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Long-term selection for protein amount over 70 generations in mice

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Summary

Based on the outbred mouse strain Fzt: Du, which has been obtained by systematic crossing of four inbred and four outbred lines, a long-term selection experiment was carried out for total protein amount (*PA*) in the carcass, starting in 1975. An unselected control line (*CO*) was kept under the same management but without continuous protein analysis. The protein amount of male carcasses at 42 days of age (P42) increased from 2·9 g in generation 0 to 5·2 g at generation 70, representing 97% of a theoretical selection limit. The total selection response amounts to 2·3 g, which is about 80% above the initial value and corresponds to $9\sigma_p$ or $12\sigma_A$. The estimated realized heritability of protein amount decreased from 0·56 to 0·03 at generation 70, which was due to an increase in phenotypic variance from 0·065 to 0·24 g^2 and a reduction in genetic variance from 0·04 to 0·01 g^2. Half the selection response was obtained after about 18 to 23 generations, a half-life of 0·25 to 0·3 N_e. The maximum selection response was 0·094 g/generation and the response was 0·01 g/generation at generation 70. The measurements of body weights at 0, 10, 21, 42 and 63 days throughout the experiment showed a strong correlated effect for all weights. The *PA* mice are one of the heaviest lines of mice ever reported, and do not differ significantly in their body composition from control mice at 42 days. The direct selection response was due primarily to increased general growth. Body weight and protein amount are phenotypically and genetically highly correlated ($r_p = 0{\cdot}82$, $r_A \approx 1$); however, selection for body weight led to fatter animals, whereas selection for protein opposed increased fatness (at least until selection age). This may be of general importance in animal breeding. The comparatively high selection response in this experiment seems due to the heterogeneity of the base population, the relatively high effective population size, and the duration of the experiment.

1. Introduction

The ubiquitous excess of fat in livestock and poultry carcasses and its consequences are described by Eisen (1989), showing that approximately one-third of these carcasses consist of fat. Fat is of concern to the consumer because of health problems, it incurs labour costs for removal, and lowers the biological efficiency of fattening animals. Reduced fat content can be achieved by dietary means, drugs (e.g. Bray, 1995), hormone application, e.g. by injection of leptin in some strains of mice (Campfield *et al.*, 1995; Pelleymounter *et al.*, 1995; Bünger & Hill, 1997) or selection. The main advantage of selection is the relative permanence of the genetic improvement achieved and often the extent of change. This has led animal breeders to emphasize selection for increased lean tissue growth rate and/or reduced fat deposition in livestock and poultry (Webb, 1986). More detailed information is needed on genetic variation in components associated with body composition. The mouse is a useful laboratory animal for such a purpose because of its short generation interval and low unit running cost.

Although considerable data are available on selection for body weight or body weight gain in mice (Roberts, 1965*a*, *b*, 1979; Eisen, 1974, 1980; Bünger et al. 1982*a*; Falconer, 1992; Hill & Caballero, 1992)

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and on correlated responses in body composition, resulting from selection for growth (Roberts, 1979; McCarthy, 1982; Malik, 1984; Bünger, 1987), few studies have involved direct selection for body composition traits (Eisen, 1989). Only two selection experiments in mice are available, in which selection for lean mass or leanness was based on chemical analysis of carcasses or whole bodies (Barkemeyer, 1984; Barkemeyer & Horst, 1990; Bünger *et al*., 1983, 1985), whereas indirect selection was practised in several studies, such as hind-leg muscle weight (McLellan & Frahm, 1973), hind-carcass weight (Eisen, 1987) and epididymal fat pad (Sharp *et al*., 1984; Eisen, 1992). There was considerable variation among the reported heritabilities and the longest experiment comprised 23 generations.

Body weight and protein weight are known to be highly correlated phenotypically, and similar genetic correlations are often assumed, but estimates of the genetic correlation are rare (Eisen, 1989). Only the estimates for the genetic correlation between lean mass and body weight at 10 weeks of age in mice, $r_A = 0{\cdot}90$–$0{\cdot}94$, are known (Beniwal, 1991). Selection for body weight is mostly accompanied by increasing fatness (for references, see above). This raises the question of what difference it makes to select for lean mass instead of body weight, when fatness and weight are so highly correlated.

The aims of this study were: (i) to describe the direct and correlated responses to selection for protein amount, (ii) to estimate the realized heritability and describe its change, and changes in genetic and phenotypic variances of the selected trait, (iii) to estimate the realized genetic correlation between body weight and protein amount, (iv) to compare correlated effects in body composition on an age basis, and (v) to compare the total response with other long-term experiments in mice, which in general involved smaller population sizes and shorter times.

2. Materials and methods

(i) *Experimental animals, housing conditions and lines*

This experiment was carried out in a semi-barrier system at the Mouse Laboratory in Dummerstorf (Germany) with feed and water available *ad libitum*. The foundation population was the outbred strain Fzt: DU, which was obtained in 1969/70 by systematic crossbreeding of four inbred and four outbred lines (Schüler, 1985). In 1975 a growth selection experiment was started that is described in detail by Bünger *et al*. (1983) and by Bünger (1987), and is briefly reviewed here. Dividing full sibs among these lines created three selection lines; sib selection (Falconer & Mackay, 1996, p. 231) was applied, so that the selected animals themselves were not measured. Litters/full sib groups were selected on the sum of the performance of two 'test males' per litter with a mean selection percentage of about 50%. These test males were randomly chosen and marked at 10 days, and were eliminated after measurements of the selection traits at 42 days in all lines.

One line (*PA*) was selected for total protein amount in the carcass, and only results from this are reported here. A second line (*BW*) was selected in the same way for body weight at 42 days. We refer to the results of this line only for the comparative aspect of the side effects of this selection on fatness. Results for line *BW* are given elsewhere (Bünger *et al*., 1990, 1993, 1994). At generations 8 and 9, a control line (*CO*) was created using the same outbred strain. *CO* was randomly selected with a similar selection percentage to the selection lines, in order to obtain a similar increase of inbreeding. In all lines the litter size was standardized to 8 (generations 0–15) or 9 (generations 16–70) pups at birth. Litters smaller than 7 pups were discarded. Litters were weaned at 21 days of age in all lines. In every generation 80 pair matings were made at an age of 63 ± 3 days.

(ii) *Measurement of selection trait*

For the determination of the selected trait in line *PA*, the two test males were usually weighed and killed by cervical dislocation at 42 days and their carcasses prepared (coat, head, digestive tract and legs were removed) and analysed by a standard chemical method (Kjeldahl procedure) for total nitrogen (N), from which the protein amount was estimated as 6·25 N. Facilities for chemical analyses of about 160 animals per generation (every 3 months over 18 years) were sometimes limited, so selection in a few generations was based on body weight or carcass weights (Table 1). Strictly this has be considered as the application of five 'different' selection criteria (X_1 to X_5. The correlation coefficients between X_1 and X_i exceed 0·8 (Table 1). The most highly correlated ($r = 0{\cdot}9$) substitute selection trait for X_1 is X_2 (Table 1) and in 58 of the 71 generations selection was for either X_1 or X_2. The protein amount in line *CO* was determined only in generation 32.

(iii) *Correlated effects in body composition*

To investigate the body composition over an age period, a cross-sectional experiment was performed (Bünger, 1987) in generation 32, using second litters, from which relevant data are summarized here. At 12 age points (0–120 days, Table 2) animals were randomly chosen, weighed, killed and (after drying and homogenization) used for chemical analyses of

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Table 1. *Overview of the information used for selection in individual generations*

Selection criterion (X_i)	Applied in generations:	Total	r
$X_1 = P_1 + P_2$	0, 1, 9, 11–15, 17, 20–37	27	1·0
$X_2 = P_1 + (P_1/CW_1)*CW_2$	4–8, 16, 18, 19, 38–55, 57–60, 70	31	0·901
$X_3 = 2P1$	3, 56, 61	3	0·852
$X_4 = (CW_1 + CW_2)*f_1$	62–65, 67–69	7	0·873
$X_5 = (BW_1 + BW_2)*f_2$	2, 10, 66	3	0·820

P_1, P_2; CW_1, CW2; BW_1, BW2 = protein amount, carcass weight and body weight for test animals 1 and 2, respectively.
f_1 = factor $1 \approx 18{\cdot}2\,\%$ (range 17·9–18·3 %, constant within generations). This corresponds to the total mean of protein content of the carcass (%) (over all available generations at time of calculation); value changes slightly with the number of generations included.
f_2 = factor $2 \approx 10\,\%$ (range 10–10·17 %, constant within generations). This corresponds to the total mean (over all available generations at time of calculation) for carcass-protein in relation (percentage) to body weight; value changes slightly with the number of generations included.
r = coefficient of correlation between X_1 and X_i, based on 1728 d.f. This is based on all full records in those 27 generations in which both animals were analysed for protein (see line 1 of table).

Table 2. *Numbers of animals* (*n*) *and samples* (*m*) *for the cross-sectional study of body composition in generation 32*

Age (days)	Control		Line *PA*		Line *BW*	
	n	*m*	*n*	*m*	*n*	*m*
0	160	8	160	8	161	8
5	105	7	90	6	100	7
10	100	10	100	10	100	10
15	54	9	54	9	54	9
21	24	6	24	6	24	6
25	27	9	27	9	27	9
30	27	9	27	9	27	9
35	20	10	20	10	20	10
42	20	10	20	10	20	10
60	20	20	20	20	20	20
80	20	20	20	20	20	20
120	21	21	26	26	25	25
Sum	598	139	588	143	598	143

protein, fat, ash and dry matter, using standard analytical methods.

To avoid influences of different levels of filling of the gastrointestinal tract, this was always removed. This preparation of the 'carcass' differs slightly from the preparation of carcasses in the selection experiment because here animals of very young ages were involved, where the removal of the coat would be impossible or very difficult. Because sexual differences can be neglected at earlier ages, females were also used to increase the sample size at younger ages (up to 15 days of age). After age 15 days only males were used. To obtain necessary sample amounts, individual samples were pooled, especially at younger ages. The numbers are given in Table 2.

(iv) *Data analysis*

For fitting generation means against generation number or the cumulative selection differential a modified exponential model (1) (Bünger & Herrendörfer, 1994) was used:

$$y = A - (A - C)\exp[-Bx/(A - C)], \quad (1)$$

where A is the theoretical final value (i.e. theoretical selection limit), B is the maximal slope at $x = 0$ (i.e. selection response in the first generation), C is the y-value at $x = 0$ (i.e. initial value) and x is an independent variable (i.e. generation or cumulated selection differential). Based on this model the half-life (HL), the time to get halfway to the limit, can easily be obtained from

$$\mathrm{HL} = (\ln 0{\cdot}5)(C - A)/B. \quad (2)$$

Model (1) was used for fitting generation means, selection response and the phenotypic variance and for estimating the 'realized heritability function' from the slope of the fitted curve relating cumulative selection response (cumSR) and cumulative selection differential (cumSD), using the first derivative of model (1) as discussed in detail by Bünger & Herrendörfer (1994). These slopes or ratios (b) yield the estimates for the realized heritability converted by (3) to a mass selection basis (Falconer & Mackay, 1996, p. 234).

$$h^2 = \frac{2b[1 + (n-1)\tau]}{n}, \quad (3)$$

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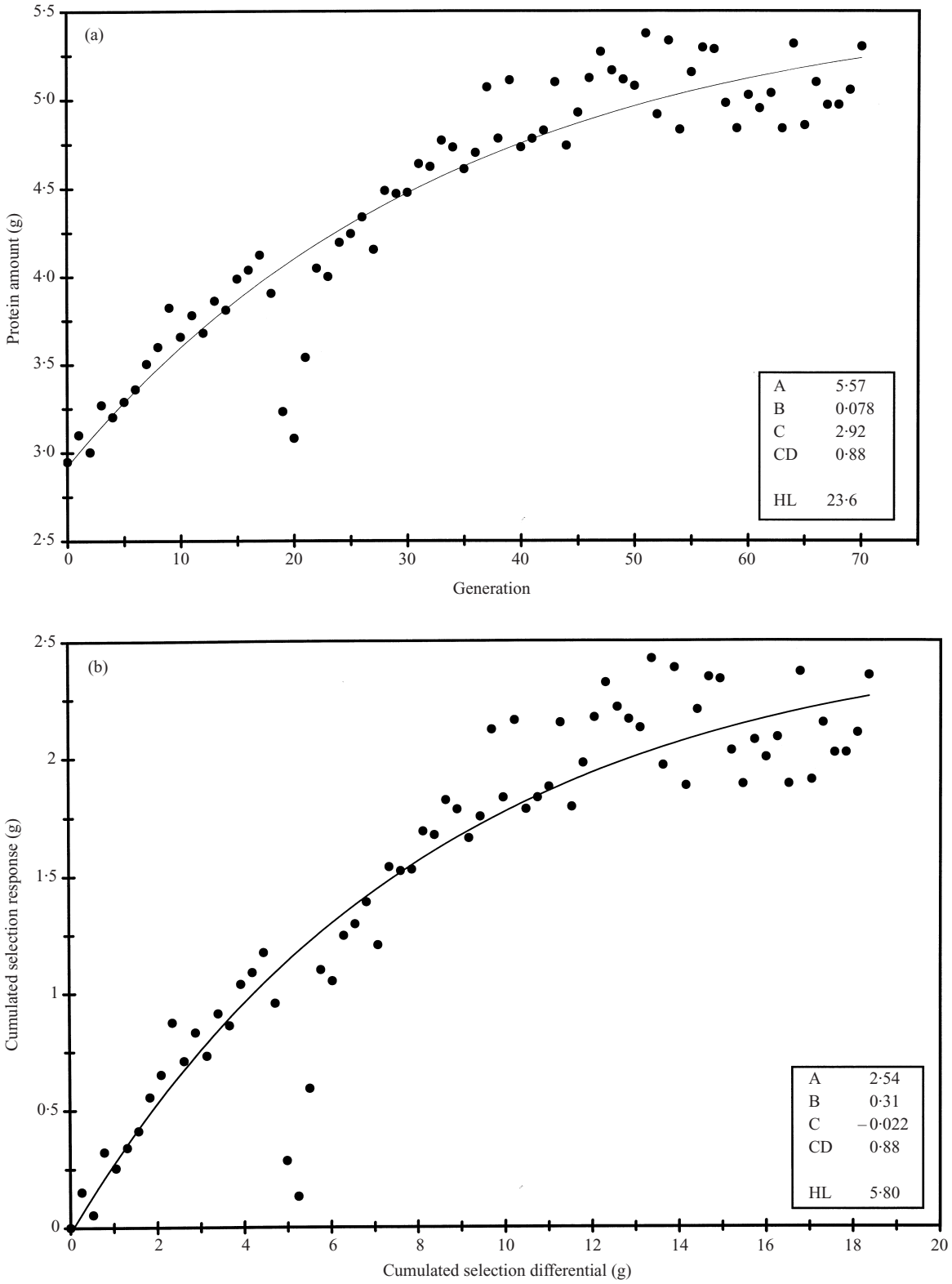

Fig. 1. Direct selection response in line *PA*. (*a*) Generation means (protein amount in the carcass of individual 42-day-old males). (*b*) Selection response versus cumulated selection differential. Fitted exponential model [model (1)]. *A*, *B*, *C*: function parameters, *A*: final value = theoretical selection limit, *B*: initial slope = maximum selection response, *C*: initial value = base population estimate, CD: coefficient of determination, HL: half-life. Parameters estimated for the upper graph excluding generations 18 to 23 were: $A = 5{\cdot}33$ g, $B = 0{\cdot}094$ g/generation, $C = 2{\cdot}90$ g, CD = 0·945, HL = 18 generations. For parameters estimated for the lower graph excluding generations 18 to 23 see Table 3.

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Table 3. *Results of fitting the cumulative selection response on cumulative selection differential*

	Full data set	Generations 18 to 23 excluded
A[a] (g)	2·54	2·35
B[a] (g/g)	0·31 $h^2 = 0{\cdot}47$[d]	0·37 $h^2 = 0{\cdot}56$[d]
C[a] (g)	−0·022	−0·03
CD[b]	0·88	0·94
HL[c] (g)	5·8 (gen 22 to 23)	4·5 (gen 18 to 19)

[a] A, B, C: parameter estimates for model (1).
[b] CD: coefficient of determination.
[c] HL: half-life.
[d] h^2 given as initial, realized values.

which for $n = 2$ gives $h^2 = b(1+\tau)$. Here, n is the number of full sibs and τ is the intraclass correlation coefficient, estimated as $\tau = 0{\cdot}53 \pm 0{\cdot}14$ (SD) from the within-generation estimates (τ_t) of the 27 generations of 'pure X_1 selection' (Table 1), based on about 65 pairs per generation. The linear regression (τ_t on generation number) showed no significant decrease ($\tau_0 = 0{\cdot}62$ and $\tau_{37} = 0{\cdot}47$). Therefore, τ_t is assumed to be constant in what follows.

The cumSR is calculated as generation mean minus the mean in generation 0 and cumSD is calculated as a realized SD, so SD is weighted according to number of offspring per litter used as parents and which have given birth to a litter in the next generation. Both are based on sums of the measured two full sibs. The phenotypic variance of full sib sum (σ^2_{pFS}) was transformed to an individual basis (σ^2_{pi}) as follows:

$$\sigma^2_{pi} = \sigma^2_{pFS}/(2\tau + 2). \quad (4)$$

The mean for proportion selected (p = number of selected families × 100/number of families measured) and selection intensity ($i = SD_{FS}/\sigma p_{FS} \pm SD$) were '$\bar{p}$' = 53·4 % ± 9·4 %; '$\bar{p}$' = 45·8 % ± 2 %; and $\bar{i} = 0{\cdot}74 \pm 0{\cdot}16$ $\bar{i} = 0{\cdot}08 \pm 0{\cdot}14$ for lines *PA* and *CO*, respectively, the values for *CO* being based on BW42 data.

For calculation of mean inbreeding coefficients per generation the program DFPREP (part of the DFREML programs; Meyer, 1988) was used. The average rate of inbreeding (ΔF) was estimated from the linear regression of $\ln(1-F_t)$ on t (generation number), excluding generations 0–4, and assuming that ΔF had reached its asymptotic rate by generation 5.

Following Hill (1980), approximate estimates for the drift (σ^2_d) and error variance (σ^2_e) were obtained from (5) and (6), which allow a rough estimate of the variance of the phenotypic mean in generation t [$V(\bar{X}_t)$] using (7):

$$\sigma^2_d = \frac{h^2[1-h^2(1-p)]\sigma^2_p}{N_e} \quad (5)$$

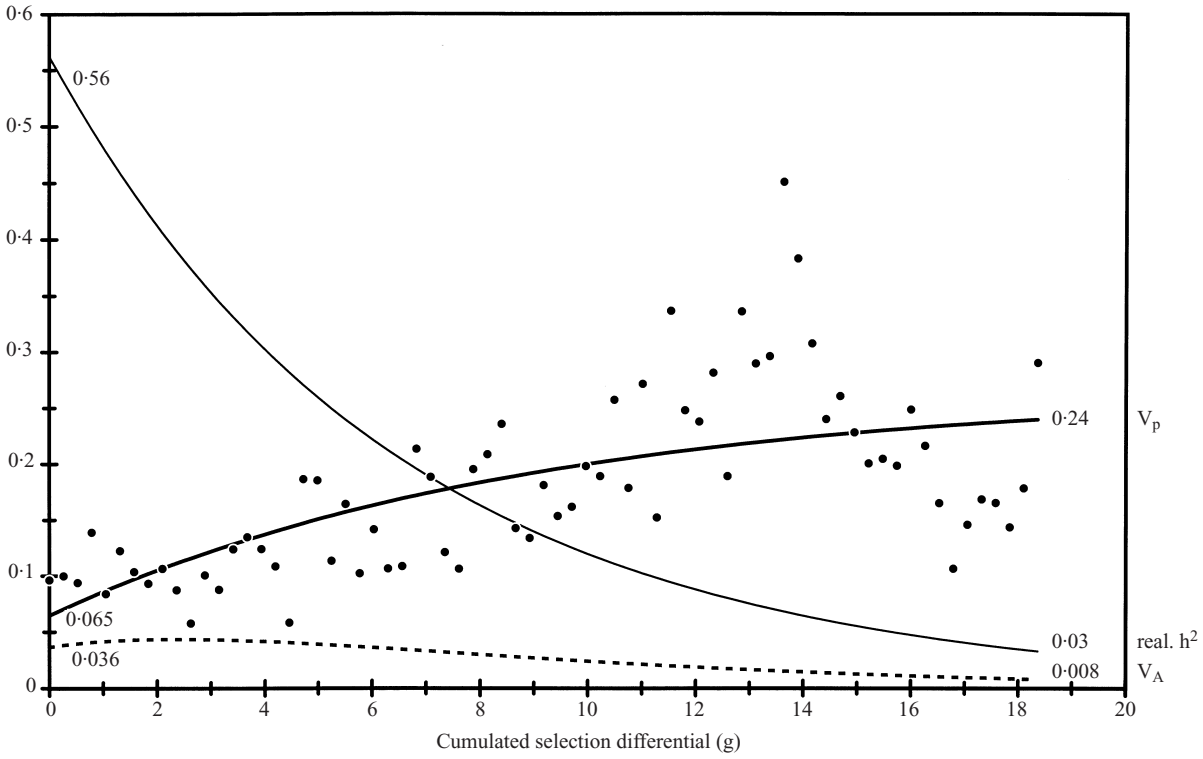

Fig. 2. Changes in realized heritability (h^2), phenotypic variance (V_p) and genetic variance (V_A). The h^2 function results from the fitted relation between cumulative selection response and cumulative selection differential (Fig. 1*b*). The values are based on the first derivative of model (1), transformed by formula (3). Parameters for the fit of V_p, using model (1): $A = 0{\cdot}27$, $B = 0{\cdot}011$, $C = 0{\cdot}065$, CD = 0·41. All values are transformed to an individual basis.

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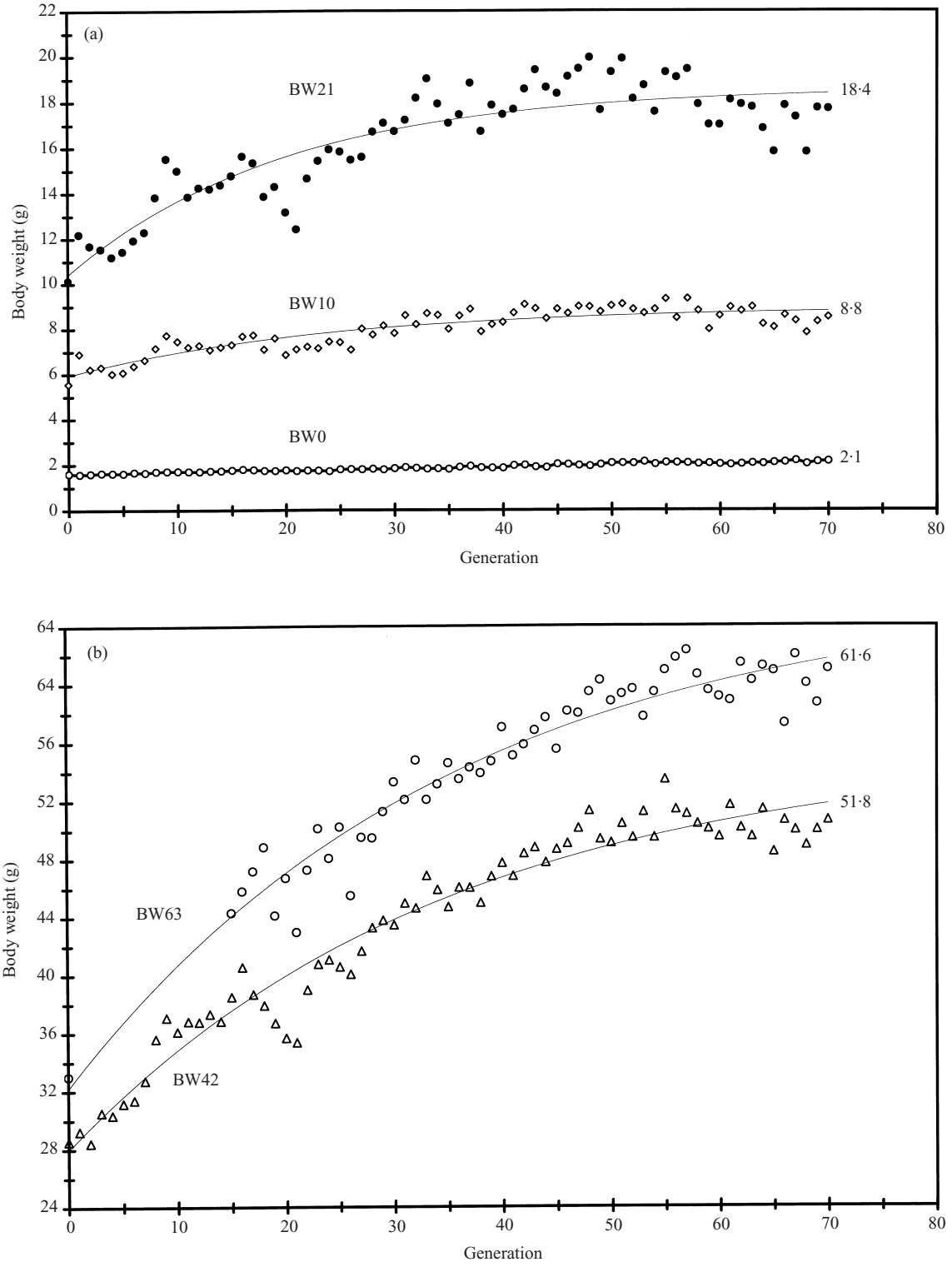

Fig. 3. Changes in individual body weight at birth, 10 and 21 days (*a*) and at 42 and 63 days (*b*) as differences from control. The difference between the means of the selected and the control lines was calculated in every generation and rescaled by adding the total mean of the control as a constant. The total means for individual body weights at 0, 10, 21, 42 and 63 days in the control line were 1·59 g, 5·94 g, 10·96 g, 28·48 g and 32·99 g respectively. BW0 is calculated from litter weight/litter size at birth (day 0). Whereas all weights were taken exactly at the given age, at mating (named as BW63) there was a small variation in age (±3 days). BW63 was not measured during the first generations up to 14. The value in generation 0 for this trait was assumed to be equal to the total mean of the control. Because the control was set up later (see Section 2.i) the means in generation 0 pooled over the three selection lines were taken as constant and used instead of control values in generations 0–7 for body weight traits. For the comparison with the control in generations 8 and 9 the body weights of the *CO* generation zero were used. Thereafter the selection line and control generations were set up almost contemporaneously (about 2 weeks difference).

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where N_e is effective population size, p is proportion selected,

$$\sigma_e^2 = \left(\frac{c^2}{N_f} + \frac{1 - c^2 - h^2/2}{nN_f}\right)\sigma_p^2 \quad (6)$$

where N_f is the number of females, n is the number of progeny recorded from each female, c^2 is the environmental correlation of full sibs and σ_p^2 is the phenotypic variance.

$$V(\bar{X}_t) = t\,\sigma_d^2 + \sigma_e^2. \quad (7)$$

c^2 is estimated as 0·29, using the estimates for h^2, V_p and τ for the beginning of this experiment, ignoring dominance.

Using the results of the cross-sectional analysis (Section 2.iii), a joint estimate of the genetic correlation (r_A) between body weight and protein amount at 42 days of age was calculated from formula (8) (Falconer & Mackay, 1996):

$$r_A = \frac{\overline{cSR_x\, cSR_Y}}{SR_x\, SR_Y}, \quad (8)$$

where SR and cSR are the direct and correlated responses, respectively.

3. Results

(i) *Direct selection response*

The steady increase in protein amount in the carcass of 42-day-old individual males (P42) was interrupted by a dramatic decrease over 3 generations after generation 18, which was obviously caused by an exceptional environmental deterioration affecting all lines (Fig. 1). Ignoring this decrease, the variation of the generation means around a trend presented by the fitted curved is obviously smaller in the first part of the experiment, up to about generation 35, than later on. Despite this disturbance, the fit of the non-linear model (1) to the observed values was good; the coefficient of determination (CD) was 0·88. The estimated initial mean value of P42 was 2·9 g (parameter C) and it increased by generation 70 to 5·2 g. This is an increase of 2·3 g (78 %) or, expressed in units of the initial individual standard deviations, $9\sigma_p$ and $12\sigma_A$ ($\sigma_p = 0{\cdot}254$, $\sigma_A = 0{\cdot}191$). The exclusion of generations 18–23 has only a minor effect on the estimated protein values in generation 70 (5·23 g vs 5·18 g) and the initial value C (Fig.1). The drift and the error variance were $\sigma_d^2 = 0{\cdot}0013$ g^2 and $\sigma_e^2 = 0{\cdot}0058$ g^2, giving an approximate standard deviation for the generation mean in generation 70 of 0·31 g.

Because the selection intensity in this experiment was relatively constant, the plot of cumSR versus cumSD showed a very similar pattern (Fig. 1 *b*). The fit was carried out (*a*) using the whole data set and (*b*) excluding the responses in generations 18 to 23. The fitted parameters are given in Table 3. Excluding these generations results in slightly lower estimates for parameter A and for the half-life. The relation between cumSR and cumSD is the basis for the estimation of the 'realized h^2 function' from their ratio at every point of the curve, fitting the relation cumSR versus cumSD by model (1) and using the first derivative of this model. These values are converted to a mass selection basis using formula (3). The realized heritability was initially 0·47 (*a*) or 0·56 (*b*); the latter is plotted in Fig. 2. The realized heritability decreased to 0·03 at the end of the experiment. There is a strong increase in the phenotypic variance up to about generation 50 (cumSD = 12–14) and an exceptionally high value in generation 52. Thereafter V_p decreases but still remains at about double the starting level (Fig. 2). The generation estimates for V_p are fitted using model (1) for the individual cumSD. The estimate for V_p at the beginning of the experiment was 0·065. V_p increased dramatically to 0·24 at the end, a 4-fold increase. While the phenotypic variance increased, the coefficient of variability (CV %) remains relatively stable. The mean CV % over the generations for the individual P42 was 9·3 % ± 1·5 % (SD) without any significant trend. Calculated as the product of realized heritability and V_p, using the smoothed values for V_p, V_A was initially 0·04 and decreased to 0·01 (Fig. 2).

The rates of inbreeding (ΔF) in lines *PA* and *CO* were 0·0073 and 0·0063 per generation, respectively, resulting in inbreeding coefficients of $F = 0{\cdot}4$ and $F = 0{\cdot}32$, respectively, at the end of this experiment, corresponding to generation 70 in line *PA* and generation 62 line *CO*. Using these estimates, the effective population sizes in these lines were about 70 and 80, respectively.

(ii) *Correlated effects on body weights*

The body weights at birth, 10, 21, 42 and 63 days of age are given in Fig. 3. There is a significant correlated positive effect of the selection on all body weights, although it is less evident for the body weights at younger ages because of the common scale used. The effect increases with age in absolute and relative terms. The fitted values, using model (1) for the mean BW0, BW10, BW21, BW42 and BW63 in generation 70, were 2·1 g, 8·7 g, 18·4 g, 51·8 g and 61·6 g, respectively, which are 0·52 g (33 %; $3\sigma_p$), 2·8 g (48 %; $5\sigma_p$), 8·0 g (77 %; $6\sigma_p$), 23·8 g (85 %; $12\sigma_p$) and 29·5 g (91 %; $13\sigma_p$), respectively, over the levels in the control line.

Because this presentation is based on the difference from the control, the decrease in growth traits at about generation 20, which affected the protein values very strongly, is almost completely removed, indi-

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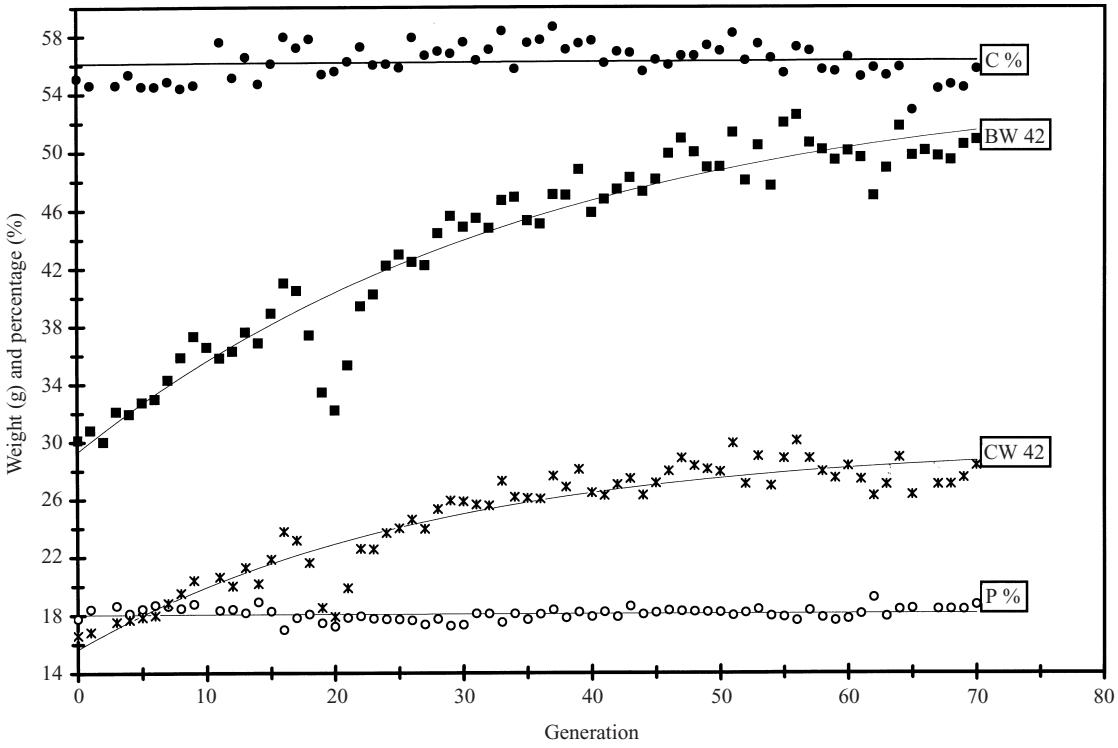

Fig. 4. Changes in individual body weight (BW42) and carcass weight (CW42) at 42 days of age and carcass and protein percentage. For further explanation see legend to Fig. 1. The development of BW42 and CW42 is fitted using model (1); percentages are fitted by simple linear regression.

cating that this was due to a negative environmental factor affecting both lines at this time.

(iii) *Correlated effect on body composition*

The direct selection response can be achieved by an increase of body weight and/or by a change in protein percentage and/or proportion of the carcass to the whole body. The initial individual BW42 and carcass weight (CW42) in line *PA* were 29·3 g and 15·7 g, respectively. Both increased similarly, by 76% and 83%, respectively (Fig. 4). The mean ratios CW42/BW42 and the mean percentage of protein (P%), and their corresponding standard deviations, were 56·2% $\pm$1·2% and 18·1% $\pm$0·44%, respectively. Neither has any significant trend, indicating that the selection response in protein weight is due to an increase in general growth without any obvious change in the protein or carcass percentage at the selection age.

To investigate correlated effects of protein selection on body composition at a wider age range (0–120 days) data from the cross-sectional experiment, carried out in generation 32, were used. One striking characteristic in all lines is the increase in fat content up to an age of about 15 days and the strong decrease thereafter (Fig. 5). The fat content in line *PA* is significantly higher than in line *CO* up to 15 days, whereas from weaning up to 42 days there is only one significant difference at 30 days. After the selection age there is an increasing divergence between *PA* and *CO*, resulting in a difference of 6·5% (17·2% vs 10·8%) at 120 days. A plot of fat content against body weight is very similar to the 'age plot', but shows that the fattening process already starts in control mice at about 25 g, whereas the intensive increase in fat content in the selection line starts at about 40 g, a weight which is the 'final weight' for the control mice in this study. This fattening process seems to be related to a 'degree of maturity'. If body weights are expressed as a percentage of the observed 'mature weight' (BW120 = 100%), and the fat content is plotted against this, the development in the two lines is very similar up to a stage where they have reached about 70–80% of their 'mature weight'. Thereafter there is a strong increase in the fat content in *PA*, whereas the fat content in *CO* shows only a small further increase. The mice of line *BW* were already much fatter than the other two lines starting at 25 days. At the selection age their fat content was 13·6%, whereas lines *PA* and *CO* had fat contents of 9·0% and 9·3%, respectively. Line *BW* mice had their highest fat content (22·3%) at 80 days. Thereafter there was a strong decrease in fat% in *BW*, so that they did not differ from *PA* at this age.

The development of the amount of fat with age is presented in Fig. 6. The maximum fat gains in *BW*, *PA* and *CO* were 0·24, 0·12 and 0·06 g/day, respectively and the age at the point of inflection for fat

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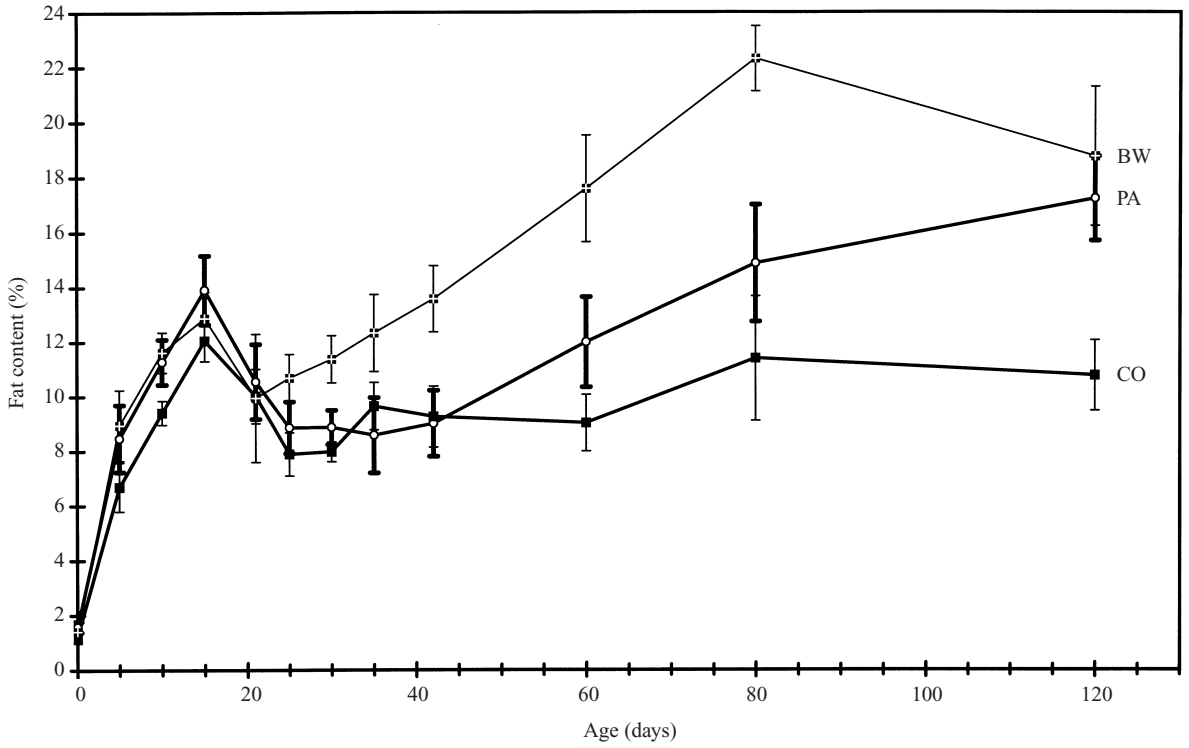

Fig. 5. Fat percentage in relation to age in protein (*PA*), body weight (*BW*) and control (*CO*) lines. Values are means with confidence intervals ($P < 0{\cdot}05$).

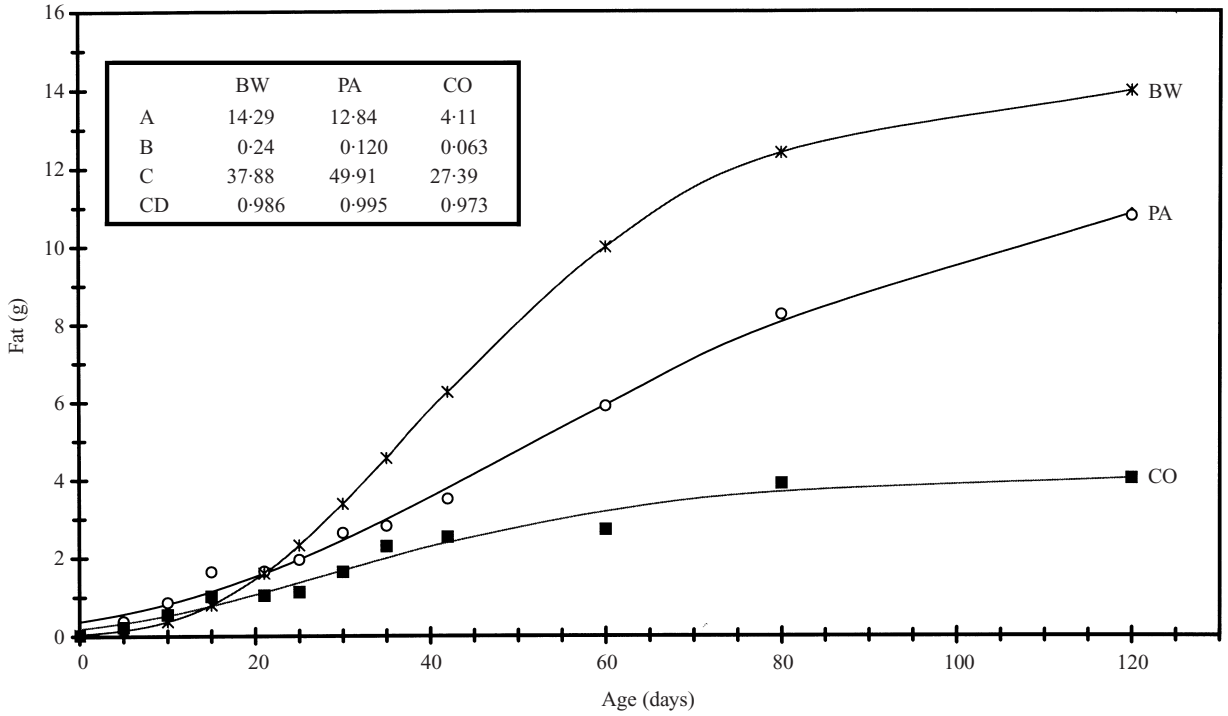

Fig. 6. Observed and fitted growth curves for fat amount in relation to age in protein (*PA*), body weight (*BW*) and control (*CO*) lines. A modified form of the GOMPERTZ model described by Bünger *et al.* (1982*b*) was used to fit data on body weights and on fat amount in this cross-sectional experiment. $Y = A\exp(-\exp[Be(C-x)/A])$, where $A =$ asymptote, mature weight; $B =$ maximum body weight gain; $C =$ age at maximum gain (age at point of inflection); and $x =$ age (days).

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Table 4. *Means* ($\bar{x}$) *with standard errors* (*SE*) *of the body weight selected line* (*BW*), *the protein line* (*PA*) *and the control and differences* (*Dif.*) *from the control in generation 32*

	Line *BW*		Line *PA*		Control (C)		'*BW*−C'		'*PA*−C'	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	Dif.	SE	Dif.	SE
BWe (g)[a]	42·7	0·84	38·82	0·53	27·5	0·62	15·2	1·0	11·3	0·8
BW42 (g)	50·7	0·48	45·81	0·87	32·3	0·56	**18·4**[b]	0·9	13·5	1·0
P (g)	7·65	0·16	6·67	0·25	4·97	0·14	2·68	0·21	**1·70**	0·29
P (%)	17·9	0·12	17·2	0·75	18·1	0·14	−0·16	0·19	−0·84	0·76

[a] BWe, body weight empty (without gastrointestinal tract).
[b] Direct and correlated selection responses are in bold and underline, respectively.

amount is about 38, 50 and 27 days, respectively. Whereas fat aggregation in the control approaches a plateau at about 4 g, the selection mice obviously still produce a considerable amount of fat. Their fat amount at 120 days is 14 and 10·8 g, respectively – a 2·5–3·5-fold difference compared with the control. A plot of the fat amount versus body weight shows that control mice begin to aggregate more fat per unit body weight than the selected mice at about 18–20 g of body weight.

(iv) *Genetic correlation between body weight and protein amount*

The phenotypic correlation between body weight and protein amount with its 95% confidence interval is $r_p = 0{\cdot}82$ (0·80, 0·83), based on more than 1700 observations in the *PA* line (Table 1). Using the results of the cross-section analysis in generation 32 for the traits at 42 days, the direct and correlated selection response for BW42 and P42 in lines *BW* and *PA* were calculated as differences from the control, and can be used to obtain a rough estimate for the genetic correlation between the traits (Table 4). From (8) we obtain $r_A = 1{\cdot}1$, indicating that r_A seems to be very close to 1. Because the protein content in line *PA* at 42 days was low (17·2%) compared with the content at 35 and 60 days (18·1% and 18·0%, respectively), the direct response for protein amount seems a little underestimated, which could have caused the r_A estimates to be somewhat over 1.

4. Discussion

(i) *Direct selection response*

(a) *Drift and effective population size*

Some selection experiments with mice have shown the impact of genetic drift on direct and correlated responses (Eisen *et al.*, 1970; Falconer, 1973; Hanrahan *et al.*, 1973; Baker *et al.*, 1975; Eisen & Pomp, 1990). Because N_e in this experiment was comparatively high, the disadvantage of having no replicates seems not to be crucial. Because of a lack of replicates for the line *PA*, there is no easily available estimate for the drift variation of this 'point estimate' of the selection response. Using the theory outlined by Hill (1974, 1977) approximate drift and the error variances were obtained: $\sigma^2_d = 0{\cdot}0013\ g^2$ and $\sigma^2_e = 0{\cdot}0058\ g^2$, giving an approximate variance of the mean after 70 generations of $V_{(\bar{x}_t)} = 0{\cdot}095$. Using twice the standard error, an approximate 5% confidence interval for the mean in generation 70 can be obtained as $5{\cdot}2 \pm 0{\cdot}6$ g, accounting for drift and the sampling variance. The major effect of the drift variance on this confidence interval is obvious. But the essential conclusions of this experiment will not be too much affected.

(b) *Total selection response, number of loci*

The protein amount of the carcass of 42-day-old individual male mice (P42) in generation 0 was 2·95 g, which corresponds very well with the estimated initial value from model (1): $C = 2{\cdot}92\ g \pm 0{\cdot}06$ (95% confidence interval). In generation 70 the P42 was 5·3 g. Because of the variation between the generation means, the final value seems to be better characterized by the fitted value $P42_{70} = 5{\cdot}2$ g, which is a total selection response of 2·3 g (about 80%) or $9\sigma_p$ or $12\sigma_A$. Obviously the selection has taken the mean far beyond the range of variation in the base population. This pattern is typical of several other selection experiments on body weight or growth rate of mice but the magnitude of the response is relatively high. This is shown by the comparison with the results of some other long-term growth selection experiments given in Table 5, including or estimating body weights at 42 days as a reference point, although different selection criteria were used. The total selection response in relation to the initial mean seems to be very variable, but selection in the high direction can roughly double the mean. The relative selection response (80%) in line *PA* is again high, but a little lower than for direct selection for body weight (Table 5, row 11). Line *BW* showed a correlated change in fat content, whereas the body composition at 42 days has not changed in *PA* (Fig. 5).

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Table 5. *Selection response or limits in some experiments on mice*

Base population[a]	Selection trait	N_e	Trait	Gen.	'Initial'	End	Selection response Abs.	%	σ_p	σ_A	h^{2b}	HL	References
OB	BW60+	< 108	BW60 m	83	22·2	43·4	21·1	95	7·6	13	0·36	16	Goodale (1938), Wilson *et al.* (1971)
			BW42 m	83	18·4	36·0	17·6						Bünger & Herrendörfer (1994)
OB	BW60+	30	BW60 m	23	23·2	40·0	16·8	72	6·6	13	0·25		MacArthur (1944, 1949)
(6 × IB)			BW42 m	23	19·3	33·2	13·9						
	BW60−/BW42−	20	BW42	38	19·0	10·0	8·0	53	4·2			4	King (1950), Roberts (1966a)
OB	BW42+	15	BW42	52	21·6	28·0	6·4	30	3·4	7·1	0·35	8	Falconer (1953, 1955)
(4 × 1B)	BW42−	15	BW42	42	21·6	11·0	10·6	49	5·6	11·8	0·35	9	Roberts (1966*a*)
OB	G21–42+/BW42+	17	BW42	53	24·5	35·0	10·5	43	4·6	8·1	0·33	7	Falconer (1960)
	G21–42−/BW42−	19	BW42	53	24·5	14·0	10·5	43	4·6	8·1	0·33	10	Roberts (1966*b*)
OB (4 × S)	BW42+		BW42	18	32·0	41·0	9·0	28					Roberts (1967)
OB (3 × S)	BW42−		BW42	18	15·0	12·0	3·0	20					
OB	BW42+	32	BW42 m	23	24·5	37·0	12·5	51	5·1	8·3	0·40		Falconer (1973)
Q-strain	BW42−	32	BW42 m	23	24·5	15·0	9·5	39	6·1	6·3			
OB	BW56+	43	BW56 m	43	29·3	57·2	27·9	95	10·3	14·1	0·53	16	Bakker (1974)
Swiss Cpb; /Se/S	BW56−	26	BW56 m	27	31·1	13·0	18·1	58	5·7	9·4	0·37		Buis (1993)[c]
			BW42 m	43	25·3	43·3	18·0						
			BW42 m	27	25·3	13·1	12·2						
OR	G21–42+	41	G21–42	24/27	13·6	24·7	11·1	82	4·7	8·2	0·35	12	Eisen (1975)
ICR			BW42	–	27·0	44·0	17·0	63					Eisen *et al.* (1988)
OB	G21–42+	33	G21–42 m	34/43	13·8	26·6	12·8	93	7·4	16·5	0·20	15	Barria & Bradford (1981)
(4 × IB)			BW42 m	33	25·0	41·0	16·0	64					Eklund & Bradford (1977*b*)
			BW70 f	31	22·8	41·9	19·1	84					Meyer & Bradford (1974)
OB (2 × IB) × OB	PLM70+/BW70+	50[d]	BW70 m	50	32·0	51·0	19·0	59	5·9	8·5	0·51	24	Sharp *et al.* (1984)
	PLM70−/BW70−		BW70 m	50	32·0	17·0	15·0	47	4·7	6·7			Beniwal *et al.* (1992*a*, *b*)
			BW42 m			41·3							Bünger & Hill (unpublished)
			BW42 m			13·8							
OB (4 IB × 4 OB)	BW42	70	BW42 m	70	27·9	58·3	30·4	109	13	18	0·67	23	Bünger *et al.* (1994)
OB	BW42+	30/Rep	BW42	20	20·0	26·0	6	30	3		0·20		Heath *et al.* (1995)
(2 × IB)	BW42−		BW42		20·0	16·0	4	20	2				

BWxx m or BWxx f: individual male or female body weight of at xx days; G21–42: gain between 21 and 42 days of age
PLM70: predicted mean mass at 70 days (PLM70 = BW70 − 8 × gonadal fat pad weight).
Approximations for BW42 from BW70, BW60 and BW56 were obtained by multiplying by 0·81, 0·83 and 0·85, respectively.
If σ_p was not given, CV = 10% was assumed and σ_p was calculated from the 'initial' mean.
Rep.: replicate.

[a] OB, IB: outbred, inbred; S: selection line.

[b] Some h^2 values are given by the authors for within-family deviations (h^2_w); however, $h^2 = h^2_w\ (1-\tau)/(1-r)$ (Falconer & Mackay, 1996) and r (coefficient of relationship) = 0·5 for full sibs and τ (intraclass correlation of full sibs) ≈ 0·5 h^2 and h^2_w are almost identical.

[c] Data for the high line (until generation 43) and the low line (until generation 26) were kindly supplied by Reinoud Buis to the senior author and fitted using model (1).

[d] N_e before generation 20 was about 100 over the three replicates; after crossing the replicates in generation 20, N_e was about 50 ($\Delta F \approx 1\%$ per generation; MBAGA, 1996).

The reported mean body weights for line *PA* in generation 70, i.e. about 52 and 62 g at 42 and 63 days, respectively (Fig. 3*b*), make these mice in absolute terms one of the 'heaviest' mouse populations known. Only mice from line *BW* (Table 5, row 11) from the same experiment are heavier. Although selection in line *PA* was on protein and not on body weight, knowing that the correlation between body weight and protein is high ($r_A \approx 1$, $r_p = 0{\cdot}82$, Table 1), the weights in line *PA* can be used to obtain a rough estimate for the '*total range*' for BW42 in laboratory mice produced by selection. The BW42 of 52 g reached as a correlated effect in generation 70 in line *PA* (Fig. 3*b*) or 58 g in line *BW* (Table 5, row 11) can probably be considered as an approximate *upper limit* for this body weight in laboratory mice. This value would be even higher if we consider the estimated theoretical selection limit for BW42 ($A = 63$ g) in line *BW* calculated by Bünger *et al.* (1994), which is a real consideration because means of recent generations (90–92) overcame this predicted limit by 0·5 g (Bünger *et al.*, unpublished data). There was no selection on low body weight in this experiment and therefore no estimate for the *lower limit* is readily available. Assuming symmetry of the selection response selection on low BW42, starting at the same level as the high selection (BW42 = 28 g), would result in a negative value if calculated on an absolute scale, or 12·4 g if calculated on log-transformed data.

This value of 12·4 g, or the limit of 11 g reached in Falconer's low line (Table 5), could be used. After repeated backcrossing of the *lit* gene, a recessive mutation causing a growth hormone deficiency in homozygous *lit*/*lit* animals, into a line selected for low body weight male mice reached a BW42 of 8 g. Such mice are still fertile and this mutation could therefore become fixed in a population (Bünger *et al.*, 1998). Taking 63 g and 8 g as upper and lower limits, respectively, the total range comes to 55 g. The ratio between the lower limit and the upper limit would be nearly 1:8. Similar ratios are known between high-weight and low-weight breeds of chicken and rabbits (Herre & Röhrs, 1973), but the ratio of heavy to light breeds of dogs is much bigger: 1:40 or 1:80 considering the Chihuahua (about 1–2 kg) and the St Bernard (about 80 kg) as 'limits' for dogs (Kaiser, 1971; Sarowsky, 1986). This higher ratio in dogs is probably due to the population size involved, the time scale of domestication and the domestication from different subspecies of the wolf in different areas in the world, possibly allowing the use of many mutations. Thus the total range for body weights in mice is impressive when reckoned in terms of variation present in the original populations but less than spectacular when compared with the achievement of dog breeders.

This calculation of the total range for BW42 in mice can be used to estimate the effective number of genes, assuming an additive model and a logarithmic scale, as the coefficient of variation is more stable than the standard deviation. The phenotypic and genetic standard deviation for BW42 can be assumed to be 2·8 and 2 g ($h^2 = 0{\cdot}5$), respectively, corresponding to 0·0433 and 0·0306 when log (base 10) transformed as described by Falconer & Mackay (1996, p. 293). The total range (8–63 g) on the log scale is 0·896, or about $21\sigma_p$ or $29\sigma_A$. Using this range ($R = 0{\cdot}896$), a genetic variance of 0·00094 (log scale) and the formula ($n = R^2/8V_A$) given by Wright (1952) and Falconer & Mackay (1996, p. 226), an estimate of the number of loci for BW42 is $n = 107$. Though such estimates are by their nature imprecise, they cover an area where little knowledge is available, especially for mammals (Roberts, 1966*a*; Eisen, 1975).

In the same way a rough estimate can be provided for the number of loci for protein. The upper limit for the protein amount can be derived from the selection response in line *PA*. This is 5·2 g (response up to generation 70) or 5·3 g (the estimated theoretical limit; Table 3), 0·724 on the log-scale. Assuming a mean protein percentage of the BW42 of 10%, as found here (Table 1, factor 2), an approximation for a lower limit for protein can be derived. Thus a lower limit for P42 can be expected at about 0·8 g (log-transformed = −0·097), resulting in a total range of 0·821. Using the additive genetic variance found in this experiment ($V_A = 0{\cdot}036$ g^2, or 0·00092 on the log scale) the approximate number of loci for protein amount is about 92.

These estimates for the 'number of growth-related loci' are much higher than the values given by Roberts (1966*a*) for BW42 of about 8–20 or by Eisen (1975) for postweaning body weight gain of about 20–40. This is not surprising because (i) the 'total range' was derived here from selection responses in different experiments and (ii) the upper limits are obtained from this experiment (*PA* and *BW*), where the selection response was very high. This high response seems to be primarily due to the comparatively high population size of about 70 in this experiment (Table 5), reflecting a more or less linear relation between $N_e i$ and the total selection response, found in some selection experiments in mice (e.g. Eisen, 1975; Kownacki & Zuk, 1986) and in *Drosophila* (Jones *et al.*, 1968). The high heterogeneity of the base population, founded by a cross of four inbred and four outbred populations, and the length of the experiment may also have contributed to the high response.

(c) *Response curve and selection limit*

The classical selection response pattern in long-term selection can be viewed as an initial linear change followed by a gradual decrease in the response until a

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selection limit is reached. Without the creation of new variation by mutation the response cannot be expected to continue indefinitely, as genes segregating in the base population become fixed (or in equilibrium in the case of overdominance) by selection and the accompanying inbreeding. A progressive decline in the rate of response is inevitable and the population reaches a 'selection limit'. A limit may be reached well before the genetic variance is exhausted if the selection favours individuals that are heterozygous at some loci, or if natural selection opposes the direction of the artificial selection (for further details see, e.g., Robertson, 1960; Bohren, 1975; Kress, 1975; Eisen, 1980; Hill, 1985; Falconer & Mackay, 1996). Though deviations from this general form are frequently encountered in practice, as discussed by Falconer & Mackay (1996), in most cases a stage is reached after which little or no further progress is made. The development of selection response in line *PA* (Fig. 1) fits this general picture very well.

From about generation 30 onwards there was a somewhat greater variation of the generation means around the trend line (Fig.1). Such a picture emerges at the end of most long-term selection experiments, where selection lines often oscillate rather violently between higher and lower weights, although over a longer period no discernible trend is apparent (Roberts, 1966*a*; Bünger & Herrendörfer, 1994). The reasons for this may be a scale effect and/or a higher general environmental susceptibility, or decreased stress resistance of selected lines (e.g. Bünger *et al*., 1994). The increase in phenotypic variance (Fig. 2) in line *PA*, however, seems to be primarily a scale effect, because the coefficient of variation for protein amount remained constant (CV = 9%). A similar picture emerged from a retrospective analysis of the 'Goodale experiment', in which selection for high BW60 was performed over 84 generations (Bünger & Herrendörfer, 1994), suggesting this to be a general picture for upward selection.

As Roberts (1966*a*) pointed out, the oscillation of the generation means near a limit makes it extremely difficult to decide what mean weight is 'the limit', and quite impossible to decide when this was reached. In such a situation it seems advantageous to fit an exponential model to the observed response curve (James, 1965), so as to provide parameters describing the outcome of long-term selection experiments: the limit (parameter *A*), the maximum selection response (parameter *B*), the total selection response and the half-life (Fig. 1).

The total selection response after 70 generations is 2·27 g and a theoretical selection plateau can be expected at about 2·43 g. The population has reached about 93% of this limit in generation 70, less than 0·2 g 'to go', but the recent selection response per generation is only 0·01 g per generation and it is further diminishing. Such an approach is of course based on the assumption of no substantial contribution of mutation to the selection response, which might be not true, because other experiments have shown that spontaneous mutations contribute to the selection response, especially in long-term experiments (Frankham, 1980; Bradford & Famula, 1984; Caballero *et al*., 1991, 1995; Keightley & Hill, 1992; Hill *et al*., 1994; Mackay *et al*., 1994). Caballero *et al*. (1995) found it impossible to give an unequivocal estimate of mutational heritability for body weight in the mouse, but it seemed likely that the total mutational heritability, including genes conferring significant fitness reduction, is at least 0·5% per generation, of which the component contributed by neutral or nearly neutral genes is about 0·1%, sufficient to contribute to long-term selection responses. But there are many selection experiments in which an obviously non-linear development or a plateau over a long time is shown (for examples see Falconer & Mackay, 1996; Bünger & Herrendörfer, 1994). The question arises why mutations did not contribute to an obvious extent to the selection response in those experiments. It could be that the fluctuations of observed means mask an underlying steady genetic change in some cases, or that many mutations may have negative pleiotropic effects on fitness (Keightley *et al*., 1993).

To estimate theoretical selection limits does not necessarily mean to consider them as fixed and everlasting limits, as evolution shows that every limit is only a temporary one; the question is the time scale over which the progress is measured and what population size is involved. But because in some populations limits are maintained over a very long time (e.g. 30 or 40 generations in the Goodale experiment: Bünger & Herrendörfer, 1994), it seems worthwhile to analyse and describe them.

Assuming an infinitesimal model, selection limits can theoretically be predicted by the response in earlier generations, as shown by Robertson (1960) for low values of $N_e i$, where the total response is $2N_e$ times the response in the first generation. This ($2 \times 70 \times 0{\cdot}094$) gives a value of over 13 g – much above the realized response in generation 70 (2·3 g) and the predicted limit (2·4 g). Because the assumption given above seems not to be true, Robertson's theory would also overestimate the expected half-life (HL $\approx$ 100) from $1{\cdot}4\,N_e$; the half-life estimated in the experiment is about 18 generations (Table 2, Fig. 1) or $0{\cdot}25\,N_e$. Similar low figures can be obtained from Table 5. In all cases the half-lives are less than N_e with a mean of about $0{\cdot}45\,N_e$, which suggests that many of the desirable alleles had been fixed by selection.

There are models describing the interaction between natural selection and artificial selection for a quantitative character in a finite population. If natural

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selection is strong enough to create a selection plateau in which genetic variance declines relatively slowly, then the total response to artificial selection prior to the plateau will be much less than that expected in the absence of natural selection and the half-life of response will be shorter (James, 1962; Nicholas & Robertson, 1980; Falconer & Mackay, 1996). Because of reduced fertility in selected lines the selection differential may be dramatically reduced in later generations. A plot of means against generations would then show an approach to a limit (Fig. 1 *a*), but a plot against cumSD would not (Fig. 1 *b*). But both approaches for line *PA* showed that the population is near a limit and the estimated limits were almost the same. The selection intensity decreased form 0·95 to 0·52 during the experiment, but this was due to a drop in the number of fertile matings from an initial value of 89% to 63%; as a consequence the proportion selected had to be increased from 42% at the beginning to about 65% at the end. If artificial selection is opposed by natural selection, it should be detectable by the realized SD being less than the expected SD or by relaxation of the artificial selection. The expected and realized cumSD at the end of the selection were 36·74 and 36·46 g, respectively and the mean expected and realized SDs were 0·522 and 0·518 g, respectively. These small differences cannot have caused the observed decrease in selection response.

The reasons for approaching a selection limit in this experiment are not clear. To reveal the causes, this line should be subjected to some further experiments. A reverse selection would 'measure' how much genetic variance still exists. Inbreeding could test whether unfavourable recessive alleles at low frequencies are of importance, and crossing of such inbred lines could possibly eliminate them and increase the total selection response, as shown by Eklund & Bradford (1977 *a*).

(d) *Realized heritability*

Using the first derivative of model (1) a 'realized heritability function' was derived from the relation between cumSR and cumSD by determining the slope relating cumSR and cumSD at every point, in a similar way to Frahm & Kojima (1966). Because cumSR and cumSD are based on full sibs, these slope values were transformed, using (3) to obtain the h^2 function on a mass selection basis. The realized heritability was initial 0·56 and decreased asymptotically. At the end of the experiment it was 0·03. Instead of assuming a constant intra-class correlation (τ) in formula (3), c^2 could be assumed to be constant. Formula (3) would then become:

$$h^2 = 2b[1(n-1)c^2]/[n-(n-1)b],$$

which for $n = 2$ gives

$$h^2 = 2b(1+c^2)/(2-b).$$

An estimate for the initial c^2 was 0·29. The initial heritability would be not much different ($h^2 = 0{\cdot}58$) and would also decrease to 0·03.

The present estimate fits very well with the general picture of heritabilities for lean traits given by Eisen (1989), summarizing seven selection experiments, including an earlier report on *PA*. The mean realized h^2 was 0·37–0·41 for different lean traits. Beniwal *et al.* (1992 *a*) estimated for the predicted lean mass in mice over all replicates up to generation 20 that $h^2 = 0{\cdot}51 \pm 0{\cdot}03$, using an animal model. In the only other selection experiment in mice in which selection was directly on protein amount, the estimated realized heritability was exceptionally low: $h^2 = 0{\cdot}18$ (Barkemeyer, 1984). Possible reasons for this low estimate are discussed elsewhere (Eisen, 1989).

(ii) *Correlated selection response*

(a) *Correlated effects on body weights*

All body weight measures show significant correlated responses, which is expected because of the reported correlations. Because of the high direct response the magnitude of the correlated effects is also very high. The body weights (Fig. 3) show these to be some of the heaviest laboratory mice reported (Table 5).

(b) *Body composition*

From measurement of protein percentage and carcass percentage at 42 days over the generations (Fig. 4) it is seen that the direct response in protein aggregation was not due to a change in the protein (18%) or carcass percentage (56%) at 42 days but to increased general growth. Barkemeyer (1984) also found an increased body and carcass weight as a side effect of selection for protein amount, but also a small positive correlated effect on protein percentage. A large variation in protein content, and an increase from about 20% to about 26–28% in both lines, indicate some methodological difficulties in this study and cast some doubt on the generality of this finding.

Selection for body weight or gain is very often accompanied by an increase in fatness (for reviews see Roberts, 1965 *a*, *b*; Eisen, 1974, 1989; McCarthy, 1982; Bünger, 1987). Because body weight and protein amount are highly correlated, very different consequences for fatness are not expected *a priori* if selection is on either one of these traits. A comparison of both selection lines with the control (Fig. 5), however, shows different correlated effects. The general picture emerging from this approach is that the fat content increased from very low levels at birth to a maximum at about 12–15 days, coinciding with the maximal milk performance of the mother, followed by a decrease which continued up to about 21 days (weaning) in line *BW* and to 35 days in lines *PA* and

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CO. Thereafter there was a more or less linear increase with different increments between the lines. This general curve for the development of fat percentage in mice in relation to age seems to be very typical for mice (Bailey *et al*., 1960; Cheek & Holt, 1963; Gestrich, 1972), reflecting the very strong influence of the mother on the energy supply of offspring at this age and showing the difficult energy situation around weaning. From birth to weaning the fat percentage of the *PA* mice is mostly significantly higher than the control, which could presumably be attributed to higher maternal performance of *PA* mothers. Thereafter, throughout the period of most intensive growth the fat content in the control and in *PA* is very similar, but is already much higher in line *BW*. After the selection age line *PA* becomes increasingly fatter, with fat contents in *PA* and *CO* of 17·2 and 10·8 %, respectively, at 120 days of age.

The selection for body weight at 42 days (*BW*) led to mice that were already fatter than the other two lines at 42 days (fat % = 13·6 %), increasing to 22·3 % at 80 days. This indicates that selection for protein amount can prevent an increase in fat content at least up to the selection age, whereas selection on body weight results in animals with a higher fat content at the age of selection. That selection for protein amount or lean mass prevents an increase in fatness up to the selection age is confirmed by the results of McLellan & Frahm (1973), Barkemeyer (1984) and Bishop & Hill (1985). Eisen (1987) found that selection for hindcarcass weight/body weight at 12 weeks was accompanied by a decreased fat percentage in hindcarcass tissue.

There has been no other study in mice that directly compared correlated effects on fat percentage of a selection on body weight and on protein amount. The different consequences for fat percentage were rather unexpected, because of the high correlation between body weight and protein amount.

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