 1960).

Total number of litters: According to Silver (1995) outbred female mice can remain fertile up to 18 months of age and bring up around 10 litters, but the

inbred mice have greatly reduced fertility already at age of 8-10 months. The total litter production of inbred female mice is much less than that of outbred females and the number of infertile matings tends to increase due to accumulated inbreeding. A regression of percentage infertile matings on generation number was estimated to be 0.2 (Siedwert et al., 2000).

In many selection experiments lines have been lost due to the inbreeding depression, e.g. when inbreeding coefficient reached 76% only three lines out of twenty survived (Falconer, 1960). But the loss of lines had an effect on the mean litter size by increasing it, since the surviving lines had the highest litter sizes. Despite the general negative inbreeding effect on the reproduction, some reports have been published of nearly unaffected fertility of females and viability of offspring over long-term inbreeding scheme (Bünger et al., 2001a). This could be explained by the loss of the families with increased infertility of the animals.

Ovulation rate: Comparison of ovulation rates made between inbred and not inbred female mice showed that there was no difference in ovulation rate after the correction for body weight (Falconer and Roberts, 1960). In case that body weight is affected by inbreeding, the ovulation rate would probably decline as well. However, in pigs the ovulation rate has been reported to suffer from inbreeding and it has been the cause for lower litter size rather than the increased losses (King and Young, 1957).

Embryonic mortality: Inbreeding tends to reduce the number of implanted eggs and also the survival of the embryos or foetuses in mice (Falconer and Roberts, 1960). The inbreeding of the mother had a smaller effect on the losses occurring after the implantation (Falconer, 1960). The losses which happen before the implantation seem to be more of a trait of the mother than the offspring. It could be assumed that inbred females might have more abnormal eggs than outbred, but no difference has been found between inbred and outbred females (Braden, 1957). The more likely explanation according to Falconer and Roberts (1960) is the impaired hormonal function which affect the implantation of the eggs to the uterine wall. The male sperm quality or reduced libido could be another reason for lower fertilisation rate. However, the inbreeding of the male seems to be small since only a minor difference between the losses before implantation of inbred or not inbred males was found (Falconer and Roberts, 1960).

After implantation the genotype or inbreeding of the embryo affects the situation additionally to the uterine environment provided by the mother.

1.2 The aims of this study

The aim of this study was to measure the correlated effects of growth selection on reproductive fitness. As the reproductive fitness of growth selected mice is also affected by the inbreeding an attempt was made to remove the effects of inbreeding in two steps: removal of the effects of inbreeding on foetuses and on the parents.

Most of the studies discussed concentrate either on growth selection or inbreeding, but only few papers have been published which examine them simultaneously (Falconer, 1971; Al-Murrani and Roberts, 1974; Bünger et al., 2001a). This study aims to examine both the effect of selection and inbreeding on reproductive fitness of mice. The reproductive fitness is studied in the first parity only using information from a range of components.

The objective of Chapter 2 was to elucidate the correlated effects of growth selection on litter size in a unique set of inbred mouse lines. In the light of these results an experiment was designed (Chapter 3) to estimate the effects of growth selection and inbreeding on the components of reproductive fitness of female mice.

The aim of Chapter 4 was to estimate the effects of growth selection on reproductive fitness in inbred mouse lines. The aim of Chapter 5 was to examine whether the components of reproductive performance of inbred mice are affected by the effects of the inbreeding and selection of foetus. The aim of Chapter 6 was to estimate the effects of growth selection on reproductive fitness on crossbred mice. Furthermore the question was addressed if the removal of the effects of inbreeding increased the reproductive performance and also if the fitness would be different between outbred medium sized mice and outbred mice with extreme sizes. To summarize, this study gives a wide picture of reproductive fitness from extreme sized highly inbred mice and ending to a non-inbred average sized mouse.

Chapter 2

Litter size as a correlated trait in inbred mouse lines

2.1 Introduction

Laboratory mice have been widely used as models for animal breeding, especially for growth (McCarthy, 1982; Bünger et al., 2001b). Body weight has been found to be a highly heritable trait and continuous response to selection has been achieved in very long term selection experiments with mice. For example one of the longest known mouse selection experiments, in Dummerstorf Germany, has been running since 1975 and has achieved a 115% increase in body weight at 42 days during 92 generations (Bünger et al., 1998). A common observation in growth selection experiments is the correlated effect on litter size, which tends to change in the same direction as body weight (Fowler and Edwards, 1960). A positive genetic correlation of 0.6 has been estimated between litter size and body weight at 6 weeks (Eisen, 1978).

In long-term experiments with small population size accumulation of inbreeding cannot be avoided. Traits such as body weight usually do not show severe inbreeding depression, but traits related with fitness do (Eisen, 1978; Roberts, 1981; McCarthy, 1982; Falconer and Mackay, 1996). For example, reduction in the litter size was estimated to be 0.5 pup per 10% increase of inbreeding coefficient (Bowman and Falconer, 1959). In the absence of inbreeding, the litter size is expected to increase with increasing body weight, but the harmful effects of inbreeding tend to reduce the litter size from the expected level.

The objective of this chapter is to elucidate the correlated effects of growth

selection on litter size in a unique set of inbred lines derived from growth selection experiments. The inbred mouse lines were all subjected to a similar selection and mating scheme during the experiment, but they had a different background history. Thus they provided a unique set of 'replicates', composed of seven heavy lines, four small lines and an unselected outbred control line. During the experiment the animals were subjected to a rapid inbreeding scheme, thus the reproductive performance was expected to suffer. The effect of inbreeding on reproduction of lines diverging in growth was assessed by studying the litter size over different time periods depending on the level of inbreeding. It was also of interest to examine the differences in litter size between females from control, high and low body weight lines over the inbreeding period.

2.2 Material and Methods

An experiment was set up during 1996 with the aim to provide a resource of highly diverged growth lines for future mapping studies (for a detailed description see Bünger et al. 2001a). In this resource experiment sets of mouse lines from several countries and with different selection backgrounds (table 2.1) were imported to the Edinburgh mouse laboratory and were subsequently subjected to rapid inbreeding over several generations. The selection history of the lines differed in terms of base population, selection trait, population size and length of the selection experiment. An unselected line was used as a control, which represented a normal sized outbred mouse and to which results from the selection lines were reflected. The history of the control line and eleven selection lines prior to the resource experiment is described below.

2.2.1 Mouse lines

Control, EDC: An outbred unselected line was kept as a control for this experiment. The control line was derived from the same base population as the Edinburgh selection lines (EDH and EDL), i.e. a cross of two inbred and one outbred lines (Hastings et al., 1997). Thus the line was a "true" control for the Edinburgh lines only and then only partially so because of the inbreeding of the selection lines. In divergently selected line pairs the high and low lines acted as

was started from crossing four inbred and four outbred lines. Full sib groups were selected on basis of their 42 day body weight and in each generation 80 pairs were mated. From generation 85, animals (32 females and 17 males) were brought to the Edinburgh mouse laboratory.

RAH: The mouse line was originally created in Raleigh, North Carolina, from an outbred base population. Initially within full-sib family selection was practised on weight gain from 21 to 42 days (Eisen, 1975b). After 23 generations selection was relaxed and later one male and one female were selected per full-sib family with 16-20 families. The mice (28 females and 27 males) were taken from generation 128 to Edinburgh mouse laboratory.

EDH and EDL: Edinburgh high and low lines were derived from the same base population as the control line (EDC, see above). The lines were divergently selected for 20 generations for predicted total lean mass of males based on an index and three replicates of each line was used with 16 full-sib families in each replicate (Sharp et al., 1984). Afterwards the replicates within selection direction were crossed and selection on 10 week body weight of both sexes continued until generation 46. Inbred lines were derived from this generation by full-sib matings (Bünger and Hill, 1999). Due to poor fertility new inbred lines of the high line were derived from generation 51.

The Edinburgh selection lines were more inbred than the other mouse lines into the experiment, since they had been on full-sib mating scheme prior to the present experiment. The EDH line animals were taken to the resource experiment after 9 generations of full-sib mating and the EDL after 13 generations. Bünger et al. (2001) estimated that in generation 14 of the resource experiment the inbreeding coefficient in the high line (EDH) was 0.989 and in the low line (EDL) 0.995.

BEH and BEL: The Berlin mouse lines started from a population of mice bought from pet shops. They first were divergently selected for protein amount in the body at 60 days using sib selection with 40 to 50 families per generation (Weniger et al., 1974). After that, another set of lines, derived from the original selection lines, was selected on an index based on body weight at 60 days and fat content with 40 pairs mated per each line. Animals were taken to the Edinburgh laboratory from generation 64. The BEH line founders (30 females

and 15 males) were selected for high body weight at 42 days and the founders for BEL (30 females and 30 males) from a line selected for low body weight.

MUH and MUL: The base population for the Munich lines was a cross of four inbred lines. After two generations of random mating the population was divided into eight selection groups divergently selected for body weight at 56 days and eight pairs were mated within each group (von Butler et al., 1984). The mice were taken to the Edinburgh mouse laboratory from generation 61 (8 males and 6 females for MUH and 52 females and 48 males for MUL).

ROH and ROL: The mouse lines were started in the Roslin institute in Edinburgh from a cross between two inbred lines which were obtained from Jackson laboratory USA, in 1985 (Heath et al., 1995). Lines were divergently selected for 42 day body weight using within family selection with six replicates for each selection direction and eight pairs mated per replicate. For each line 50 females and males were imported to the Edinburgh mouse laboratory.

The sudden increase of the litter size in ROH line (e.g. table 2.2) was unusual and unexpected. The possibility that the line was contaminated with another line around generation 13 could not be ruled out. Another reason for the observed pattern might have been a new mutation in the line. Unfortunately it was not possible to tell what was the reason behind the sudden increase of the litter size. The results for the line are shown nevertheless in order to make an attempt to understand the change in litter size.

2.2.2 Experimental procedure

The first animals from the above described mouse lines created generation 0 of the resource experiment and their offspring were used as donor females and males (gen 1) for embryo transfer (Bünger et al., 2001a). Since some of the animals in generation 3 were still born from embryo transfer, that generation was not included in the data analysis. Figure 2.1 describes the history of the selection lines apart from the Edinburgh lines. The EDC was kept as an outbred line and no selection on body weight was practised, and in the selection lines, EDH and EDL, full-sib mating scheme had been practised already before the beginning of the resource experiment.

After the line establishment (from gen 4 onwards, figure 2.1) a moderate within

family selection on 70 day body weight was practised to counterbalance the effect of relaxed selection (gen 0-4) and to maintain the body weight at previous level. In generation 8 a brother-sister mating scheme was started. Several sublines were kept within the selection lines to avoid loss of lines due to reduced fertility caused by rapid inbreeding. Bünger et al. (2001a) estimated that the inbreeding coefficient in generation 14 was on average 78.5% in the selection lines, but higher in Edinburgh lines.

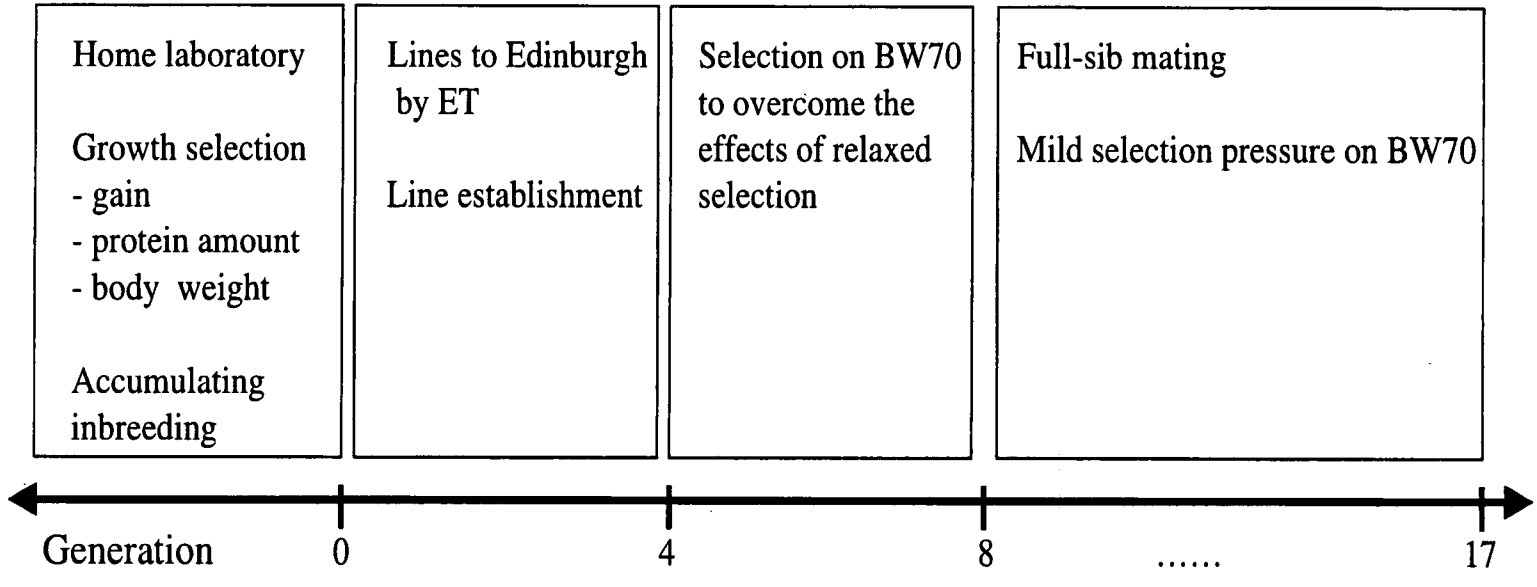
The last generation included in the data analysis was generation number 17. The animals in generation 4 were born at the beginning of 1997 and the animals in generation 17 were born at the end of 2000.

2.2.3 General management

Mice were fed on a standard expanded breeding diet from weaning onwards (Rat and Mouse No 3, Special Diet Services, Witham, Essex, UK). The lightning system was 12/12 hours. The temperature was kept at 21° C ($\pm 1°C$).

The matings were made at an age of 12 weeks using either single mating or harem matings. The mated pairs were kept in M3 cages (internal size 490cm^2 x 12cm, Kent Plastics Ltd). The litters were adjusted to a maximum of 12 pups and were weaned at 21 days. After weaning the same sex offspring of one or several dams, depending on the litter size, were housed in plastic MB1 cages (internal size 960cm^2 x 12cm, Kents Plastic Ltd). For the selection and control lines both female and male body weights were routinely taken at 42 and 70 days of age.

Figure 2.1: The history of the selection lines



2.2.4 Data analysis

The body weight at 70 days (BW70) and litter size at birth (LS) were studied from generation 4 to generation 17. Development of the traits over the generations was analysed by least square and by regression analysis. The least square means and regression coefficients were calculated with the GLM prodecure (SAS, 1996). The model for the least square means of 70 day body weight (BW70) was

$$\text{BW70} = \text{mean} + \text{line}_i + \text{gen}_j + \text{sex}_k + \text{litter} + \text{line*gen} + \text{line*sex} + \text{gen*sex} + \text{line*gen*sex} + \text{error}$$

where line (i=1...12) was the mouse line, gen was the generation (j=4...17), sex (k=1,2) was the sex of the offspring in generation j. Litter was the common environmental effect, i.e. the litter within line and generation. All two-way and three-way interaction terms between generation, line and sex were included. In most of the analyses the significance of the interaction terms was low, but they were kept in the models when possible, i.e. no non-estimable effects arose.

The litter size at birth was considered as the trait of the mother. The model for litter size did not include an effect for the litter, since it was not regarded as substantial. The model for litter size (LS) in the least square analysis is shown below, where the terms are as in the BW70 model.

$$\text{LS} = \text{mean} + \text{line}_i + \text{gen}_j + \text{line*gen} + \text{error}$$

To examine the changes in the traits during inbreeding regression coefficients of body weight at 70 days and litter size on generation number were estimated. The regressions were estimated with the GLM procedure (SAS, 1996). Models used in the regression analysis were

$$\text{BW70} = \text{mean} + \text{line}_i + \text{sex}_k + \text{line*sex} + \beta * \text{gen} + \text{error}$$

$$\text{LS} = \text{mean} + \text{line}_i + \beta * \text{gen} + \text{error}$$

Regressions were calculated for all groups and all lines for the whole duration of the experiment and also for three different time periods, the first for the time with minimal inbreeding (gen 4-7), the second for the beginning of full-sib mating period (generations 8-11) and for the third for the time with continuos full-sib mating (gen 12-17). In generation eight brother-sister mating was practised for the first time, so the inbreeding coefficient started increasing rapidly. Generation

eleven was the last included in the initial period of rapid inbreeding, and chosen as a cut off point, because initially non-inbred parents would have reached an inbreeding coefficient of 50 percent and after that the increase of inbreeding slows down (Falconer and Mackay, 1996).

2.3 Results

Body weight and litter size averaged over generations 4-17: The mean body weight averaged over sexes and generations (4-17) in the control line was 29.5g, in the high lines 50.2g and in the low lines 17.3g (table 2.2). The differences between the mean body weights of the lines were large, for example the heaviest lines was 4.6 fold heavier than the smallest line. There was large variation in body weights among the high lines, with a range of average body weight from 38g to 70g, while the low body weight lines formed a more homogeneous group. Among the high body weight lines there were three lines with significantly higher body weight than any other high lines. Unfortunately these extreme lines had no low body weight line pair, unlike the rest of the high lines. In the divergent line pairs derived from the same base population the average difference in body weight between the high and low lines ranged from 21g to 34g (table 2.3). The Edinburgh high and low selection lines each diverged from the control to a similar extent by on average 14g.

The litter size averaged over generations (4-17) was highest in the control line, being on average 0.8 pups higher than in the high lines and on average 4.3 pups higher than in the low lines (table 2.2). Two of the extreme lines (DUH and RAH) had on average 1 pup more than the control line. The divergent line pairs had a difference in litter size from 1.3 pups to 3.8 pups and EDH and EDL had 1.4 and 5.2 pups respectively less than the control (table 2.3).

Table 2.2: Least square mean of body weight at 70 days averaged over sexes and litter size for overall time (gen 4-17), slow inbreeding period (gen 4-7), start of rapid inbreeding (gen 8-11) and continuation of rapid inbreeding (gen 12-17). Standard errors are in brackets.

	Body weight (g) at 70d				Litter size at birth			
line	4-17	4-7	8-11	12-17	4-17	4-7	8-11	12-17
Control	29.48	28.41	29.32	30.04	10.24	10.03	9.99	10.70
	(0.14)	(0.28)	(0.20)	(0.17)	(0.19)	(0.37)	(0.32)	(0.27)
High	50.16	49.52	48.73	51.97	9.43	9.58	9.47	9.24
	(0.12)	(0.21)	(0.21)	(0.18)	(0.08)	(0.14)	(0.15)	(0.12)
Low	17.28	18.20	16.99	16.89	5.96	6.37	5.69	5.81
	(0.18)	(0.29)	(0.36)	(0.27)	(0.10)	(0.18)	(0.18)	(0.13)
DAH	58.37	60.58	55.37	58.89	9.08	8.70	10.24	8.57
	(0.14)	(0.36)	(0.29)	(0.28)	(0.20)	(0.38)	(0.37)	(0.30)
DUH	69.55	66.78	66.82	73.63	11.22	12.50	10.97	10.74
	(0.14)	(0.27)	(0.28)	(0.27)	(0.22)	(0.34)	(0.44)	(0.29)
RAH	56.31	53.41	54.48	60.69	11.39	11.27	11.67	11.59
	(0.13)	(0.29)	(0.26)	(0.25)	(0.20)	(0.31)	(0.31)	(0.31)
EDH	43.00	43.11	42.03	42.98	8.85	8.75	9.13	8.70
	(0.14)	(0.28)	(0.29)	(0.28)	(0.18)	(0.36)	(0.35)	(0.25)
BEH	53.03	51.96	53.26	53.50	8.31	8.90	8.06	8.27
	(0.18)	(0.39)	(0.36)	(0.33)	(0.20)	(0.31)	(0.37)	(0.32)
MUH	39.94	40.15	39.01	39.62	7.57	7.17	8.31	7.19
	(0.15)	(0.35)	(0.30)	(0.27)	(0.18)	(0.35)	(0.34)	(0.26)
ROH	38.05	36.87	35.85	42.28	9.55	9.24	8.56	10.40
	(0.12)	(0.23)	(0.33)	(0.22)	(0.18)	(0.31)	(0.37)	(0.27)
EDL	15.21	15.13	14.12	15.55	5.03	5.21	4.96	4.86
	(0.17)	(0.35)	(0.45)	(0.33)	(0.16)	(0.34)	(0.31)	(0.22)
BEL	18.59	20.32	18.68	17.19	7.02	7.61	7.05	6.63
	(0.21)	(0.29)	(0.35)	(0.26)	(0.17)	(0.33)	(0.34)	(0.25)
MUL	17.64	18.89	17.48	16.99	5.85	5.73	5.65	6.16
	(0.14)	(0.31)	(0.37)	(0.28)	(0.17)	(0.33)	(0.33)	(0.24)
ROL	17.25	18.04	16.61	17.50	6.04	6.90	5.32	5.91
	(0.15)	(0.31)	(0.44)	(0.31)	(0.17)	(0.33)	(0.33)	(0.24)

Models:

BW70 = mean + $\text{line}_{i=1...12}$ + $\text{sex}_{j=1...2}$ + $\text{gen}_{k=4...17}$
+ litter-effect + all interactions (line, sex, gen) + error

LS = mean + $\text{line}_{i=1...12}$ + $\text{gen}_{k=4...17}$ + line*gen + error

Body weight and litter size development from gen 4 to 17: The development of body weight in all lines is shown in figure 2.2. In the control line the body weight at seventy days increased by 2g during the period from generation 4 to 17 (table 2.4). The selection on body weight in the other lines was practised only to maintain their body weight and therefore it was rather mild. However, in both directions the regression of body weight on generation number was significant over generations, being 0.4 g/gen in the high lines and -0.1 in the low lines. Five out of seven high lines had a significant positive regression of body weight on generation number and two out of four low lines had a significant negative regression.

Table 2.3: The mean divergence of body weight and litter size over generations 4-17 between high and low lines pairs. Standard errors in brackets.

Divergence	BW70, g	LS
EDH - EDL	27.80	3.82
	(0.22)	(0.24)
EDH - EDC	13.52	-1.39
	(0.20)	(0.26)
EDL - EDC	-14.27	-5.21
	(0.22)	(0.25)
BEH - BEL	34.44	1.29
	(0.28)	(0.26)
MUH - MUL	22.31	1.72
	(0.21)	(0.25)
ROH - ROL	20.81	3.51
	(0.19)	(0.25)

The litter size over the generations had a less clear picture than the body weight (figure 2.3). The litter size of the control line had a positive trend most likely due to increased body weight, but the regression on generation number was not significant (table 2.4). Some of the heaviest lines outperformed the control line, but not in all generations due to small sample size per generation and consequent erratic behaviour of the average litter size. In general the low lines had the lowest litter size. In both selection directions the regression of litter size on generation number was negative. The extemely large DUH line had a reduction of nearly 0.2 pups per generation while the regression of body weight was 0.7g per generation, thus it seemed to suffer most from the inbreeding depression.

Figure 2.2: Least square means of body weight at 70 days (g) for all lines averaged over sexes over the generations

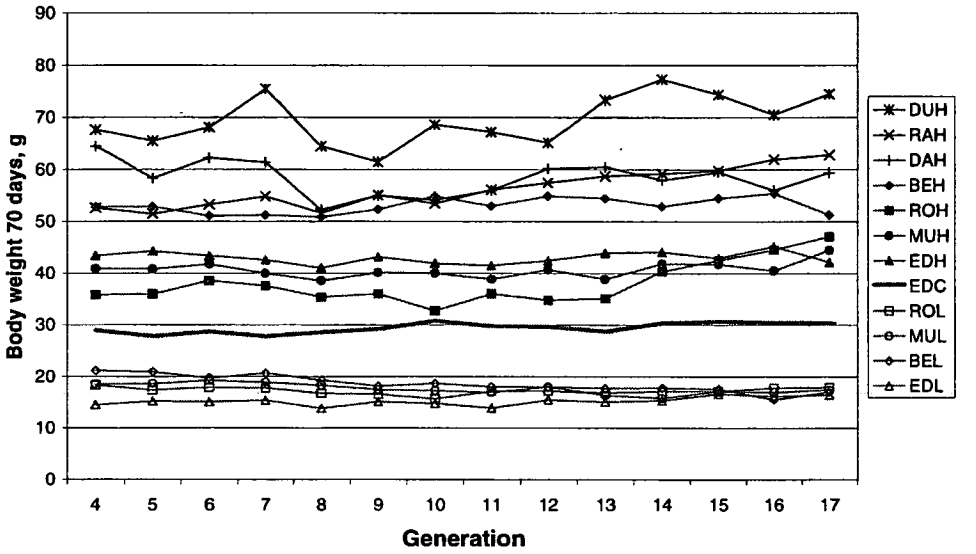
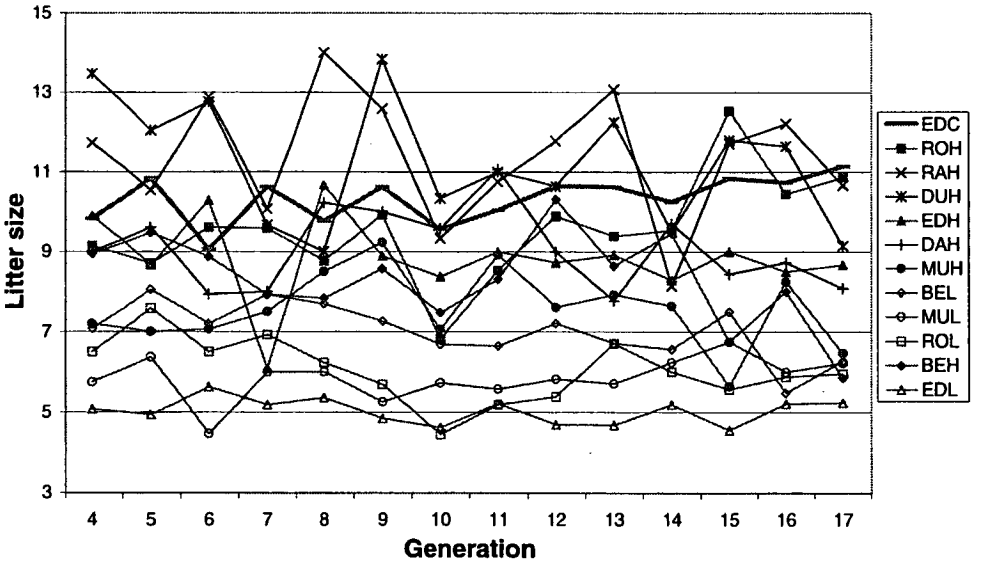


Figure 2.3: Least square means of litter size for all lines over the generations



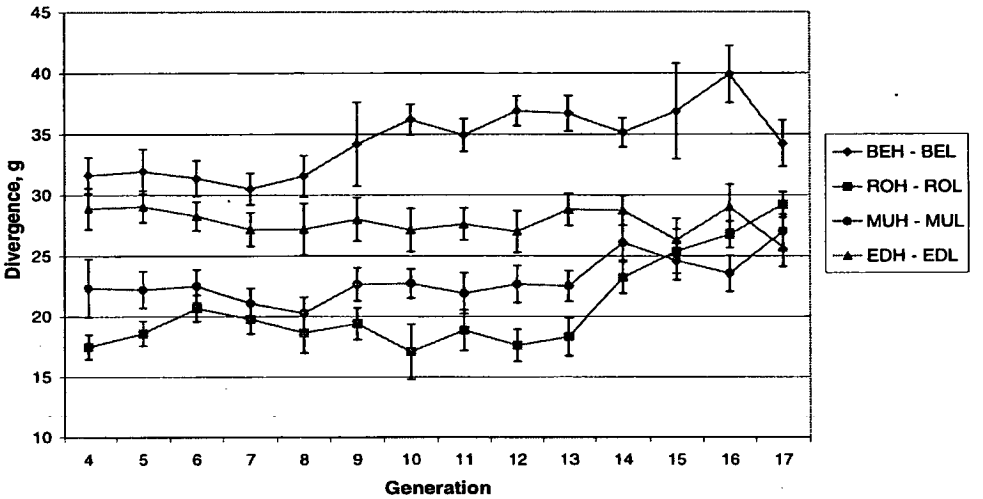
The divergence between high and low pairs from gen 4 to 17: The divergence in body weight between high and low lines from the same base population is shown in figure 2.4. Throughout the period (gen 4 to 17) the high lines were heavier than their line pairs, as expected and the divergence differed among the pairs of lines. During the initial period of counterbalancing selection on body weight (gen 4-7) the divergence between pairs remained at the same level and after that, during the rapid inbreeding scheme, increased. However, in the Edinburgh lines the body weight divergence remained at the same level throughout the period, possibly because these lines were already inbred at the start of the experiment.

The divergence in the litter size showed a more erratic pattern than body weight, but the number of observations of litter size was much smaller than of body weights (figure 2.5). In most generations the divergences between the lines did not differ significantly. A regression of generation mean for divergence on generation number was calculated for each line pair in order to examine whether the divergence was consistent over all line pairs. For three of the line pairs it was slightly negative (between -0.01 and -0.08) and not significantly different from zero. The only line pair in which litter size divergence significantly increased was the Roslin pair with regression coefficient of 0.24 (s.e. 0.07). However, the divergence was affected by the suspected contamination or mutation in ROH line.

Litter size for control, high and low lines over the generations: All the high body weight lines were clearly heavier than the control line throughout the experiment. Because of the positive genetic correlation between body weight and litter size, the heavy females were expected to have higher litter size than the average size controls. However, this was not observed, not even in the beginning of the experiment where the level of accumulated inbreeding was not expected to affect the litter size (figure 2.6). In later generations, when full-sib mating scheme was practised the average litter size of the high lines further diverged from the control line. A similar decrease was observed in the average low line litter size (figure 2.6). Actually the reduction in litter size was expected to be much larger, because the inbreeding coefficient was so rapidly increased in the selection lines with the exception of Edinburgh lines.

Body weight and litter size at different stages of inbreeding: The litter size was shown to be slightly reduced during the full-sib mating period in

Figure 2.4: The High - Low divergence for body weight at 70 days over generations with 95% confidence intervals

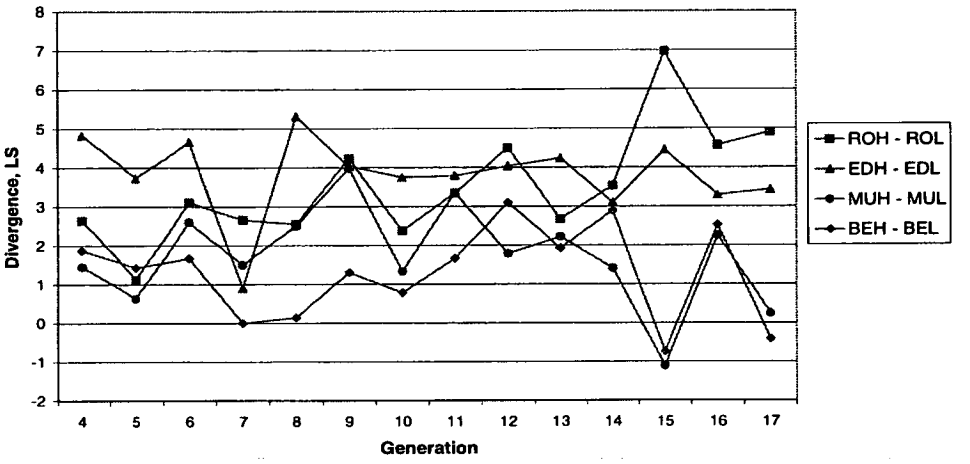


both high and low selection directions. The different time periods of inbreeding were analysed separately to compare the changes in body weight and in litter size between the periods and to draw conclusions of the inbreeding effects on litter size. In the first time period the accumulated inbreeding from the growth selection experiments (time in home laboratory, see figure 2.1) was present and it was assumed to be mild except in the Edinburgh lines. The two last time periods covered the beginning and continuation of full-sib mating scheme where increase of inbreeding coefficient was accelerated. The regression coefficients over the time periods for all lines are listed in table 2.4.

The regression of body weight on generation number for the randomly mated control line varied slightly depending on the stage of the experiment (table 2.4). Litter size remained at the same level independent of the stage of the experiment. The control line was used as an environmental control for the selection lines, thus the regressions in certain time periods were compared between the control and the selection lines.

Time before rapid inbreeding, gen 4-7: During the first four first generations the regression of body weight on generation number was not significant in both

Figure 2.5: The High - Low divergence for litter size over generations

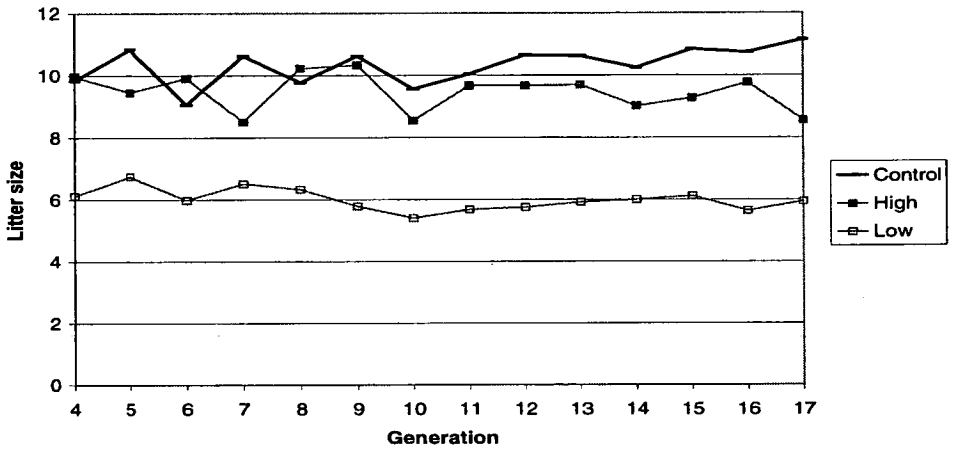


selection directions, which is likely because mild selection on body weight was practised only to overcome the relaxed growth selection. The high lines had a negative regression of litter size on generation number and in the low lines the litter size remained nearly constant.

The individual high lines had a range of regressions of body weight on generation number from -0.55g/gen to 1.94g/gen (table 2.4). There were three outstanding regressions on generation number in lines DUH, ROH and BEH, the first two of them with a very large increase in body weight and BEH having a negative regression coefficient (table 2.4). Apart from the two extreme lines with increased body weight, no other lines had significantly different regression coefficient from the control line. In the low body weight lines the regressions were not significantly different from zero or from each other or from the control line. The regressions were of both positive and of negative sign ranging from -0.2 to 0.2 g/gen.

The regression of litter size on generation number in control line was not significantly different from the selection line regressions, but the absolute value was always smaller than in the high lines and nearly always smaller than in the

Figure 2.6: The least square means of litter size for control, high body weight line and low body weight line females over generations 4 to 17.



low lines. Most of the high body weight lines had a decreasing trend of litter size during this period and most of the low lines an increasing trend. The large negative regression coefficient in EDH line was due to a unexplained drop in litter size in generation 7. Among the high lines the ones with significant decrease in litter size were lines with a decrease in body weight during the same time period. In the low lines the litter size changed mainly in the opposite direction to body weight.

Start of rapid inbreeding, gen 8-11: During the immediate period after the start of rapid inbreeding a clear change in body weight was seen in both directions, much stronger in high body weight direction than in low body weight. The body weight increased on average by 1.7g in the high lines per generation and decreased by 0.5g in the low lines. In both directions the litter size decreased during this period by a rather similar amount (nearly 0.3 pups/gen on average), while in control line no changes were observed.

At this time period all individual high lines increased in average body weight, especially the extreme heavy lines. The remaining four high lines had a positive but not significant regression coefficients of body weight on generation number

and it was similar among the lines. In the individual low lines the regressions of body weight were not significantly different from zero and all apart one were of negative sign (table 2.4). Also the outbred control line had an increase in the body weight during this time period and the regression was similar in the four moderately heavy lines.

The first four generations of full-sib mating (gen 8-11) seemed to lead to reduction of the litter size, since nearly all individual high and low selection lines had a negative litter size development during this time period. In the high lines the correlated changes in litter size did not follow the changes in body weight. Despite an increase in weight the litter size dereased in all but one line, which was most likely caused by the accelerated accumulation of inbreeding. In the low direction changes in body weight and litter size happened in the same direction and the decrease in litter size was larger than in any other time period considered here.

Time period of rapid inbreeding, gen 12-17: In the last period the full-sib mating scheme continued over six generations and the inbreeding coefficient was assumed to increase slowlier than in the previous time period. The high lines still increased in body weight on average, the regression on generation number being 1.4g/gen. In the low line the body weight remained constant. A small and non significant change in the litter size was observed in both directions, a decrease of litter size per generation in high lines and an increase in low lines.

Compared with the control line, most of the high lines had much larger absolute values of the regression estimates of body weight. During this time body weight decreased in some lines, e.g. DAH and BEH, and in some lines, e.g. ROH and MUH, body weight increased around 2g per generation. The response in the previously clearly responding DUH line had ceased at this time period. From the low lines only one line decreased in body weight by 0.3g per generation. The rest of the lines had smaller positive regressions of body weight on generation number and two of them very similar regressions to the control line.

Table 2.4: Regression of body weight at 70 days and litter size on generation number for overall time (gen 4-17), slow inbreeding (gen 4-7), start of rapid inbreeding (gen 8-11) and continuation of rapid inbreeding (gen 12-17). Standard errors are given in brackets and coefficients marked with bold are significantly different from zero.

	Body weight at 70d, grams				Litter size at birth			
line	4-17	4-7	8-11	12-17	4-17	4-7	8-11	12-17
Control	**0.18**	-0.20	**0.37**	**0.25**	0.08	0.05	0.00	0.09
	(0.03)	(0.22)	(0.15)	(0.11)	(0.04)	(0.33)	(0.24)	(0.14)
High	**0.38**	0.04	**1.69**	**1.38**	**-0.06**	**-0.48**	**-0.30**	-0.12
	(0.06)	(0.20)	(0.17)	(0.11)	(0.02)	(0.15)	(0.13)	(0.06)
Low	**-0.13**	0.02	-0.46	0.03	-0.04	0.05	-0.27	0.07
	(0.04)	(0.25)	(0.29)	(0.16)	(0.02)	(0.18)	(0.16)	(0.07)
DAH	-0.07	0.41	**0.88**	**-0.57**	-0.05	-0.67	-0.27	-0.06
	(0.04)	(0.39)	(0.25)	(0.16)	(0.05)	(0.45)	(0.33)	(0.17)
DUH	**0.67**	**1.94**	**1.45**	0.32	**-0.19**	-0.63	0.16	-0.20
	(0.04)	(0.28)	(0.28)	(0.17)	(0.05)	(0.42)	(0.38)	(0.16)
RAH	**0.81**	0.32	**1.46**	**0.90**	-0.01	-0.17	**-1.30**	-0.16
	(0.04)	(0.22)	(0.21)	(0.15)	(0.05)	(0.32)	(0.33)	(0.17)
EDH	-0.03	-0.29	0.22	-0.07	-0.05	**-0.95**	-0.56	-0.03
	(0.04)	(0.24)	(0.23)	(0.18)	(0.05)	(0.36)	(0.31)	(0.14)
BEH	**0.14**	**-0.55**	0.53	-0.44	**-0.13**	**-0.33**	-0.03	**-0.81**
	(0.06)	(0.26)	(0.32)	(0.24)	(0.05)	(0.30)	(0.37)	(0.17)
MUH	**0.12**	-0.25	0.28	**1.73**	-0.04	0.15	-0.14	-0.17
	(0.05)	(0.33)	(0.22)	(0.16)	(0.05)	(0.39)	(0.30)	(0.15)
ROH	**0.66**	**0.97**	0.36	**2.58**	**0.14**	0.21	-0.38	**0.29**
	(0.04)	(0.20)	(0.26)	(0.13)	(0.04)	(0.31)	(0.33)	(0.15)
EDL	**0.10**	0.22	-0.19	0.27	-0.02	0.12	-0.06	0.10
	(0.05)	(0.28)	(0.37)	(0.20)	(0.04)	(0.34)	(0.28)	(0.12)
BEL	**-0.34**	-0.17	-0.35	**-0.32**	-0.11	0.14	-0.37	-0.23
	(0.04)	(0.23)	(0.25)	(0.15)	(0.04)	(0.33)	(0.31)	(0.15)
MUL	**-0.17**	0.13	-0.50	0.04	0.05	-0.11	-0.07	0.10
	(0.04)	(0.24)	(0.34)	(0.18)	(0.04)	(0.31)	(0.31)	(0.14)
ROL	-0.02	-0.19	0.05	0.21	-0.08	0.04	-0.43	-0.02
	(0.04)	(0.23)	(0.37)	(0.16)	(0.04)	(0.32)	(0.29)	(0.14)

Models within group:
BW70 = mean + line + sex + line*sex + β * gen + error
LS = mean + line + β * gen + error

Models within line:
BW70 = mean + sex + β * gen + error
LS = mean + β * gen + error

All except one high line showed a reduction in the litter size during the rapid inbreeding period (table 2.4). The only one where significant correlated change, 0.3 pups/gen, in litter size was seen was ROH, which also had the highest regression on body weight at the given time period, which was unexplained. The largest reduction was observed in BEH line of 0.8 pups/gen, which caused the litter size of BEH to drop below its line pair BEL in the last generations (figure 2.5). In the low lines the regressions of litter size remained non significant. The BEL line showed more correlated changes in the litter size than the others. However, it is not possible to say if the reduction in the litter size was due to the reduced body weight or due to inbreeding.

2.4 Discussion

This chapter has described the body weight and litter size over period of inbreeding in growth diverging mouse lines. The lines differed greatly in body weight, the largest high line being on average nearly five times as heavy as the smallest of the low lines. These inbred mouse lines were derived from several growth selection experiments to be utilised in mapping studies and provided a unique set of 'replicates' (Bünger et al., 2001a). Due to the positive genetic relationship between body weight and litter size the heaviest animals were expected to have the largest litter size. However, the high level of inbreeding accumulated during the experiment was expected to affect the litter size of these lines. The aim of the study was to analyse the correlated response in litter size in a set of large and small mice during slow and accelerated accumulation of inbreeding.

The high line animals were on average 20.7g heavier and the low line animals 12.2g lighter than the controls. Based on the estimate of the positive genetic correlation (0.6) between body weight and litter size (Eisen, 1978), largest litters were expected to be seen in the heaviest animals. However, this was not observed in the study. The average litter size in high lines was 0.8 pups less than in the control, though much higher than the low line average. Only in two extereme high lines, which were over two times heavier than the control did the average litter size diverge by one pup. Eisen (1977) estimated the regression of litter size on body weight to be around 0.3 pups/g. By using this estimate, the litter size

for high lines would be expected to be on average 6 pups more than in control. In the low lines the predicted litter size was just 0.5 pups higher than the observed litter size. Despite the different body weights used by Eisen (1977), 42 day body weight, and in this experiment, 70 day body weight, it seems clear that the litter size in the selection lines has been affected by another factor.

The results of two of the high lines need some attention. Firstly, the ROH line, in which a sudden increase in body weight was observed, while the others had a more constant body weight. The change in body weight could not be explained, but possible reasons would be a line contamination or perhaps a new mutation which occurred at generation 13. Also the litter size increased rapidly, possibly following the increase in body weight or might have been caused new variation in the line. Since no genotypic data was available, the reason or details behind changes in ROH line remain unknown and the results only show what a large impact introduction of new variation in an inbred line might have on traits like body weight and litter size. Secondly, the BEH line, in which a low litter size was observed. In that line a mutation in myostatin gene has been reported (Bünger et al., 2001a), which increased the muscularity and decreased the fatness. This mutation might have an effect on the reproductive performance of the line.

The use of the EDC line as a control line needs to be clarified. EDC was not a proper control, but the best available line to be used as some kind of control line in this experiment. First of all, it was derived from a different base population than all other lines except the Edinburgh lines (EDH and EDL). Secondly, its use as a environmental control might not be realistic, since the response to environmental conditions is not necessarily similar in inbred animals and in outbred animals. Thus, EDC was mainly seen as a representative of a line with medium sized not inbred animals rather than a control line in a strict sense.

The experiment involved rapid accumulation of inbreeding, which would be likely to cause a reduction of litter size. However, only a minor changes in the litter size over the generations was observed. For the overall period the litter size was reduced by on average 0.1 pup per generation in both high and low lines. In the experiment during the initial period the lines were assumed to have only mild level of inbreeding accumulated during the past growth selection experiments and from generation 8 onwards the accumulation of inbreeding was accelerated by

full-sib mating scheme. Thus the litter size should have reduced greatly at least in the beginning of the full-sib mating period due to the increasing inbreeding coefficient. A reduction of litter size by 0.5 pups per 10% increase in inbreeding coefficient has been estimated (Bowman and Falconer, 1959), which was not the case in this study. Actually in several lines the litter size was decreasing more from generation to generation during the period when only mild inbreeding was present than during the full-sib mating period.

A possible explanation for such mild effect of full-sib mating would be that the animals had already high inbreeding coefficient prior to the full-sib mating scheme and therefore the increase of inbreeding coefficient had not been large. It was known that the Edinburgh lines were more inbred than the other lines in the beginning of the experiment and thus might show less reduction in litter size during rapid inbreeding than the other lines. This was not observed in the EDH line, which had one of the largest reduction in litter size of the high lines during the first four first generations of full-sib mating. The EDL line had a smallest change in the litter size of the low lines during that period, but not significantly smaller. Therefore, it does not seem likely that the high starting level of inbreeding would be a reason for the small inbreeding effect observed in this study.

Infertility is another known harmful effect of inbreeding and might cause loss of families (Falconer, 1971; Roberts, 1981; McCarthy, 1982; Bünger et al., 1993). As shown by Bünger et. al. (2001a) the number of families in all lines was greatly reduced during the full-sib mating period, i.e. from generation 8 to generation 14. The loss of families leads to involuntary selection for reproductive performance by emphasising those families in which the reproductive fitness is not greatly affected by the inbreeding depression. For example, Falconer (1971) reported only 4 surviving families out of 20 after 7 generations of inbreeding and 3 of the surviving families showed only a little inbreeding depression in the litter size. The reduction of families possibly explains the very small effect of inbreeding on the litter size, since the inbreeding effects could have been suppressed by the selection on litter size and also by the selection on body weight. However, the selection on body weight was not very intense, also because of the reduction of families. Within family selection was practised, but the reduction of families led to the use

of more individuals from each remaining family.

During the first four generations of the experiment (gen 4-7) the mean litter size of high lines was half a pup lower than in the control, but the difference was not significant. The mild inbreeding level of selection lines in the beginning of the experiment might not be sufficient explanation for the smaller litter size in the heavy animals than in the average size control. However, the only lines derived from the same base population than the control were the Edinburgh lines. EDH was more inbred than the other lines, but its litter size diverged from the control by approximately the same amount as did the other high lines apart from the two extremes. As discussed before the observed litter size in the low line differed much less from the predicted litter size, while the high lines had a completely different value than could have been expected. Additionally to the inbreeding effect the effect of body composition might explain some of the 'too small' litter size of the high lines, especially the fatness (Roberts, 1978). Bünger et. al. (2001a) estimated the average fat percentage to be 9.5% in the high lines and 3.8% in the low lines. The high line litter size might be affected by the fatness of the lines and might also be more sensitive to the harmful effects of inbreeding, possibly because of the metabolic disturbances related to fatness (Roberts, 1978).

The general conclusion of this study was that litter size was not greatly reduced by the inbreeding over the generations against the expectations. However, there might have been some other harmful effects of inbreeding affecting litter size development, such as reduction of families due to increased infertility. However, this experiment was not designed to examine the reproductive performance and thus did not provide data about the other factors, like infertility problems. In order to further study the effects on inbreeding on reproductive fitness an experiment was designed using the same mouse lines than described in this chapter and also using wider range of components of litter size. The experiment should also clarify the reasons behind the differences in litter size of large, average and small animals.

Chapter 3

Experimental study on reproduction of growth selected lines: design and methods

3.1 Introduction

Reproductive performance has been studied mainly by analysing litter size either directly selecting for litter size (e.g. Falconer, 1971; Vangen, 1999) or as a correlated trait (e.g. Fowler and Edwards, 1960; Barria and Bradford, 1981; Brien et. al., 1984). In Chapter 2 litter size development of seven large and four small mouse lines before and during intensive inbreeding period was described, and these lines are now used in further study. In this experiment the reproductive performance was examined in more detail including a wider range of fertility traits, such as infertile matings, ovulation rate and embryonic losses. The components of litter size should reveal the background behind the observed differences in litter size between large and small females.

The objective of the whole experimental work was to examine the effect of growth selection on reproductive fitness in inbred female mice. It consisted of three parts: part one concentrated on the reproduction of growth diverging inbred lines, part two on the reproduction of inbred parents with crossbred foetuses and part three on the reproduction of crossbred parents with crossbred foetuses. Additionally to the inbreeding effects the two last parts of the experiment examined the effect of counterbalanced selection in the foetuses, by mating high and low line pairs, and in the parents, by producing two-way crosses of high and low lines.

3.2 Material and Methods

The material for the experiment came from a resource experiment which is still running at the Edinburgh mouse laboratory. The mouse lines used in this experiment were described in Chapter 2 and also by Bünger et al. (2001a). The line codes, origin and selection direction are listed in table 2.1.

The experiment ran over three generations from May 2000 to March 2001. Females used in the first two experiments were contemporaneous with generations 14, 15 and 16 females of the resource experiment (see Chapter 2). Therefore, all the selection lines had experienced eight or more generations of rapid inbreeding additionally to the cumulated inbreeding during the long term selection experiment. After eight generations of full brother-sister mating the inbreeding coefficient would be above 0.8 (Falconer and Mackay, 1996). The true inbreeding coefficient of these lines was higher due to the long term selection history and accumulated inbreeding prior to the full-sib mating scheme. Bünger et al. (2001a) estimated that the inbreeding coefficient at generation 14 was over 0.9, being highest in the Edinburgh lines. The last experiment utilising crossbred parents was contemporaneous with generation 17.

3.2.1 Experiments

Table 3.1 shows the type of parents and offspring in terms of selection and inbreeding at different stages of the experiment. The term "offspring" refers here to a potential offspring, which however remained at foetus level during the experiment. The mating codes shown in the table were designed so that they reflect the genotype of offspring. Figure 3.1 shows a diagram of the matings in all the experiments.

Experiment I involved parents with the full effect of inbreeding and selection present (table 3.1). The aim was to estimate the components of reproductive fitness in large and small mice with a high level of inbreeding. The results were expected to partition the differences in litter size between the divergent growth lines observed in Chapter 2.

The brother sister pairs were taken randomly from the spare animals which were not used in the resource experiment. Usually all available pairs were used to

maximize the number of observations. Some mouse lines had poor fertility and the number of pairs remained rather low.

In **experiment II** line crossing was practised. The inbreeding and selection effects of the parents remained the same as in experiment I, but from the offspring the inbreeding effects were removed. Furthermore, the selection effect on offspring was counterbalanced, i.e. high and low lines were crossed so that the offpsring were genetically a mixture of high and low growth genes (table 3.1). The aim was to examine the effect of offspring inbreeding and selection on reproductive performance of the mother.

In the first part lines were crosses within the same selection direction, e.g. a high body weight female with a high body weight male from a different line. In the second part high and low lines were crossed. All crosses were made with the restriction that no matings were made between lines derived from same base population (e.g. EDH and EDL) to avoid any inbreeding at offspring level. The pairs were randomly selected from the spare animals which remained after the animals were picked for the resource experiment and for the first part of the current experiment. An effort was made to keep the line contributions as equal as possible in each type of crosses.

Experiment III, was done to remove the effects of the inbreeding from parents and offspring. In addition to that selection effects were counterbalanced in some parents and offspring, so that all combinations would be available (table 3.1). The aim was to estimate the reproductive fitness traits in non inbred mice and draw conclusions about the inbreeding and selection effects by comparing the results with the previous experiments.

The two-way crossed parents were created by using pairs from generation 15 of the resource experiment for second litters. Reproduction at second parity was not analysed, since it was made only to create the parental generation necessary for the last experiment. The two-way crosses were made in a similar manner to those in experiment II. A full description of the procedure will be given in chapter 6.

Figure 3.1: A schematic figure of the mating procedures in experiments I, II and III. The "line" refers to a growth selected mouse line.

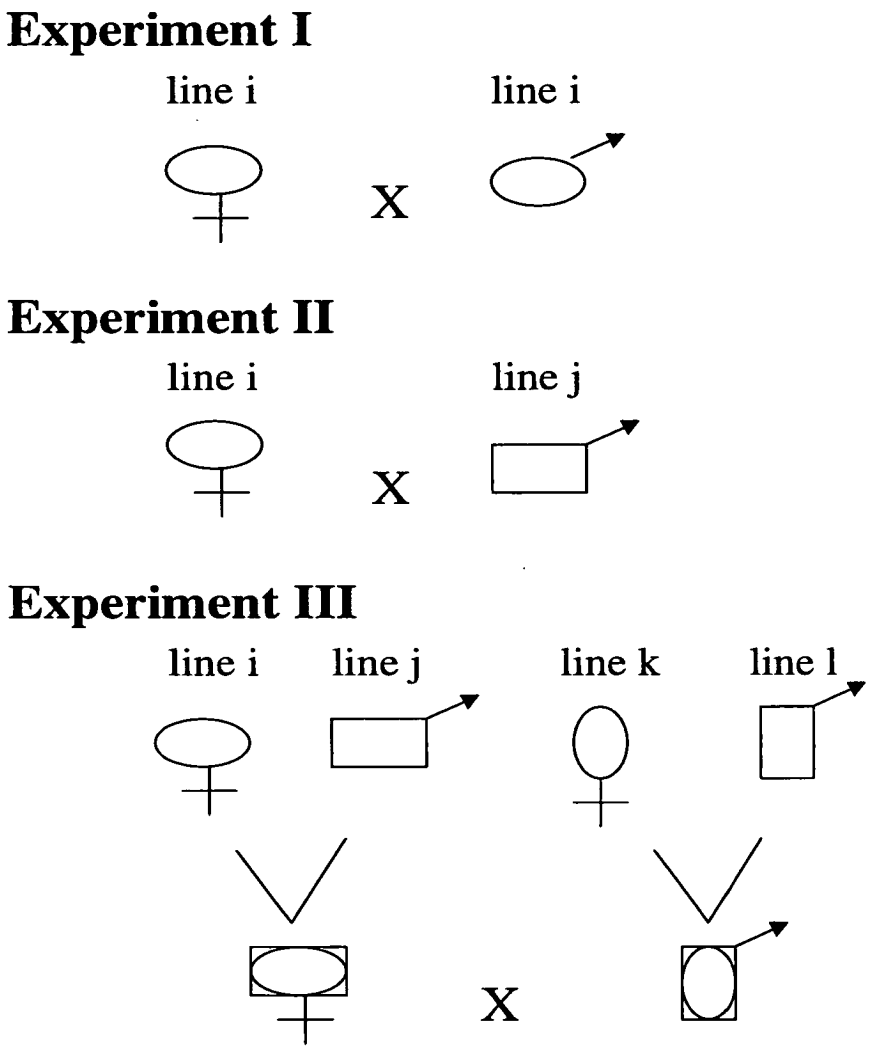


Table 3.1: The number of the experiment, the code for mating group, type of mating (female lines with subscript i and k and male lines with j and l, i, j, k, l= 1 ... 11) and the effect of inbreeding and selection in parents and offspring

	code	mating	parents	offspring
I	H L	Hi x Hi Li X Li	inbreeding + selection	inbreeding + selection
II	HH LL	Hi x Hj Li x Lj	inbreeding + selection	no inbreeding + selection
	HL LH	Hi x Lj Li x Hj	inbreeding + selection	no inbreeding + counterbalanced selection
III	HHHH LLLL	HiHj x HkHl LiLj x LkLl	no inbreeding + selection	no inbreeding + selection
	HHLL LLHH	HiHj x LkLl LiLj x HkHl	no inbreeding + selection	no inbreeding + counterbalanced selection
	HLLH LHHL	HiLj x LkHl LiHj x HkLl	no inbreeding + counterbalanced selection	no inbreeding + counterbalanced selection

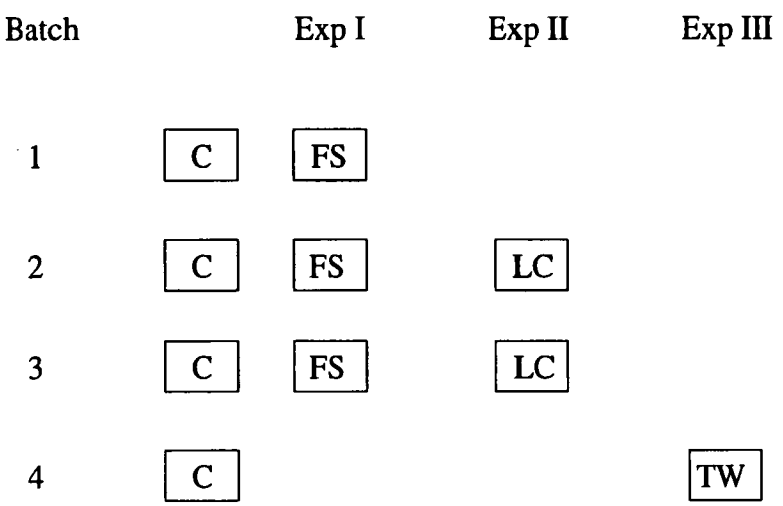
3.2.2 Batches

The first experiment with inbred parents and inbred offspring was replicated over three batches (figure 3.2). The second experiment with crossbred offspring was done in batches two and three. The last part was done on its own in batch four due to the limited space in the mouse laboratory. For each batch new animals were taken from the resource experiment, since no offspring were kept. Throughout the experiment an unselected and non inbred control line (EDC) was kept and handled in a similar manner as the selection lines to connect the batches and to control for the environmental fluctuations.

3.2.3 Housing and feeding

The housing and feeding was done in a similar manner to that in the resource experiment (Chapter 2). The only exception was the cage size, which was smaller M2 cage (internal size 330cm^2 x 12cm, Kent Plastics Ltd.). The mice were kept mainly in the same room of the animal laboratory to provide equal environmental conditions.

Figure 3.2: Batches and experiments. C = control, FS = full sib matings, LC = line crosses and TW = two-way crosses



3.2.4 Procedure from mating to dissection

Matings for one batch were made for all the lines within a seven week period in the same order as in table 2.1. The control line animals were mated randomly avoiding brother sister pairs. The mice were mated at an age of ten to twelve weeks and the females were weighed on the day of mating. Male weights were not recorded, except in experiment III where no prior knowledge of the weights was available. Harem matings were practised, so that usually two females were paired with one male, but on a few occasions one male was mated to one or three females. For ten mornings following the pairing, the females were checked for the presence of a vaginal plug, which is an easily observed sign of copulation in mice. If there was not a sufficient number of plugs per line, say less than 50% females plugged, they were checked for another week. The day of a vaginal plug was counted as day zero of the gestation and once the plug was observed the females were not checked again so as not to affect the pregnancy. Most of the pairs were kept together until the day 18 of pregnancy when the females were dissected.

There was no certainty about the correct dissection day for females on which no vaginal plug was observed. To find an appropropriate dissection day (day 18 of gestation) for unplugged females, all female mice were weighed eighteen days after the mating day. A proportional increase in body weight since mating date was then calculated. This measure was compared with the plugged females and used to estimate the right day for dissection. Females were already at such an age that substantial growth did not occur at this stage without pregnancy. Around gestation days 16-17 a typical increase in body weight was about 60%-90% since pairing day, depending on the line.

All dissections were carried out by the same person throughout the experiment. The mice were killed by cervical dislocation and dissected immediately. Dissection was not carried out if the animal was sick or had already given birth. The uterine horns were first checked for resorptions, which can be seen as dark small lumps in the uterine horn (see picture A.1 in appendix 1). The resorptions are eggs, which have been implanted to the uterine horn but were not capable of developing to a foetus (Austin and Short, 1985). The foetuses were then dissected out from the uterine horns, checked, counted and decapitated. Foetuses, which were clearly further developed than resorptions but had died at some point of gestation, were recorded as a mole (see picture A.2 in appendix 1).

The ovaries were removed and cleaned from the surrounding fat. The ovaries have a pink colouring and the corpora lutea can be seen on the surface as darker red spots (see picture A.3 in appendix 1). The number of corpora lutea was counted by using a microscope with a magnifying power of 12. The counts were mainly done by two people, the first by the dissector and the second by a "blind-reader" who did not have any information on the number of foetuses found from uterus or the line of the mouse. In case of a disagreement, a mean number of corpora lutea counts was taken.

3.2.5 The traits

The components of reproductive performance which were recorded during the dissections were ovulation rate (OR), number of live foetuses (LF), number of dead embryos and foetuses, i.e. resorptions (RE) and moles (MO). They were recorded separately for the left and right uterine horns observed from the ventral

side. However, analysis of variance did not detect any mean differences between left and right side and the results were pooled together. Hence, for example, the ovulation rate refers to pooled ovulation rate unless otherwise mentioned. The number of lost eggs was separated into two parts, pre-implantation losses (PRE) and post-implantation losses (POS). The pre-implantation losses were counted as the difference between ovulation rate and number of eggs implanted to the uterine horns, i.e. [OR - (LF + RE + MO)]. Post-implantation losses were the sum of resorptions and moles (RE + MO).

Pre- and post-implantation losses are dependent on the ovulation rate, so to adjust the losses proportional to OR they were also expressed in relative terms. Relative pre-implantation losses (PRE%) were calculated proportional to the ovulation rate as PRE/OR. Relative post-implantation losses (POS%) were calculated similarly (RE+MO)/OR. Post-implantation losses were also considered as the ratio of dead embryos or foetuses to number of eggs attached, (RE+MO)/(RE+MO+LF). It will be specified in the text which method has been used to describe the relative losses after implantation.

The time in days from pairing to observation of vaginal plug was counted to assess the changes in cycle length or libido. The number of infertile matings was recorded. There were two types of infertile matings, one where the reason was failure to ovulate and the other where ovulation happened, but no pregnancy was achieved. The pregnant and not pregnant females were analysed separately and in the main analysis only the pregnant ones were included.

Chapter 4

Experimental study I: The effect of growth selection on the reproductive performance of inbred mice

4.1 Introduction

Chapter 2 discussed the correlated response of growth selection on litter size and its change during inbreeding. In the light of the observations from that study an experiment was designed to further analyse, on a wider range of traits, the components of reproductive fitness of the mouse lines. Ovulation rate and embryonic mortality are the main components of litter size (Falconer and Mackay, 1996). Ovulation rate, like litter size, has a positive genetic correlation with body weight (Land, 1970) and the regression coefficient has been estimated as 0.24 ova/g (Falconer and Roberts, 1960). However, embryonic mortality has not shown a constant positive correlation with body weight and it has also a negative genetic correlation with ovulation rate (Bowman and Roberts, 1958; Bradford, 1969).

The experimental animals had been subjected to an intensive inbreeding mating scheme, brother-sister matings, for at least seven generations. Traits related with reproductive fitness are in general sensitive to inbreeding depression and are thus expected to suffer under such mating scheme (Falconer and Mackay, 1996). Ovulation rate, which tends to change in the same direction as body weight, would be expected to show inbreeding depression if body weight does (Falconer

and Roberts, 1960). Embryonic mortality has been observed to increase with accumulating inbreeding due to altered hormonal factors which are also related to body composition like fatness (Land, 1970; Durrant et al., 1980). Another component of reproductive fitness affected by inbreeding is the number of fertile matings. Due to infertility, families have been lost in several selection experiments (Falconer, 1971; Roberts, 1978; Roberts, 1981; McCarthy, 1982; Dietl et al., 2001).

The objective of the experiment I was to estimate the effect of growth selection on components of reproductive fitness in inbred female mice. The mouse lines used in this study (see Chapter 2 and Bünger et. al. 2001a), provided a unique opportunity to study the components of reproductive fitness in inbred animals with a wide range of body weights. As all the mouse lines had a similar inbreeding coefficient the hypothesis was that the differences in the reproductive performance might be due to the differences in body size. The aim was to describe the components of reproductive fitness in inbred, large and small, mice and to estimate the effects of body weight selection on the components. Within large and small size groups, several lines ('replicates') were available, thus the study was not restricted just to one set of divergent lines, and therefore more general conclusions can be made about the relationship between reproductive performance and body weight in inbred mice.

4.2 Material and methods

Mouse lines (seven high lines, four low lines and an outbred control) from a resource experiment (described in Chapter 2) were used in the present reproductive fitness experiment. The inbreeding coefficient in all selection lines was over 0.9 in the beginning of this study (Bünger et al., 2001a). The animals were sampled from generations 14, 15 and 16 of the resource experiment and here the samples are called batches I, II and III. The experiment ran from May 2000 to March 2001.

The general experimental procedure was explained in Chapter 3. All matings were brother-sister matings to maintain the high level of inbreeding in the offspring. The mating pairs were picked randomly from the spare animals of the

as the ratio of pregnant females to all mated females. Also number of days from matings to occurrence of vaginal plug was calculated (Days). A more detailed description of the dissection procedure and the traits was given in Chapter 2.

Only pregnant females were included in the main data analysis, since this was the most important group having observations for each trait. Females which failed to become pregnant were analysed separately. The data were grouped according to the selection objective, i.e. high body weight (H), low body weight (L). The control line was maintained with minimal inbreeding scheme and no selection on body weight was practised.

The approximate normality of the distributions of the traits within lines was tested with the Genstat 5 (Genstat, 1993) NORMTEST procedure which gives statistics from Anderson-Darling, Cramer Von Mises and Watson tests. The figures describing the traits within groups (C, H, L) were presented as Box-and-Whisker plots in Minitab Release 12.1 (www.minitab.com) because of the informative nature of this presentation. In the Box-and-Whisker plot the middle line of the box is the median of the data. The upper and lower limits of the box show the 75 % and 25 % quartiles (Q3 and Q1). The whiskers show how the data are distributed by showing the upper and lower adjacent values. The upper adjacent value was calculated by $Q3 - 1.5(Q3 - Q1)$ and the lower similarly $Q1 - 1.5(Q3 - Q1)$. This gives approximately the area in which two standard deviations from the mean lies. The data points outside of the range are marked with stars.

The least square means were calculated by SAS generalized linear model (GLM) procedure (SAS, 1996). The basic model for least square analysis of body weight and all reproduction traits was

$$Y = \text{mean} + \text{batch}_i + \text{line}_j + \text{batch}_i{*}\text{line}_j + \text{error},$$

where batch is the number of batch $i = 1 \ldots 3$, line is the mouse line (either selection line or control) $j = 1 \ldots 12$ and batch*line is the interaction term between the mouse line and batch number. In some analysis the interaction term was dropped, because some of the lines were not present in all batches and the inclusion of the interaction term led to non-estimable effects. In this data set the animals were mated at 10 and 12 weeks of age and the age at mating had no significant effect on any of the traits considered.

The analysis was made both between and within the selection objectives. The differences between several group means were analysed by ANOVA procedure in Genstat (Genstat, 1993). The homogeneity of variances was tested at the same time by using Bartlett's test. ANOVA is not a suitable method of testing differences in means if the distributions are significantly different from the normal or if the variances are very heterogeneous (Ranta et al., 1989). Therefore a non-parametric Kruskall-Wallis statistics was used to confirm the results from analysis of variance. The Kruskall-Wallis test is similar to an analysis of variance, but does not assume normality and is based on the ranks of the data (Ranta et al., 1989). Pairwise tests between line or group means were performed by the GLM procedure in SAS and the t-test was used to detect differences between the two means (SAS, 1996).

Divergence between high and low body weight line pairs, e.g. EDH and EDL, which were derived from the same base population, were calculated by using the least square means. Standard errors of the least square means were utilised in calculations of the confidence intervals. The control line was used as a reference point in within selection objective analysis, where differences between lines in one size group were analysed. Use of a reference point makes the comparisons between the high and low body weight groups easier. When divergence from the control was examined, the confidence interval was calculated with the Welch test. When the variances are not equal and the sample size is small (say under 30) Welch test is one way to find the appropriate degrees of freedom, since it utilises the information on the estimated variances and sample sizes (Ranta et al., 1989).

An important question in this experiment was the relationship between body weight and reproduction traits. To study that relation a regression analysis of the reproductive traits on body weight was performed using SAS (1996). The constant in the regression model was not fitted through zero, because only the range of body weights within a group or line was of interest. Body weight was fitted in the model as a covariate both on the original scale and transformed to natural logarithms, since there was a concern that scale effect might affect the linearity of the regression when body weights were high and thus a transformation was required.

The regressions on body weight were estimated within groups, so that only

animals belonging to e.g. the high body weight group were included in the analysis. The batch number and the line within group were the fixed terms in the model. The group regression was an overall estimate of the relationship consisting of both genetic and environmental part.

Furthermore, the regression analysis was repeated on a line by line basis in order to study how constant the relationship between the reproduction traits and body weight was across the lines within similar size groups. The regression coefficient estimated for each line was called an environmental regression. The genetic regression was estimated using the line means within a size group, e.g. mean BW and OR, as data points.

Any differences between the regression coefficients were tested by t-test. First a difference between the coefficients was calculated, $b_1 - b_2$ and t-test value by dividing the difference with the square root of the sum of squared standard errors of the regression coefficients.

4.3 Results

A total of 109 females from the control line, 255 from the high body weight lines and 169 from the low body weight lines were dissected (table 4.1). In the main analysis only pregnant females were included, i.e. 106 observations from the control line, 207 from high body weight line and 134 from the low line (table 4.2). There were between 15 and 43 observations from pregnant females per selection line, ranging from 5 to 20 observations per batch per line. The BEH line was used only in batch I because of limited number of brother sister pairs, but no full-sib pairs in batch I were available from the RAH line.

In figure 4.1 a histogram of weights in control, high and low groups is plotted. In the control the body weight of females at mating (BW) was approximately normally distributed in the control group (Anderson-Darling test, p=0.6). The body weight at mating was normally distributed also in the individual selection lines, but not when the high and low groups were considered. The figure 4.1 illustrates the variability of body weights in the study, ranging from the 11g females in the low group to 80g females in the high group. There was no overlap between high and low lines, but the control overlapped with the high lines.

Table 4.2: Number of pregnant females in each selection direction and mouse line over all three batches

Control		High		Low	
	n	line	n	line	n
		DAH	28		
		DUH	26		
		RAH	30		
EDC	106	EDH	34	EDL	27
		BEH	15	BEL	33
		MUH	32	MUL	31
		ROH	42	ROL	43
	106		207		134

4.3.1 Descriptive statistics

The means of the groups (high, control, low) were compared to examine the average differences between the size groups. The control group was kept as outbred, thus in the between group comparisons the control represented outbred and medium sized mice. The line mean differences within high and low groups were tested to draw conclusions about similarity of the traits in inbred mice with the same selection objective.

Body weight at mating The mean body weights at mating in the groups were significantly different, the high line mean being nearly twice the control mean and three times the low line mean (figure 4.2 and table 4.3). The difference between high body weight mean and control mean was 19g and between low body weight group mean and control 13g (table 4.4). Figure 4.2 shows also the distribution of the transformed body weight in the groups. After the transformation the variation of the high line group mean became smaller relative to the variation in control group.

The least square means of body weight at mating (table 4.3) varied among the high lines from 37.3g in the MUH line to 58.5g in the DUH line, i.e. 21g difference between the heaviest and lightest. There were three lines (DUH, RAH, DAH) with extreme body weights which all exceeded 50g, while the average of the remaining four lines was 40g. Among the four moderately heavy lines the body weights between the lines did not differ significantly. The coefficients of variation

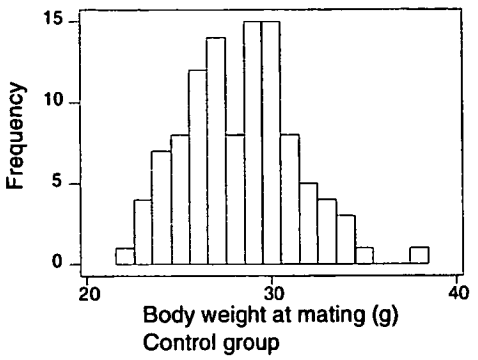
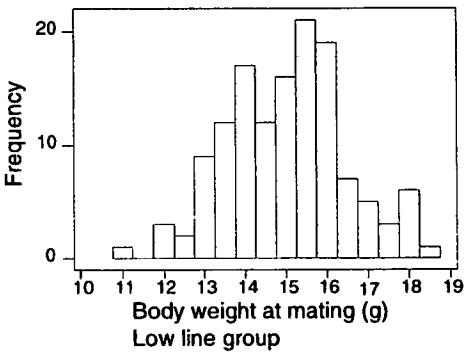
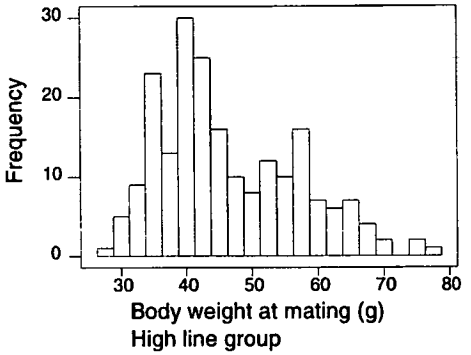


Figure 4.1: The distribution of female body weight (g) at mating in control, high and low line groups





in high lines ranged from 7% to 17% and the mean CV% of the lines was 11%, which was similar to the one in control.

In the low line groups the least square means of body weight varied from 14.5g (EDL) to 15.5g (ROL) (table 4.3). The line means did not differ from each other significantly. The low line group was the least variable group with the average of line CV% being 9.4% (table 4.5).

Ovulation rate The mean ovulation rates were significantly different among the groups (figure 4.2 and table 4.3). On average the high body weight females outperformed the control females. The difference between high and control was on average 5.5 ova and between control and low groups 4.3 (table 4.4). From figure 4.2 it can be seen that the plots for body weight and ovulation rate are quite similar, high body weight connected with high ovulation rate and similarly low body weight with low ovulation rate.

The least square means of the ovulation rate in the high lines ranged from

16.4 ova (BEH, result only from batch one) to 22.6 (DUH). As can be seen in figure 4.2, the high body weight group had more extreme values (marked with stars) than the other groups. These observations were from the heaviest three lines. The coefficient of variation was larger for ovulation rate than for the body weight (table 4.5). The CV% averaged over lines was 22% with a range from 14% to 30%.

Ovulation rate of the low body weight females ranged from 7.2 to 9.9 ova (table 4.3). There was a 2.7 ova difference between highest ovulating line and lowest ovulating line within a low line group, compared with 8.7 ova difference in the high body weight group. The CV% in the low lines were on average at same level as in control, apart from one line with larger coefficient.

Pairwise comparisons between the line means were made within a size group to examine the differences between the lines. In the high body weight group a total of 9 out of 21 line OR comparisons were found to be significant at 5% level, while 19 of the comparisons were significant for body weight. In the low line group only one line (BEL) differed significantly from the others.

The differences in line means using the control mean as reference point are shown in figure 4.3 for the high line group and in figure 4.4 for the low line group. Some of the significances detected in the pairwise tests were not seen in the figures, because the confidence intervals were calculated for the divergence between the control and the line mean, while pairwise test compared the lines. All the heaviest three high lines had significantly larger ovulation rate than the control. The divergence in absolute terms was similar between the groups.

Figure 4.2: The Box-and-Whisker -plots of mating weight (g), transformed mating weight (ln(g)) ovulation rate, number of live foetuses and relative pre- and post-implantation losses (PRE% and POS%) of the pregnant females in control, high and low body weight groups.

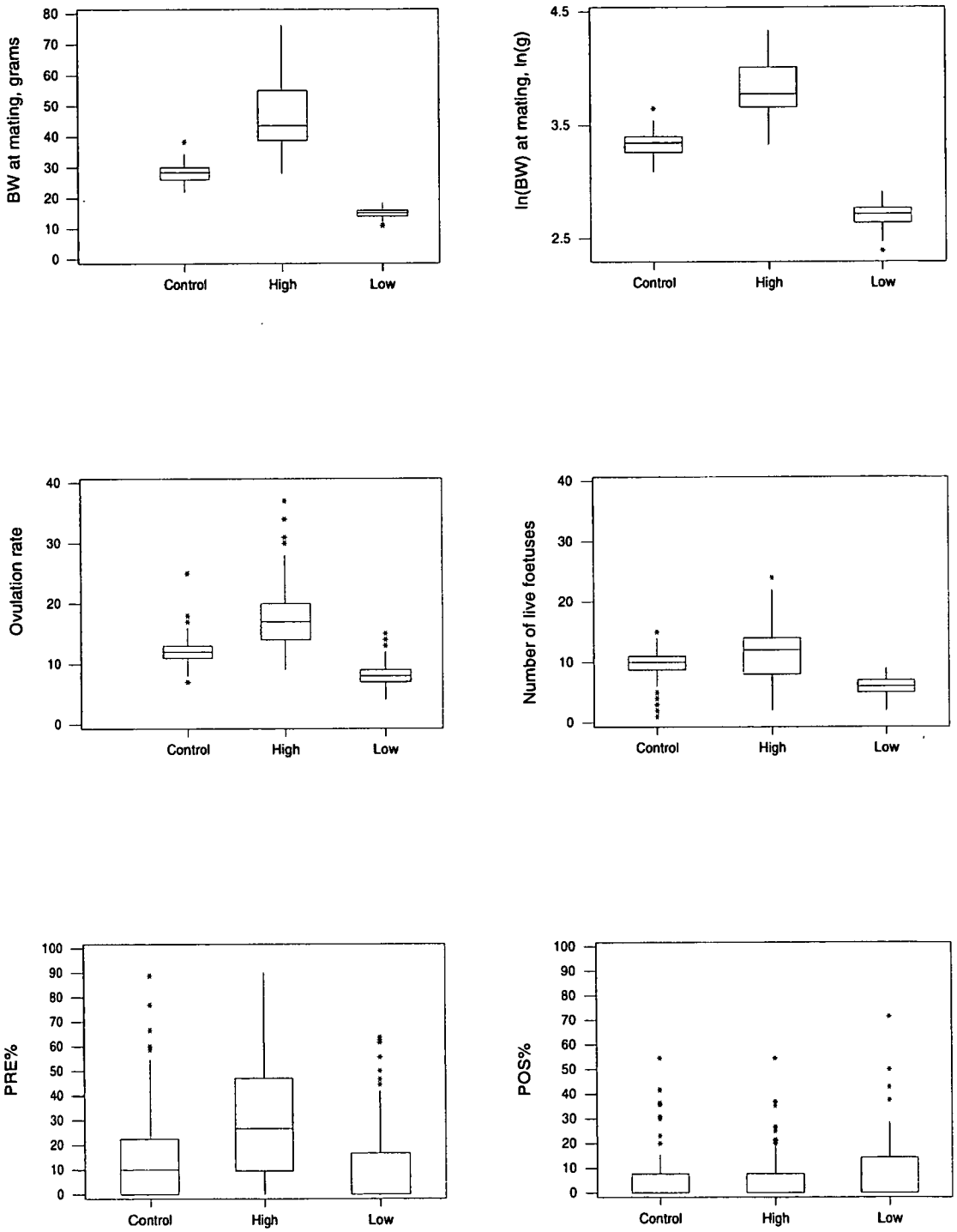


Table 4.3: The least square means for female body weight at mating, ovulation rate, number of live foetuses and losses. Only pregnant females included. Standard errors are given in the brackets

	n	BW, g	OR	LF	PRE	POS	LF%	PRE%	POS%
Control	106	28.09	12.34	9.59	2.21	0.55	77.7	16.5	4.6
		(0.38)	(0.27)	(0.32)	(0.33)	(0.11)	(2.1)	(1.9)	(1.0)
High	207	47.42	17.77	11.04	5.89	0.84	62.1	31.7	7.2
		(0.31)	(0.22)	(0.25)	(0.27)	(0.09)	(1.9)	(1.5)	(0.9)
Low	134	15.09	8.03	6.30	1.10	0.63	78.5	11.6	9.3
		(0.37)	(0.26)	(0.29)	(0.32)	(0.10)	(2.0)	(1.7)	(1.1)
DAH	28	54.68	18.65	12.06	5.34	1.25	64.8	28.4	6.8
		(0.75)	(0.53)	(0.63)	(0.64)	(0.22)	(4.1)	(3.8)	(1.9)
DUH	26	58.51	22.59	10.70	11.36	0.52	48.8	48.9	2.3
		(0.83)	(0.58)	(0.70)	(0.71)	(0.25)	(4.5)	(4.2)	(2.1)
RAH *	30	54.88	18.76	11.32	6.75	0.69	60.3	36.0	3.7
		(1.08)	(0.72)	(0.82)	(0.90)	(0.24)	(5.1)	(4.3)	(2.4)
EDH	34	41.32	18.36	13.35	4.57	0.43	73.7	23.8	2.4
		(0.75)	(0.53)	(0.64)	(0.64)	(0.22)	(4.1)	(3.8)	(1.9)
BEH **	15	47.35	16.38	9.96	5.67	0.75	61.1	34.6	4.6
		(1.58)	(1.05)	(1.20)	(1.32)	(0.35)	(8.4)	(6.1)	(3.4)
MUH	32	37.31	16.86	9.07	6.69	1.10	55.6	38.0	6.4
		(0.70)	(0.49)	(0.59)	(0.60)	(0.21)	(3.8)	(3.5)	(1.8)
ROH	42	38.18	14.68	11.55	1.98	1.15	79.1	12.8	8.2
		(0.65)	(0.46)	(0.55)	(0.56)	(0.19)	(3.6)	(3.3)	(1.7)
EDL	27	14.48	7.31	5.43	0.82	1.06	73.6	11.6	14.8
		(0.81)	(0.57)	(0.69)	(0.70)	(0.24)	(4.4)	(4.1)	(2.1)
BEL	33	15.10	9.87	7.24	2.26	0.37	76.7	19.6	3.6
		(0.76)	(0.53)	(0.64)	(0.65)	(0.22)	(4.1)	(3.8)	(1.9)
MUL	31	14.99	7.51	6.19	0.60	0.72	83.3	7.1	9.6
		(0.70)	(0.50)	(0.60)	(0.60)	(0.21)	(3.8)	(3.5)	(1.8)
ROL	43	15.52	7.24	6.22	0.60	0.42	86.4	8.0	5.7
		(0.61)	(0.43)	(0.51)	(0.52)	(0.18)	(3.3)	(3.1)	(1.6)

Models y = mean + batch + group + group(line) + error
y = mean + batch + line + batch*line + error

* = results from only two batches
** = results from only one batch

BW = body weight of female at mating
OR = ovulation rate
LF = number of live foetuses
PRE = pre-implantation losses
POS = resorptions and moles
LF% = live foetuses as a proportion of OR
PRE% = pre-implantation losses as prop. of OR
POS% = post-implantation losses as prop. of OR

Table 4.4: The divergence between high and low groups means and the divergence from the control. Standard errors in brackets.

group	n	BW, g	OR	LF	PRE	POS	LF%	PRE%	POS%
H - L		32.33	9.74	4.74	4.79	0.21	-16.4	20.1	-2.1
		(0.48)	(0.34)	(0.38)	(0.42)	(0.13)	(2.8)	(2.3)	(1.4)
H - C		19.36	5.49	1.45	3.76	0.28	-15.6	15.5	1.2
		(0.51)	(0.36)	(0.41)	(0.44)	(0.14)	(2.7)	(2.4)	(1.5)
L - C		-13.00	-4.25	-3.29	-1.03	0.07	0.8	-4.6	3.3
		(0.55)	(0.39)	(0.43)	(0.47)	(0.15)	(2.9)	(2.6)	(1.6)

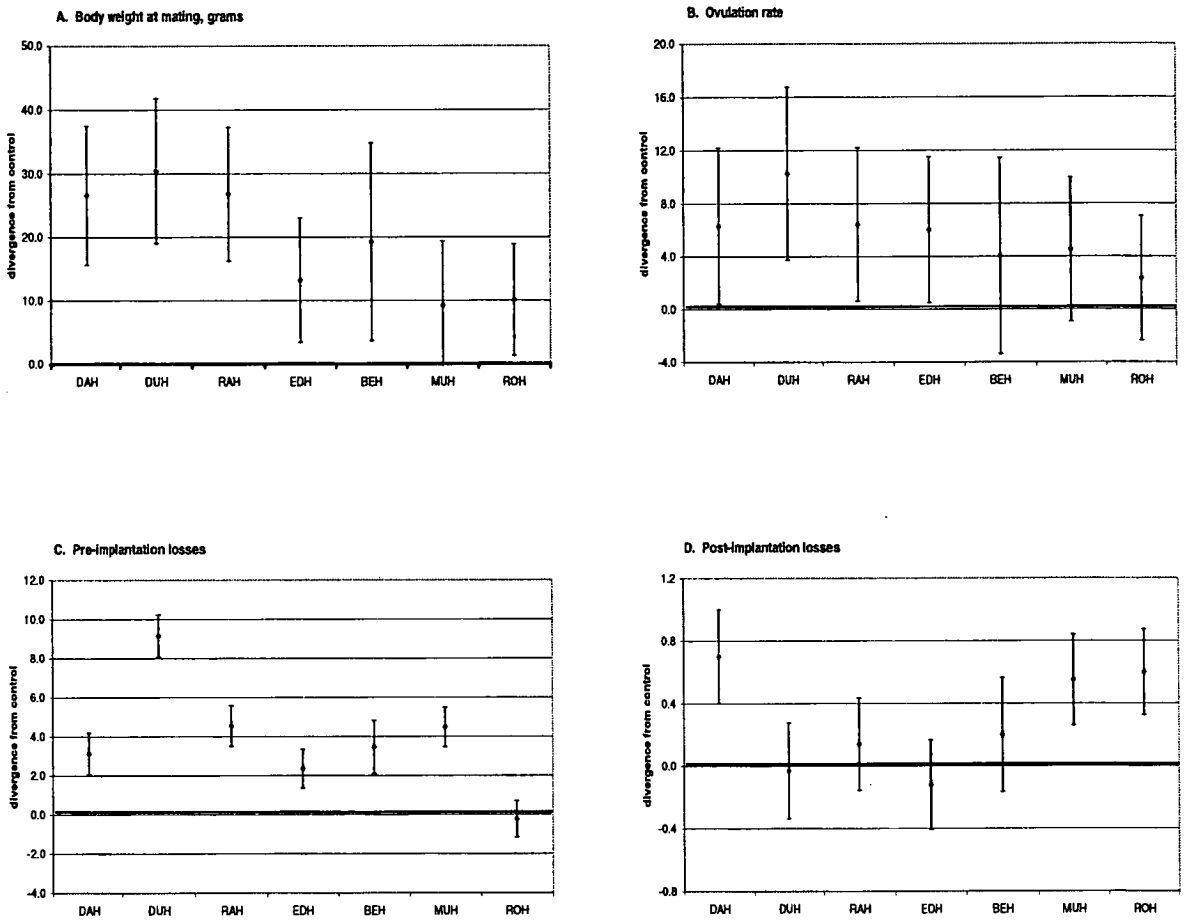
Model: mean + batch + group + group(line) + error

Table 4.5: Coefficient of variation (%) in the control, high and low groups and within each line.

	n	BW, g	OR	LF	PRE	POS
Control	106	10.5	17.6	28.3	145.7	178.9
DAH	28	12.9	25.1	35.6	80.0	147.0
DUH	26	9.4	29.9	48.1	69.4	191.3
RAH	30	16.5	14.3	52.2	81.3	155.7
EDH	34	8.2	22.3	28.6	102.7	179.2
BEH	15	10.5	16.8	35.9	51.3	185.7
MUH	32	7.4	27.4	40.8	64.3	128.6
ROH	42	14.6	17.7	27.8	133.5	110.3
EDL	27	8.7	16.5	31.9	133.1	119.6
BEL	33	9.6	24.7	25.0	113.4	225.7
MUL	31	9.6	18.8	25.5	177.1	178.7
ROL	43	9.5	15.0	17.5	125.5	182.1

Number of live foetuses The number of live foetuses in the uterus was highest in the high body weight lines and lowest in the low body weight lines (figure 4.2). The differences between groups were much smaller compared to the figure of ovulation rate in the groups, but still significant. The difference between high and low ovulation rate was 9.7 ova, but just 4.7 live foetuses (table 4.4). The average number of live foetuses in the control line was 1.5 foetuses lower than in the high line group and 3.3 embryos larger than in the low line group. The coefficient of variation increased within all lines compared with CV% of ovulation rate, most in the rather heterogeneous high line group (table 4.5). The most constant group

Figure 4.3: Divergence from the control based on the least square means for high body weight lines with batch and line in the model. 95% confidence interval was calculated by using Welch test. The zero line represents the control line, which was used as a reference point.

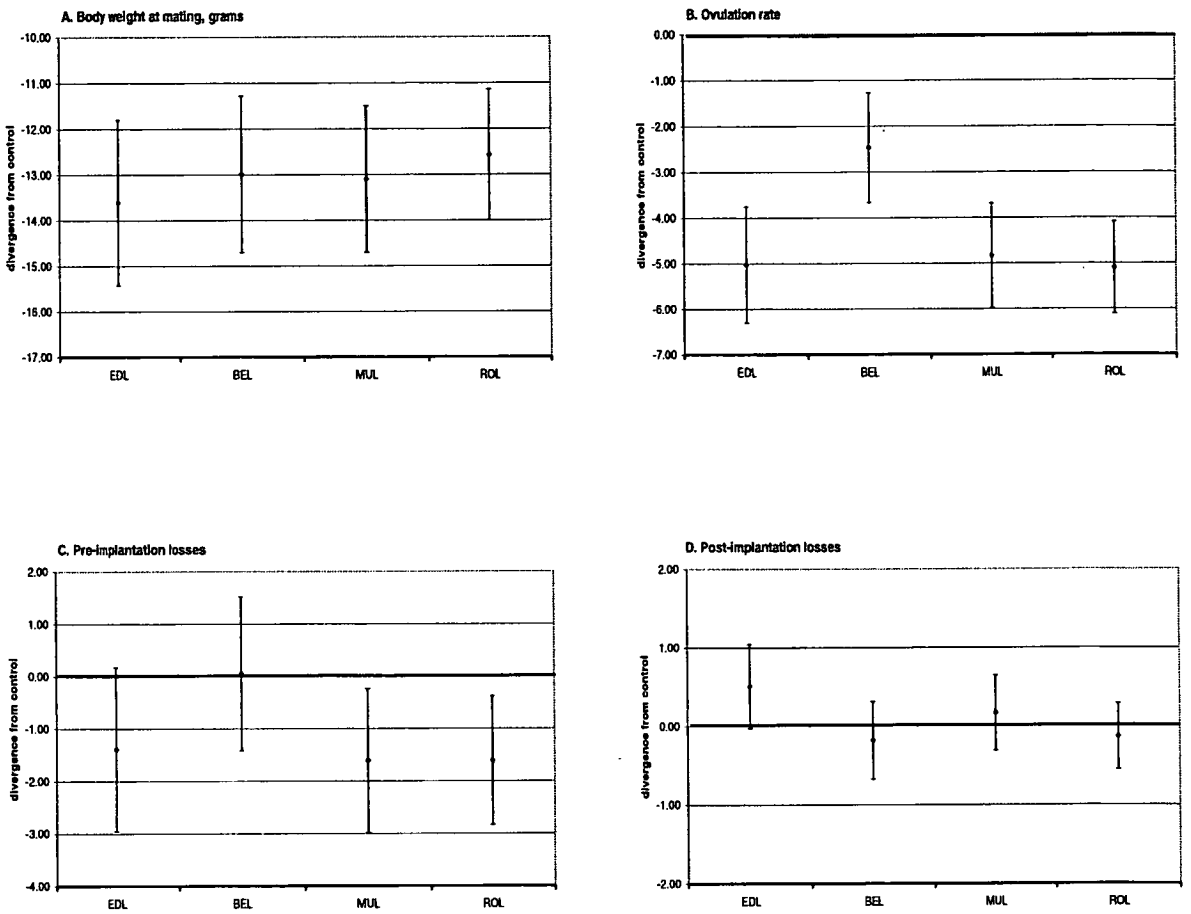


seemed to be the low line group.

Among the high body weight females the highest number of live foetuses was found in EDH line and the lowest in MUH, the difference being 4.3 foetuses, which is considerably more than the difference in ovulation rate, 1.5 ova. In the low body weight group the difference was 1.8 between the largest and lowest mean number of live foetuses (table 4.3). In both of the groups only few significantly different line means were observed in the pairwise analysis.

The number of live foetuses depends on the ovulation rate, thus it is more informative to express the number as a proportion of the ovulation rate (LF%).

Figure 4.4: Divergence from the control based on the least square means for low body weight lines with batch and line in the model. 95% confidence interval was calculated by using Welch test. The zero line represents the control line, which was used as a reference point.



This value gives a percentage of eggs developing to live foetuses. The high lines had a higher number of live foetuses than the control, but the survival percentage was significantly lower (table 4.4). However, the low line group had as good LF% as the outbred control group. The survival of eggs varied between 49% and 79% in the high line group, while in the low line group the range was from 74% to 86% (table 4.3). Among the high lines the LF% was the lowest in the heaviest mice, but similar relationship with the weight was not observed in the low line group.

Pre- and post-implantation losses The absolute values of pre-implantation losses were significantly higher in the high body weight group than in the control

or in the low body weight group (table 4.3). The difference in pre-implantation losses between the high and low mean was 4.8 ova, while the control line females had on average one more egg lost than the females from the low body weight group (table 4.4). The coefficient of variation of the pre-implantation losses was high in all lines (table 4.5).

The post-implantation losses were on average largest in the high line group and smallest in the control (table 4.3). The divergence from control in average post-implantation losses was not found to be different between high and low body weight groups (table 4.4).

Similarly to the life foetuses the losses are more comparable between the groups if they are expressed relative to the ovulation rate. In PRE% differences between the groups are clear, but in POS% not so clear (figure 4.2). However, the low line had on average larger POS% than control (significant at 4% level) and slightly higher POS% than in the high line group (table 4.4).

In the high line group the PRE% varied from 13% to 49%, being on average largest in the heaviest females (table 4.3). The low line group was more homogeneous with PRE% ranging from 7% to 12%. The more equal behaviour within low line group compared with the high line group can be seen by comparing the figures 4.3 and 4.4. The divergence from the control for pre- and post-implantation losses was nearly equal between the low lines, while there was a variation between the lines in the high line group.

In the high body weight lines 204 females out of 207 had at least one egg shed from ovaries, but which did not develop into a foetus. This might be affected by the difficulty of counting some of the high body weight corpora lutea, since the ovaries were not as clear in the high body weight females as in the low body weight females or controls. However, the occurence of pre-implantation losses in the group of high lines was very high (99%) compared with 54% occurence of pre-implantation losses in the low body weight females and 71% in control.

In general there were rather few post-implantation losses, particularly foetuses which died in the later stages of the gestation, i.e. moles. A percentage of females having lost one or more foetuses was calculated (figure 6.1). About 5% of the control females had moles and around 28% had resorptions. The low lines had on average a smaller proportion of females with resorptions (32%), than the high

line females (38%) and the losses per female were fewer (except in EDL line). The number of females having lost foetuses in later stage (moles) was large in the low lines (around 6-8%) compared with the 5% in the control. However, ROL line did not have any moles at all (figure 6.1 B). The occurence of moles varied in the high body weight group from 3% up to 12%.

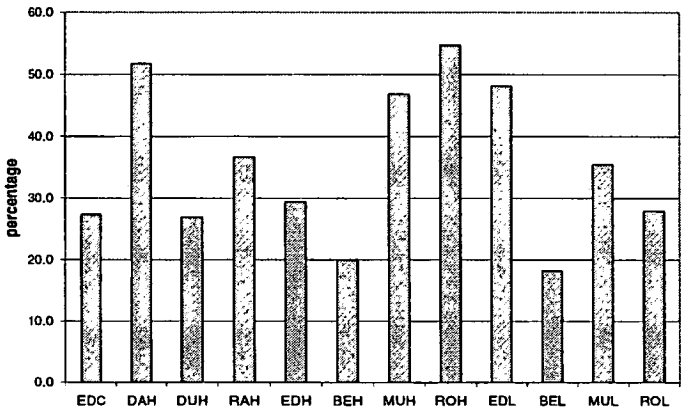
4.3.1.1 Divergently selected line pairs

The control line used in this study was a "true" control only for the Edinburgh lines, since they were derived from the same base population. In the absence of a real control, the high and low line pairs derived from the same population can act as controls for each other. Four divergently selected line pairs from the same base were available. The Berlin high and low pair was compared only for the first batch, since there were no observations from BEH in later batches. Thus the Berlin comparisons are not contemporaneous with the others.

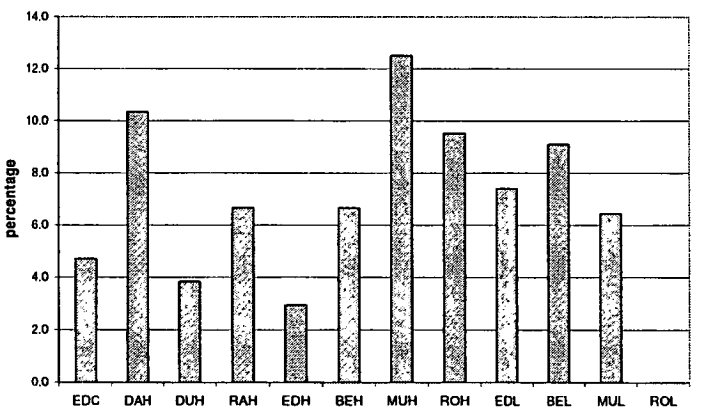
The most diverged pair in terms of body weight was the Berlin high and low (30g), Edinburgh pair the next (27g) and the other two line pairs had a similar divergence (22g) (table 4.6). The scale effect transformation to natural logarithms, did not affect the results. For Edinburgh lines the body weight divergence from the control was equal in both directions, but after transformation the low line divergence from the control was double the high line divergence.

Figure 4.5: The percentage of females with one or more resorptions (figure A) and moles (figure B) from the total number of dissected pregnant females in each line.

A.



B.



The ovulation rate diverged the most in Edinburgh lines by 11.1 ovas and the divergence from the control was similar in both directions. A large divergence of OR was expected because of the difference in body weights. Suprisingly, the Berlin line pairs had the lowest divergence in OR, despite the large body weight divergence. This was caused by the significantly larger OR in the BEL line compared with other low lines, while BEH had an intermediate ovulation rate in its group.

The divergence in number of live foetuses was significantly higher in Edinburgh lines than the other pairs. The other pairs did not diverge significantly. The survival of eggs to foetuses (LF%) was 74% in both Edinburgh lines, while in the other pairs the low line had always better survival of the eggs. In two of the pairs (Edinburgh and Roslin) the divergence of PRE seemed to behave in an opposite manner to the divergence of LF, i.e. large divergence in LF together with small divergence in PRE. In general the high body weight lines had more pre- and post-implantation losses than their low body weight line pair.

Table 4.6: Comparisons of the high and low body weight lines which are derived from the same base population. Only pregnant females included.

	BW (g)	ln(BW)	OR	LF	PRE	POS
range of s.e.	0.7-1.3	0.2-0.3	0.5-0.9	0.6-1.0	0.6-0.9	0.2-0.3
EDH-EDL	26.8	1.05	11.05	7.92	3.75	-0.63
EDH-EDC	13.2	0.39	6.02	3.76	2.36	-0.12
EDL-EDC	-13.6	-0.66	-5.03	-4.16	-1.39	0.51
MUH-MUL	22.3	0.91	9.35	2.88	6.09	0.38
ROH-ROL	22.7	0.89	7.44	5.33	1.38	0.73
BEH-BEL	30.8	1.15	4.62	2.10	2.18	0.36

4.3.2 Pregnancy rates and fertility problems

The pregnancy rate was calculated as a ratio of pregnant females to all mated females (table 4.7). The pregnancy rate of control animals was 97%, which was significantly higher than in the high line group (82%) and in the low line group (79%). The high and low body weight group pregnancy rates did not differ significantly from each other. The pregnancy rate varied from 63% to 94% among the high lines (table 4.7) with the lowest percentage in the extremely heavy DUH

line. The average pregnancy rate in the low body weight group was especially affected by MUL line which had significantly lower pregnancy rate compared with the other low body weight lines.

In this experiment it was not possible to estimate the effect of the male on the pregnancy rate. One way to take into account the male was to include only those females which had an observed vaginal plug. In this case it was sure that the mating had occurred, i.e. the male was capable of copulation. However, this method does not address the question of the possible effect of reduced sperm quality. Among plugged females the pregnancy percentage was similar in all groups, varying from 94% in the high body weight group to 99% in the control. Problems with libido could therefore explain the different pregnancy rates among all females and those who had evidence of copulation, despite the fact that some of the vaginal plugs were not observed. However, the number of not observed vaginal plugs among pregnant females was rather low.

Table 4.7: Number of females with vaginal plug among all dissected females (N), the pregnancy rate (PR %) and the number of pregnant (P=1) and non-pregnant ovulating females (P=0) among those plugged. The total number of pregnant and non pregnant females is shown in brackets. The number of females with vaginal plug observation but in an early stage of gestation when dissected (Early) and average days from mating to vaginal plug observation (Days)

			VP=1				
line	N	PR%	n	P=1 (tot)	P=0 (tot)	Early	Days
Control	109	97	90	88 (106)	2 (3)	6	3.7
High	255	82	179	165 (207)	14 (48)	34	4.3
Low	169	79	110	105 (134)	5 (35)	5	4.3
DAH	34	85	27	23 (28)	4 (6)	7	3.5
DUH	41	63	23	21 (26)	2(15)	2	5.7
RAH	32	94	26	25 (30)	1 (2)	4	3.8
EDH	38	89	24	22 (34)	2 (4)	6	4.3
BEH	18	83	10	10 (15)	0 (3)	8	3.5
MUH	42	76	26	26 (32)	0(10)	1	5.8
ROH	50	84	43	38 (42)	5 (8)	6	3.7
EDL	30	90	27	25 (27)	2 (3)	4	3.8
BEL	47	70	26	24 (33)	2(14)	0	4.0
MUL	47	66	25	25 (31)	0(16)	0	6.1
ROL	45	96	32	31 (43)	1 (2)	1	3.2

Fertility problems: Two types of fertility problems were observed: group of ovulating females which did not become pregnant and a group of females which did not ovulate at all. The reason for failure of the first group remains unknown, since the the infertility might be due to male or female. It was not known what caused the non functioning ovaries or if they had ever had any function.

Out of all dissected females 1% in the control and 5% in the low line group were not pregnant but had been ovulating. The percentage was largest in the high line group, 11%. DUH, the extremely heavy line, had around 40% not pregnant and ovulating females. In the low line group rather few ovulating and not pregnant females were dissected per line.

The percentage of non-ovulating females was similar in control (3%) and high line (3%) groups, while in the low line group 16% of all dissected females were not ovulating. Two of the low lines had a problem with not functioning ovaries namely BEL and MUL. If the not ovulating females were excluded from data, the pregnancy rate in the low line increased to 94% and was significantly higher from the high body weight group (88%), but still lower than in the control.

Another sign of fertility failure arised from the possibly terminated pregnancies before full term. This group could be divided into two parts, i.e. those females who were not pregnant when dissected and those who had a vaginal plug (VP) observation but were not at a late (15-18 days) gestation stage on the estimated day of dissection. Some of the non-pregnant females had a vaginal plug observation, thus probably these pregnancies were terminated for some reason and on the day of dissection the condition of the uterus was intepreted as empty (table 4.7). For the females who were not at the end of gestation as expected on the basis of the vaginal plug observation, it was not possible to say if those pregnancies at early stages would have lasted to full term. Most of the early stage pregnancies were observed in the high line group. This suggests that high lines females might have some difficulties to carry the pregnancies to full term.

Cycle length: The normal cycle length of female mice is around five days (Snell, 1941), thus copulation could be expected to happen within those five days from pairing if both parties have a normal reproductive performance (cycle or libido). The number of days from mating to observation of vaginal plug was calculated in order to examine the differencies between outbreds and inbreds. The control

line females were plugged on average 3.7 days after the mating (table 4.7). The average was 4.3 days in both high and low line groups, which indicates that inbreeding might have an effect on the cycle or the libido. When inbred lines were considered individually there were clear differencies between the lines. Some of the selection lines had times from pairing to copulation over the normal five days, which further suggests abnormal copulation behaviour.

4.3.3 Regression analysis

In order to study the relatiosnhip between the body weight and components of reproductive performance a regression analysis was done. First the regressions were examined in size groups and later in individual lines within size groups.

4.3.3.1 Regressions on body weight in groups

Ovulation rate: All the regressions of ovulation rate on body weight were significantly different from zero, but the regression coefficients on body weight were not different between the low line group and the control. The estimate was on average 0.3 ova/g for the low line group and the control (table 4.8). In the high line group the regression was 0.1 ova/g and significantly lower than in the other groups. After log transformation of the body weight, the regressions were equal in high and low line groups and not significantly different from the regression estimate of the control.

The divergence between control and low line group was around 13g and 4 ova (table 4.4), which would be explained by the observed regression. In the high line group the divergence observation does not agree so well with the regression estimate. The divergence from the control was 19.4g and 5.5 ova (table 4.4), thus a higher regression coefficient than 0.1 would be needed to explain the difference.

When the ovulation rate was plotted against body weights for all high body weight females, different patterns were seen (figure 4.6). The females with average body weight had stronger relationship between body weight and ovulation rate than those with very heavy body weight. Due to the similar body weights in all low body weight females such patterns as in the high body weight group were not seen when data were plotted (figure 4.7). It was suspected the lower regression in high body weight group might be due to the scale effect, therefore a transfor-

Table 4.8: Within group regression coefficients on the female body weight (g) at mating with the standard errors. The coefficients which were significant at 5% level are marked with bold.

Model A: batch + line + β * BW

	OR	LF	LF%	PRE	POS
C	**0.28** ± 0.07	**0.21** ± 0.09	-0.07 ± 0.75	0.06 ± 0.10	0.01 ± 0.04
H	**0.11** ± 0.05	0.01 ± 0.05	-0.32 ± 0.30	0.07 ± 0.06	0.03 ± 0.02
L	**0.35** ± 0.10	0.13 ± 0.10	-1.59 ± 1.21	**0.22** ± 0.10	0.00 ± 0.07

Model B: batch + line + β * ln(BW)

	OR	LF	LF%	PRE	POS
C	**8.0** ± 2.1	**6.1** ± 2.7	-0.9 ± 21.4	1.6 ± 2.9	0.3 ± 1.2
H	**5.5** ± 2.3	2.1 ± 2.7	-9.5 ± 14.4	2.5 ± 3.0	0.9 ± 0.8
L	**5.2** ± 1.5	1.9 ± 1.5	-23.7 ± 17.9	**3.3** ± 1.4	0.0 ± 1.0

mation for the body weight was done. When natual logarithm transformed body weights were used the regressions between the groups were not significantly different on 5% level according to the t-test. The transformation had largest effect for the high body weight group, as expected (table 4.8).

Number of live foetuses and losses: The regressions of the number of live foetuses in control and of pre-implantation losses in low line group on body weight were significant (table 4.8). After body weight transformation the regressions of live foetuses were equal in high and low line groups and smaller than in the control. Otherwise changes in the body weight had no significant effect on the remaining traits. However, the increase in body weight was connected with increasing number of live foetuses and pre-implantation losses. The regression of pre-implantation losses on body weight was clearly highest in the low body weight group, but not significantly different from the other groups. The low body weight females lost 0.2 ova/g, which is over double the losses in the other lines. However, because the group was selected for low body weight regression of losses can be seen as negative, i.e. one gram reduction in body weight reduces the losses by 0.2 ova. The body weight had the smallest effect on the post-implantation losses, i.e. post-implantation losses were not affected by the changes in body weight.

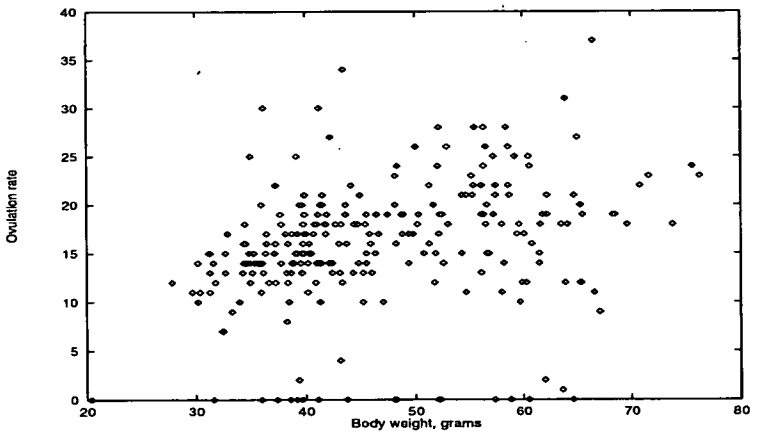
The regression coefficients of ovulation rate on body weight were much larger

in all groups than the regression of number of live foetuses. For the total data set regression of LF% on body weight was -0.3 (s.e. 0.2), which indicates that the larger the body weight the less foetuses are achieved from shed eggs. The regression was not significant in any group, but all coefficient were negative and larger among the inbred lines than in the control.

4.3.3.2 Regressions on body weight within the high line group

Regression on body weight was estimated for each line separately to further assess the relationship between body weight and reproductive traits (table 4.9). The regression coefficients of ovulation rate on body weight varied from 0.1 to 0.5 ova/g. The line regressions did not differ from each other at the 5% level, nor from the control line. The log transformation of body weight did not alter much the results already observed from the untransformed data set. The regression coefficient of the DUH line, one of the extremely heavy lines, was most affected by the scale effect. The regression on ln(BW) became larger than in other lines so that it significantly differed from the control line regression but not from the other selection lines.

Figure 4.6: Ovulation rate plotted against the body weight (g) at mating for high body weight group females



The average within line regression of ovulation rate, environmental regression, was 0.3 ± 0.1 ova/g and for transformed body weight 14.2 ± 2.6 ova/ln(g). Both of these values were much larger than the overall regression for high body weight

group (0.1 ± 0.1 and 5.5 ± 2.3). To further examine the situation a between line regression of ovulation rate on body weight, genetic regression, was calculated using the line means of the traits as data points. The between line regression on body weight was 0.2 ± 0.1 and on transformed body weight 10.2 ± 3.9, thus the average within and between line regressions were of similar magnitude. The lower regression obtained from the group data was mostly due to two heavy lines with slightly lower ovulation rate than expected (DAH and RAH). The genetic regression (between line) was not significantly different from the estimate for the control line and suggest that relationship between ovulation rate and body weight is rather similar in outbred controls and in inbred heavy lines.

Table 4.9: The regression coefficients of ovulation rate, number of live foetuses and losses on body weight at mating with standard errors for the high body weight lines. The coefficients which were significant at 5 % level are marked in bold.

Model A: batch + β^* BW + e

	OR	LF	PRE	POS
Control	**0.28** ± 0.07	**0.21** ± 0.09	0.06 ± 0.10	0.01 ± 0.04
DAH	0.09 ± 0.14	-0.13 ± 0.13	0.16 ± 0.12	0.07 ± 0.06
DUH	**0.43** ± 0.10	0.30 ± 0.18	0.13 ± 0.14	-0.01 ± 0.02
RAH	**0.17** ± 0.06	-0.09 ± 0.14	0.23 ± 0.13	0.04 ± 0.03
EDH	**0.54** ± 0.23	-0.04 ± 0.24	0.56 ± 0.29	0.02 ± 0.06
BEH	**0.32** ± 0.11	**0.47** ± 0.14	-0.21 ± 0.12	0.06 ± 0.06
MUH	0.35 ± 0.30	0.30 ± 0.26	0.05 ± 0.30	0.00 ± 0.09
ROH	**0.21** ± 0.08	0.03 ± 0.11	0.18 ± 0.12	0.01 ± 0.05

Model B: batch + β^* ln(BW) + e

	OR	LF	PRE	POS
Control	**8.0** ± 2.1	**6.1** ± 2.7	1.6 ± 2.9	0.3 ± 1.2
DAH	5.5 ± 7.6	-6.8 ± 6.9	8.8 ± 6.6	3.5 ± 3.1
DUH	**26.1** ± 6.1	18.1 ± 10.9	8.3 ± 8.6	-0.3 ± 1.5
RAH	**9.8** ± 3.2	-3.9 ± 8.0	11.7 ± 7.5	1.9 ± 1.5
EDH	**22.9** ± 8.8	1.2 ± 8.9	21.4 ± 11.8	0.3 ± 2.2
BEH	**13.2** ± 4.8	**19.9** ± 6.4	-9.5 ± 5.0	2.8 ± 2.5
MUH	13.7 ±11.3	11.3 ± 9.9	2.2 ± 11.2	0.2 ± 3.7
ROH	**8.1** ± 3.1	1.3 ± 4.1	7.0 ± 4.4	-0.1 ± 2.0

The regression of number of live foetuses on body weight differed significantly from zero only for two lines (table 4.9). None of the line regressions differed from the control when 95% confidence intervals were considered. The transformation of body weight resulted in a large increase in the regression coefficient in DUH line compared with the other lines. The regression coefficient on untransformed body weight was the same in DUH and MUH lines, but after transformation there was a difference of 6.8 LF/ln(g).

The average within line regression was 0.1 ± 0.1 LF/g on body weight and 5.8 ± 3.0 LF/ln(g) on transformed body weight. Both of these estimates were larger than the overall high line group regressions of LF (table 4.9), as seen also for regression of ovulation rate. The between line regression calculated from the line means on body weight was -0.02 ± 0.03 LF/g and on transformed body weight 0.8 ± 3.4 LF/ln(g).

Regressions of the losses were all non-significant and did not differ either from that in the control or from each other. In particular, the regressions of post-implantation losses were neglible. The losses did not seem to be affected at all by the scale effect like OR and LF.

There were two lines which behaved in a different manner to the others, namely DUH and EDH. The regression coefficients of ovulation rate were much higher than for the other lines and the same was observed after the transformation of body weight. Among the four moderately heavy lines EDH had the highest mean ovulation rate and number of live foetuses, but second highest body weight. The heavy DUH line had very large mean ovulation rate. Some of the heaviest females had wide range in ovulation rate, which most likely had an effect on the fit of the linear regression line. However, the number of observations per line was quite small and therefore more detailed analysis within individual line would not be sensible.

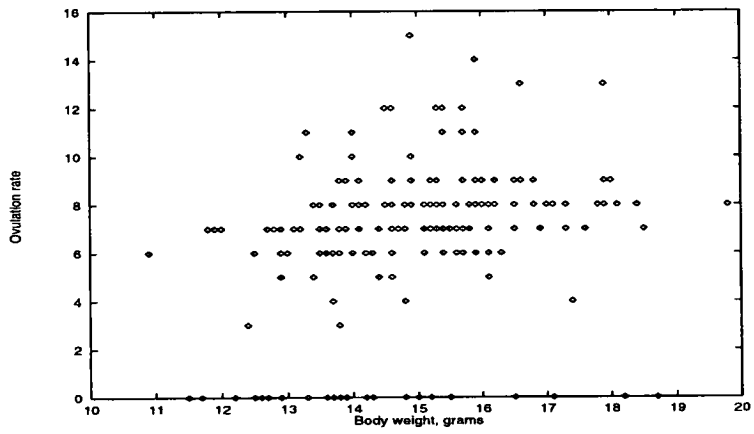
4.3.3.3 Regressions on body weight within low line group

All the regressions of ovulation rate on body weight were positive, as expected, i.e. the larger the body weight the higher the ovulation rate (table 4.10). The regression coefficients of ovulation rate on body weight were not significantly different between the lines or from the control. The transformation of body

weight did not affect the results, which was expected as there was only little variation on body weight within or between the lines.

The average within line regression of ovulation rate on body weight for low lines was 0.4 ova/g, which is similar to the one observed for the whole low line group. The average regression on transformed body weight was also similar but slighly larger (5.9 ova/ln(g)) than the one for whole low line group (5.2). The between line regressions calculated using the mean values of each line were 0.3 ± 0.1 on body weight and 5.1 ± 1.5 on transformed body weight.

Figure 4.7: Ovulation rate plotted against the body weight (g) at mating for low body weight group females



Three of the lines had a significant regression of number of live foetuses on body weight, but this dropped to two lines when transformation for the body weight was made. Only one of the lines had a strong negative regression of LF and the rest a positive one. The average regression coefficient over lines was 0.1 LF/g on body weight and 3.0 LF/ ln(g) on transformed body weight. The between line regression of number of live foetuses was not significantly different from zero on body weight or transformed body weight. This agrees with the result from the low line group regression analysis.

The BEL line had very much larger regression coefficient of pre-implantation losses on body weight than the other lines. This was due to a few heavy BEL females who had large losses and consequently low number of live foetuses. It was also the only line with significant regression of LF% on body weight, -9.7 (s.e.

Table 4.10: The regression coefficients of ovulation rate, number of live foetuses and losses on body weight at mating with standard errors for the low body weight lines. The coefficients which were significant at 5 % level are marked in bold.

Model A: batch + β^* BW + e

	OR	LF	PRE	POS
Control	**0.28** ± 0.07	**0.21** ± 0.09	0.06 ± 0.10	0.01 ± 0.04
EDL	0.28 ± 0.18	0.20 ± 0.30	-0.06 ± 0.20	0.15 ± 0.19
BEL	0.56 ± 0.28	**-0.46** ± 0.21	**0.97** ± 0.27	0.05 ± 0.09
MUL	0.35 ± 0.19	**0.70** ± 0.22	-0.08 ± 0.18	-0.27 ± 0.20
ROL	**0.38** ± 0.11	**0.26** ± 0.12	-0.03 ± 0.08	0.15 ± 0.09

Model B: batch + β^* ln(BW) + e

	OR	LF	PRE	POS
Control	**8.0** ± 2.1	**6.1** ± 2.7	1.6 ± 2.9	0.3 ± 1.2
EDL	3.9 ± 2.4	2.6 ± 4.1	-0.7 ± 2.7	1.9 ± 2.5
BEL	**8.6** ± 4.2	-6.3 ± 3.2	**14.2** ± 4.1	0.7 ± 1.3
MUL	5.0 ± 2.8	**10.1** ± 3.2	-1.2 ± 2.6	-3.9 ± 3.0
ROL	**5.8** ± 1.7	**3.9** ± 1.9	-0.3 ± 1.2	2.3 ± 1.3

2.5). Apart from the BEL line, the other low body weight lines did not differ from each other or from the control line regression. All the other selection lines had a small negative regression of PRE on body weight.

4.3.3.4 Regressions on ovulation rate

The number of live foetuses obviously depends on the number of ova shed and the same applies for the pre-implantation losses. Within group regression analysis was done to study this relationship and possible differences between the groups (table 4.11). A regression of live foetuses on ovulation rate was highly significant in all groups, ranging between 0.3 and 0.4, being the lowest in control and highest in the low body weight group. However, the within group regressions were not significantly different from each other. An increase in ovulation rate was connected with a large increase in pre-implantation losses in all groups, thus there was not much gain in terms of the number of live foetuses from more shed eggs.

The within line regressions of number of live foetuses on ovulation rate were all positive in both high and low line groups (table 4.12). Among the high lines,

Table 4.11: Within group regression coefficients on the ovulation rate with the standard errors. The coefficients which were significant at 5 % level are marked with bold.

Model: batch + line + β * OR

	LF	PRE	POS
Control	**0.29** ± 0.12	**0.73** ± 0.10	-0.02 ± 0.05
High	**0.35** ± 0.08	**0.65** ± 0.08	0.01 ± 0.02
Low	**0.37** ± 0.08	**0.60** ± 0.07	0.03 ± 0.06

the regression coefficients had a wide range from 0.1 to 1.1 LF/ova and among the low lines from 0.1 to 0.8 LF/ova. The average of the high and low line coefficients were both around 0.6 (s.e. 0.1), i.e. much larger than the group regressions.

The within group regressions of pre-implantantion losses on ovulation rate were significant and varied from 0.6 lost eggs per ova in low body weight group to 0.7 in control. Post-implantation losses did not change significantly together with the number of ovas shed, thus the loss of foetuses seems to happen more independently of the female than the losses of eggs.

Only in one line, BEH, was an increased ovulation rate connected with decreasing pre-implantation losses. In all heavy lines, except BEH, the pre-implantation losses increased with ovulation rate with an average regression of 0.4 PRE/ova (table 4.12). Among the heaviest three lines the change of pre-implantation losses was smaller than the change in number of live foetuses, while in the other high lines the regression of PRE was much larger than the regression of LF. In the low lines the coefficients were variable between the lines, having an average of 0.4 PRE/ova. Post-implantation losses had on average small regression coefficients on ovulation rate, being either positive or negative.

In all line pairs the regression coefficients of LF on OR were of different magnitude. In two of those pairs the regression in the high lines was small and in the low line large, e.g. in the Edinburgh pair 0.1 for the high line and 0.8 for the low line. Thus in the small lines the increase in ovulation rate was profitable in terms of number of live foetuses, while in the heavy lines fewer foetuses per extra ova were gained. The exception was the Berlin pair, where the heavy line was associated with an increasing number of foetuses due to increasing OR.

Table 4.12: The within line regressions on ovulation rate with standard errors. The coefficients marked with bold were significant at 5% level

Model: batch + β^* OR + e

	LF	PRE	POS
Control	**0.29** ± 0.12	**0.73** ± 0.10	-0.02 ± 0.05
DAH	**0.56** ± 0.15	**0.40** ± 0.16	0.04 ± 0.08
DUH	**0.95** ± 0.22	0.09 ± 0.23	-0.04 ± 0.04
RAH	0.68 ± 0.40	0.29 ± 0.41	0.03 ± 0.08
EDH	0.12 ± 0.23	**0.91** ± 0.17	-0.03 ± 0.04
BEH	**1.08** ± 0.25	-0.23 ± 0.25	0.15 ± 0.11
MUH	0.29 ± 0.16	**0.65** ± 0.13	0.06 ± 0.06
ROH	0.25 ± 0.20	**0.79** ± 0.18	-0.02 ± 0.10
EDL	**0.84** ± 0.29	0.16 ± 0.22	0.01 ± 0.21
BEL	0.13 ± 0.14	**0.87** ± 0.12	0.00 ± 0.05
MUL	**0.66** ± 0.21	**0.49** ± 0.15	-0.15 ± 0.20
ROL	**0.73** ± 0.11	0.18 ± 0.10	0.09 ± 0.11

4.4 Discussion

This chapter has described components of reproductive fitness and the effect of growth selection on the components in inbred mouse lines. All selection lines had a high inbreeding coefficient (above 90%) at the time of the experiment and the high level of inbreeding in foetuses was maintained by practising full-sib matings. The aim was to examine a range of components of reproductive fitness and their relationship with body weight under the effect of inbreeding. Three size groups were studied, i.e. the high and low body weight groups and an outbred control group with average size. Within group analysis was done to study how constant the relationship between reproductive traits and body weight was in a set of lines with similar size.

The female body weights ranged from 11g to 80g and the high line group was on average twice as heavy as the control and three times heavier than the low line group. As expected, the heavy animals had the largest ovulation rate. The average within line regression, i.e. environmental regression, of ovulation rate on body weight was similar, approximately 0.3 ova/g, in both high and low body weight groups and constant between the lines within a group. The genetic

regression was 0.2 ova/g in the high line group and 0.3 ova/g in the low line group. Similar estimates of genetic regressions have been reported in the literature, e.g. 0.2 ova/g (Falconer and Roberts, 1960) and 0.4 ova/g (Land, 1970). Inbreeding did not seem to affect the relationship between ovulation rate and body weight, since the estimates were not significantly different between the inbred lines and the control, which has also been observed by Falconer and Roberts (1960).

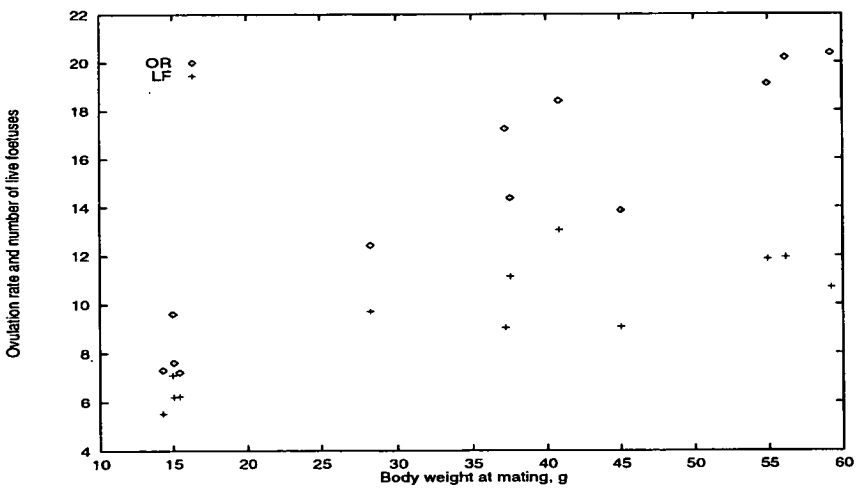
Number of live foetuses was highest in the high line group and lowest in the low line group. However, the difference in number of foetuses was much smaller than the difference in ovulation rates, especially in the high line group. The high level of inbreeding in the high and low groups could have been assumed to affect the reproductive performance, but only the high line group showed reduced survival rate from egg to foetus compared with the control. In high lines the losses mainly happened before implantation, while in low lines approximately the same amount of losses happened before and after implantation. Inbreeding alone was not a likely explanation for the larger losses in the high lines, since similar patterns were not observed in the equally inbred low lines. During the dissections the heaviest lines were observed to have an excess amount of fat. The fatness of the lines was analysed by Bünger et. al. (2001a) and they reported fat percentages of 11% in DUH line and 17% in RAH line, while the average in high lines was 9.5%. Fatness has been has been reported to have an impact on the reproduction due to the altered hormonal functions (Roberts, 1981). Thus the body composition, possibly combined with harmful effects of inbreeding, could explain partly the larger losses in high lines.

The regression coefficients of number of live foetuses on body weight were equal in high and low line groups, but smaller than in control. The coefficients between lines were not as similar as for the ovulation rate in either high or low group, which was also observed about the regressions of pre-implantation losses. This observation was supported by results of Land (1970), who concluded that a consistent positive genetic relationship between body weight and ovulation rate was found, but less consistent negative relation between body weight and embryo survival. Thus the survival of eggs before implantation seemed to be more line specific, but on average reduced by either the inbreeding or growth selection when compared to the control line. Figure 4.8 illustrates the relation of ovulation rate

and number of live foetuses to body weight over a range of weights.

The inbred growth lines had a lower pregnancy rate than the control line and no clear difference between the high and low groups was observed. However, the pregnancy rates were found to be at a similar level in the low line group and in the control, if the non ovulating females were excluded. For some reason two of the low lines had a high occurence of females with not functioning ovaries, while in the high lines the females were suffering more difficulties in carrying the pregnancy to full term. The pregnancy rates found from the inbred lines, on average 80%, were higher than the estimates in the literature, e.g. Snell (1941) reported a 80-90% pregnancy rates in outbred mouse stocks and for inbred lines much lower coefficients have been reported (Fowler and Edwards, 1960). Estimating of impact of the male on pregnancy rates was not possible in this experiment, but some indication was found about general lower libido in the selection groups. Fowler and Edwards (1960) reported a large mouse line where the infertility was due to the males, while the female fertility was unaffected. However, the contribution of males reproductive fitness is likely to be much smaller than the one from females, e.g. ovulation rate seems to be completely a female trait.

Figure 4.8: Mean ovulation rate and number of live foetuses from each line plotted against the mean body weight of the line



Few families were found among the selection lines in which all dissected sibs suffered from impaired fertility. The amount of available full sibs was usually only two or three females per family which were kept in the same cage with one of their full brothers. Based on this information it is difficult to draw any conclusions about the infertility in families. The fertility problems in males and females separately could have been tested by mating sibs with mice from other families or from the outbred control line. The number of whole families suffering from infertility was very low and this testing could not have been made with the amount of animals available. Obviously the parents were not infertile, but in some lines there might be an unfavourable combination of genes which caused infertility in the offspring.

Some changes which were observed between the lines could have been due to a small sample size from each line per batch. Also the sampling technique might have an effect. In all families the best animals, highest or lowest body weight, were never picked for this experiment. All sick animals were culled, but still some animals with lesser fitness might have been picked for the experiment.

Another fertility problem was observed in the Edinburgh high body weight line, i.e. several early abortions were observed in all batches. The situation was worst in the first batch where five females had an early abortion which happened between 15 and 18 days after mating. In the second batch two cases were observed and in the third three cases. No live pups were found in the cages. In the resource experiment, which ran parallel to this one, occurence of early abortions was not reported.

The litter size of the mouse lines in contemporaneous generations (gen 14-16) with this experiment was on average 10.7 pups for the control line, 9.4 for the high line and 5.9 for the low line. The number of live foetuses was on average 11.1 in the high lines and 6.3 in the low lines. An estimate of the expected litter size can be calculated by multiplying the average number of live foetuses by pregnancy rate, which would result in an average estimated litter size of 9.0 pups for the high lines and 5.0 for the low lines. The small differences between the realised litter size and the estimated litter size were most likely due to differences in experimental procedures and the small sample sizes.

The results from this experiment showed that the ovulation rate has a strong

positive relationship with body weight, which might not be affected by inbreeding and seems to be similar in high and low body weight lines. The reproductive fitness components which seemed to show most inbreeding depression were the number of losses and pregnancy rate, especially in the heavy mouse lines. However, the survival of the eggs had a weaker and less consistent relation with the body weight than ovulation rate. The lower survival rate of eggs to foetuses in selection lines might be due to the inability of the female to carry the foetuses to full term possibly because of inbreeding depression or it might be caused by the inbreeding depression of the embryo itself. In this experiment it was not possible to separate the effect of growth selection from the effect of inbreeding, thus more studies are needed on this matter. In following two chapters experiments, which concentrate on both effects on offspring and in parental level, are described.

Chapter 5

Experimental study II: Effects of foetus inbreeding and selection on reproductive performance of inbred mice

5.1 Introduction

Previous chapter addressed the question of the effects of growth selection on components of reproductive fitness in inbred mice. However, it was not possible to separate the effects of inbreeding and growth selection. This chapter will utilise the same mouse lines and examine both effects separately on the foetus level in inbred growth diverging mothers.

Some of the components of reproductive fitness like ovulation rate are assumed to be dependent on the genotype and phenotype of the mother only, while others are influenced by the viability of the foetuses (Austin and Short, 1985). Several causes for losses have been suggested, e.g. fertilisation failure, production of abnormal eggs, hormonal reasons, crowding of the uterine horn and viability of the foetuses (Falconer and Roberts, 1960; de A. Ribeiro et al., 1996). Thus, the pre-implantation losses might be affected mainly by the parents and the viability of implanted foetuses by both parents and the genotype of the foetus. Austin and Short (1985) suggested that the mothers have a surveillance system which enables them to reject the abnormal foetuses after the time of implantation. The effect of any factors on the number of live offspring a female produces comes from two sources, the parental level and the foetus level. Therefore, the degree

of inbreeding of the foetuses might affect the intrauterine survival.

The aim of this experiment was to study if the removal of the effects of inbreeding of the foetuses by crossbreeding increases the offspring production of inbred female mice, which were either from high or low growth selection background. Line crossing of the mouse lines was done to remove the effects of inbreeding from the foetuses and to allow comparisons between inbred mothers with inbred (Chapter 4) and with non inbred foetuses. Within selection direction analysis addressed also the question whether the offspring production was different when the mating partners were of the same size or of different size. The line crosses were made in such way that the inbred mother from different size groups were carrying foetuses with genetically high, low or intermediate growth potential.

5.2 Material and methods

In this experiment a set of mouse lines diverging in growth was used. The selection history of the lines was described in Chapter 2 and in more detail by Bünger et. al. (2001a). The experimental procedure and methods were described in Chapter 3. The present study was a second part of the reproductive fitness experiment, with an aim to concentrate on effects at the foetus level.

The experiment was running over two batches, which were contemporaneous with the batches II and III from the first experiment with full-sib matings (see figure 3.2 in Chapter 3). Females from experiment I and experiment II, which were from same families, were randomly placed in one of the experiments. For example, if there were three full-sisters available from one family, one would have been mated with the full-brother (Experiment I, Chapter 4), the second with males from another line with the same direction of selection (DS) and the last with male from a line with a different selection direction.

5.2.1 Mating groups

The effects of inbreeding were removed from the foetuses by crossing inbred growth selection lines and the selection effects were counterbalanced by crossing lines with different DS (table 5.1). The aim was to create a balanced mating design, so that each line combination would have an approximately equal num-

ber of matings (table 5.2). This was not fully achieved due to the lack of spare animals from the resource experiment, especially from BEH line. The matings were set up so that one male was placed with two females. Females from the same family, i.e. full-sibs, were used across the groups (high line mate or low line mate) rather than male lines because the number of available full-sibs was low per family.

In total four mating groups were formed in experiment II (table 5.1), i.e. matings between pairs of similar size, $high_i$ x $high_j$ (HH) and low_i x low_j (LL), and matings between pairs of different size, $high_i$ x low_j (HL) and low_i x $high_j$ (LH). In the mating group codes the first letter refers to the line of the female (H=high and L=low body weight) and the second letter to the line of the male. Females were crossed only with males that were not from the same base population in order to avoid any inbreeding on foetus level (table 5.2). The number of mated pairs in each mating group is shown in table 5.3. The average body weights of females and males in each mating group is shown in figure 5.1. As no body weights of the males was taken in experiment II the appropriate male weights were derived from the data set used in Chapter 2 and weighted by the line contributions in each group to demonstrate body weight differences in the line crosses.

Table 5.1: The experiment, the code for mating group, the type of mating (i and j refer to the mouse line, in high line group i, j =1...7 and in low line group i, j =1...4) and the effects of inbreeding and selection present in parents and offspring

Exp.	Code	Mating	Parents	Foetuses
I	H L	H_i x H_i L_i x L_i	inbreeding + selection	inbreeding + selection
II	HH LL	H_i x H_j L_i x L_j	inbreeding + selection	no inbreeding + selection
II	HL LH	H_i x H_i L_i x L_i	inbreeding + selection	no inbreeding + counterbalanced selection

Data from experiment I was used in comparisons. The mating groups from the first experiment (Chapter 4) with full-sib matings were coded as H (high body weight) and L (low body weight). The H and L groups from batches II and III were contemporaneous with animals in this experiment, therefore results from batch number one was not used in the comparisons. The results of the outbred control line (EDC) were also included only from batches II and III.

Table 5.2: Number of mated pairs per line cross

	Male											
Female	DAH	DUH	RAH	EDH	BEH	MUH	ROH	EDL	BEL	MUL	ROL	tot
DAH	-	2	2	3	2	2	2	4	4	4	4	29
DUH	0	-	0	2	2	2	2	2	0	2	2	14
RAH	0	2	-	2	0	2	2	2	2	2	2	16
EDH	3	0	2	-	2	4	3	-	4	4	3	25
BEH	0	1	1	0	-	0	0	0	-	0	0	2
MUH	2	2	2	2	2	-	4	2	3	-	2	21
ROH	4	3	2	4	4	4	-	4	4	4	-	33
EDL	1	2	2	-	4	2	2	-	4	2	2	21
BEL	1	3	4	3	-	2	2	2	-	3	1	21
MUL	5	3	3	5	4	-	4	4	4	-	4	36
ROL	1	2	2	4	4	4	-	3	3	2	-	25
tot	17	20	20	25	24	22	21	23	28	23	20	

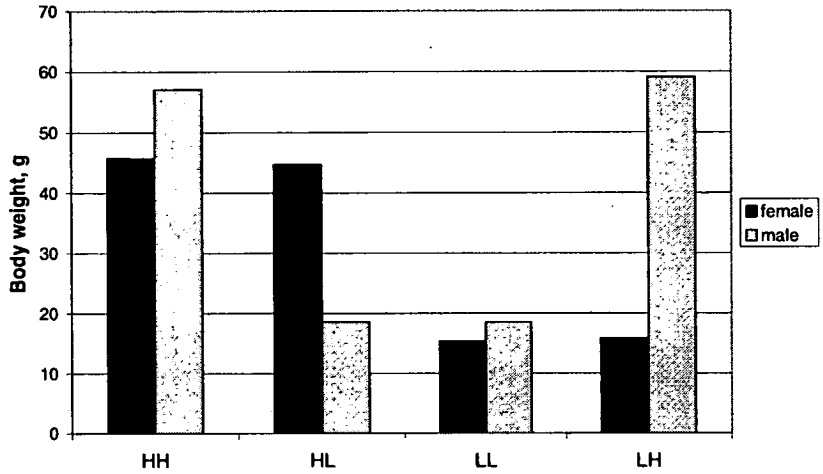
Table 5.3: Number of pairs in mating groups

	Male			
Female	Control	High	Low	
Control	82	-	-	82
High	-	80	60	140
Low	-	69	34	103
	82	149	94	

5.2.2 Data analysis

The traits included in the analysis were female body weight at mating (BW, g), ovulation rate (OR), number of live foetuses (LF), number of pre-implantation losses (PRE), number of post-implantation losses (POS) and pregnancy rate (Preg%). The number of live foetuses and losses were also expressed relative to ovulation rate (LF%, PRE% and POS%). For more detailed description of the traits see Chapter 3. The emphasis of the data analysis was on the survival of

Figure 5.1: Average female weight at mating (g) and male weight at 70 days (g) in each mating group



the eggs shed from ovaries until birth as the line crossing was assumed to affect only the survival of the foetuses and not the parents.

The least square means for the whole data set were estimated using the GLM procedure (SAS, 1996) with a model

$$Y = \text{mean} + \text{batch}_i + DS_j + \text{line}_k(DS_j) + \text{group}_l(DS_j) + e$$

where the batch is the batch number (i=1...2), DS is the direction of selection (j=1...3), line is the line of the female within the DS (k = 1...7 in high body weight group and k = 1...4 in low body weight group) and group is the mating group (l = 1...3) within DS, which corresponds to the crossbreeding effects. Including both of the parental lines into the model caused some problems in the data analysis as the diallelic crossing scheme was unbalanced which led to non estimable least square means. The individual male lines were not significant terms in the models, but the the size group of the male was and this was included in the models by fitting the mating group.

The main interest of the study was to compare the means of females from the same DS between different mating groups. The females were grouped according to DS in three groups, i.e. control, high and low. The differences in means between

inbred and crossbred foetuses within DS were tested by ANOVA or by pairwise t-tests (SAS, 1996). The group of crossbred offspring consisted of matings made between pairs from the same DS and different DS to analyse the effect of selection on foetuses. The main model for within selection direction analysis was

$$Y = \text{mean} + \text{batch}_i + \text{group}_j + \text{line}_k + e$$

where batch is the batch number (i=1 ... 2), group is the mating group (j=1 ... 3), i.e. the full-sib mating, line crossing of same DS or different DS pairs, and the line is the mouse line of the female (in high line group k =1 ... 7 and in low line group k=1 ... 4). In some analyses a covariate was fitted in the model, either body weight or ovulation rate, in order to make comparisons between the mating groups. The use of a covariate will be specified together with the results.

Regression analysis within mating groups was done to estimate the relationship between body weight or ovulation rate and the components of reproductive fitness in particular pairs. The regressions on body weight or ovulation rate were calculated by the GLM procedure (SAS, 1996). The regression coefficients on body weight were also calculated on log transformed body weight in order to take into account the scale effect. In the regression analysis it was possible also to fit the male line and the interaction between male and female line in the above model.

Effects of inbreeding and selection: The effects of inbreeding and selection were estimated from the least square means for the mating groups. The females in the mating groups were treated similarly, i.e. inbred and same selection direction, therefore the comparison for example between L, LL and LH groups was straightforward. The effect of inbreeding and selection was estimated by subtracting e.g. the LL and LH line means from the L group mean. The difference between L and LL means estimated the effect of the inbreeding of the foetus. The difference between LL and LH estimated the effect of selection of the foetus, by comparing the foetuses with genes for low growth and foetuses with counterbalanced selection effects (mixture of high and low growth genes). The joint effect of selection and inbreeding of foetuses was calculated by subtracting the LH from the L group mean. The statistical significance of the effects was tested by t-test.

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{\frac{s_1^2}{n} + \frac{s_2^2}{n}}}$$

In the above formula the $\overline{x}$ is the mean of the group, i.e. in the numerator is a difference between two means. In the denominator is the standard error of the difference, where s^2 is the variance and n the number of observations for both groups.

5.3 Results

In all analyses only pregnant females were included unless otherwise mentioned. The total number of pregnant females from the high body weight line was 89 and about 70 percent of them were mated with another high body weight male and the rest with a low body weight male (table 5.4). Among the low lines the total number of pregnant females was 83 and around 40 percent were from a mating with another low line male. Only a subset of the data from experiment I, i.e. H and L groups from batches II and III, was included in this analysis, i.e. 129 and 70 observations, respectively, and 79 observations from control.

Table 5.4: Number of pregnant females from line crosses in batches II and III and in mating groups including females from experiment I (H and L)

	Line crosses			Mating groups					
female	I	II	total	H	L	HH	LL	HL	LH
EDC	44	35	79						
DAH	5	8	13	21		10		3	
DUH	0	8	8	12		6		2	
RAH	0	7	7	30		4		3	
EDH	7	11	18	21		12		6	
BEH	0	2	2	0		2		0	
MUH	11	4	15	21		11		4	
ROH	12	14	26	24		17		9	
EDL	8	9	17		13		8		9
BEL	8	7	15		18		6		9
MUL	22	10	32		23		11		21
ROL	6	13	19		25		8		11

Body weight at mating: The average body weight in the control group was 29g, in the high line groups 48g and in the low line groups 15g. The least square means of body weights of females between mating groups were equal in both high and low line groups (table 5.5). This was expected due to the sampling method of the animals, e.g. the females in H group were sampled from the same spare animals of the resource experiment, in a similar manner and at the same time as the females in HH and HL groups.

Ovulation rate: The ovulation rates in the high, low and control groups were different, the mean ovulation rate being 12.7 ova in the control, 17.6 ova in the high lines and 8.2 ova in the low lines.

The mean ovulation rate was different between the high line mating groups ($p<0.001$), which was an unexpected result taking into account their similar body weights and the similar inbreeding coefficient of the females (table 5.5). Pairwise comparisons of the ovulation rate between the mating groups showed that the HL group had a significantly lower mean OR than the other groups, differing from the H group by nearly three eggs and from the HH by nearly two eggs. Comparison between H and HH least square means with a t-test resulted in a significance level of 5.3%. The results from the high line group suggest that the ovulation rate was not a trait of the female alone, but also affected by the mating partner.

Among the low body weight females the mean ovulation rate was not significantly different between the mating groups.

Table 5.5: The least square means for female body weight at mating, ovulation rate and number of live foetuses and losses, live foetuses and losses relative to ovulation rate and the corrected mean when ovulation rate fitted as covariate in the model. Standard erros shown in brackets. Pregnant females included from experiments I (Chapter 4) and II.

								% of ovulation rate			with OR as covariate		
Exp.	group	n	BW, g	OR	LF	PRE	POS	LF%	PRE%	POS%	LF	PRE	POS
	C	79	28.81	12.73	9.69	2.34	0.71	77.69	16.44	5.87	9.89	3.22	0.67
			(0.47)	(0.35)	(0.40)	(0.42)	(0.13)	(2.43)	(2.31)	(1.06)	(0.40)	(0.40)	(0.14)
I	H	129	48.38	18.85	11.85	6.03	0.96	66.24	28.65	5.11	10.24	2.58	0.97
			(0.58)	(0.42)	(0.49)	(0.51)	(0.16)	(2.98)	(2.83)	(1.30)	(0.59)	(0.58)	(0.20)
II	HH	62	48.84	17.90	11.33	5.95	0.62	65.03	31.17	3.80	9.03	4.04	0.72
			(0.64)	(0.47)	(0.54)	(0.57)	(0.17)	(3.30)	(3.13)	(1.44)	(0.61)	(0.60)	(0.21)
II	HL	27	47.88	16.18	9.61	5.80	0.76	61.59	33.95	4.46	8.57	4.66	0.56
			(0.92)	(0.68)	(0.78)	(0.82)	(0.25)	(4.76)	(4.52)	(2.08)	(0.85)	(0.83)	(0.29)
I	L	79	15.12	8.03	6.28	1.08	0.68	78.81	12.40	8.80	9.24	3.92	0.63
			(0.48)	(0.35)	(0.41)	(0.43)	(0.13)	(2.48)	(2.35)	(1.08)	(1.29)	(1.27)	(0.44)
II	LL	33	15.55	8.22	6.57	1.36	0.29	81.44	14.67	3.89	10.25	3.08	0.45
			(0.74)	(0.54)	(0.62)	(0.65)	(0.20)	(3.80)	(3.61)	(1.66)	(2.14)	(2.10)	(0.73)
II	LH	50	15.65	8.25	7.08	0.92	0.25	86.45	10.95	2.60	10.72	2.11	0.96
			(0.61)	(0.45)	(0.51)	(0.54)	(0.16)	(3.13)	(2.97)	(1.37)	(1.42)	(1.40)	(0.49)

Models:
mean + batch + DS + DS(line) + DS(group) + e
mean + batch + DS + DS(line) + DS(group) + β * OR + e

DS = selection direction

BW = body weight of the female, g
OR = ovulation rate
LF = number of live foetuses
PRE = pre-implantation losses
POS = post-implantation losses

Number of live foetuses: The control group had an average 9.7 live foetuses, high line group 10.9 and the low line group 6.6. The high line group was expected to have the largest number of live foetuses as it has the largest ovulation rate. The mean number of live foetuses was corrected for ovulation rate by fitting a common regression on OR for all data (table 5.5). After all groups were assumed to have similar ovulation rate, no significant differences between any groups were observed in the number of live foetuses. However, the number of live foetuses relative to OR, LF%, was lowest in the high lines (64%) and similar in the low line group (82%) and in the control (78%).

Table 5.6: Least square means when ovulation rate was fitted in the model as a covariate for each selection direction (DS) separately. Standard errors of the estimates are shown in brackets.

group	LF	PRE	POS
C	9.64	2.34	0.71
	(0.30)	(0.27)	(0.15)
H	11.80	5.64	0.97
	(0.61)	(0.62)	(0.17)
HH	11.86	5.91	0.63
	(0.67)	(0.67)	(0.19)
HL	10.76	6.59	1.06
	(1.22)	(1.23)	(0.34)
L	6.27	1.16	0.66
	(0.16)	(0.14)	(0.10)
LL	6.60	1.17	0.33
	(0.25)	(0.22)	(0.16)
LH	6.92	0.97	0.21
	(0.21)	(0.18)	(0.13)

Model within DS (control, high or low):
mean + batch + group + female line + β * OR + e

The differences between the mating groups were calculated both from the least square means in table 5.5 and from least square means where OR was fitted as a covariate in the model, which was done separately for each selection direction (table 5.6). Only two statistically significant contrasts were found, because of rather small number of observations and consequently high standard errors (table 5.7).

Among the high line mating groups the females with inbred foetuses (H) and females with crossbred foetuses from same size matings (HH) had a very similar

number of live foetuses, but one live foetus less was obtained from matings between different size partners (HL) after correction for ovulation rate (table 5.7). The differences in foetus numbers between mating groups were also analysed as LF%, which takes into account the differences in ovulation rates. The group with inbred offspring (H) had a slightly higher value than the group with crossbred foetuses, HH, but there was a difference of 4.6% between H and HL group (table 5.5). The effect of foetus inbreeding was not clear and it was not possible to draw conclusions on the effect of counterbalancing selection, because the small mating partner had a reducing effect on the number of live foetuses.

Table 5.7: The effect of inbreeding (i) and selection (s) on number of live foetuses, pre- and post-implantation losses calculated from the LS-means (see tables 5.5 and 5.6). Significant contrasts at 5% level are marked in bold.

					regression on OR fitted		
contrast	effect	LF	PRE	POS	LF	PRE	POS
H-HH	i	0.52	0.08	0.34	-0.06	-0.27	0.34
		(0.73)	(0.76)	(0.23)	(0.91)	(0.91)	(0.25)
H-HL	i+s	2.24	0.23	0.20	1.04	-0.95	-0.09
		(0.92)	(0.97)	(0.30)	(1.36)	(1.38)	(0.38)
HH-HL	s	1.72	0.23	-0.14	1.10	-0.68	-0.43
		(0.95)	(1.00)	(0.30)	(1.39)	(1.40)	(0.39)
L-LL	i	-0.29	-0.28	0.39	-0.33	-0.01	0.33
		(0.74)	(0.78)	(0.24)	(0.30)	(0.26)	(0.19)
L-LH	i+s	-0.80	0.16	**0.43**	**-0.65**	0.19	0.45
		(0.65)	(0.78)	(0.21)	(0.26)	(0.23)	(0.16)
LL-LH	s	-0.51	0.44	0.04	-0.32	0.20	0.12
		(0.80)	(0.85)	(0.26)	(0.33)	(0.28)	(0.21)

Among the low lines an expected trend was seen in the foetus number, i.e. when inbreeding effects from the foetuses were removed, the number of live foetuses increased; and a further increase was observed when the selection effect of the foetuses was counterbalanced (table 5.7). When the ovulation rate was fitted as a covariate within the selection direction, the difference between L and LH group means was significant (p=0.01). No significant differences in LF% were detected between the mating groups in low body weight females (p=0.07), but a similar trend was seen as above (table 5.5). The full-sib mated group L mean was 5 percentage points below the mean of the crossbred groups. In the pairwise comparisons between the mating group LS-mean of L and LH groups differed on

5% significance level by nearly 8%. The LH group had even significantly higher LF% (p=0.03) than the control (table 5.5). The difference between the crossbred groups was 5%.

The average of high and low line means was expected to be approximately the same as the mean of the outbred control. The mean of groups with inbred foetuses (H and L) was 9.1 (s.e. 0.3) which was not different from the control mean of 9.7 (table 5.5). The other means were expected to be higher than the control mean, because of the foetus heterosis, but the mating partner effect in the high group line crosses kept the means below the control mean. Thus, it was better to compare the means of LF% than the actual numbers of live foetuses. The mean LF% of H and L groups was around 73% (s.e. 1.9), mean of HH and LL 74% (s.e. 2.5) and mean of HL and LH 75% (s.e. 2.8). The control still had the largest survival percentage of eggs to foetuses (78%), but the positive effect of removing inbreeding effects and counterbalancing the selection effects on the foetuses was seen from these group means.

Pre-implantation losses: The average pre-implantation losses expressed relative to OR was 31% in the high body weight group, 16% in the control and 13% in the low body weight group. The low and control groups did not differ significantly from each other. The least square means of the number of pre-implantation losses corrected for ovulation rate were not different between the groups (table 5.5).

Between the mating groups of the high body weight females the losses were equal across the groups (table 5.6) and also when the losses were considered proportional to the ovulation rate (table 5.5). However, higher losses were found in the group where mating partners were of different size (table 5.7). The removal of the effects of foetus inbreeding did not greatly affect the pre-implantation losses, but in the mating group with different size partners the losses were nearly one ova more than in other groups.

The PRE% were slightly different between the low body weight mating groups, the smallest percentage of losses being in the LH group and the highest in the LL group (table 5.5). The correction for ovulation rate within the low line group made the difference between L and LL groups disappear, but still the smallest PRE% was observed in the LH group (table 5.6). Therefore, the removal of the effects of foetus inbreeding did not seem to have an effect on the pre-implantation

losses while the counterbalancing of selection effect seemed to reduce the losses slightly (table 5.7).

The mean relative pre-implantation losses of H and L groups was 21%, which was not significantly higher than in the control line (16%). The means of line crosses (HH and LL, HL and LH) were both around 23%, i.e. slightly higher losses than in the group with inbred offspring. The mating partner effect on the high body weight group was influencing these results, thus they were not such as could have been expected.

Post-implantation losses: The post-implantation losses were around 0.7 dead foetuses or 5% dead foetuses from the number of eggs shed in all groups (table 5.5). The losses after implantation in all groups were much smaller than the losses before implantation.

The mean post-implantation losses did not differ at the 5% level among the high body weight females between the mating groups. However, the highest relative post-implantation losses were found in the H group, with 0.7% higher than in the HL group and 1.3% higher than in the HH group (table 5.5). After the ovulation rate correction within the high body weight group, the highest number of post-implantation losses was observed in the HL group (table 5.6). Therefore, the removal of foetus inbreeding had a positive effect on the survival of implanted foetuses and the selection effect on foetus survival was again confounded with the mating partner effect.

The relative post-implantation losses differed significantly (p=0.02) among the low body weight mating groups (table 5.5). The least post-implantation losses were observed in the groups with crossbred foetuses and lowest in the group where selection effects on foetuses were counterbalanced (table 5.6). The inbreeding effect on foetus was similar, i.e. 0.3 dead foetuses, to that in the high line group (table 5.7). The selection effect of foetus was about one third of the inbreeding effect.

The occurrence of resorptions (early death) and especially moles (late death) was quite rare, thus the actual number of females with at least one post-implantation loss was analysed (table 5.8). In the high body weight groups over 40% of the dissected females had one or more resorptions, but the differences were not significant between the mating groups. The highest occurrence of early deaths was

observed in the group where foetuses were inbred, while most of the late deaths occurred in the group where large females were mated with a small male. The occurence of resorptions in the low body weight groups was around 12% higher in L group than in the groups with crossbred foetuses, but the difference was not significant. Late deaths were observed only when the foetuses were inbred.

Table 5.8: Number of females with resorptions (RE) and moles (MO) in each mating group and the percentage of females with RE or MO

group	n	RE	% (s.e.)	MO	% (s.e.)
C	79	26	32.9 (5.3)	4	5.1 (2.5)
H	129	60	46.5 (4.4)	11	8.5 (2.5)
HH	62	26	41.9 (6.3)	4	6.5 (3.2)
HL	27	11	40.7 (9.5)	3	11.0 (6.2)
L	79	25	31.6 (5.3)	6	7.6 (3.0)
LL	33	7	21.2 (7.2)	0	-
LH	50	10	20.0 (5.7)	0	-

The mean POS% of full-sib mated groups (H and L) was 7%, i.e. 1% higher than the control mean. The means of high and low groups with the same size or with different size mating partner were both around 4%. This further illustrates the point that inbreeding of foetuses has a negative impact on the losses which happen after the implantation.

5.3.1 Individual high body weight lines in mating groups H, HH and HL

The lines within high line groups were examined separately to examine if they were consistent with the results from the high line pooled data. A model for the least square analysis included the batch number, the female line, mating group and the interaction between the female line and the mating group. The individual male lines were not included in the model, since they were not significant for the traits, but the size difference between the mates, i.e. the mating group, was included. The number of observations per line in mating groups was very small, thus most of the differences remained statistically non significant and only the trends were looked at.

Females from the lines were used in all three mating groups, thus the mean body weight at mating was expected to be the same between the groups. Some small differences were detected, but only in the DAH line were the differences significant (table 5.9). In most of the lines the ovulation rate was lower in the mating group where the male was much smaller than the female. Because of the slightly different body weights in the mating groups, ovulation rate was analysed with a model including the body weight as a covariate. In four lines the HL group had significantly lower mean ovulation rate than the other two groups at the 5% level (figure 5.2). The differences seemed to become larger when the average size difference between the mates increased.

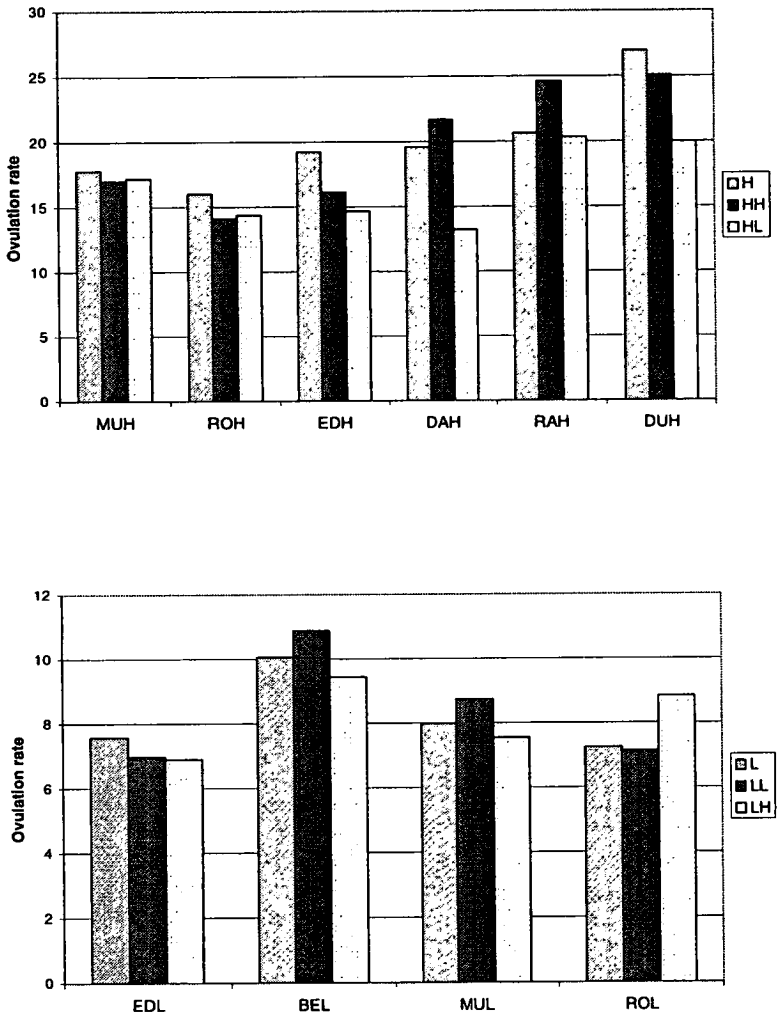
The LF% in most lines was largest when foetuses were not inbred, except in the EDH and ROH lines, and higher when matings were made between similar size pairs from different lines. Most of the differences were accounted for the pre-implantation losses, which in groups with crossbred foetuses were mainly higher when mating pairs were of different size. POS% was in general the largest in groups with inbred embryos.

Table 5.9: Least square means for all pregnant high body weight females in mating groups with standard errors in brackets.

line	group	n	BW (g)	OR	LF	PRE	POS	LF%	PRE%	POS%
DAH	H	21	56.37	19.83	11.02	7.36	1.44	55.04	37.39	10.79
			(1.22)	(0.85)	(1.00)	(1.09)	(0.26)	(5.40)	(5.39)	(2.50)
	HH	10	53.29	21.86	14.33	7.01	0.52	67.90	29.50	3.05
			(1.77)	(1.23)	(1.45)	(1.58)	(0.38)	(7.82)	(7.81)	(3.62)
	HL	3	46.65	13.26	6.72	5.52	1.03	50.33	40.95	9.39
			(3.23)	(2.25)	(2.65)	(2.89)	(0.70)	(14.28)	(14.26)	(6.61)
DUH	H	12	57.19	25.38	11.53	13.01	0.85	46.21	50.06	5.83
			(1.61)	(1.12)	(1.32)	(1.44)	(0.35)	(7.14)	(7.13)	(3.30)
	HH	6	61.14	23.79	11.67	11.38	0.74	50.42	46.29	5.18
			(2.32)	(1.61)	(1.90)	(2.07)	(0.50)	(10.25)	(10.24)	(4.75)
	HL	2	63.11	19.79	8.17	9.55	2.08	41.40	48.49	15.23
			(3.97)	(2.77)	(3.26)	(3.55)	(0.86)	(17.57)	(17.55)	(8.13)
RAH	H	30	55.91	20.13	11.99	7.35	0.79	59.41	36.63	6.11
			(1.03)	(0.72)	(0.84)	(0.92)	(0.22)	(4.55)	(4.55)	(2.11)
	HH	4	53.18	22.79	12.92	9.80	0.08	56.51	42.91	0.00
			(2.83)	(1.97)	(2.32)	(2.53)	(0.61)	(12.49)	(12.48)	(5.78)
	HL	3	60.66	19.79	12.50	6.55	0.74	65.79	30.29	4.01
			(3.25)	(2.26)	(2.67)	(2.91)	(0.70)	(14.38)	(14.37)	(6.66)
EDH	H	21	41.73	19.61	14.18	4.91	0.52	73.35	22.81	4.36
			(1.24)	(0.86)	(1.02)	(1.11)	(0.27)	(5.74)	(5.47)	(2.53)
	HH	12	42.76	15.63	10.61	4.51	0.51	68.34	30.95	8.86
			(1.61)	(1.12)	(1.32)	(1.44)	(0.35)	(6.62)	(7.13)	(3.30)
	HL	6	42.40	14.93	9.06	5.52	0.36	59.52	38.32	4.12
			(2.29)	(1.59)	(1.88)	(2.04)	(0.49)	(10.11)	(10.09)	(4.68)
BEH	H	0	-	-	-	-	-	-	-	
			-	-	-	-	-	-	-	
	HH	2	48.01	13.29	11.67	1.05	0.58	86.55	9.53	3.42
			(3.97)	(2.77)	(3.26)	(3.55)	(0.86)	(17.57)	(17.55)	(8.13)
	HL	0	-	-	-	-	-	-	-	
			-	-	-	-	-	-	-	
MUH	H	21	37.80	17.95	9.61	7.04	1.30	55.69	37.13	12.95
			(1.22)	(0.85)	(1.00)	(1.09)	(0.26)	(5.41)	(5.40)	(2.50)
	HH	11	39.88	16.91	10.47	5.57	0.87	62.76	31.77	12.02
			(1.70)	(1.18)	(1.39)	(1.52)	(0.37)	(7.50)	(7.49)	(3.47)
	HL	4	35.90	16.10	8.92	6.73	0.46	55.61	41.74	3.92
			(2.80)	(1.95)	(2.30)	(2.51)	(0.61)	(12.39)	(12.37)	(5.73)
ROH	H	24	40.63	15.58	12.74	1.77	1.07	82.97	10.08	7.58
			(1.16)	(0.80)	(0.95)	(1.03)	(0.25)	(5.10)	(5.10)	(2.36)
	HH	17	41.38	13.94	10.05	2.98	0.90	69.68	23.10	9.87
			(1.36)	(0.95)	(1.12)	(1.22)	(0.29)	(6.02)	(6.01)	(2.78)
	HL	9	40.30	14.51	10.83	2.82	0.86	75.86	18.39	7.36
			(1.87)	(1.30)	(1.53)	(1.67)	(0.40)	(8.26)	(8.25)	(3.82)

Model: mean + batch + female line + group + female line*group + e

Figure 5.2: The ovulation rate for each mouse line in different mating groups. Data corrected for body weight within groups.



5.3.2 Individual low body weight lines in mating groups L, LL and LH

Similarly to the high line group, a model was fitted with batch, female line, mating group and the interaction term between female line and mating group to estimate the least square means for each line (table 5.10). The male line was not found to be important, but the size group of the male was.

Table 5.10: Least square means for all pregnant low body weight females in mating groups.

line	group	n	BW (g)	OR	LF	PRE	POS	LF%	PRE%	POS%
EDL	L	13	14.73	7.53	5.50	0.83	1.20	71.84	11.87	18.87
			(0.41)	(0.44)	(0.47)	(0.38)	(0.25)	(4.81)	(4.11)	(3.39)
	LL	8	15.01	6.96	5.16	1.39	0.40	73.05	21.32	5.64
			(0.52)	(0.57)	(0.60)	(0.49)	(0.31)	(6.12)	(5.23)	(4.32)
	LH	9	15.14	7.00	5.84	0.98	0.18	84.17	13.49	2.87
			(0.49)	(0.53)	(0.56)	(0.46)	(0.30)	(5.78)	(4.94)	(4.08)
BEL	L	18	15.01	9.74	7.15	2.30	0.28	75.93	20.98	4.61
			(0.35)	(0.38)	(0.40)	(0.33)	(0.21)	(4.13)	(3.53)	(2.91)
	LL	6	14.15	10.56	7.27	2.71	0.57	68.83	25.59	6.83
			(0.61)	(0.66)	(0.70)	(0.57)	(0.37)	(7.12)	(6.09)	(5.03)
	LH	9	16.13	9.89	8.06	1.42	0.41	84.11	12.77	4.19
			0.49	0.53	0.56	0.46	0.30	(5.78)	(4.94)	(4.08)
MUL	L	23	15.26	7.90	6.21	0.78	0.91	79.23	8.83	12.19
			(0.31)	(0.33)	(0.35)	(0.29)	(0.19)	(3.60)	(3.08)	(2.55)
	LL	11	15.84	8.34	7.34	0.62	0.38	87.59	6.67	5.77
			(0.46)	(0.50)	(0.53)	(0.43)	(0.28)	(5.42)	(4.63)	(3.82)
	LH	21	15.75	7.59	6.82	0.57	0.20	90.52	6.97	2.69
			(0.32)	(0.35)	(0.37)	(0.30)	(0.19)	(3.77)	(3.22)	(2.66)
ROL	L	25	15.92	7.13	6.21	0.63	0.30	87.47	8.30	4.47
			(0.30)	(0.32)	(0.34)	(0.28)	(0.18)	(3.47)	(2.96)	(2.45)
	LL	8	16.02	7.04	6.59	0.36	0.09	94.25	4.46	1.51
			(0.52)	(0.57)	(0.60)	(0.49)	(0.31)	(6.12)	(5.23)	(4.32)
	LH	11	16.28	8.76	7.57	0.97	0.22	86.27	10.94	2.94
			(0.45)	(0.49)	(0.51)	(0.42)	(0.27)	(5.25)	(4.49)	(3.71)

Model: mean + batch + female line + group + female line*group + e

Body weights did not differ significantly between the mating groups in any lines. A mating partner effect on the ovulation rate was not observed in the low lines (figure 5.2). The LF% was larger in all lines for the groups with crossbred offspring and with one exception larger when the selection effects of foetuses were counterbalanced, i.e. matings were made between different size pairs. The losses were mainly pre-implantation, but the difference between PRE% and POS% was

smaller on average than in the high line group. In two lines the PRE% was smaller in the L group than in LL and in the other two lines the percentage was larger in LL than in LH. POS% was on average largest when foetuses were inbred and smallest when the selection effects on foetuses were counterbalanced.

5.3.3 Pregnancy rates and non-ovulating females

5.3.3.1 Between mating groups

The pregnancy rate of females from all the mated females was significantly higher in the control females than in the selection line females (table 5.11). When the pregnancy rate was considered without taking the male line into account the high body weight females had the lowest pregnancy rate.

The highest success rate for pregnancy was achieved when females were mated with a male of their own size. In both DS groups the pregnancy rate was around 30% lower when the mate was of different size than of the same size (table 5.11). In the LL group, Preg% was rather high for an inbred female being similar to the control group and higher than in the L group (84%, s.e. 3). Preg% in the HH group was slightly smaller than the one of H group (82%, s.e. 3).

The lower pregnancy rates among different size mates might have been due to physiological reasons which prevent copulation, since the size differences were very large between the mates (figure 5.1). This question was addressed by studying the number of females with vaginal plug, i.e. the sign of copulation. Among the high line females mated with low line males 40% of the pairs had an observation of a vaginal plug, while in the same size group it was nearly 75%. In the low line group 62% of the pairs had a plug when mated with the large males and 88% when mated with another low line male. Thus in both DS groups the copulation was less frequent with a different size partner.

The time from pairing to copulation was examined to study if the mating partner affected the female cycle or the libido of pairs. The days were the same in the high line group independent of the mating partner, but in the low line group copulation took one day longer on average when the partner was large (table 5.11). The shortest times were observed in the control and in the LL group. The number of days to copulation was similar in the full-sib mating groups H and L.

The survival of foetuses to full term was studied by analysing the estimate of

Table 5.11: Number of matings (n), pregnancy rates (Preg%), number of females with vaginal plug (n_{VP}) and time from pairing to vaginal plug (Days) within selection direction and within line for mating groups (HH, HL, LL and LH). Standard errors of the means in brackets.

	Total				High male				Low male			
female	n	Preg%	n_{VP}	Days	n	Preg%	n_{VP}	Days	n	Preg%	n_{VP}	Days
Control	82	96.3	67	3.9								
		(2.1)		(0.3)								
High	140	64.3	84	4.7	80	77.5	60	4.5	60	46.7	24	4.5
		(4.1)		(0.4)		(4.7)		(0.5)		(6.5)		(0.5)
Low	103	80.6	73	4.5	69	72.5	43	4.9	34	97.1	30	3.9
		(3.9)		(0.4)		(5.4)		(0.5)		(2.9)		(0.6)
DAH	29	44.8	13	6.8	13	76.9	10	6.3	16	18.8	3	8.7
DUH	14	57.1	6	9.2	8	75.0	4	10.8	6	33.3	2	6.0
RAH	16	43.8	3	4.0	8	50.0	3	4.0	8	37.5	-	-
EDH	25	76.0	23	5.7	14	85.7	14	3.8	11	63.6	9	6.1
BEH	2	100.0	2	4.5	2	100.0	2	4.5	-	-	-	-
MUH	21	71.4	16	3.0	14	78.6	12	3.2	7	57.1	4	2.5
ROH	33	78.8	21	3.6	21	81.0	15	3.4	12	75.0	6	4.0
EDL	21	81.0	17	5.1	13	69.2	9	6.0	8	100.0	8	4.0
BEL	21	71.4	13	6.4	15	60.0	7	7.9	6	100.0	6	4.7
MUL	36	88.9	29	4.0	24	87.5	19	3.8	12	91.7	10	4.3
ROL	25	76.0	14	3.1	17	64.7	8	3.5	8	100.0	6	2.5

gestation day on the day of dissection. Counting from the vaginal plug observation, the dissection was always made at day 18 of gestation. However, some of the pregnancies had been terminated and a new pregnancy had started, which was observed from the unexpectedly early stage of gestation on the day of dissection. The control line had on average 5% of not full term pregnancies and HH and LL groups 13% and 10% respectively, which was similar to that in H and L groups. In the HL and LH groups around 25% of terminated pregnancies were observed. A terminated pregnancy involves the loss of all embryos or foetuses in the uterus, thus it was not likely to be caused by the effects of the foetus since usually the mothers with crossbred foetuses had less losses. Possibly some unknown complications arose when mated pairs had very different body sizes.

5.3.3.2 Within mating groups

The pregnancy rate among the high body weight lines varied from 44 to 100% and among the low body weight lines from 71 to 89% (table 5.11). In all lines the pregnancy rate and the number of females with a vaginal plug observation as a proportion of all mated females was lower when mated pairs were of different size. The difference in Preg% between mating groups was highest among the heaviest females, in which the size difference between the mates was the largest. Among the low body weight females smaller differences were observed between the mating groups than in the high lines.

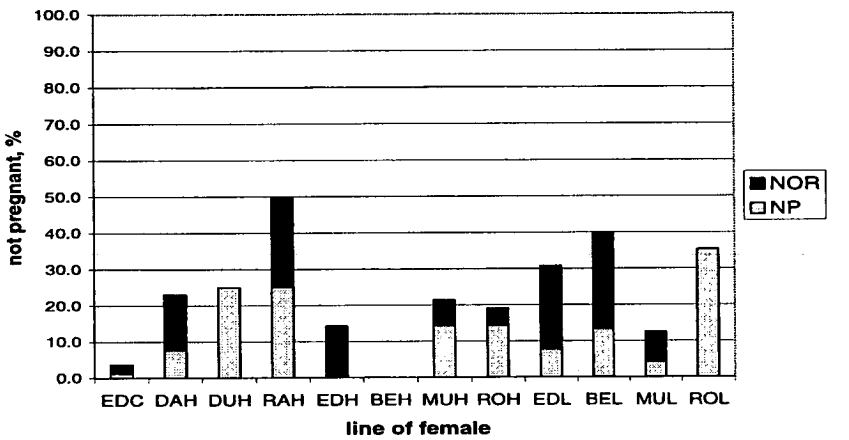
The non pregnant females were divided in two groups, i.e. those who were not ovulating and those who ovulated but did not become pregnant (figure 5.3). In all lines the number of not ovulating females rose when the male was of different size. This was seen particularly clearly in the low females, most of the failed pregnancies were caused by failure in ovulation when mated to a large male. In contrast, when low females were mated with low line males no failure in the ovulation rate was observed.

There was a concern that the male lines might differ in the success of getting the female pregnant. The pregnancy rate was considered as a female trait, but the importance of the male line was studied by comparing the pregnancy rates attached to each male line. When all high or low males were considered together there was no difference in their success independent of the size of the female. Among the high lines females only some differences in Preg% attached to the male was observed. The lowest pregnancy percentage was observed in BEL males, while just 35% DUH, RAH and EDH males had 100% success. The mates of low females had a similar pregnancy rate independent of their size.

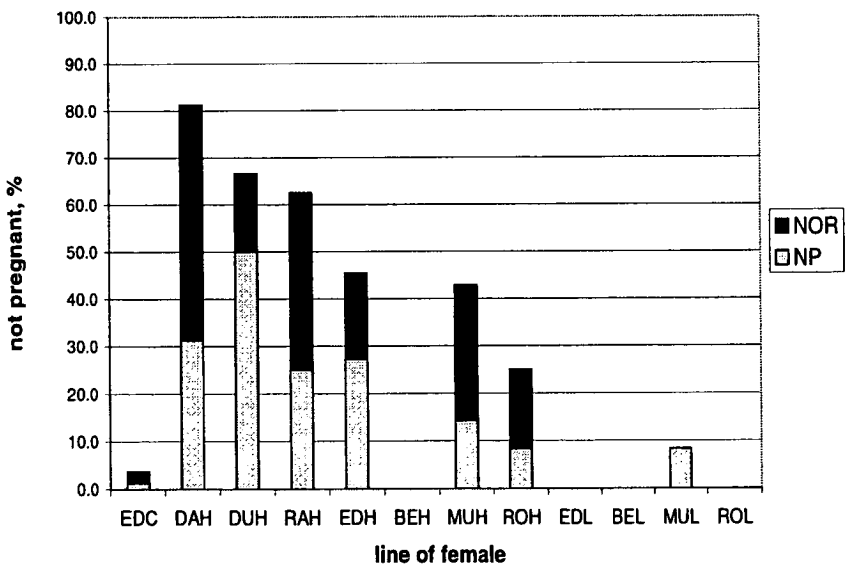
The time from pairing to copulation differed significantly between the high lines and also between the low lines. The range was from 3 days to 10 days in the high females and from 3 to 8 days in low females. The differences between the mating groups were not consistent: in some lines the time was longer when mates were of same size and in some lines when mates were of different size. In the high line group on average more days were observed among the heaviest three lines than the remaining four lines in both mating groups.

Figure 5.3: The percentage of not pregnant females for all lines when mated with a high body weight male (figure A) and a low body weight male (figure B). NOR = not ovulating and NP = not pregnant but ovulating.

A.



B.



5.4 Discussion

This experiment studied how the inbreeding and selection of foetuses affect the offspring production of inbred females. It also addressed the question of the effect on reproduction when mating pairs of different and of the same size. The results were analysed within groups of large and small female mice from seven high growth lines and four low growth lines (Bünger et al., 2001a) and were contemporaneous with the females used in Experiment I (Chapter 4). As this unique set of mouse lines was used instead of just one line or divergent pair, it was possible to make more general conclusions about the effects.

The removal of effects of inbreeding on the offspring increased the number of live foetuses in both large and small inbred females. In the high line females the effect of foetus inbreeding was rather small, while in low line females the effect was approximately half a foetus. Nearly all individual lines were consistent with this observation. The average increase in the number of live foetuses was 0.4 pups and 1.5% increase in the survival rate of the eggs. Estimates of litter heterosis in the literature are similar, e.g. change of the litter inbreeding coefficient from 90% to 0% resulted in litter heterosis of 0.97 pups (Falconer, 1971) and from 50% to 0% a litter heterosis of 0.5 pups (Roberts, 1960).

The factors related to pre-implantation losses, e.g. fertilisation rate and implantation rate, might have been affected more by the parents than the foetus. However, the removal of inbreeding effects on foetuses decreased the pre-implantation losses in the low line group. Among the individual lines the decreasing effect of foetus inbreeding on pre-implantation losses was seen in some lines and in others an increase was observed. The observations were not consistent, which most likely was due to the very small sample size within the lines. Therefore, no convincing evidence was found to suggest that the pre-implantation losses were actually greatly affected by inbreeding of the foetus.

The offspring inbreeding had an effect on the post-implantation losses in both high and low lines and the pattern was seen in most of the lines. The losses of foetuses appeared mainly in the early stage of the gestation, i.e. resorptions, being larger in the high body weight females. Deaths in the late stage of gestation, i.e. moles, were not observed at all in low body weight females when the

effects of offspring inbreeding was removed. The reduction in the litter size by inbreeding has been reported to be mainly due to the decreased fertility of the mothers and the viability of the offspring (Falconer, 1960). Thus, the decrease of post-implantation losses after removing the inbreeding effects from foetuses was expected due to an increase in viability.

The effect of counterbalancing the selection effects on offspring were confounded by a mating partner effect, especially on the high body weight females, while in the low lines it further increased the number of live foetuses compared with the full-sib mated females. In the low body weight females both losses decreased after the selection effects were counterbalanced, but the change was smaller than that due to inbreeding effects. The foetuses which had genes for low growth were expected to be smaller than the crossbred LH embryos (Güneren et al., 1996). This could have caused some difficulties for the low body weight females due to the more crowded uterus. Evidence for this was not seen in the number of live foetuses or the losses, but the percentage of low line females who had terminated pregnacies was 10-20% higher than in the groups where matings were made between similar sized partners. Thus, the foetuses of extremely different pairs might not be as viable as those from a mating between less extreme pairs, despite the fact that in both cases the offspring were not inbred.

The pregnancy rates were analysed between same and different size mates in order to examine if there were any problems in conception. In both high and low body weight groups and in all lines a lower pregnancy rate was observed when the pairs were of different size, possibly due to lower libido which could have been caused by lower stimulation from a different size mate. The difference between the mates was up to a six fold in some cases, so there could be also physiological constraints related with the fertilisation process.

The effect of mating partners on the ovulation rate of the females was an unexpected result. Because of the sampling technique of the animals, no differences between body weights within the mating groups were observed and therefore differences in ovulation rates were not expected. However, a lower ovulation rate was clearly observed among the high line females mated with a small male and the difference in ovulation rate seemed to become larger when the size difference between mates became larger. Even more surprising was the increase in

non-ovulating females with different size mates compared to those with same size mates. This was seen in most of the lines in both selection directions. In other studies the female mating preference has been observed to affect significantly the offspring production, e.g. matings with preferred male (based on mate preference tests) resulted in a litter in 93% of all matings and matings with not preferred male only 71% (Drickamer et al., 2000). Mating success have been shown to be affected by major histocompatibility complex genes (MHC) so that females more often abort offspring or inhibit the fertilisation when mated with males with MHC alleles similar to their own (Pen and Potts, 1999). The results from this experiment suggest that the females could also regulate their ovulation rate or even stop ovulating when paired with a non preferred male.

Many of the results in this study have not been statistically significant due to the very small number of females, especially pregnant females, per group or line. The group means might have been also affected by the different line contributions in each mating group. However, this was taken into account for the female side by including the female line in the models. The male lines contributed fairly equally, except that some of the average heavy lines were used less in the LH crosses than in the HH crosses. However, no significant differences in offspring production were found between the male lines. The minor effect of the male on the reproductive performance of the female was reported also by Falconer (1960), Bateman (1966) and Eisen (1977).

This study has addressed the question of whether the reproductive performance would be higher when the effects of offspring inbreeding are removed. To answer this question the hypothetical litter size for a group of mice was estimated as a product of the number of live foetuses and the pregnancy rate (table 5.12). In the high lines the litter size remained at the same level independent of the offspring inbreeding. In the low lines an increase of 1.5 pup was obtained due to larger number of live foetuses and higher pregnancy rate, the latter being an interesting result since the females were similarly inbred and line crossing was not expected to affect the pregnancy rate.

The second main question of this study was whether the litter size would be affected by whether the mated pairs were of the same size or different size. Matings between differently sized pairs were clearly not profitable for the litter

Table 5.12: The number of live foetuses (LF), pregnancy rate (Preg%) and the estimated litter size ("LS") in mating groups

Group	LF	Preg%	"LS"
C	9.6	97	9.3
H	11.0	82	9.0
HH	11.3	78	8.8
HL	9.6	47	4.5
L	6.3	79	5.0
LL	6.6	97	6.4
LH	7.1	73	5.1

size (table 5.12). In the high lines, the effects of mating partner decreased both the number of live foetuses and the pregnancy rate, reducing the litter size to half of that achieved from other high female groups. In the low lines the increase in litter size as a result of the removal of the effects of offspring inbreeding was lost because of the low pregnancy rate. The control line represents the matings between average size outbred animals, which seem to have the most potential in terms of offspring production.

The conclusion of this experiment is that the offspring inbreeding or selection effects on offspring have a small impact on the reproductive performance of the mother. However, the results were confounded with the mating partner effect and the pure effects of the foetus inbreeding or selection cannot be separated. The male effect has usually been reported to be negligible, but in this experiment the size of the male clearly affected the reproductive performance of the female. The next step in the study of the reproductive fitness will involve examining the removal of inbreeding effects and counterbalancing the selection effects on the parents. The above mentioned issues can be then studied in large, medium-size and small outbred females.

Chapter 6

Experimental study III: Reproductive performance of crossbred mice.

6.1 Introduction

Experiment II (Chapter 5) addressed the question of selection and inbreeding effects of the foetus on the reproductive performance of inbred mother. Experiment III will take a further step and analyse the effects on the mother itself. The decline in litter size generally found to be partially due to the inbreeding of offspring and partially to the inbreeding of the parents (Roberts, 1960). When crossbreeding of mice has been done, a larger part of the heterosis on litter size, around 70%, has been observed to be attributable to the mother (Falconer, 1971). In a study of Falconer and Roberts (1960) the increase in litter size due to heterosis was not caused by an increase in ovulation rate, but by a decrease in pre-implantation losses. The post-implantation losses were not expected to be greatly affected by the maternal inbreeding, since they are more related to the viability of offspring as discussed in Chapter 5.

The aim of experiment III was to estimate the effects of growth selection on crossbred mice and to examine the effects of inbreeding and growth selection on the components of reproductive performance. The inbreeding effects were studied by comparing the reproductive performance of inbred females (data from experiments I and II) with the two-way crosses in different size groups, i.e. heavy and small. The question was addressed of whether the removal of inbreeding effects in mothers would increase the litter size and would the increase be similar

in different size females. The effects of growth selection on reproduction were studied by comparing the reproductive performance of large, medium size and small outbred females. The selection effects of the mothers were counterbalanced to achieve a special kind of control, i.e. medium sized mother with heterosis effects, which was compared with the extremes in order to estimate the effects of growth selection on first parity reproductive fitness.

6.2 Material and Methods

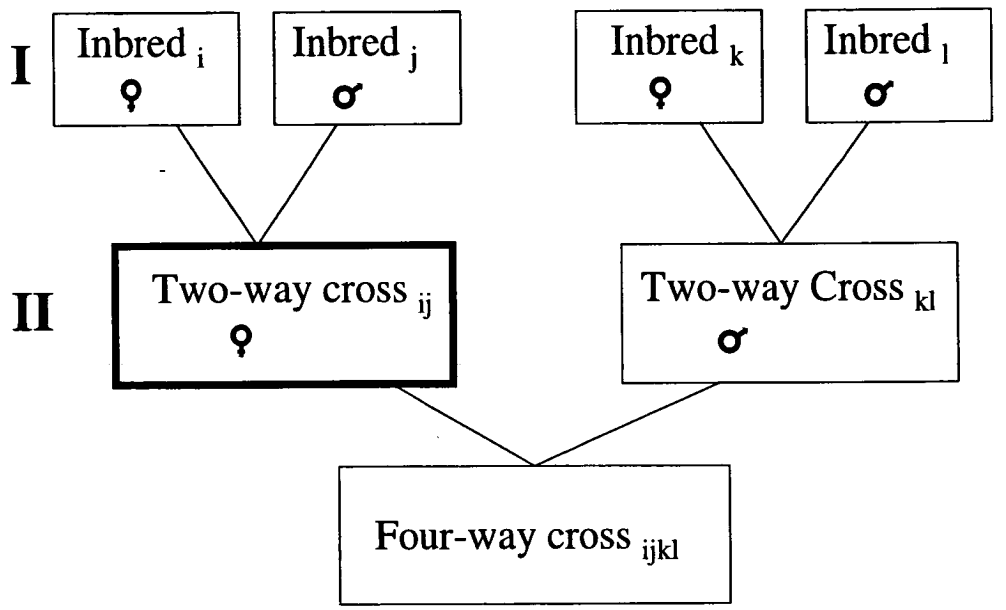
This chapter describes the last part of the experimental study designed to examine the effects of inbreeding and selection on reproduction, which was explained in Chapter 3. The purpose of this part was to produce a set of two-way crosses, i.e. outbred animals, and mate them so that the foetuses were four-way crosses. These results were then compared to those obtained from earlier stages of the reproductive fitness experiment (Chapter 4 and 5).

Table 6.1: The number of matings and the average age of females in days for each mating group

group	n	age
C	36	85
HLxLH	50	105
LHxHL	73	114
HHxHH	59	111
HHxLL	43	112
LLxLL	48	121
LLxHH	76	114

The seven high and four low body weight mouse lines, described in Chapter 2, were used in crosses. Six different mating groups (table 6.1) were created for this experiment, i.e. crossbred high (low) body weight females mated with crossbred high (low) body weight males and crossbred females and males with counterbalanced selection effects by mating pairs of different size (figure 6.2). Together with the six mating groups the outbred control line, EDC, was also examined in this experiment. The codes for mating groups consist of four letters, which refer to the selection direction of maternal and paternal grandparents in respective order.

Figure 6.1: The mating scheme for the experiment. Phase I describes the production of two-way cross offspring and phase II the production of four-way crosses. The dissection results were collected from two-way cross females. Subscripts refer to the genotype of an individual.



6.2.1 Crossing scheme

The different phases in production of two-way crosses are shown in figure 6.1. The parents for the phase I animals came from the resource experiment (Chapter 2 and Bünger et. al. 2001a). After pups for the resource experiment were weaned, the parents (animals from generation 14, Chapter 2) were allowed to produce second and a few also third litters. These litters were inbred, but no data of the litter size was used in this study, which concentrated only on the first parity. The litters were adjusted to 12 pups in order to minimize the pre-weaning effects on reproduction. The pups were weaned at 21 days and after that housed in MB1 stock cages (internal size 960 cm^2 x 12 cm; Kent Plastics Ltd.) with up to 15 animals in the same cage.

Table 6.2: Number of dissected two-way cross females (ij) presented in a form of a mating table of inbred mouse lines (i and j). The male lines in crosses (kl) are not specified, but presented as a type, either HH or LL. All HL female line crosses were mated with LH males. Letters i, j, k and l refer to subscripts in table 6.1. An example of how to read the table is given in the text.

fem. line (i)	male type (kl)	H male (j)							L male (j)			
		DAH	DUH	RAH	EDH	BEH	MUH	ROH	EDL	BEL	MUL	ROL
DAH	tot.	-	0	6	0	0	0	0	0	0	2	0
	HH			3								
	LL			3								
DUH	tot.	0	-	0	6	0	0	1	0	0	0	1
	HH				4			1				
	LL				2							
RAH	tot.	5	0	-	0	3	3	8	0	6	0	5
	HH	2				1	3	3				
	LL	3				2		5				
EDH	tot.	0	0	0	-	2	3	6	-	0	6	-
	HH					1	3	4				
	LL					1		2				
BEH	tot.	2	0	0	0	-	3	7	2	-	2	5
	HH	1					2	3				
	LL	1					1	4				
MUH	tot.	0	2	0	0	2	-	5	0	0	-	5
	HH		2			1		3				
	LL					1		2				
ROH	tot.	0	0	7	9	11	12	-	16	0	0	-
	HH			5	5	7	6					
	LL			2	4	4	6					

fem. line (i)	male type (kl)	L male (j)				H male (j)						
		EDL	BEL	MUL	ROL	DAH	DUH	RAH	EDH	BEH	MUH	ROH
EDL	tot.	-	13	5	10	5	0	0	-	11	3	2
	HH		9	2	6							
	LL		4	3	4							
BEL	tot.	7	-	12	10	6	0	0	7	-	7	0
	HH	3		9	6							
	LL	4		3	4							
MUL	tot.	13	8	-	10	0	0	0	7	4	-	0
	HH	7	4		6							
	LL	6	4		4							
ROL	tot.	12	11	2	-	0	0	0	5	8	9	-
	HH	8	4	1								
	LL	4	7	1								

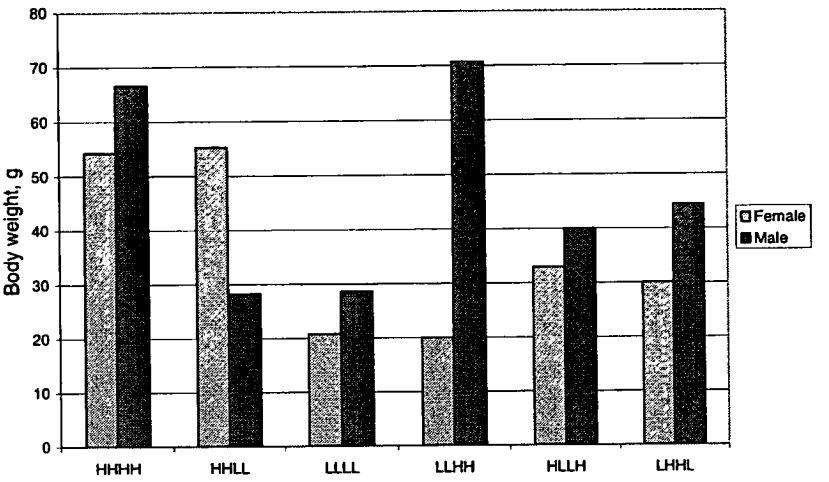
When the phase I animals were around 12 weeks old matings were set up to produce crossbred offspring (figure 6.1). Usually two females were placed with one male. Animals from lines derived from the same base population were not allowed to mate in order to keep the two-way crosses completely non-inbred. Around 18 days after pairing the pairs were separated. The handling of litters was made as explained above and also in Chapter 2.

The two-way crosses (phase II in figure 6.1) were mated at a minimum age of ten weeks. However, some of them were mated up to one month older due to the limited amount of space and lack of suitable partners at the correct age (table 6.1). The two-way crosses were mated so that no matings were made between animals with the same lines of parents or from same base population so as to avoid inbreeding. For example MUHxROH females were not mated with any males with either MUH, MUL, ROH or ROL lines among their parents (table 6.2)

At the time of pairing both female and male body weights were taken. Males were weighed since there was no prior knowledge of the mean weight of such crossbred animals (figure 6.2). From the next morning after pairing the females were checked for vaginal plugs to estimate the correct day for dissection, i.e. day 18 after the observation of a vaginal plug. The procedure after mating was similar throughout the whole experiment as explained in Chapter 3.

The contributions from each line to the mating groups were not equal despite the effort, in particular the number of HL females was quite low due to the low success in high line females of getting second litters and also because of a problem of getting litters from matings where the pair was of very different size (table 6.2). In table 6.2 the female line (i) refers to those born from second litters (phase I in figure 6.1). Also the male lines from phase I are given in the table. The number of mated crossbred females (phase II) and the group of the male (HH, LL) is shown in the tables. For example the first row in the table 6.2 shows that a total of six DAHxRAH females were mated and the second two lines shows that three of them were mated with another high x high male and three with a low x low male. Another set of matings was done between pairs of different size (e.g. H x L) to counterbalance the selection. The crossbred HL (LH) females were all mated to LH (HL) males. For example the first line on the right hand side box in the table 6.1 shows that two DAH females were mated with a MUL male.

Figure 6.2: The average female and male weight (g) in each mating group



6.2.2 Data analysis

The main traits included in the analysis were female body weight at mating (BW, g), ovulation rate (OR), number of live foetuses (LF), number of pre-implantation losses (PRE), number of post-implantation losses (POS) and pregnancy rate (Preg%). The number of live foetuses and losses were also expressed relative to ovulation rate (LF%, PRE% and POS%). For more detailed description of the traits see Chapter 3.

The least square means were estimated by GLM procedure (SAS, 1996) with models described below. The differences between means of groups were tested by ANOVA and pairwise comparisons between means (t-test) were made by *pdiff* procedure (SAS, 1996). Regression analysis on body weight and on ovulation rate was done by GLM procedure (SAS, 1996) using similar models as for least square means, but all models for regression analysis will be specified with the results.

Two-way crosses: First the two-way crossed females and the control were analysed in order to examine the effect of growth selection on reproductive fitness. The general model for least square means was

$$Y = \text{mean} + \text{size}_i + \text{size}_i(\text{group}_j) + \text{group}_j(\text{line}_k) + \beta * \text{age} + e$$

where size (i=1 . . . 3) is the size group of the female, i.e. heavy (HH), average (C, HL, LH) or small (LL), group is the mating group (j=1 . . . 2 and for C, HL and LH j=1 . . . 3), line is the mouse line of the parents of the female (k=1 . . . 7 for high lines and k=1 . . . 4 for low lines) within a mating group and age is the age of the female at mating in days. The parental lines of the male did not have a significant effect on the traits, but the size of the male did. This was taken into account by including the mating group in the model, i.e. the different size mating partner.

In order to analyse the differences between mating groups within a size group the least square means were calculated within size groups with the model

$$Y = \text{mean} + \text{group}_i + \text{group}_i(\text{line}_j) + \beta_1 * \text{age} + \beta_2 * \text{BW} + e$$

where the terms are like explained above. Body weight was used as a covariate in order to equalise the body weights within size groups and to make the reproductive traits more comparable within the size group.

Data from all experiments: The data from experiments I and II were included in the further analysis to estimate the effect of inbreeding of females. The overall data was divided into three different size categories, due to the large differences in body weight. The size groups were large, medium and small females (table 6.3). Within each size group were either three or five mating groups. For example within the large group the mating groups were full-sib matings (H), inbred females crossbred with same size or different size male (HH or HL) and crossbred females mated with same size or different size males (HHHH or HHLL). The control and crossbred counterbalanced selection females were kept in the same group since they were not to be subjected to inbreeding and no selection was done for the growth. The basic models within size group and within DS group were

$$Y = \text{mean} + \text{batch} + \text{DS(group)} + \text{DS(line)} + \beta * \text{age} + e$$

$$Y = \text{mean} + \text{batch} + \text{group} + \text{line} + \beta * \text{age} + e$$

where batch is the number of batches (1-4) and the other terms are as in the above models. Some modifications were made to the above "full" model and those will be specified in the text. For example the term DS(line) was dropped in some analysis, because inclusion of all terms led to inestimable least square means. Also the regression on body weight was included in some situations within size

groups and within DS group.

Table 6.3: The size groups, selection direction (DS) groups, mating groups and batch number from which data were collected for each mating group.

size	DS	mating group	mating (fem x male)	batch
average	C	C	control	1,2,3,4
	HL	HLLH	H_iL_j x L_kH_l	4
	LH	LHHL	L_iH_j x H_kL_l	4
large	H	H	H_i x H_i	1,2,3
		HH	H_i x H_j	2,3
		HL	H_i x L_j	2,3
	HH	HHHH	H_iH_j x H_kH_l	4
		HHLL	H_iH_j x L_kL_l	4
small	L	L	L_i x L_i	1,2,3
		LL	L_i x L_j	2,3
		LH	L_i x H_j	2,3
	LL	LLLL	L_iL_j x L_kL_l	4
		LLHH	L_iL_j x H_kH_l	4

Effects of inbreeding and selection: First the effect of inbreeding and selection of the parents was examined by using least square means which were calculated for each size group and corrected for both age and weight within the size group. The means were mainly analysed based on the type of the female (inbred, selected). The comparisons were made between females of similar size, e.g. H and HH females, of which one was inbred and the other was crossbred.

Further analysis was made by calculating the means in the cells in table 6.4. The change in reproductive traits due to inbreeding or selection was estimated from the average of high and low body weight group least square means for each type of females, which would only take into account the presence of inbreeding and/or selection. Both effects were shown separately in mothers and their offspring. The magnitude of change was measured as the percentage reduction of the inbred mean from the crossbred mean, i.e. $100 * \frac{\overline{outbred} - \overline{inbred}}{\overline{outbred}}$. The control line (EDC) was not included in this table, but was compared previously with the average of HL and LH females. In the table the average of HL and LH means was used as a control, i.e. animals without any inbreeding and with medium size.

Table 6.4: Inbreeding and selection effects of the females and the foetuses, the number of the experiment from which the results were obtained and the mating groups in each cell.

	Females		
Embryos	inbreeding + selection	no inbreeding + selection	no inbreeding counterbal. selection
inbreeding + selection	I H, L		
no inb. + selection	II HH, LL	III HHHH, LLLL	
no inb. + counterbal. selection	II HL, LH	III HHLL, LLHH	III HLLH, LHHL

6.3 Results

6.3.1 Two-way crosses, selection effects

First the two-way crosses were analysed separately to study the reproduction of non inbred parents. On the parent level there were two groups, those with a selection effect and those with counterbalanced selection effects, i.e. medium sized parents in mating groups HLLH and LHHL. A total of 54 female line combinations were created to achieve an approximately equal number of observations (only pregnant females were included in the main analysis) from each mating group. The average age at matings in days and the range of ages is shown in table 6.5.

Table 6.5: The number of dissected pregnant females in mating groups, the mean age of the females and the range of ages (days).

fem	male group						
group	C	HH	LL	HL	LH	age	min - max
C	36	-	-	-	-	85	84 - 86
HH	-	55	35	-	-	108	93 - 142
LL	-	65	43	-	-	118	81 - 146
HL	-	-	-	-	49	105	91 - 128
LH	-	-	-	72	-	114	94 - 141

Body weight of the females at mating: The two-way crosses were mated at older age than control females (table 6.5) and the body weights differed between the age groups. Therefore, the means were corrected for the age effect.

HL and LH females had an average body weight of 32g ranging from 19g to 54g (table 6.6). They were on average heavier than the control (table 6.7) which had an average body weight of 27g, either due to heterosis or due to the age. The body weights of HH females varied from 31g to 102g and the least square mean was about 59g. The smallest were the LL females with an average weight of 20g and the range of body weights was from 16g to 29g.

Within each size group (large, medium sized and small) the body weights differed between the mating groups significantly apart for the LL females (table 6.6). The difference between the medium sized females (HL and LH) was only 4g, while in the high body weight group the difference between the mating groups was 11g (table 6.7). This could be explained by a small sample size and unequal line contributions to crosses. The divergence from the medium sized females (HL,LH) was not symmetrical for HH and LL females, the high lines being 27g heavier and the low lines being 12g lighter. After the log transformation of body weight the divergence from the medium sized was 0.6 ln(g) for the heavy females and 0.5 ln(g) for the light females.

Ovulation rate: The mean ovulation rate of the medium sized animals was 14.6 ova, which was larger than the control line mean of 11.9 ova (tables 6.6 and 6.7). The mean OR was also different between the mating groups of average size (HL and LH females), but after body weight correction no difference was observed between them and the mean difference from EDC was reduced to 1.5 ova (table 6.8). The high lines diverged from the medium sized females by 6 ova (table 6.7) and by 5 ova after means were corrected for body weight (table 6.8), while the low line divergence was 4 ova.

The means in mating groups within large or small females were not significantly different (table 6.7). Among the large females the difference between groups was larger, 2.54 (s.e. 1.14), when the body weights of the groups were equalised. Thus, in the outbred females the size of the mating partner did not seem to affect the ovulation rate as it did in the inbred females.

Table 6.6: Least square means of body weight and reproductive traits for outbred females. The first two letters of the mating group refer to the female type. Standard errors shown in brackets.

								% of ovulation rate		
group	n	BW, g	ln(BW)	OR	LF	PRE	POS	LF	PRE	POS
C	36	28.32	3.335	11.90	9.96	1.22	0.73	83.65	9.93	6.43
		(0.85)	(0.021)	(0.46)	(0.47)	(0.45)	(0.17)	(3.20)	(3.00)	(1.36)
HLxLH	49	34.06	3.519	15.26	12.06	2.64	0.57	79.65	16.30	4.52
		(0.94)	(0.024)	(0.51)	(0.52)	(0.50)	(0.19)	(3.51)	(3.30)	(1.49)
LHxHL	72	30.26	3.395	13.99	11.78	1.79	0.42	84.10	12.81	3.10
		(0.78)	(0.020)	(0.42)	(0.43)	(0.41)	(0.16)	(2.91)	(2.74)	(1.24)
HHxHH	55	64.52	4.112	20.07	16.05	3.08	0.94	81.50	13.88	4.63
		(0.89)	(0.022)	(0.48)	(0.50)	(0.47)	(0.18)	(3.35)	(3.14)	(1.42)
HHxLL	35	53.71	3.961	20.74	15.74	4.62	0.38	76.15	21.62	2.22
		(1.43)	(0.036)	(0.77)	(0.80)	(0.76)	(0.29)	(5.36)	(5.04)	(2.28)
LLxLL	43	20.24	3.013	10.65	7.92	2.51	0.22	74.42	23.58	1.99
		(0.71)	(0.018)	(0.39)	(0.40)	(0.38)	(0.15)	(2.67)	(2.51)	(1.14)
LLxHH	65	19.90	2.989	10.26	8.00	1.88	0.38	77.52	18.75	3.73
		(0.59)	(0.015)	(0.32)	(0.33)	(0.31)	(0.12)	(2.20)	(2.06)	(0.93)

Model: mean + size + group(size) + line(group) + β * age + e

size = large (HH), medium sized (C, HL, LH) and small (LL) females
group = mating group
line = parental lines of the female
age = age at mating, days

n = number of pregnant females
BW = body weight of female at mating in grams
OR = ovulation rate
LF = number of live foetuses
PRE = pre-implantation losses
POS = post-implantation losses

Table 6.7: Contrasts between and within size groups calculated from least square means in table 6.6. The mean over female type is marked e.g. (HL,LH) as a mean of HLxLH and LHxHL groups. Significant contrasts at 5% level marked in bold and standard errors are shown in brackets.

contrast	BW, g	ln(BW)	OR	LF	PRE	POS	LF%	PRE%	POS%
HLxLH - LHxHL	**3.80**	**0.124**	**1.27**	0.28	0.85	0.15	-4.45	3.49	1.42
	(1.22)	(0.031)	(0.65)	(0.67)	(0.65)	(0.25)	(4.56)	(4.29)	(1.94)
HHxHH - HHxLL	**10.81**	**0.151**	-0.67	0.31	-1.54	0.56	5.35	-7.74	2.41
	(1.68)	(0.042)	(0.91)	(0.94)	(0.89)	(0.34)	(6.32)	(5.94)	(2.69)
LLxLL - LLxHH	0.34	0.024	0.39	-0.08	0.63	-0.16	-3.10	4.83	-1.74
	(0.92)	(0.023)	(0.50)	(0.52)	(0.49)	(0.19)	(3.46)	(3.25)	(1.47)
EDC - (HL,LH)	**-3.84**	**-0.122**	**-2.73**	**-1.96**	-1.00	0.23	1.77	-4.63	2.62
	(1.05)	(0.026)	(0.80)	(0.81)	(0.79)	(0.30)	(5.57)	(5.23)	(2.37)
(HH) - (HL,LH)	**29.96**	**0.582**	**5.78**	**3.98**	**1.63**	0.16	-3.06	3.19	-0.39
	(1.04)	(0.026)	(0.80)	(0.82)	(0.79)	(0.31)	(5.55)	(5.22)	(2.36)
(LL) - (HL,LH)	**-12.09**	**-0.456**	**-4.18**	**-3.96**	-0.03	-0.20	-5.91	6.61	-0.95
	(0.76)	(0.019)	(0.71)	(0.72)	(0.69)	(0.27)	(4.88)	(4.59)	(2.07)
(HH) - (LL)	**39.05**	**1.036**	**9.95**	**7.94**	**1.66**	0.36	2.86	-3.42	0.57
	(0.96)	(0.024)	(0.52)	(0.54)	(0.51)	(0.20)	(3.60)	(3.38)	(1.53)

Table 6.8: Body weight corrected least square means of reproductive traits for outbred females. The first two letters of the mating group refer to the female type.

						% of ovulation rate		
group	n	OR	LF	PRE	POS	LF	PRE	POS
C	36	12.57	10.05	1.57	0.95	79.91	12.11	7.98
		(0.64)	(0.70)	(0.71)	(0.27)	(5.10)	(4.76)	(2.12)
HLxLH	49	14.19	11.09	2.35	0.74	78.25	15.52	6.22
		(0.51)	(0.56)	(0.57)	(0.21)	(4.10)	(3.82)	(1.70)
LHxHL	72	14.00	11.97	1.74	0.29	85.60	12.38	2.02
		(0.45)	(0.49)	(0.50)	(0.19)	(3.58)	(3.34)	(1.49)
HHxHH	55	17.83	14.71	1.83	1.28	83.22	9.65	7.13
		(0.76)	(0.91)	(0.87)	(0.34)	(4.80)	(4.75)	(1.98)
HHxLL	35	20.37	15.52	4.41	0.44	76.41	20.93	2.66
		(0.86)	(1.00)	(0.95)	(0.37)	(5.26)	(5.20)	(2.17)
LLxLL	43	10.43	7.74	2.47	0.22	74.15	23.68	2.18
		(0.28)	(0.25)	(0.25)	(0.11)	(2.49)	(2.27)	(1.08)
LLxHH	65	10.27	8.04	1.86	0.38	77.84	18.43	3.74
		(0.23)	(0.21)	(0.21)	(0.09)	(2.10)	(1.91)	(0.91)

Model within size (large, medium sized and small female):
mean + group + line + β_1 * age + β_2 * BW + e

Number of live foetuses: The mean number of live foetuses in HL and LH females was 11.9, which was two foetuses higher than in the control (EDC). The difference in number of foetuses was not significant when the medium sized group means were corrected for body weight (table 6.8). The LF% was lower in the HL females than in control or LH females. However, the difference from control was small and not significant when compared to mean of HL and LH (table 6.7). This was expected since they were all outbred and not of extreme size.

The average LF of the extremes, high or low body weight females, was similar to the LF of the medium sized (HL/LH) females, i.e. 11.9. The extremes had on average lower LF% (77%), than the medium sized (82%) but the differences were not statistically significant possibly due to the small number of observations and consequently high standard errors. Another factor, which might affect the results was unequal line contributions to the crosses.

Between the high line mating groups, a difference less than half a pup was observed, but the LF% was nearly 5% higher in the group with similar size of mating pairs compared with the different size pairs in the high line group (ta-

ble 6.7). The small females had 3% higher LF% when mated with the large males than with the small males. The size of the mating partner did not seem to be very important for the survival of eggs to foetuses in the two-way cross females.

Pre- and post-implantation losses: The medium size females had the lowest losses on average over all groups and among them the control line had lowest value of PRE and highest POS (table 6.8). The large females had an average 3% larger PRE% than the average of HL and LH, while in the small females it was 6%. The HL females had over two times more post-implantation losses than LH, when means were adjusted for the body weights, this could be explained by the low number of females with dead foetuses and possibly also the unequal line contributions.

The average high and low pre-implantation losses were 3 eggs or 19% as relative to ovulation rate, which were higher than in the medium sized females, 2 eggs and 15%. The post-implantation losses were similar between the extremes and the medium sized females, i.e. around 0.5 eggs and 3%.

The PRE% were not significantly different between the mating groups in size group (tables 6.7). The HH females had twice as many losses when mated with a small male than when mated with a large male, while in the LL females the difference was opposite and smaller (table 6.8). The post-implantation losses were smaller in all groups than the pre-implantation losses and the differences between groups were more likely to be due to chance than selection effects.

6.3.2 Inbreeding effects

The traits were compared within size groups between inbred and outbred females in order to estimate the effects of inbreeding on reproduction. In this part all data from Experiments I, II and III were included (n=974). The mating groups within female type (e.g. HHxHH and HHxLL) were pooled together and females referred to by types, i.e. DS groups (see table 6.3).

The two-way crosses were mated at considerably older age than the inbred females, thus in all models the age correction was included (table 6.9). The age correction did not remove the differences in body weights between the inbred and outbred females from the same size group. The two-way crosses were 5g heavier among the high lines and 4g heavier among the low lines. The further reason for

lower body weight of inbred females could be either heterosis in two-way crosses or the unequal line contributions in the groups.

In order to make the inbred and outbred means more comparable for the reproduction traits they were corrected for body weight (table 6.11). First the body weigth corrections were made within a DS group, which corrects the means for each type of female over the mating groups and the means were compared accross size groups. The second body weight correction was made within size group, e.g. H and HH females together, and it allowed comparisons between inbred and outbred with equalised body weight.

Table 6.9: The type of female (DS), experiments providing data, age at mating in days and the least square means with standard errors in brackets.

DS	exp.	n	age	min	max	BW, g	OR	LF	LF%	PRE%	POS%
C	I-III	142	85	74	91	28.06	12.19	9.67	80.0	14.9	5.1
						(0.62)	(0.28)	(0.29)	(1.8)	(1.7)	(0.7)
HL	III	49	105	91	128	32.23	14.15	10.98	79.2	17.8	3.0
						(1.51)	(0.67)	(0.70)	(4.4)	(4.2)	(1.8)
LH	III	72	114	94	141	29.55	14.23	11.73	83.6	13.7	2.7
						(1.43)	(0.64)	(0.66)	(4.1)	(3.9)	(1.7)
H	I-II	296	81	50	113	45.35	16.41	10.69	66.2	29.1	4.8
						(0.64)	(0.28)	(0.30)	(1.9)	(1.7)	(0.7)
HH	III	90	109	93	142	50.69	17.97	13.72	76.3	20.2	3.6
						(1.11)	(0.50)	(0.51)	(3.2)	(3.1)	(1.3)
L	I-II	217	75	57	112	15.48	7.53	6.61	85.2	10.1	4.8
						(0.66)	(0.29)	(0.30)	(1.8)	(1.8)	(0.8)
LL	III	108	118	81	140	19.62	10.49	7.84	76.7	21.9	1.4
						(1.10)	(0.49)	(0.50)	(3.2)	(3.0)	(1.3)

Model: mean + batch + size + size(group) + β * age + e

Ovulation rate: The ovulation rate for each type of female corrected for body weight within a size group is shown in figure 6.3. The OR in the control was significantly lower than the mean of the medium sized two-way crosses by 1.3 ova with standard error of the difference of 0.4 (table 6.11).

In both high and low groups the two-way crosses had larger ovulation rate than the inbred ones (figure 6.3), but the differences were not statistically significant when the body weights were corrected (table 6.12). The mean ovulation rate of the inbred high and low females was 12, i.e. 2 ova smaller than the mean of outbred females (table 6.10), but the inbred ones were also lighter by 5g on

average (table 6.9). The means for OR were not corrected for the BW and if the effect of BW was taken into account the body weight differences between the cells would explain most of the differences in OR (regression around 0.3 ova/g). The OR among inbred females was affected by the mating partner effect discussed in Chapter 5 and exclusion of those means reduced the OR difference to one egg (table 6.10). The effect of inbreeding on ovulation was also confounded with the effect of age and consequent increase in body size and possibly also changes in body composition. Thus, the data seems to suggest that inbreeding has no substantial effect on the ovulation rate.

Figure 6.3: Least square means of OR, PRE, POS and LF in different types of female corrected for body weight within size groups.

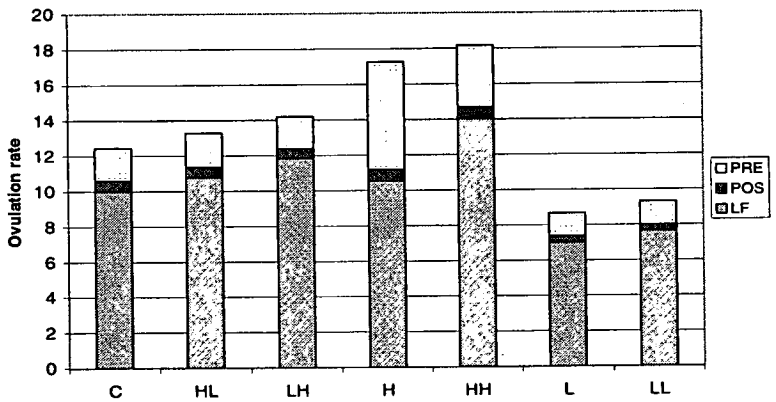


Table 6.10: Ovulation rate with standard error in brackets averaged over different type of high and low females calculated from age corrected means (table 6.9).

	Female		
Foetus	inbreeding + selection	no inbreeding + selection	no inbreeding counterbal. sel.
inbreeding + selection	12.81 (0.23)		
no inb. + selection	12.04 (0.77)	14.48 (0.49)	
no inb. + counterbal. sel.	11.05 (0.42)	13.99 (0.49)	14.19 (0.46)
	11.97 (0.30)	14.24 (0.35)	14.19 (0.46)

Table 6.11: The least square means for ovulation rate, number of live foetuses and losses calculated with two different models, both corrected for age and body weight, one within selection direction (DS) and the other within size (C+HL+LH, H+HH and L+LL). Standard errors are shown in brackets.

		Model A, within DS							**Model B, within size**						
						% of ovulation rate							% of ovulation rate		
DS	n	OR	LF	PRE	POS	LF	PRE	POS	OR	LF	PRE	POS	LF	PRE	POS
C	142	12.28	9.68	1.99	0.60	79.90	15.09	5.01	12.45	10.01	1.84	0.60	81.64	13.51	4.84
		(0.16)	(0.23)	(0.24)	(0.10)	(1.81)	(1.64)	(0.80)	(0.31)	(0.37)	(0.35)	(0.13)	(2.56)	(2.35)	(1.08)
(HL,LH)	121	14.32	11.64	2.13	0.56	81.08	14.58	4.34	13.75	11.31	1.88	0.56	84.00	13.04	4.46
		(0.34)	(0.29)	(0.34)	(0.19)	(2.18)	(2.01)	(1.02)	(0.30)	(0.35)	(0.34)	(0.12)	(2.46)	(2.25)	(0.97)
H	296	16.51	10.23	5.63	0.65	62.63	33.17	4.20	17.25	10.54	6.07	0.65	62.14	33.84	4.01
		(0.34)	(0.41)	(0.43)	(0.11)	(2.24)	(2.22)	(0.67)	(0.34)	(0.39)	(0.40)	(0.10)	(2.09)	(2.07)	(0.64)
HH	90	18.81	15.10	3.11	0.84	80.31	14.51	5.17	18.16	14.03	3.49	0.65	77.80	18.61	3.59
		(0.71)	(0.77)	(0.78)	(0.28)	(4.12)	(4.01)	(1.66)	(0.57)	(0.65)	(0.67)	(0.17)	(3.47)	(3.43)	(1.07)
L	217	8.05	6.60	1.20	0.35	83.33	12.20	4.47	8.66	7.00	1.30	0.36	81.85	13.72	4.43
		(0.14)	(0.15)	(0.13)	(0.08)	(1.61)	(1.35)	(1.03)	(0.17)	(0.19)	(0.17)	(0.08)	(1.92)	(1.70)	(1.03)
LL	108	10.29	7.90	2.09	0.30	76.45	20.50	3.04	9.31	7.65	1.32	0.34	82.67	13.52	3.81
		(0.18)	(0.17)	(0.16)	(0.07)	(1.63)	(1.48)	(0.71)	(0.28)	(0.33)	(0.28)	(0.14)	(3.26)	(2.88)	(1.75)

Model A within DS:
mean + batch + group + line + β_1 * age + β_2 * BW + error

Model B within size:
mean + DS + DS(line) + DS(group) + β_1 * age + β_2 * BW + error

Table 6.12: The contrasts between inbred and non-inbred females calculated from means in table 6.11. Contrasts significantly different from zero at 5% level marked in bold and standard errors given in brackets.

contrast	BW, g	OR	LF	LF%	PRE%	POS%
H - HH	-	-0.91	**-3.41**	**-15.66**	**15.23**	0.42
		(0.66)	(0.75)	(4.05)	(4.01)	(1.25)
L - LL	-	-0.65	-0.64	-0.82	0.20	0.62
		(0.33)	(0.38)	(3.78)	(3.34)	(2.03)
H - (HL,LH)	14.5	**2.19**	**-1.41**	**-18.45**	**18.59**	-0.14
		(0.48)	(0.50)	(3.13)	(2.99)	(1.22)
L - (HL,LH)	-11.3	**-6.27**	**-5.04**	2.25	-2.38	0.13
		(0.37)	(0.33)	(2.71)	(2.42)	(1.45)

Live foetuses: The number of live foetuses corrected for body weight was smaller in the control line than in the HL and LH females by an average of 1.3 foetuses (figure 6.3). The control line had accumulated some inbreeding during the resource experiment and it was not a true control for all of the lines, therefore the LFs were not strictly comparable.

The higher number of live foetuses in two-way crosses than in inbreds was seen in both high and low line groups (figure 6.3), but was more clear among the high lines (table 6.12). The LF% shows that the survival of eggs to embryos had clearly increased due to removal of inbreeding effects in the heavy females. However, in the inbred low line females the LF% was 3% higher than in the two-way crossed high line females (table 6.11) and even 2% higher than in the HL and LH females (table 6.12). Thus removal of inbreeding effects affected the foetus production in the high lines, but in the low lines no such large effect was observed.

When the number of live foetuses was averaged over high and low body weight females for each type of female, i.e. the presence of selection or inbreeding effects (table 6.13), the pattern was consistent over groups even when the body weight differences were taken into account. The number of live foetuses increased significantly when inbreeding effects were removed and a further small increase was observed when the selection effects were counterbalanced. The same was observed within certain type of embryos, i.e. reading the table in rows. The number of foetuses in the inbreds was 22% smaller than in the two-way crosses. Here only the cells in top left corner and the bottom right corner were compared, because the mating partner had a reducing effect on the number of embryos.

Table 6.13: Mean number of live foetuses with standard errors in brackets averaged over different type of high and low females calculated from age corrected means (model in table 6.9).

	Female		
Foetus	inbreeding + selection	no inbreeding + selection	no inbreeding counterbal. sel.
inbreeding + selection	8.90 (0.24)		
no inb. + selection	8.72 (0.40)	10.90 (0.51)	
no inb. + counterbal. sel.	8.20 (0.43)	10.66 (0.51)	11.36 (0.48)
	8.61 (0.21)	10.78 (0.36)	11.36 (0.48)

Table 6.14: The mean pre-implantation losses calculated as in table 6.13

	Female		
Foetus	inbreeding + selection	no inbreeding + selection	no inbreeding counterbal. sel.
inbreeding + selection	3.15 (0.49)		
no inb. + selection	2.76 (0.41)	2.98 (0.52)	
no inb. + counterbal. sel.	2.40 (0.89)	2.90 (0.53)	2.45 (0.49)
	2.77 (0.37)	2.94 (0.37)	2.45 (0.49)

Table 6.15: The mean post-implantation losses calculated as in table 6.13

	Female		
Foetus	inbreeding + selection	no inbreeding + selection	no inbreeding counterbal. sel.
inbreeding + selection	0.77 (0.07)		
no inb. + selection	0.42 (0.12)	0.37 (0.15)	
no inb. + counterbal. sel.	0.46 (0.13)	0.21 (0.15)	0.39 (0.14)
	0.55 (0.06)	0.29 (0.11)	0.39 (0.14)

Losses: The post-implantation losses were smaller in all groups compared with pre-implantation losses and were not different between inbred and outbred females (figure 6.3 and table 6.11). Pre-implantation losses differed significantly only between inbred and outbred females in the high line group, the inbreds having 2.6 eggs more lost before implantation. The inbred low line females had very low number of losses and the PRE% was even slightly lower than the mean of HL and LH females (table 6.12).

In tables 6.14 and 6.15 a decreasing trend was seen for the losses when inbreeding effects were removed. The highest average pre-implantation losses of high and low lines was observed when they were inbred (top left corner cell), which was 28% higher than the value in the bottom right corner. The average of high and low means from two-way crosses was larger than the average of inbred high and low females due to the low number of losses in inbred low line females. For the post-implantation losses the large reduction in the average values was observed when the offspring inbreeding effects were removed, i.e. a drop from 0.8 to 0.4 dead foetuses. However, the difference between inbred females with inbred foetuses and outbred females with counterbalanced selection effects was the same. The comparison within non-inbred embryos and between different mothers suggest that the maternal inbreeding was affecting the post-implantation losses less than the inbreeding of the foetuses.

6.3.3 Pregnancy rates

Pregnancy rate (Preg%) was calculated as a percentage of pregnant females over all females mated (table 6.16). The differences between the pregnancy rates were analysed between inbred and outbred females within size group to study the effect of inbreeding on the trait. In Chapter 5 it was observed that matings between different size mates were not succesful, thus the Preg% was calculated also for each mating group in order to study if the situation would be the same in outbred females as in inbred females. The failure in pregnancy was divided in two classes (figure 6.4). Firstly, those who did not ovulate, which was considered as more serious fertility failure. Secondly, those who ovulated but for unknown reason did not become pregnant. Additionally the days from pairing to copulation were calculated to study the possible differences in cycle length or libido.

First the pregnancy rate was analysed as a 0/1 trait to examine which factors affect the trait. The pregnancy rates between size groups were significantly different and also between mating groups within size. Significant differences were observed between the parental lines, apart from the grandfathers (lines j and l in figure 6.1). The age of the animals was not a significant covariate in the model when medium sized animals were analysed together or the DS groups were analysed separately.

Control, HL and LH: The highest pregnancy rates were observed among the average size females and there was no difference in the Preg% between control, HL and LH females (table 6.16). The percentage of females with zero ovulation rate was much smaller in average size females than in the other groups (figure 6.4). The average time from pairing to mating was 2.5 days among these females.

Table 6.16: Number of observations, pregnancy percentage (P%), number of non-ovulating females (OR=0) and the average days from pairing to vaginal plug in each mating group. Standard errors are shown in brackets.

		Pregnancy rate %		OR=0	Days	
Group	n	DS	group	%, DS	DS	group
C	145		97.9 (2.9)	1.4		2.7 (0.3)
HLxLH	50		98.0 (4.9)	2.0		2.4 (0.5)
LHxHL	73		98.6 (4.1)	0.0		2.5 (0.4)
H	255	75.4 (1.8)	81.6 (2.2)	11.1	2.7 (0.2)	2.8 (0.2)
HxH	80		77.5 (3.9)			3.0 (0.4)
HxL	60		46.7 (4.5)			1.8 (0.4)
HHxHH	59	88.2 (3.5)	93.2 (4.5)	8.8	1.8 (0.4)	1.9 (0.5)
HHxLL	43		81.4 (5.3)			1.6 (0.5)
L	169	79.8 (2.2)	79.3 (2.7)	13.2	3.0 (0.2)	2.5 (0.3)
LxL	34		97.1 (6.0)			5.6 (0.6)
LxH	69		72.5 (4.2)			3.2 (0.4)
LLxLL	48	87.1 (3.2)	89.6 (5.0)	8.9	2.4 (0.3)	2.3 (0.5)
LLxHH	76		85.5 (4.0)			2.4 (0.4)

High body weight group: In the high body weight group there was a range of pregnancy rates from 47% to 93% (table 6.16). The inbred females had a significantly lower pregnancy rate than the outbred ones, by 13%. However, the mating partner had a significant effect on the pregnancy rate among the inbred females and thus the overall mean for H group was confounded by the mating

partner effect. When only matings between same size pairs were considered the two-way crosses still had a higher Preg% by 11%. The mating partner effect was also observed within the two-way crosses. In the outbred groups the difference between Preg% of same size pairs and different size pairs was 12%, while in the inbreds it was around 30%. Despite the small sample sizes, this might suggest that the outbred animals were less sensitive to external factors as the size of mating partner.

The failure to ovulate was higher among the inbred females than the outbred by 2% (table 6.16). Of the pregnancy failures on average 45% was due to zero ovulation rate in inbred females and 75% in outbred females. Among the inbred females the number of not ovulating females doubled when the mating partner was of different size and a smaller increase in ovulation rate failure was seen in the crossbred females (figure 6.4).

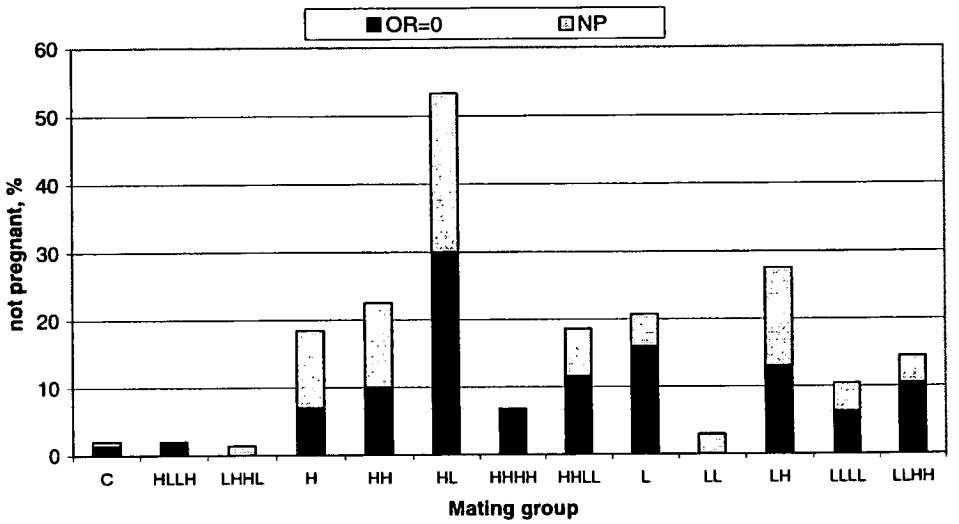
The mean number of days from pairing to vaginal plug was significantly higher among the inbred than the outbred females, by nearly one day. The size of the mating partner did not affect the time among the two-way crosses, while in the inbreds those with a different size partner mated one day faster.

Low body weight group: The range of pregnancy rates in the low line group was from 73% to 97%, with the highest Preg% observed in inbred females (table 6.16). The average pregnancy rate was significantly higher in the two-way crosses than in the inbred by 7%. The lowest pregnancy rates were observed in groups where pairs were of different size for both inbred and outbred females.

Slightly fewer crossbred females than inbred had zero ovulation rates, but in the LL mating group no ovulation failure was observed (figure 6.4). In inbred females 64% of pregnancy failures were due to zero ovulation rate and similarly, 62%, in the two-way crosses. In the two-way crosses the increase in percentage of not pregnant females was due to increased ovulation failure only in the LLxHH group.

Time to copulation was on average slightly higher for the inbred females than the outbreds. However, the average difference was only half a day and not significant (table 6.16). There were differences within the inbred females: time to copulation was over two fold higher in LL females than L females, while the crossbred females had equal times.

Figure 6.4: Percentage of not pregnant females in each mating group either due to failure to ovulate (OR=0) or due to unknown reason (NP).



6.3.4 Regression on body weight

In order to study the relationship between body weight and reproduction traits (OR and LF), and its similarity in inbred and in outbred females, the regression coefficients were calculated on body weight and log transformed body weight for each DS group separately (table 6.17). The regression on age was added to the model, but it did not change the results apart from a minor increase in the coefficients in HH and LL groups.

The regression coefficients of ovulation rate on weight in outbred females were similar on the log scale across the size groups and also between H and L groups. However, the outbred groups had larger estimates of regression coefficients than the inbred groups. The same was observed on regressions of number of live foetuses.

The coefficients within size groups were tested pairwise by calculating the divergence and the standard error for the divergence from table 6.17. There was no significant differences between the regressions of ovulation rate or number of live foetuses on BW among the average size females, i.e. control, HL and LH.

Table 6.17: Regression of ovulation rate on body weight and on ln transformed body weight for each DS group. Significant coefficients on 5% level marked in bold.

	OR		LF	
DS	Regr. on BW coeff. (se)	Regr. on ln(BW) coeff. (se)	Regr. on BW coeff. (se)	Regr. on ln(BW) coeff. (se)
C	**0.27** (0.06)	**7.8** (1.7)	**0.21** (0.08)	**5.8** (2.3)
HL	**0.36** (0.13)	**11.6** (4.2)	**0.29** (0.11)	**10.5** (3.4)
LH	**0.26** (0.08)	**8.1** (2.5)	**0.22** (0.08)	**7.2** (2.5)
H	**0.09** (0.04)	**4.7** (2.0)	0.03 (0.05)	2.4 (2.3)
HH	**0.16** (0.04)	**8.7** (2.2)	**0.11** (0.04)	**5.9** (2.4)
L	**0.32** (0.07)	**4.7** (1.1)	**0.21** (0.08)	**3.1** (1.2)
LL	**0.38** (0.09)	**7.7** (1.9)	**0.37** (0.08)	**7.6** (1.7)

Model within DS: mean + group + line + β * BW + e

In the high body weight group the regression of ovulation rate for inbred H females (0.1) was nearly half of the regression coefficient of crossbred HH females (0.2). On the log scale the difference between the coefficients was 4.0 (s.e. 2.9). The regression of number of live foetuses was nearly three fold higher in crossbred than inbred females, but after transformation the difference was only two fold, being 3.5 (s.e. 3.3). In the low body weight group the inbred females had smaller regression coefficients than the crossbred. The difference in regression estimates for OR was 3.0 (s.e. 2.2) and for LF 4.5 (s.e. 2.1) from transformed data. Thus, the increase in body weight might be slightly stronger connected with the ovulation rate and number of live foetuses in outbred females than in inbred females, but the data does not give statistically significant evidence of that.

6.4 Discussion

This chapter has focused on the reproductive performance of outbred females and comparisons between inbred and outbred females. The aim was to study if the reproductive fitness is affected by growth selection and/or inbreeding and which components were changed.

The outbred medium sized females had an ovulation rate equal to the average of large and small females, but the number of live foetuses was slightly larger. Thus, the medium sized females seemed to be more efficient than the extremes in terms of getting live foetuses from the eggs released from the ovaries.

Inbreeding did not have a large effect on the ovulation rate when corrected means were compared and also the regressions of ovulation rate on body weight were only slightly larger among the outbred females. The results from the analysis between means agree with Roberts and Falconer (1960) who did not observe differences in ovulation rates between inbred and outbred females. In their study of unselected mouse lines, the inbreeding effect on body weight was balanced by the effect of pre-weaning environment and body weights remained constant while inbreeding was practised. Thus, they suggested that the ovulation rate would decline together with the body weight, but might not decline due to inbreeding on its own.

The removal of inbreeding effects from parents clearly had a positive effect on foetus production, but more so in the heavy females. The average increase in number of live foetuses was 22% or 3 foetuses when inbreeding effects were removed (table 6.13). The estimates of heterosis on litter size in the literature agree well with this figure being around 3 pups (Roberts, 1960; Falconer, 1971). Most of the reduction in number of live foetuses was caused by the inbreeding of mothers rather than foetuses, as seen in other studies as well (Roberts, 1960; Falconer and Roberts, 1960). Pre-implantation losses increased mainly because of the maternal inbreeding and whereas post-implantation losses were mainly affected by the inbreeding of the foetuses.

Many of the above results in this study suffered from lack of statistical significance due to a small number of observations. They might have been affected also by the unequal line contributions to the different groups and by the older

mating age of the two-way crosses. The effect of age at mating was a concern in this study. The age was connected with increasing body weight, possibly due to fattening of animals at older age. This could in turn affect the relationship between body weight and ovulation rate, so it would be interesting to have regression estimates on fat free body weight to study this issue in detail. However, in this study the age differences were of magnitude one month and therefore no extreme age differences were present.

Inbreeding has been reported to affect the length of reproductive life so that independently of their past reproduction history most inbred female mice were found to show greatly reduced fertility by the age of 8 – 10 months (Silver, 1995). However, the oldest mice in this study were up to four months old and therefore were not approaching age of menopause. The litter size has been reported to remain rather constant in outbred and inbred mouse populations up to 300 days and after that gradually decrease (Grüneberg, 1943).

The percentage of pregnant females from all matings was not different between the sizes (e.g. H and L), but the differences occured between the inbred and non inbred females. As expected, pregnancy rates were higher among the outbred females and highest among the medium sized females. The pregnancy rates in the outbred females were similar than the average in outbred rodent populations, i.e. 98% (Nalbandov, 1976).

The mating partner of different size had an effect on the ovulation rate and pregnancy rate of the females. The mating partner effect was seen in both inbred and outbred females, but was more clear in the inbred females (Chapter 5). In the HHxLL mating group the difference between mean female weight and mean male weight was 27g, so on average the female was twice as heavy than the male; while in the LLxHH mating group the difference was on average 50g, hence the females were on average just one quarter of the weight of the male. Thus, the size difference between partners was substantial as was among the inbred females. However, the inbred females might be more sensitive to environmental factors like stress and therefore showed larger mating partner effect.

To conclude the results of the effect of selection and inbreeding on reproductive fitness the hypothetical litter size for a group of mice is estimated in all mating groups as a product of pregnancy rate and number of live foetuses (table 6.18).

Table 6.18: The number of live foetuses (LF), pregnancy rate (Preg%) and the reproductive output for group of mice ("LS") in mating groups

Group	LF	Preg%	"LS"
C	10.0	97	9.7
H	11.0	82	9.0
HxH	11.3	78	8.8
HxL	9.6	47	4.5
L	6.3	79	5.0
LxL	6.6	97	6.4
LxH	7.1	73	5.1

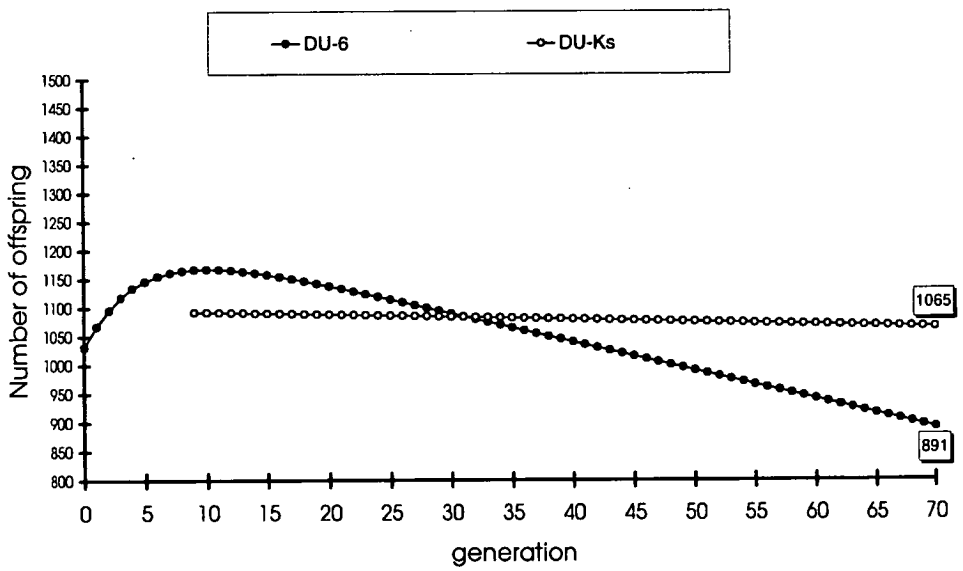
Group	LF	Preg%	"LS"
HLxLH	12.1	98	11.9
LHxHL	11.8	99	11.7
HHxHH	16.1	93	15.0
HHxLL	15.7	81	12.7
LLxLL	7.9	90	7.1
LLxHH	8.0	86	6.9

Among the inbred females, medium sized and outbred control line had higher litter sizes than the large females. However, this was confounded by the inbreeding effects. Among the outbred females the medium sized actually had smaller litter size than the large females, as would be expected taking into account the consistent positive relationship between ovulation rate and body weight. The mean litter size of large and small females was 10.4 and the average of medium sized females was 11.8, i.e. nearly one and half pups higher. Therefore, the reproductive fitness of medium sized outbred females seemed to be more efficient than that of the extremes. The inbred females had clearly lower fitness than the outbred, but the fitness was also lower in extreme sized females compared with the medium sized females.

since it was not possible to separate the inbreeding effects from the effects connected with the establishment of inbred lines, which involves a reduction of family number and a certain selection for fertility.

Other components of reproductive fitness might have been changed either due to the effects of selection or due to inbreeding and thereby affected the litter size. However, there were no data available on other reproduction traits than litter size. The reduction of reproductive output was demonstrated in the heavy DU-6 line which was clearly responding to selection over 70 generations (Bünger et al., 1993). In the DUH-6 line the litter size increased as a correlated trait to growth selection, but the number of fertile matings dropped from 80% to 60% causing an overall reduction in the offspring production. An experimental study was needed to estimate the effects of inbreeding and selection separately on range of components of reproductive fitness.

Figure 7.1: Development of expected offspring from 100 matings per generation in line selected for 42 day body weight DU-6 and in the control line DU-Ks (Bünger et al., 1993).



The aim of experiment I (Chapter 4) was to estimate the effect of growth selection on the components of reproductive fitness in inbred mice. The components were studied from the same inbred high and low growth lines that were discussed above. As there were several 'replicates' for high growth and low growth lines, the study was not restricted to a certain line or pair of high and low lines and so it allowed more general conclusions to be made about the relationship between reproductive fitness and body weight in inbred mice.

The relationship between body weight and ovulation rate was similar, 0.3 ova/g, independent of the size of the female, while the relationship between number of live foetuses was less consistent. The number of live foetuses was higher in the heavy females than in the light ones, but the difference was much smaller than for the ovulation rate. In order to make comparisons despite the differences in ovulation rate, the survival rate from egg to foetus was estimated. The high line average survival rate was 62%, while in the low lines it was 79%, i.e. at similar level in the outbred control line. The losses were mainly attributable to the losses before implantation and no differences were observed between the size groups in percentage of dead foetuses, i.e. post-implantation losses.

The percentage of successful matings was observed to be around 80% in both high and low lines and 97% in the control, but the high line females seemed to suffer more from fertility problems such as difficulties to carry the pregnancies to full term. The pregnancy rate was higher than would be expected of a group of inbred females.

The inbreeding coefficient of the lines was assumed to be high (over 90%), but no marker data was available to calculate the realised degree of inbreeding. It might have been possible that the lines were not as homozygous as expected. Natural selection might have been acting on the lines so that the most unfit animals with harmful recessive gene combinations were not cabable of surviving or reproducing. Thus more variation might have been present within the lines than was assumed, which might explain for example the above mentioned unexpectedly high pregnancy rate.

The high line females had a potential for large reproductive performance, having an ovulation rate of 17 ova, but an estimated reproductive output taking into account the losses and the pregnancy rate was only 9 pups. In the low

line females the ovulation rate was 8 and the estimate of 'litter size' 5 pups, i.e. slighty better relative performance than in the heavy females. The selection and inbreeding effects were still confounded, so the effects of inbreeding had to be removed in order to separate the effects. This was undertaken in the two following experiments.

The aim of experiment II (Chapter 5) was to study if crossbreeding of the foetuses increases the reproductive performance of inbred mothers. Line crosses were set up between the inbred growth lines in order to remove the inbreeding effects of foetuses. Experiment II also addressed the question whether the line crosses between similar size pairs and different size pairs would have an effect on the reproduction through the genotype and phenotype of the foetuses.

The removal of inbreeding effects increased slightly the number of live foetuses, more so in the low line females by half a foetus an average. The increase was mainly attributable to lower death rate, i.e. post-implantation losses, of the crossbred foetuses. This was expected since viability of the foetuses was assumed to be affected by both the parents and the genotype of the foetus (Austin and Short, 1985).

The effect of counterbalancing the selection, i.e. mating pairs of different size (high x low), on viability of the foetuses was not clear. In the high lines the survival of crossbred foetuses was lower when mate was small and in the low lines the survival was better when the mate was large, but the results were not consistent accross the lines. Against expectations the mating partner had an effect on the ovulation rate of the females. In high lines the females with a small partner had a lower ovulation rate, in both size groups the number of non-ovulating females increased when partner was of different size and also the pregnancy rates were lower. Thus, the different size males did not seem to stimulate the females.

The aim of experiment III (Chapter 6) was to estimate the effect of growth selection on reproductive fitness of crossbred females. The question of the effect of removing the inbreeding of the parents on reproduction was addressed by comparing data from inbred and outbred females.

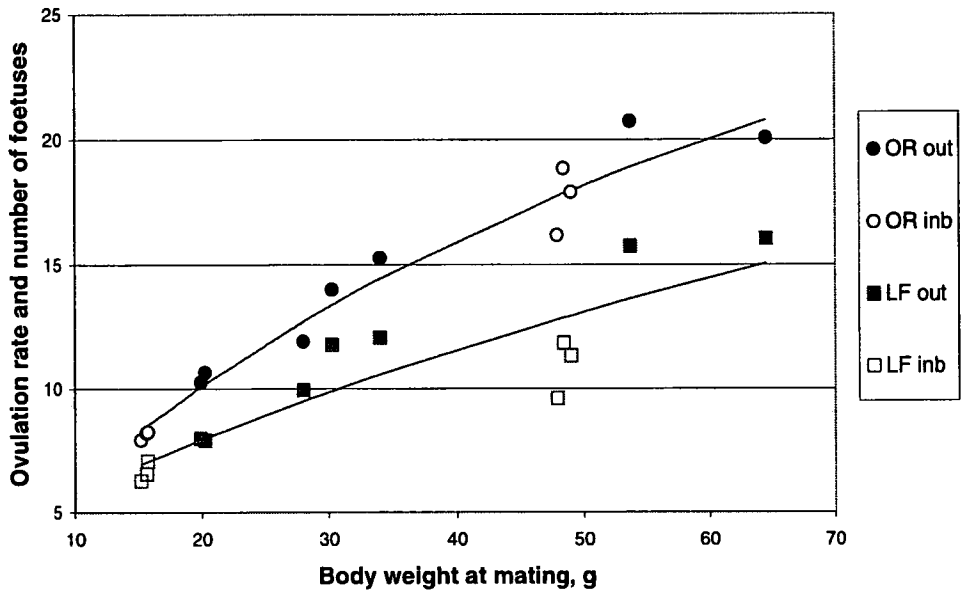
The ovulation rate of the medium sized outbred females was approximately the average of large and small females, but the number of live foetuses was slightly higher than the average of high and low due to lower pre-implantation losses. The

reproductive output for a group of mice, which took into account the losses and pregnancy rates, was 12 pups, which was two pups higher than the average of the extremes. Thus, the medium sized animals may well be more fit than the extremes.

Inbreeding did not greatly affect the ovulation rate and relation between ovulation rate and body weight was similar for both inbred and outbred females (figure 7.2). However, the number of live foetuses was much larger, three foetuses on average, in outbred females than in inbred females. The higher survival rate of foetuses was mainly attributable to lower pre-implantation losses in outbred mothers. Also the pregnancy rates were clearly higher among the outbred females than the inbred females. The results agree with the general observation that litter size during inbreeding has been affected mainly by fitness of the mothers and partly by the viability of foetuses (Falconer, 1960). To summarise the effect of inbreeding on offspring production the estimated litter sizes were compared. The outbred heavy females had 6 pups higher litter size than the inbreds and in the small outbred females two pups larger litter than the inbreds. In relative terms the litter size was 25% smaller in heavy inbred females than in outbred and in the low lines nearly 30% smaller.

To summarise the results of all experiments the means of the ovulation rate and number of live foetuses from inbred and outbred females were plotted against the body weight (figure 7.2). The fitted lines were estimated by an exponential model developed by Bünger et. al. (1998). Ovulation rate follows the body weight development in similar manner in all females, except when it was affected by the mating partner. That might suggest a pleiotropic gene action between body weight and ovulation rate and also an additive gene action, since the means of crossbreds were in the middle of the extremes. The fitted line of number of live foetuses diverged more from the ovulation rate when body weight increased, which indicates the lower embryonic survival in heavy animals. Thus, an increase in the offspring production would level off sooner than the ovulation rate. The distance between the curves would increase with increasing body weight as the regression between body weight and ovulation rate would hold over the whole range with substantially smaller increase in number of live foetuses. The harmful effects of inbreeding would be seen clearly if a line was fitted for inbred and

Figure 7.2: The ovulation rate (OR) and number of live foetuses (LF) plotted against body weight at mating (g). Lines of fitted values were estimated by exponential model (Bünger et al., 1998) using data from all mating groups.



outbred females separately, the line for inbred females would be much further away from the line for ovulation rate and also with smaller slope. Both of the examined effects had a large impact on the reproductive fitness, growth selection increasing the potential for offspring production and inbreeding suppressing it.

For a breeder the efficiency of the females would be economically important. The reproduction efficiency for certain type of females was calculated by dividing the estimated litter size (pregnancy rate * number of live foetuses) by the metabolic body weight ($BW^{3/4}$). The efficiency coefficients were clearly smaller for the large females among inbreds, i.e. 0.49 for H and 0.66 for L groups. Among the outbred females the HL/LH group were the most efficient (0.85) compared with the HH (0.66) and LL (0.75) groups. These calculations support the observation, that the small sized inbred females had reproduction approximately on the same level than outbred large females, while the crossbred average sized females outperformed all other groups.

7.2 Future research

In connection with many results can be suspected that the fatness of the animals might affect the regression of ovulation rate on body weight. Therefore, it would be important to estimate the effects of body composition on reproductive fitness. For example the regression of ovulation rate on fat free body mass might differ from the regression estimate on body weight and thus the regression on fat free body mass might be more reliable estimate.

An unexpectd mating partner effect was observed among the inbred females, but the result was not clearly confirmed with the data from outbred females. A further study to validate the results of mating partner effect would be needed with larger number of animals. If similar observations were seen, the question to be studied would be the cause of the effect. It might be due to physiological reasons, i.e. pairs so divergent that they cannot mate anymore, or it might be due to the female preferences and some kind of regulation system of her own physiology based on those.

The range of traits of reproductive fitness was wide in this study, but the results considered only the litter size at the first parity. To complete the picture of effects of growth selection and inbreeding on reproductive fitness, a further study should be done about the length of the reproductive life. The questions to be addressed would be whether the litter size, ovulation rate, number of live foetuses and pregnancy rates over several parities are affected by the body size and how large is the effect of inbreeding.

Appendix A

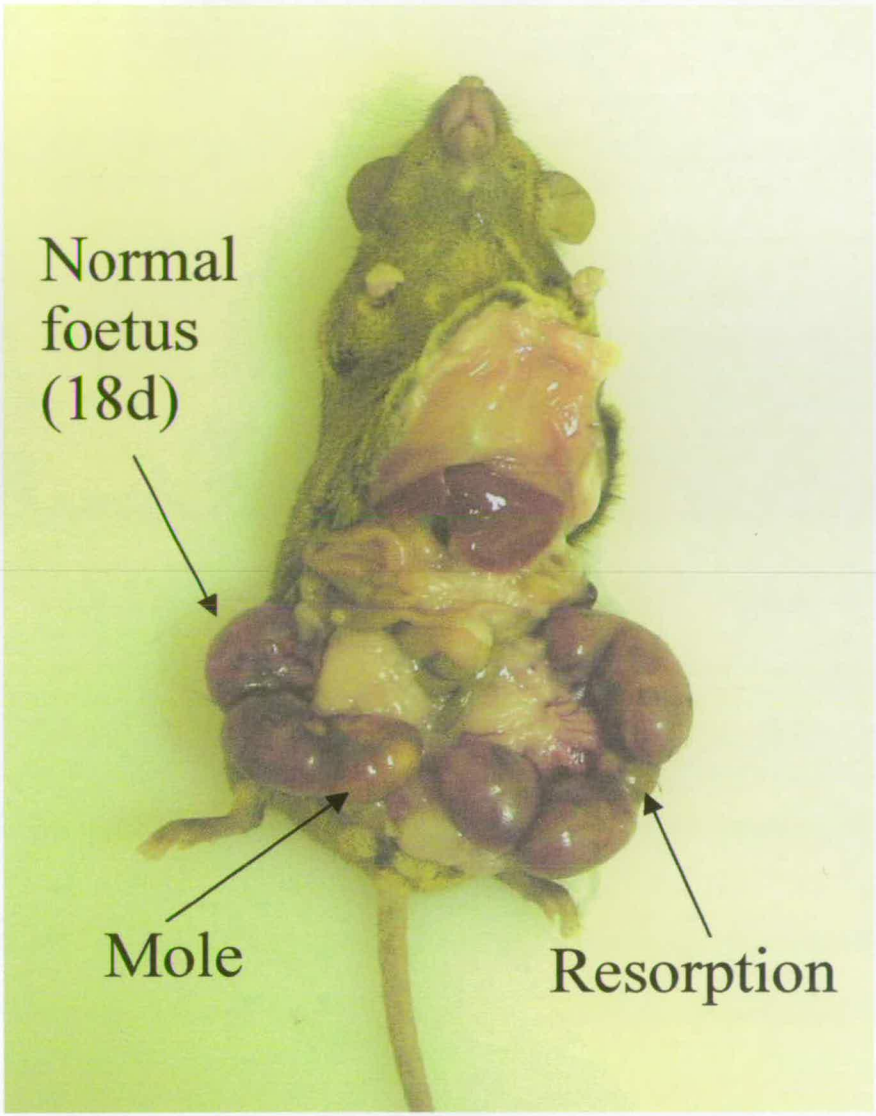
Pictures

Figure A.1: A ventral view of dissected pregnant mouse at day 18 of gestation. In the figure foetuses which died at early stage of gestation (mole) and at late stage of gestation (resorption) are marked with arrows. The others in uterine horns are normal live foetuses.

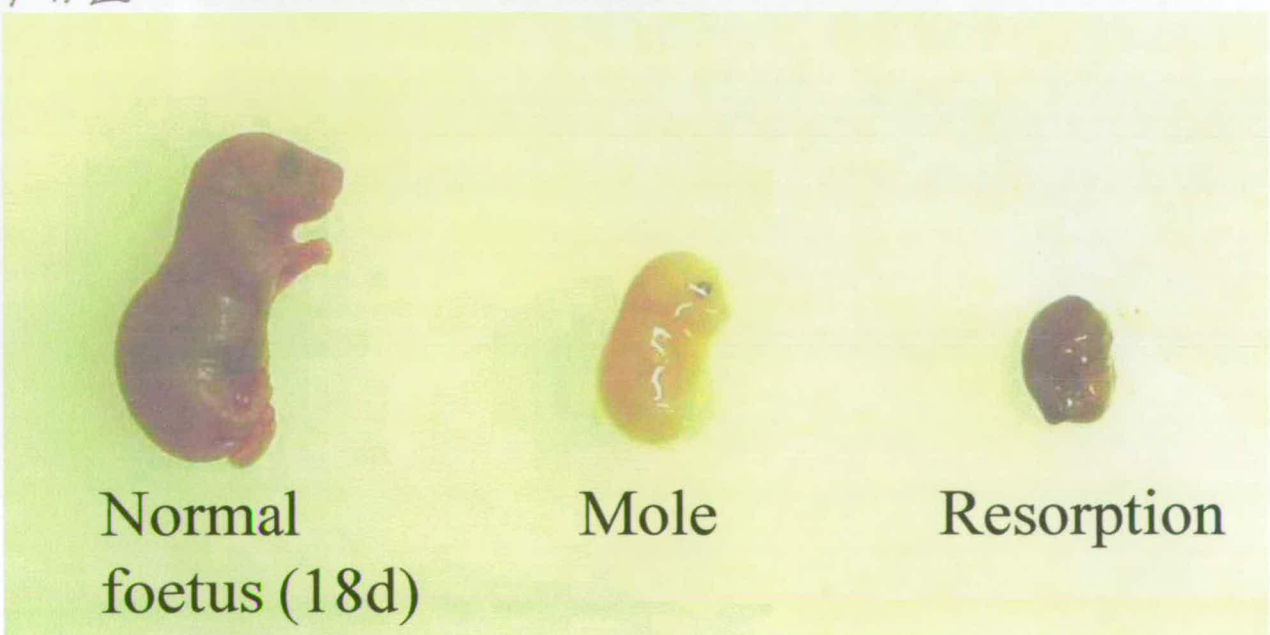
Figure A.2: Normal foetus at 18 days of age, i.e. day before birth, a foetus which died at late stage of gestation and a foetus which died after early stage of gestation were taken from the female in picture A.

Figure A.3: A mouse ovary (with match-head for scaling purposes) which is situated in the top of each uterine horn. The red dots on the surface of ovary, corpora lutea, were counted in order to get the ovulation rate

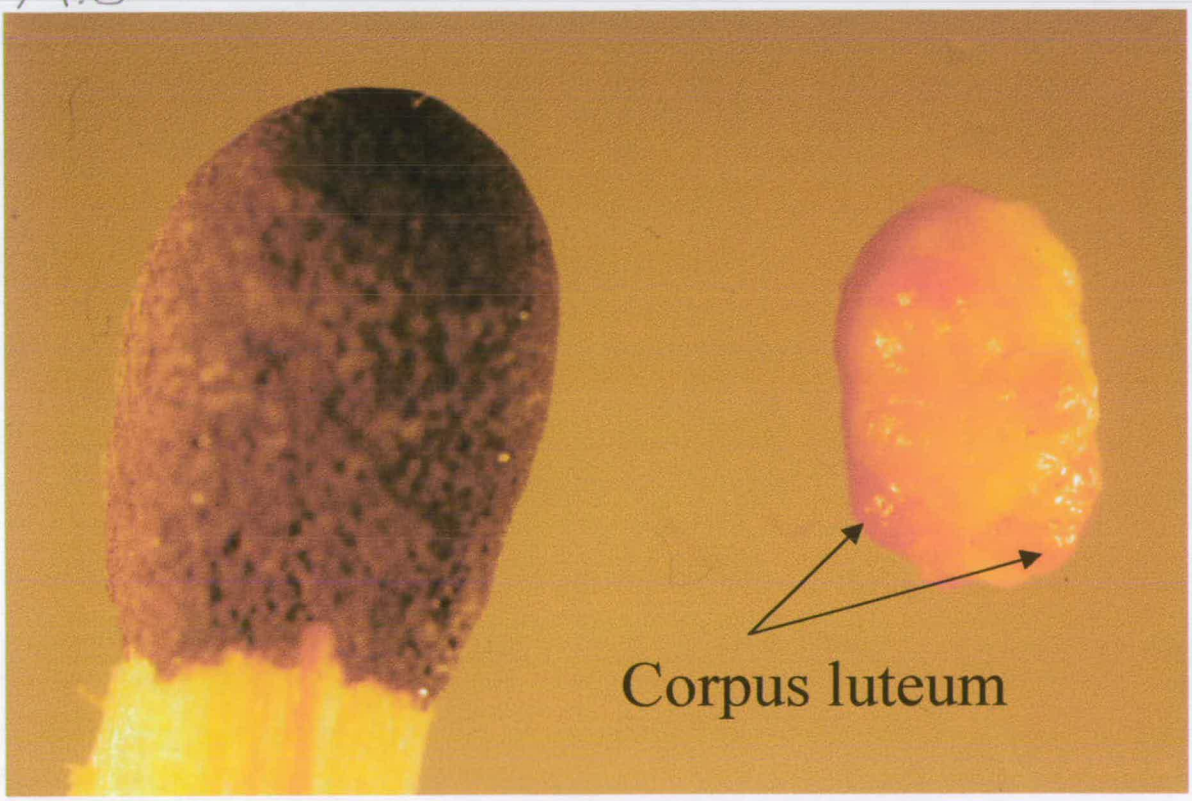
A.1


Normal
foetus
(18d)
Mole
Resorption

A.2.


Normal
foetus (18d)
Mole
Resorption

A.3



Appendix B

Data from the experiments

Table B.1: Summary of the results from the experiment I, with data from control and high body weight lines (Chapter 4)

		Not pregnant					Pregnant					
		OR = 0		Ovulating								
line	batch	n	BW	n	BW	OR	n	BW	OR	LF	PRE	POS
EDC	1	-	-	-	-	-	27	26.61	11.52	9.44	1.85	0.22
	2	1	23.80	-	-	-	44	28.23	12.23	10.00	1.52	0.70
	3	1	31.20	1	27.60	16.0	35	29.43	13.29	9.31	3.26	0.71
DAH	1	1	58.80	-	-	-	7	50.27	16.86	14.43	1.71	0.71
	2	-	-	1	59.40	17.0	9	59.68	17.33	10.00	5.22	2.11
	3	1	48.20	3	54.03	16.6	12	54.08	21.75	11.75	9.08	0.92
DUH	1	1	57.30	9	62.72	11.2	14	60.84	16.07	10.00	6.00	0.07
	2	1	52.30	1	58.00	11.0	5	57.54	28.40	8.40	19.80	0.20
	3	2	62.55	1	58.00	18.0	7	57.16	23.29	13.71	8.29	1.29
RAH	1	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	2	60.80	18.0	10	50.99	20.90	11.70	8.40	0.80
	3	-	-	-	-	-	20	58.74	19.85	12.05	7.05	0.75
EDH	1	1	37.40	1	39.40	2.0	13	38.77	16.69	12.08	4.08	0.54
	2	-	-	-	-	-	5	43.50	18.20	13.80	4.20	0.20
	3	2	40.40	-	-	-	16	41.70	20.19	14.19	5.44	0.56
BEH	1	3	47.93	-	-	-	15	45.03	13.87	9.07	4.27	0.53
	2	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-
MUH	1	1	20.40	-	-	-	11	35.78	15.82	8.00	7.18	0.64
	2	1	38.60	1	38.70	13.0	8	38.49	14.75	9.75	3.50	1.50
	3	4	36.35	3	36.40	8.3	13	37.67	20.00	9.46	9.39	1.15
ROH	1	-	-	2	35.40	10.0	18	33.04	12.67	9.11	2.33	1.22
	2	-	-	3	42.40	10.3	7	40.29	15.71	13.00	1.43	1.29
	3	-	-	3	38.93	13.3	17	41.21	15.65	12.53	2.18	0.94

Table B.2: Summary of the results from the experiment I, with data from low body weight lines (Chapter 4)

		Not pregnant					Pregnant					
		OR = 0		Ovulating								
line	batch	n	BW	n	BW	OR	n	BW	OR	LF	PRE	POS
EDL	1	1	11.70	2	13.50	4.0	14	13.96	7.00	5.50	0.86	0.64
	2	-	-	-	-	-	5	14.76	6.80	4.80	0.60	1.40
	3	-	-	-	-	-	8	14.71	8.13	6.00	1.00	1.13
BEL	1	5	13.06	-	-	-	15	14.95	9.27	6.93	2.07	0.27
	2	4	16.20	1	19.80	8.0	5	15.54	10.80	7.80	2.40	0.60
	3	4	13.70	-	-	-	13	14.82	9.54	7.00	2.31	0.23
MUL	1	10	13.73	1	13.70	6.0	8	14.39	6.75	6.13	0.25	0.38
	2	1	18.20	2	13.75	3.5	11	16.14	7.27	6.27	0.64	0.36
	3	1	14.80	1	18.50	7.0	12	14.46	8.50	6.17	0.92	1.42
ROL	1	1	13.60	-	-	-	18	14.74	7.22	6.22	0.44	0.56
	2	-	-	-	-	-	10	15.90	7.50	6.10	0.90	0.50
	3	-	-	1	17.40	4.0	15	15.93	7.00	6.33	0.47	0.20

n = number of observations
BW = body weight of female at mating
OR = ovulation rate
LF = number of live foetuses
PRE = pre-implantation losses
POS = post-implantation losses (resorptions and moles)

Table B.3: Summary of the results from the experiment II, with data from high body weight lines (Chapter 5)

			Not pregnant					Pregnant					
			OR = 0		Ovulating								
line	batch	group	n	BW	n	BW	OR	n	BW	OR	LF	PRE	POS
DAH	2	HH	1	66.5	0	-	-	4	51.0	21.0	16.5	3.5	1.00
		HL	3	54.6	4	53.2	7.3	1	51.1	18.0	11.0	6.0	1.00
	3	HH	1	45.9	1	54.5	17.0	6	55.1	22.5	12.8	9.5	0.17
		HL	5	49.1	1	53.1	21.0	2	44.8	11.0	4.5	5.5	1.00
DUH	2	HH	0	-	0	-	-	0	-	-	-	-	-
		HL	0	-	0	-	-	0	-	-	-	-	-
	3	HH	0	-	2	65.0	14.5	6	61.9	24.0	11.5	11.8	0.67
		HL	0	-	3	71.7	17.0	2	63.9	20.0	8.0	10.0	2.00
RAH	2	HH	0	-	0	-	-	0	-	-	-	-	-
		HL	0	-	0	-	-	0	-	-	-	-	-
	3	HH	2	59.7	2	60.9	22.0	4	53.9	23.0	12.7	10.3	0.00
		HL	3	53.6	2	56.6	21.0	3	61.4	20.0	12.3	7.0	0.67
EDH	2	HH	1	39.0	0	-	-	5	41.7	15.8	10.2	4.8	0.80
		HL	1	36.0	3	43.7	10.7	2	42.3	13.5	9.5	4.0	0.00
	3	HH	1	39.1	0	-	-	7	43.7	15.6	10.9	4.4	0.29
		HL	1	44.2	0	-	-	4	42.5	15.8	8.8	6.5	0.50
BEH	2	HH	0	-	0	-	-	0	-	-	-	-	-
		HL	0	-	0	-	-	0	-	-	-	-	-
	3	HH	0	-	0	-	-	2	48.8	13.5	11.5	1.5	0.50
		HL	0	-	0	-	-	-	-	-	-	-	-
MUH	2	HH	0	-	0	-	-	8	38.7	17.6	11.4	5.5	0.75
		HL	0	-	0	-	-	3	33.5	16.0	11.0	4.3	0.67
	3	HH	1	41.8	2	37.3	13.0	3	41.9	14.7	8.3	5.0	1.33
		HL	2	38.2	1	42.3	14.0	1	41.7	16.0	3.0	13.0	0.00
ROH	2	HH	0	-	0	-	-	6	38.8	16.0	11.8	3.2	1.00
		HL	0	-	0	-	-	6	38.3	14.2	11.5	2.0	0.67
	3	HH	1	63.5	3	41.8	11.7	11	43.1	12.9	9.0	3.1	0.82
		HL	2	39.5	1	43.7	4.0	3	43.6	15.0	9.7	4.0	1.33

Table B.4: Summary of the results from the experiment II, with data from low body weight lines (Chapter 5)

			Not pregnant					Pregnant					
			OR = 0		Ovulating								
line	batch	group	n	BW	n	BW	OR	n	BW	OR	LF	PRE	POS
EDL	2	LL	0	-	0	-	-	5	15.0	7.0	5.0	1.8	0.20
		LH	2	11.6	0	-	-	3	13.6	5.7	4.7	0.7	0.33
	3	LL	0	-	0	-	-	3	15.1	6.7	5.3	0.7	0.67
		LH	1	15.8	1	15.7	3.0	6	15.9	7.8	6.5	1.2	0.17
BEL	2	LL	0	-	0	-	-	5	13.6	10.4	7.2	3.0	0.20
		LH	1	15.1	1	15.7	8.0	3	14.5	8.0	7.7	0.3	0.00
	3	LL	0	-	0	-	-	1	16.7	10.0	7.0	1.0	2.00
		LH	3	14.0	1	14.9	8.0	6	17.0	11.0	8.3	2.0	0.67
MUL	2	LL	0	-	1	15.2	6.0	11	15.8	8.0	7.2	0.5	0.27
		LH	0	-	0	-	-	11	15.9	6.9	6.5	0.4	0.09
	3	LL	0	-	0	-	-	0	-	-	-	-	-
		LH	2	13.7	1	15.0	2.0	10	15.6	8.3	7.2	0.8	0.30
ROL	2	LL	0	-	0	-	-	3	15.1	5.7	5.7	0.0	0.00
		LH	0	-	3	17.4	8.0	3	16.6	9.3	8.7	0.7	0.00
	3	LL	0	-	0	-	-	5	16.6	8.0	7.2	0.6	0.20
		LH	0	-	3	15.9	3.7	8	16.2	8.8	7.3	1.1	0.38

n = number of observations
BW = body weight of female at mating
OR = ovulation rate
LF = number of live foetuses
PRE = pre-implantation losses
POS = post-implantation losses (resorptions and moles)

Table B.5: Summary of the results from the experiment III, with data from control and HH and HL two-way crosses (Chapter 6)

		Not pregnant					Pregnant					
		OR = 0		Ovulating								
female	batch	n	BW	n	BW	OR	n	BW	OR	LF	PRE	POS
EDC	4	0	-	0	-	-	36	27.2	12.0	9.9	1.3	0.75
DAHRAH	4	3	91.2	1	85.4	16	2	92.6	21.5	14.5	6.5	0.50
DAHMUL	4	0	-	0	-	-	2	47.3	17.5	13.5	4.0	0.00
DUHEDH	4	3	65.7	0	-	-	3	49.3	20.3	15.7	4.7	0.00
DUHROH	4	0	-	0	-	-	1	84.5	13.0	12.0	0	1.00
DUHROL	4	1	62.6	0	-	-	0	-	-	-	-	-
RAHDAH	4	2	93.75	1	91.0	15	2	94.8	25.5	16.5	7.5	1.50
RAHBEH	4	0	-	0	-	-	3	67.5	20.0	17.0	3.0	0.00
RAHMUH	4	0	-	0	-	-	3	69.4	20.3	18.0	0.7	1.67
RAHROH	4	0	-	0	-	-	8	51.9	16.4	12.9	3.0	0.50
RAHBEL	4	0	-	0	-	-	6	33.8	14.5	13.0	0.8	0.67
RAHROL	4	0	-	0	-	-	5	27.2	10.2	10.0	0.0	0.20
EDHBEH	4	0	-	0	-	-	2	50.1	23.5	21.5	1.5	0.50
EDHMUH	4	0	-	0	-	-	3	47.3	20.3	18.0	1.7	0.67
EDHROH	4	1	47.4	0	-	-	5	43.8	15.8	13.8	0.4	1.60
EDHMUL	4	0	-	0	-	-	6	36.3	16.5	11.3	4.5	0.67
BEHDAH	4	0	-	0	-	-	2	47.0	18.5	16.5	0.5	1.50
BEHMUH	4	0	-	0	-	-	3	47.9	22.3	15.3	6.3	0.67
BEHROH	4	0	-	0	-	-	7	42.7	14.9	13.7	0.6	0.57
BEHEDL	4	0	-	0	-	-	2	37.4	13.5	13.0	0.5	0.00
BEHMUL	4	0	-	0	-	-	2	50.9	13.0	8.0	5.0	0.00
BEHROL	4	0	-	0	-	-	5	30.8	12.6	11.6	0.6	0.40
MUHDUH	4	0	-	0	-	-	2	65.5	18.5	17.0	1.0	0.50
MUHBEH	4	0	-	0	-	-	2	31.0	10.5	8.0	2.5	0.00
MUHROH	4	0	-	0	-	-	5	36.6	12.4	7.2	4.8	0.40
MUHROL	4	0	-	0	-	-	5	29.2	13.4	6.4	6.2	0.80
ROHRAH	4	0	-	1	63.2	14	6	60.7	15.5	14.5	0.5	0.50
ROHEDH	4	0	-	0	-	-	9	43.1	17.8	16.0	0.9	0.89
ROHBEH	4	0	-	0	-	-	11	49.0	16.6	14.8	1.4	0.45
ROHMUH	4	0	-	0	-	-	12	46.5	15.7	8.9	5.4	1.33
ROHEDL	4	0	-	0	-	-	16	28.5	14.1	13.1	0.5	0.50

Table B.6: Summary of the results from the experiment III, with data from LH and LL two-way crosses (Chapter 6)

		Not pregnant					Pregnant					
		OR = 0		Ovulating								
female	batch	n	BW	n	BW	OR	n	BW	OR	LF	PRE	POS
EDLDAH	4	0	-	0	-	-	5	31.8	14.8	13.4	0.2	1.20
EDLBEH	4	0	-	1	29.2	13	10	28.3	12.7	10.3	1.8	0.60
EDLMUH	4	0	-	0	-	-	3	27.7	12.0	9.7	1.7	0.67
EDLROH	4	0	-	0	-	-	2	26.5	12.0	9.0	2.5	0.50
EDLBEL	4	0	-	0	-	-	13	20.4	12.9	10.9	1.2	0.77
EDLMUL	4	0	-	0	-	-	5	19.1	8.8	4.0	4.2	0.60
EDLROL	4	0	-	1	21.4	12	9	19.4	9.8	9.1	0.3	0.33
BELDAH	4	0	-	0	-	-	6	35.9	19.8	17.3	2.0	0.50
BELEDH	4	0	-	0	-	-	10	28.6	15.2	13.7	0.9	0.60
BELMUH	4	0	-	0	-	-	7	29.6	16.4	14.3	2.0	0.14
BELEDL	4	1	17.7	0	-	-	6	18.7	11.7	10.3	0.8	0.50
BELMUL	4	3	20.4	2	21.8	11	7	22.6	12.7	8.1	4.0	0.71
BELROL	4	0	-	1	19.2	10	9	19.4	10.0	9.0	0.7	0.33
MULEDH	4	0	-	0	-	-	7	30.2	11.4	9.6	1.4	0.43
MULBEH	4	0	-	0	-	-		4 30.9	12.8	11.8	0.8	0.25
MULEDL	4	2	22.2	0	-	-	11	19.8	8.6	4.9	3.5	0.18
MULBEL	4	0	-	1	18.7	12	7	22.1	11.4	7.3	4.0	0.14
MULROL	4	4	20.3	0	-	-	16	20.4	7.8	6.3	1.5	0.00
ROLEDH	4	0	-	0	-	-	5	29.9	13.0	11.6	1.2	0.20
ROLBEH	4	0	-	0	-	-	8	29.6	11.6	10.6	0.6	0.38
ROLMUH	4	0	-	0	-	-	9	32.3	12.2	9.0	3.0	0.22
ROLEDL	4	0	-	0	-	-	12	19.2	10.1	9.5	0.3	0.25
ROLBEL	4	0	-	0	-	-	11	21.3	12.2	11.0	0.9	0.27
ROLMUL	4	1	24.7	0	-	-	1	21.9	7.0	3.0	4.0	0.00

n = number of observations
BW = body weight of female at mating
OR = ovulation rate
LF = number of live foetuses
PRE = pre-implantation losses
POS = post-implantation losses

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