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Abstract

The objective of this study was to evaluate selection responses and heterotic effects in mouse line crosses after ten generations of selection. Four mouse lines were analyzed: G, L, W and C, selected for growth (body weight at 42 days [BW42]), tail length at 42 days [TL], litter size at birth [LS], and the control line, respectively. After 10 generations of selection the first set of crosses was created; in generation 12, backcrosses and three-way crosses were made. In the crosses the following traits were analyzed: body weight at 21, 42, 63 days, tail length at 42 days, litter size and litter mass at birth. Additive genetic effects of all lines were significant for BW (at all three measurement times) and TL. Heterosis was found for BW42 for the WxC combina- tion, whereas the CxL combination tended to have a BW42 lower than expected from the line means. The same effect was observed for the CxG cross at day 63 with the effect increasing with age. With the exception of a maternal heterotic effect in the GxL cross, there was no significant effect on reproductive traits. The results show that 10 generations of line separation with selec- tion on different traits (rather than divergent selection on a single trait) are enough to create tic differences between the lines which result in a significant amount of heterosis for some parameters.

Disciplines

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The effects of line crossing following selection in mice

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Summary

The objective of this study was to evaluate selection responses and heterotic effects in mouse line crosses after ten generations of selection. Four mouse lines were analyzed: G, L, W and C, selected for growth (body weight at 42 days [BW42]), tail length at 42 days [TL], litter size at birth [LS], and the control line, respectively. After 10 generations of selection the first set of crosses was created; in generation 12, backcrosses and three-way crosses were made. In the crosses the following traits were analyzed: body weight at 21, 42, 63 days, tail length at 42 days, litter size and litter mass at birth. Additive genetic effects of all lines were significant for BW (at all three measurement times) and TL. Heterosis was found for BW42 for the WxC combination, whereas the CxL combination tended to have a BW42 lower than expected from the line means. The same effect was observed for the CxG cross at day 63 with the effect increasing with age. With the exception of a maternal heterotic effect in the GxL cross, there was no significant effect on reproductive traits. The results show that 10 generations of line separation with selection on different traits (rather than divergent selection on a single trait) are enough to create genetic differences between the lines which result in a significant amount of heterosis for some parameters.

Introduction

Over the last centuries two main breeding approaches have been employed for farm animal genetic improvement programs: selection and crossbreeding. By definition, selection leads to a reduction of genetic variability whereas crossbreeding stimulates genetic diversity. The mouse is perceived as a suitable experimental model for livestock breeding due to its short generation interval and high reproductive ability. Relatively high evolutionary conservation of the genome between mouse and livestock species has been noted, which manifests itself in mutations of the same genes resulting in corresponding phenotypes. For instance, mutations in the myostatin gene cause hypermuscularity and decreased fat content in mice (*McPherron et al., 1997; Bünger et al., 2004*) and other species (*McPherron & Lee, 1997; Mosher et al., 2007; Dall'Olio et al., 2010*). Also, single genes for ovulation rate have been detected in both mice (*Spearow et al., 1999*) and livestock (*Davis, 2005*) and numerous other regions of conserved synteny

between mice and mammalian farm animals exist (*Anderson, 2001*). Moreover, as Casellas (*2011*) concluded, the inbred strains of mice are essential animal models for laboratory research, in which genetic uniformity is required.

Hill (*2011*) commented that the role of new selection experiments in the genomic era might decrease, however existing selection lines still provide essential information on the genetic architecture of quantitative traits. There are numerous examples of effective selection in mice (*Bünger et al., 2001*). There also have been selection experiments conducted to evaluate heterosis in mice, showing both positive and negative effects (*Roberts, 1965*). Heterosis depends on the differences in allele frequencies between parental populations at crossing, the magnitude of interaction within a locus (dominance) and among loci (epistasis), as well as specific parental genetic effects (mainly maternal). In animal populations (contrary to plant breeding), diallel mating schemes have been rarely employed, and have focused on the most efficient crossing schemes (*Garcia-Casco et al., 2012*). Most crossbreeding programs in animals use a crossbred (F1) female as a dam of the final product to utilize maternal heterosis. It is however not well established to what extent genetic differences induced by short term selection could be utilized in crossbreeding.

The objective of this study was to evaluate selection response and heterotic effects in mouse line crosses after 10 generations of selection. The results will show if a short term directional selection for simple traits followed by line crossing could generate significant amounts of heterosis.

Material and methods

Animals

The data were collected on four mouse lines with a common origin. The base population was created from 40 males and 40 females collected from pet shops, and then rotationally mated through 32 generations and randomly mated in subsequent generations. From generation 65 of this line, phenotypic selection was started in three directions: for increasing body weight at 42 days (G line), increasing tail length at 42 days (L line) and increasing litter size at birth (W line). A control line (C line) was also kept in parallel with the selection lines. The active population varied between 20 and 40 pairs. A detailed description of the selection procedure applied was described by *Bünger et al.* (2004). In total, 8661 individuals (4373 males and 4288 females) were included the breeding experiment. After the measurements of litter size and mass, litters were standardized to 9 pups (excessive pups were removed to allow uniform expression of growth potential). After 10 generations of the breeding experiment a first set of crosses was created (male x female): CxG, GxC, CxL, CxW. In generation 12 of the breeding experiment backcrosses and three-way crosses were produced: CxCG, GxCG, LxCG, WxCG, CxCL, GxCL, LxCL, WxCL, CxCW, CxGC, and some additional two-way crosses: GxC. In generation 13, two of the three-way crosses were repeated: CxGC, WxGC. The same scheme of creating two- and three- way crosses was repeated in generations 15 to 18. The following traits were analyzed: body weight at 21, 42 and 63 days, tail length at 42 days, litter size and litter mass at birth. Animals were kept in Macrolon cages (type 2 by EBECO, E. Becker u. Co GmbH, Castrop-Rauxel, Germany) on standard litter (Altromin type, S 80150 by Altromin Spezialfutter GmbH u Co. KG, Lage, Germany). They were weaned and separated by sex at day 21. In every generation, matings were made at an age of 63±3 days. Mice were fed *ad libitum* a pelleted food based on a standard formula (Zuchtfutter für Ratten und Maeuse Nr 1314 by Altromin Spezialfutter GmbH u Co. KG, Lage, Germany). Temperature varied between 20 and 24^{0}C and relative humidity was 50-65%. All experimental procedures were conducted in conformity with guidelines for the care and use of laboratory animals at the Humboldt University in Berlin (Germany) including control of health status of the mice.

Methods

Realized heritabilities were estimated from 10 generations of selection as a linear regression of selection response on selection differential. For each cross a genotype contribution from each purebred line was calculated (Table 1) and additive genetic, maternal genetic, individual heterotic and maternal heterotic effects were estimated based on the following model:

Table 1. Expected contribution of genetic effects to phenotype of the lines used in regression analysis to estimate additive direct (A), additive maternal (Am), dominance individual (D) and dominance maternal (M) effects of each line (line code included after underscore).

Line	A -C	A -G	A –L	A -W	Am -C	Am -G	Am -L	Am -W	D -CG	D -CL	D -CW	D -GL	D -GW	D -WL	M -CG	M -CL	M -CW	M -GL	M -GW	M -WL
CG	0.5	0.5	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
CxCG	0.75	0.25	0	0	0.5	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0	0
GxCG	0.25	0.75	0	0	0.5	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0	0
LxCG	0.25	0.25	0.5	0	0.5	0.5	0	0	0	0.5	0	0.5	0	0	1	0	0	0	0	0
WxCG	0.25	0.25	0	0.5	0.5	0.5	0	0	0	0	0.5	0	0.5	0	1	0	0	0	0	0
CL	0.5	0	0.5	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
CxCL	0.75	0	0.25	0	0.5	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0
GxCL	0.25	0.5	0.25	0	0.5	0	0.5	0	0.5	0	0	0.5	0	0	0	1	0	0	0	0
LxCL	0.25	0	0.75	0	0.5	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0
WxCL	0.25	0	0.25	0.5	0.5	0	0.5	0	0	0	0.5	0	0	0.5	0	1	0	0	0	0
CW	0.5	0	0	0.5	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
CxCW	0.75	0	0	0.25	0.5	0	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0
GxCW	0.25	0.5	0	0.25	0.5	0	0	0.5	0.5	0	0.5	0	0	0	0	0	1	0	0	0
LxCW	0.25	0	0.5	0.25	0.5	0	0	0.5	0	0.5	0	0	0	0.5	0	0	1	0	0	0
WxCW	0.25	0	0	0.75	0.5	0	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0
GC	0.5	0.5	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
CxGC	0.75	0.25	0	0	0.5	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0	0
GxGC	0.25	0.75	0	0	0.5	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0	0
LxGC	0.25	0.25	0.5	0	0.5	0.5	0	0	0	0.5	0	0.5	0	0	1	0	0	0	0	0
WxGC	0.25	0.25	0	0.5	0.5	0.5	0	0	0	0	0.5	0	0.5	0	1	0	0	0	0	0
GL	0	0.5	0.5	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
CxGL	0.5	0.25	0.25	0	0	0.5	0.5	0	0.5	0.5	0	0	0	0	0	0	0	1	0	0
GxGL	0	0.75	0.25	0	0	0.5	0.5	0	0	0	0	0.5	0	0	0	0	0	1	0	0
LxGL	0	0.25	0.75	0	0	0.5	0.5	0	0	0	0	0.5	0	0	0	0	0	1	0	0
WxGL	0	0.25	0.25	0.5	0	0.5	0.5	0	0	0	0	0	0.5	0.5	0	0	0	1	0	0
GW	0	0.5	0	0.5	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
CxGW	0.5	0.25	0	0.25	0	0.5	0	0.5	0.5	0	0.5	0	0	0	0	0	0	0	1	0
GxGW	0	0.75	0	0.25	0	0.5	0	0.5	0	0	0	0	0.5	0	0	0	0	0	1	0
LxGW	0	0.25	0.5	0.25	0	0.5	0	0.5	0	0	0	0.5	0	0.5	0	0	0	0	1	0
WxGW	0	0.25	0	0.75	0	0.5	0	0.5	0	0	0	0	0.5	0	0	0	0	0	1	0

Table 1 cont

Line	A -C	A -G	A –L	A -W	Am -C	Am -G	Am -L	Am -W	D -CG	D -CL	D -CW	D -GL	D -GW	D -WL	M -CG	M -CL	M -CW	M -GL	M -GW	M -WL
C	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LC	0.5	0	0.5	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
WC	0.5	0	0	0.5	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
G	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
W	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
CxLC	0.75	0	0.25	0	0.5	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0
LxLC	0.25	0	0.75	0	0.5	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0	0
WxLC	0.25	0	0.25	0.5	0.5	0	0.5	0	0	0	0.5	0	0	0.5	0	1	0	0	0	0
GxLC	0.25	0.5	0.25	0	0.5	0	0.5	0	0.5	0	0	0.5	0	0	0	1	0	0	0	0
LW	0	0	0.5	0.5	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
CxLW	0.5	0	0.25	0.25	0	0	0.5	0.5	0	0.5	0.5	0	0	0	0	0	0	0	0	1
GxLW	0	0.5	0.25	0.25	0	0	0.5	0.5	0	0	0	0.5	0.5	0	0	0	0	0	0	1
LxLW	0	0	0.75	0.25	0	0	0.5	0.5	0	0	0	0	0	0.5	0	0	0	0	0	1
WxLW	0	0	0.25	0.75	0	0	0.5	0.5	0	0	0	0	0	0.5	0	0	0	0	0	1
CxWC	0.75	0	0	0.25	0.5	0	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0
GxWC	0.25	0.5	0	0.25	0.5	0	0	0.5	0.5	0	0	0	0.5	0	0	0	1	0	0	0
LxWC	0.25	0	0.5	0.25	0.5	0	0	0.5	0	0.5	0	0	0	0.5	0	0	1	0	0	0
WxWC	0.25	0	0	0.75	0.5	0	0	0.5	0	0	0.5	0	0	0	0	0	1	0	0	0
WG	0	0.5	0	0.5	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
CxWG	0.5	0.25	0	0.25	0	0.5	0	0.5	0.5	0	0.5	0	0	0	0	0	0	0	1	0
LxWG	0	0.25	0.5	0.25	0	0.5	0	0.5	0	0	0	0.5	0	0.5	0	0	0	0	1	0
WxWG	0	0.25	0	0.75	0	0.5	0	0.5	0	0	0	0	0.5	0	0	0	0	0	1	0
GxWG	0	0.75	0	0.25	0	0.5	0	0.5	0	0	0	0	0.5	0	0	0	0	0	1	0
WL	0	0	0.5	0.5	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
CxWL	0.5	0	0.25	0.25	0	0	0.5	0.5	0	0.5	0.5	0	0	0	0	0	0	0	0	1
GxWL	0	0.5	0.25	0.25	0	0	0.5	0.5	0	0	0	0.5	0.5	0	0	0	0	0	0	1
LxWL	0	0	0.75	0.25	0	0	0.5	0.5	0	0	0	0	0	0.5	0	0	0	0	0	1
WxWL	0	0	0.25	0.75	0	0	0.5	0.5	0	0	0	0	0	0.5	0	0	0	0	0	1

Note on basic symbols: G, and L, and W - lines selected for increasing body weight at 42 days; tail length and litter size, respectively; C- control line; x – crossing.

$$y_{ijkl} = \mu + s_i + g_j + \sum_{k=1}^{18} b_k X_{ijkl} + e_{ijkl},$$

where:

y_{ijkl} – is the observation on the *ijlk-th* animal of *i-th* sex born in the *j-th* generation and *k-th* genetic group (pure or crossbred line),

μ is the overall mean,

s_i – is the fixed effect of *i-th* sex,

g_j – is the fixed effect of *j-th* generation,

b_1 to b_3 are the partial regression coefficients representing the additive effects of the lines;

b_4 to b_6 are the maternal effects of the lines,

b_7 to b_{12} are the individual heterotic effects,

b_{13} to b_{18} are the maternal heterotic effects,

X_{ijlk} is the proportion of genotypes for ijkl-th individuals,

e_{ijkl} is the residual effect.

The parameters were estimated by the use of the PROC GLM of SAS (*2002-2010*). Heritability of the traits before and after the same selection experiment was previously analyzed using REML with the animal model by Wolc *et al.*(*2006*) and Wolc *et al.* (*2009*).

Results

Response to direct selection

The trait averages for consecutive generations under selection are given in Table 2. Selection on body weight in the G line resulted in a significant increase for this trait of almost 0.7 g per generation. The average BW42 increased from 24.71 g in generation 1 to 31.88 g in generation 11, with the realized heritability estimate of 0.41. In the line selected for tail length (L line) the increase in tail length was 0.16 cm per generation. The difference between 11th and 1st generation was 1.38 cm which is 15% of the tail length in L line at the beginning of the experiment. Realized heritability for tail length was 0.34. In the W line a highly significant increase in litter size was observed of 0.16 pup per generation even though the estimate of heritability (h^2) was low (0.07). The increase of 1.14 pups per litter over 10 generations of selection accounted for 14% of the initial litter size.

Correlations between traits

Pearson correlations between the recorded traits within lines are listed in Table 3. BW42 was strongly positively correlated with BW63 within all lines and also positively but to a lesser extent with BW21. The genetic component of this correlation can be confirmed by the highly significant changes of BW21 and BW63 in the G line selected for BW42. All body weight measurements were also positively correlated with tail length. The response in body weight and tail length was not symmetrical: L line increased by 19% in BW42 and 15% in TL whereas G line increased by 29% in BW42 but only by 6% in TL. Big litters had bigger total birth weight, but individual body weights at young age (at 21 days) were negatively affected which was compensated later in life.

Table 2. Trait averages for consecutive generations under selection and linear regression coefficients of traits per generation

LINE	GEN	LS			LW			BW21			Tail			BW42			BW63		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	STD	N	Mean	SD	N	Mean	SD
C	1	37	8.22	2.79	37	13.38	3.95	264	13.45	1.85	0[a]			230	25.38	3.14	230	27.97	3.78
C	2	35	9.03	2.26	35	14.93	4.30	282	12.76	1.71	0[a]			281	24.84	3.30	280	28.24	3.82
C	3	33	8.70	2.49	33	13.80	3.55	254	12.53	1.61	0[a]			252	25.23	2.95	251	28.51	3.66
C	4	35	7.91	3.36	35	12.60	4.76	243	13.16	1.56	0[a]			242	25.11	2.95	241	28.38	3.50
C	5	34	8.26	2.38	34	12.35	3.32	252	12.54	1.52	0[a]			251	24.84	3.07	250	27.96	3.55
C	6	40	8.58	2.65	40	13.40	3.82	305	12.20	1.75	0[a]			304	24.57	3.11	304	27.57	3.65
C	7	36	9.03	2.97	36	13.83	4.09	280	12.54	1.52	0[a]			279	25.18	2.72	278	28.22	3.44
C	8	35	9.51	1.74	35	14.73	2.63	289	12.44	1.41	0[a]			288	25.19	3.08	288	28.18	3.96
C	9	30	9.57	2.84	30	14.19	3.50	230	11.81	1.70	0[a]			229	24.83	2.88	229	27.45	3.47
C	10	35	9.37	3.07	35	14.13	3.66	262	12.44	1.55	0[a]			262	25.24	2.91	261	27.64	3.79
C	11	24	10.38	2.73	24	15.64	3.61	201	12.47	1.60	0[a]			201	25.08	3.08	201	28.60	3.72
b			0.17			0.13			-0.09						0.00			-0.03	
p-value			0.0017			0.0896			<0.0001						0.9057			0.1971	
G	1	24	9.75	2.82	24	14.69	4.69	182	12.78	1.83	156	9.20	0.43	156	24.71	3.21	156	27.42	3.83
G	2	22	8.59	2.95	22	15.04	6.05	165	13.17	1.60	138	9.47	0.75	138	26.07	3.15	138	29.50	3.90
G	3	24	9.50	3.02	24	15.62	4.25	183	12.84	1.51	158	9.08	0.53	158	26.43	3.25	158	30.10	3.96
G	4	22	8.41	3.95	22	13.59	5.80	145	13.71	1.87	119	9.54	0.42	119	27.61	3.28	119	30.98	4.14
G	5	22	8.36	3.06	22	14.27	4.44	163	13.61	1.95	136	9.85	0.45	136	27.66	3.41	136	31.35	4.21
G	6	23	9.17	2.84	23	15.95	4.57	183	13.60	1.66	147	9.54	0.63	147	28.39	3.52	147	32.64	4.51
G	7	19	8.89	3.70	19	15.72	6.24	138	14.32	1.33	112	9.70	0.44	112	29.60	3.45	112	34.30	4.69
G	8	23	9.09	2.48	23	16.16	3.67	176	13.14	1.78	148	9.38	0.58	148	29.58	3.26	148	33.73	4.07
G	9	25	9.00	3.80	25	15.88	5.53	170	13.48	2.22	142	9.25	0.41	142	30.26	3.48	142	34.89	4.49
G	10	20	7.35	3.13	20	13.26	5.46	113	14.63	1.93	93	9.71	0.42	93	31.36	4.04	93	36.22	5.11
G	11	12	9.25	4.14	12	16.43	6.56	73	14.35	2.52	62	9.72	0.44	62	31.88	3.86	62	37.14	4.65
b			-0.08			0.07			0.13			0.03			0.68			0.89	
p-value			0.2553			0.5254			<0.0001			<0.0001			<0.0001			<0.0001	
L	1	24	9.42	2.06	24	14.98	2.77	200	12.81	1.48	166	9.31	0.51	166	25.16	2.85	166	27.87	3.53
L	2	24	9.83	2.68	24	15.20	3.83	189	12.23	1.46	157	9.63	0.47	157	25.07	3.07	156	28.34	3.94
L	3	22	10.00	2.83	22	16.11	3.41	181	12.48	1.98	155	9.60	0.51	155	25.76	3.38	155	29.33	4.00

Table 2 cont

LINE	GEN	LS			LW			BW21			Tail			BW42			BW63		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	STD	N	Mean	SD	N	Mean	SD
L	4	21	9.52	2.86	21	15.56	3.55	165	13.41	1.58	139	10.16	0.58	139	26.53	3.16	138	29.58	3.79
L	5	25	10.00	1.83	25	16.40	2.88	216	13.06	1.31	177	10.56	0.53	177	26.26	3.33	176	29.88	4.24
L	6	25	10.20	2.86	25	16.50	4.14	203	13.18	1.30	171	10.12	0.60	171	26.99	3.43	171	30.57	4.18
L	7	23	8.61	3.13	23	15.00	4.97	159	15.02	1.95	135	10.75	0.42	135	29.59	3.36	135	33.51	4.12
L	8	21	9.33	3.62	21	15.92	4.70	152	14.24	2.14	133	10.58	1.13	134	28.81	3.84	134	32.53	4.82
L	9	22	10.32	3.96	22	17.32	5.78	156	14.13	1.79	130	10.59	0.45	130	30.39	3.58	130	34.66	4.40
L	10	20	9.40	3.69	20	16.44	5.60	141	15.28	1.83	125	10.99	0.44	125	29.81	3.42	125	34.01	3.96
L	11	15	10.73	3.08	15	19.53	5.10	111	15.09	2.00	92	10.69	0.58	92	29.99	3.60	92	34.10	4.21
b			0.03			0.25			0.29			0.16			0.6			0.73	
p-value			0.0686			0.0058			<0.0001			<0.0001			<0.0001			<0.0001	
W	1	24	8.17	3.06	24	13.47	4.47	172	13.34	1.65	151	9.30	0.54	151	24.61	3.15	151	27.88	3.68
W	2	24	8.67	1.88	24	13.76	2.72	189	12.45	1.19	162	9.39	0.63	162	24.61	2.92	162	27.53	3.67
W	3	26	9.27	2.38	26	14.86	3.79	211	12.32	1.42	175	9.28	0.49	175	24.77	2.96	173	28.17	3.63
W	4	25	8.12	2.65	25	13.62	4.31	168	12.92	1.21	145	9.69	0.58	145	25.11	3.05	145	27.83	4.12
W	5	26	8.23	3.68	26	13.48	5.52	177	12.42	2.00	150	9.78	0.50	150	24.73	3.21	150	27.62	3.92
W	6	24	8.92	3.02	24	14.91	4.51	182	12.32	1.49	158	9.40	0.43	158	24.50	3.06	158	27.35	3.71
W	7	24	7.88	2.91	24	13.66	4.86	162	13.56	1.12	142	9.59	0.52	142	25.68	2.96	142	28.86	3.65
W	8	21	9.62	3.15	21	15.20	4.34	156	12.27	1.62	128	9.19	0.55	128	25.18	2.99	128	28.33	3.67
W	9	25	10.40	2.75	25	16.70	4.40	199	11.58	1.36	165	9.01	0.58	165	25.03	2.87	165	28.24	3.86
W	10	25	9.80	2.43	25	16.53	3.33	199	12.89	1.39	165	9.64	0.44	165	25.76	2.92	165	29.36	3.64
W	11	13	9.31	2.90	13	15.28	4.85	90	12.56	1.13	81	9.18	0.43	81	26.08	3.05	81	29.85	3.83
b			0.16			0.28			-0.04			-0.009			0.12			0.16	
p-value			0.007			0.0015			0.0002			0.0448			<0.0001			<0.0001	

Note on symbols: C- control line; G, L, W – lines selected on body weight at 42 days (grams), tail length (cm) and litter size (pups), respectively. GEN- generation; LS – litter size; LW – litter weight, BW21 – body weight at day 21, tail – tail length at day 42, BW42 – body weight at day 42, BW63 – body weight at day 63; SD – standard deviation; b – linear regression coefficient; p-value for the hypothesis of regression coefficient equal to 0; [a]/ - tail length was not measured.

Table 3. Phenotypic correlation coefficients and their p-values between the analyzed traits within lines (C and L line above diagonals; G and W line below diagonals)

		C line					
		LS	LW	BW21	Tail	BW42	BW63
	LS		0.863	-0.427	.	-0.087	-0.073
			<.0001	<.0001	.	<.0001	<.0001
	LW	0.912		-0.19033	.	0.01725	0.029
		<.0001		<.0001	.	0.36	0.1196
	BW21	-0.460	-0.261		.	0.487	0.433
		<.0001	<.0001		.	<.0001	<.0001
G line	Tail	0.031	0.120	0.263		.	.
		0.2478	<.0001	<.0001		.	.
	BW42	-0.155	-0.023	0.502	0.405		0.911
		<.0001	0.3854	<.0001	<.0001		<.0001
	BW63	-0.132	-0.004	0.434	0.362	0.939	
		<.0001	0.8716	<.0001	<.0001	<.0001	
		L line					
		LS	LW	BW21	Tail	BW42	BW63
	LS		0.920	-0.315	-0.001	-0.079	-0.055
			<.0001	<.0001	0.9714	0.0018	0.0284
	LW	0.936		-0.065	0.177	0.077	0.091
		<.0001		0.0051	<.0001	0.0021	0.0003
	BW21	-0.353	-0.212		0.498	0.589	0.531
W line		<.0001	<.0001		<.0001	<.0001	<.0001
	Tail	0.090	0.169	0.353		0.570	0.547
		0.0003	<.0001	<.0001		<.0001	<.0001
	BW42	-0.047	0.022	0.436	0.416		0.939
		0.058	0.3792	<.0001	<.0001		<.0001
	BW63	-0.011	0.050	0.377	0.371	0.921	
		0.6667	0.0454	<.0001	<.0001	<.0001	

Number of recorded individuals – as given in Table 2

Note on symbols: LS – litter size; LW – litter weight, BW21 – body weight at day 21, Tail – tail length at day 42 , BW42 – body weight at day 42, BW63 – body weight at day 63.

Crossing

The regression analysis revealed significant positive additive effects of all selected lines for all three body weight measurements and tail length (Figure 1). For these traits, except for body weight at day 21, the strongest additive effects were estimated in lines that were directly selected for these particular traits. It should be stressed that in the case of line L (selected on tail length) the additive effects on body weight were relatively large and increased proportionally with age of the animals. On the other hand, indirect effects (effects other than direct additive effect) of lines G and W on tail length were considerably smaller and not significant. The positive W line effect on reproductive traits was not significantly different from 0. Similar to additive effects, the maternal effect of G and L lines was positive for body weight and tail length (Figure 2). The maternal effects for reproductive traits were not significant. However, when selection was focused on reproductive traits, the maternal effects were negative not only for consecutive measurements of body weight (expect for BW21) and tail length, but also for litter size and litter mass. It may indicate negative relationships between direct and maternal additive genetic effects for reproductive traits. Individual and maternal heterotic effects are shown on Figures 3-4. In general, both crossbreeding effects for respective traits were relatively similar. The estimates of heterotic effects for body weight and tail length were mostly negative, although variation among different cross-variants has been observed. In contrast to additive effects of pure lines, large individual and maternal heterotic effects were demonstrated for reproductive traits, except for some cross combinations. The largest positive heterosis was estimated for the litter weight of GxL progeny (especially maternal heterosis, which was statistically significant).

Heterosis was found for body weight at 42 days in the cross between W and C lines, whereas the CxL combination tended to have body weight averages lower than expected from the line means. The same was observed for the CxG cross at 63 days with the effect increasing with age. Again, with the exception of a maternal effect in the GxL cross, there was no significant effect on reproductive traits but it is worth noting that all except the CxL line combinations tended to have better reproduction than purebred lines

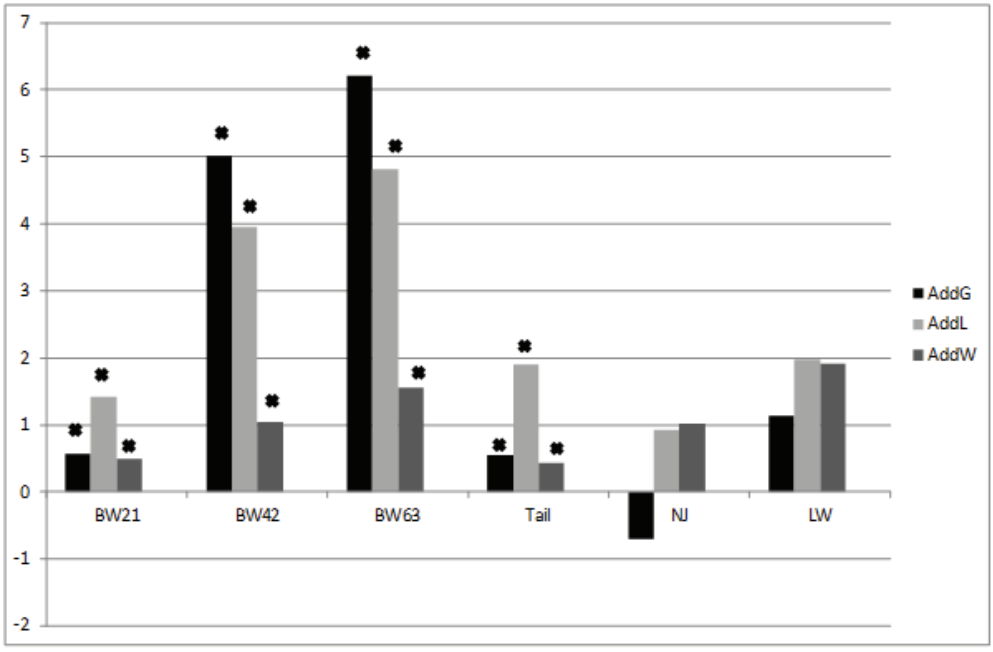

Figure 1. Additive (direct) line effects (AddG, AddL, AddW) expressed relative to the control line on body weight (in gramms) at 21 (BW21), 42 (BW42), 63 (BW63) days of life, tail length – Tail (in cm), litter size – NJ (pups) and litter weight – LW (in gramms), * - for $p < 0.05$.

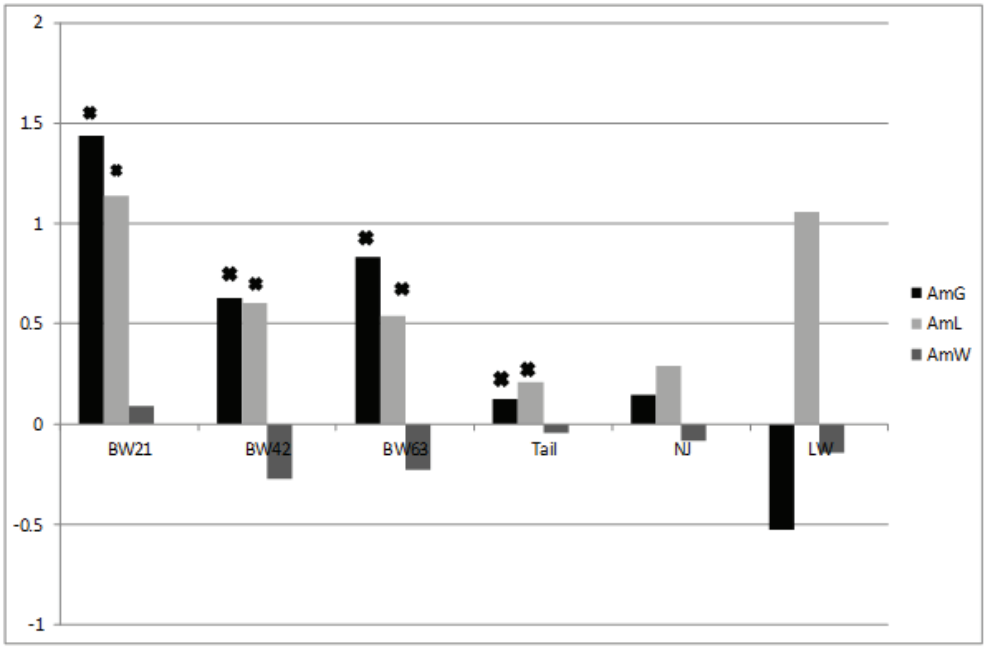

Figure 2. Maternal additive line effects (AmG, AmL, AmW) expressed relative to the control line on body weight (in gramms) at 21, 42, 63 days of life, tail length - Tail (in cm), litter size (pups) and litter weight (in gramms), * - for $p < 0.05$.

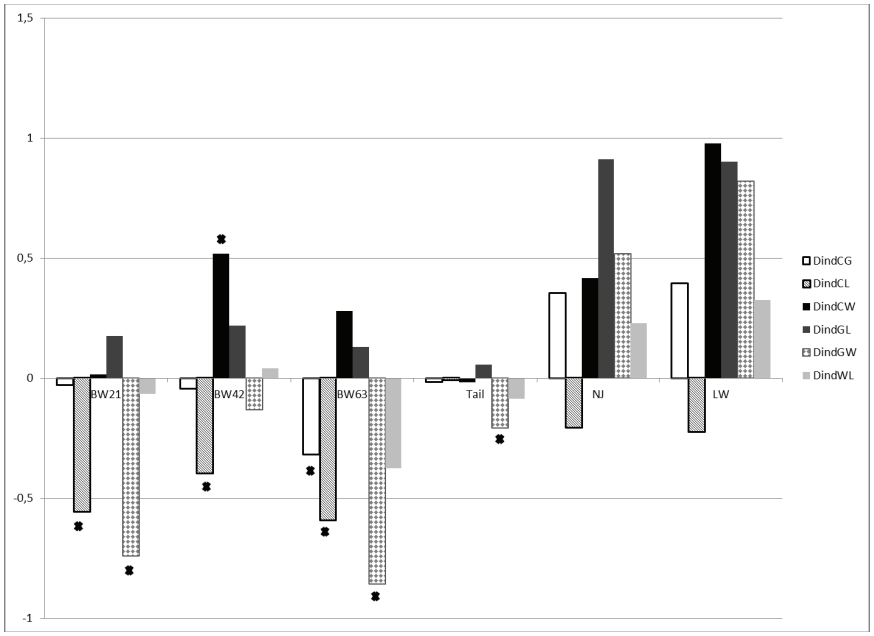

Figure 3. Individual heterotic effects (DindCG, DindCL, DindCW, DindGL, DindGW, DindWL) expressed relative to the control line on body weight (in gramms) at 21, 42, 63 days of life, tail length - Tail (in cm), litter size (pups) and litter weight (in gramms), * - for $p < 0.05$.

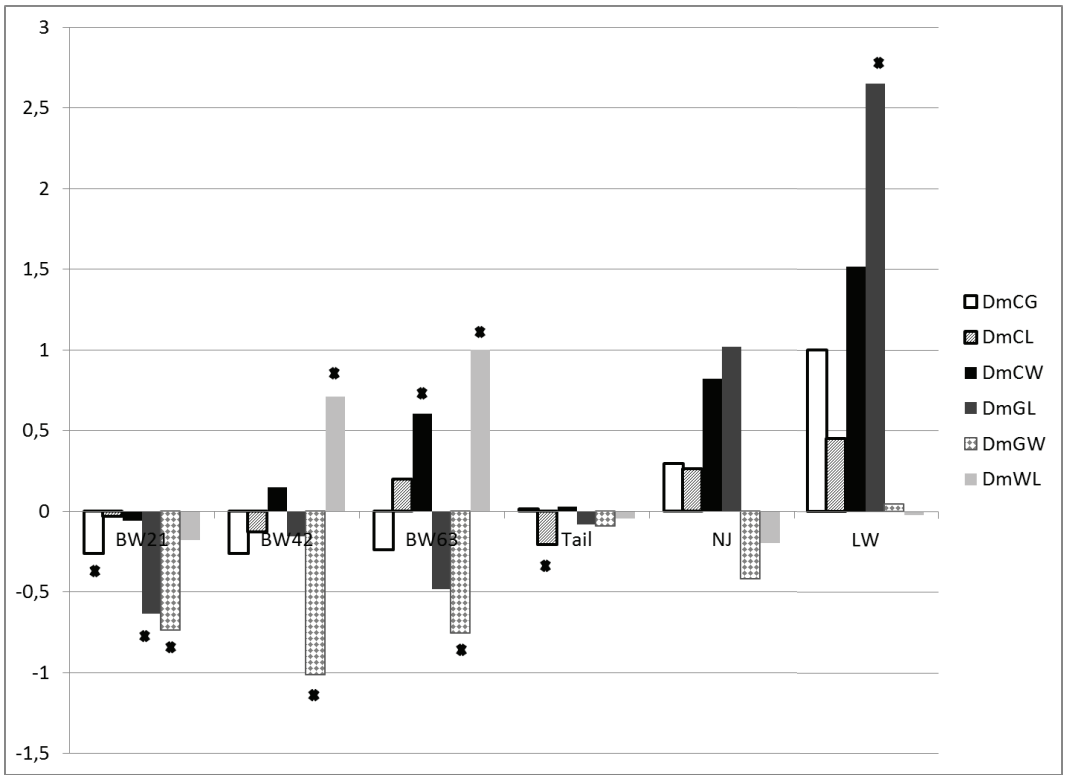

Figure 4. Maternal heterotic effects (DmCG, DmCL, DmCW, DmGL, DmGW, DmWL) expressed relative to the control line on body weight (in gramms) at 21, 42, 63 days of life, tail length - Tail (in cm), litter size (pups) and litter weight (in gramms), * - for $p < 0.05$.

Discussion

It is well known that the main criterion for effectiveness of applied selection in a closed animal population without environmental changes and non-overlapping generations is the realized heritability coefficient. The major determinants of response to selection are genetic variability of the studied population, accuracy of the information sources (in this case own phenotype thus square root of heritability) and intensity of selection. Generally, estimated realized heritabilities for the three traits analysed in this study correspond with results obtained by other authors. Moderate heritability estimates for body weight are influenced by a complex architecture of this trait. Body weight is a composite trait aggregating both fat and non-fat tissue. According to the literature it is influenced by both direct and maternal effects. As mentioned earlier a number of single loci with a large effect on body weight have been identified. Hence, heritability of this trait varies across populations, as well as with the models and methods of analysis. However, according to most studies it is relatively high for both mice (*Wolc et al., 2006*; *Wolc et al., 2009*) and livestock species (*Utrera & Van Vleck, 2004*). Ten generations of selection led to significant additive differences between the lines selected for traits with moderate or medium heritability confirming numerous previous studies on the effectiveness of selection for body weight in mice (*Beniwal et al., 1992*). On the other hand, genetic drift could have also contributed to divergence of the lines. Realized heritabilities for mouse body weight obtained in the present study were smaller than both REML (*Schlote et al., 2005*) and Bayesian estimates (*Wolc et al., 2009*) reported for the same mouse populations using REML with the animal model. The difference in the estimates may be influenced by changes in allele frequencies across generations. As reported by Moreno et al. (*2012*) an estimate of realized heritability can be affected by environmental changes over time.

A moderate heritability estimate was obtained for tail length. It corresponds with the complex nature of this character. Many decades ago, single loci which affected tail length were described (*Barnett, 1965*). In our study, tail length was considerably correlated with body weight although interestingly the response in one direction was stronger than in the other: selection for tail length led to an increase of body weight but the same was true to a lesser extent in the opposite direction.

A number of studies have been conducted for reproductive traits. Although single genes with larger effects on these characters exist, the heritabilities are

usually low. This corresponds with results obtained in the present study. In the study by Holt *et al.* (*2005*) a decline in additive genetic variance over generations of selection was observed in a line selected for reduced litter size; this agrees with a model allowing for genes with larger effect changing in frequency. Other studies in mice (*Beniwal et al., 1992*) and chickens (*Wolc et al., 2010*) further justify the questioning of infinitesimal model assumptions (genetic determination of traits by a very large number of genes with very small effects). Longer term experiments are needed to achieve a stable significant response in reproductive traits (*Holt et al., 2005*).

Bakker *et al.* (*1976*) reported heterotic effects for body weight in a cross with control or among selected populations accounting for about 5% deviations from the mid-parent value. A similar scale of heterosis was observed by Bhuvanakumar *et al.* (*1985*) but only for a body weight measurement on which direct selection was performed. Also Eaton (*1953*) noted that the magnitude of heterosis may be age dependent. In our study, the heterosis estimates were generally consistent for mice of different age. Negative estimates of crossbred performance compared to the parental average may suggest epistatic interactions; favorable allele combinations for body weight traits were established in the selection lines, which were broken by line crossing (*Marani, 1968*).

A tendency to a positive response to crossing was observed for fertility traits. Some authors reported maternal effects on reproductive traits in mammals (*Koivula et al., 2009*) and birds (*Szwaczkowski et al., 2000*). In contrast to our results, Hörstgen-Schwark *et al.* (*1984*) estimated negative direct heterosis for female fertility and litter size in a diallel cross of litter size and body weight selected mice lines. Nagai (*1971*) found significant heterosis for mouse litter mass but not for litter size. The results of molecular genetics approaches (for example, finding overdominant QTLs) will provide more insight into the background of heterosis and ways for it to be utilized (*Melchinger et al., 2007*). Brunsch *et al.* (*1999*) showed heterosis in litter size on mouse chromosome 19. Our results show that 10 generations of line separation with selection on different traits (rather than divergent selection on a single trait) are enough to create genetic differences between the lines which resulted in a significant amount of heterosis.

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