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REPRINTS AND REFLECTIONS

A third component causing random variability beside environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals?[1,2]

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Summary This paper is a review of experiments, performed in our laboratory during the past 20 years, designed to analyse the significance of different components of random variability in quantitative traits in laboratory rats and mice. Reduction of genetic variability by using inbred strains and reduction of environmental variability by highly standardized husbandry in laboratory animals did not remarkably reduce the range of random variability in quantitative biological traits. Neither did a tremendous increase of the environmental variability (i.e., living in a natural setting) increase it. Therefore, the postnatal environment cannot be that important as the source of random variability.

Utilizing methods established in twin research, only 20–30% of the range of the body weight in inbred mice were directly estimated to be of environmental origin. The remaining 70–80% were due to a third component creating biological random variability, in addition to the genetic and environmental influences. This third component is effective at or before fertilization and may originate from ooplasmic differences. It is the most important component of the phenotypic random variability, fixing its range and dominating the genetic and the environmental component.

The Gaussian distribution of the body weights observed, even in inbred animals, seems to be an arrangement supporting natural selection rather than the consequence of heterogeneous environmental influences. In a group of inbred rats, the males with the highest chance of parenting the next generation were gathered in the central classes of the distribution of the body weight.

Keywords Components of variance of body weight, Monozygotic twins, Centralizing selection, Heritability, Ooplasma

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Thirty years ago we started trying to reduce the large variability of qualitative and quantitative biological traits in laboratory animals by the standardization of genotypes, environmental conditions and of the state of health of the animals. A reduction of the variability should raise the validity of experimental research in laboratory animals. This paper reviews our experimental results over the past 20 years in order to analyse the significance of different components of variability of quantitative traits in laboratory rats and mice. Fixed effects and random variability are distinguished. This paper focuses on the components of random variability.

The paper is divided into three parts, the first of which describes experiments demonstrating the small effect of standardization on the random variability of quantitative traits. The second describes twin experiments designed to estimate the environmentally caused component of random variability directly and gives evidence for a third component of random variability. The third part shows the participation of the body weights' random distribution in the processes of natural selection.

Effects of standardization on random variability

Fixed effects and random variability

Different forms of variability could be distinguished. The left-hand side of Fig. 1 shows the kidney weights of about 1160 adult rats - selected as a model for quantitative traits-estimated in 58 groups of rats. Each group contained about 20 animals. The groups differed for age (81- and 121-days-old), genotype, animals per cage (one or four), state of health (specific-pathogen-free or infected by *M. pulmonis)* and environmental conditions. They lived either in cages in highly standardized animal rooms or freely in a wild, fenced-in area. Two different kinds of variability were seen: fixed effects and random variability. Fixed effects refer to the polar distribution of the results belonging to different groups of animals. Random variability refers to the range within the groups, frequently resembling a Gaussian distribution.

The success of standardization was limited. Standardization reduces fixed effects which are mainly caused by genetic differences, age, sex and, to a minor degree, by the environment. Random variability resists the various efforts at standardization.

The following considerations focus on the random variability. What are the true reasons for the random variability? Why does it resist standardization? This paper gives evidence as to why we should discard the well-liked hypothesis that random variability is only caused by genotypic and environmental differences. We consider this as incomplete. Quantitative geneticists use the expression 'environment' for all non-genetic variation which influences an individual after fertilization. The so called 'environmental' component contains two components, one of which is inborn and may originate from differences in the cytoplasm, in the vitelline membrane of the

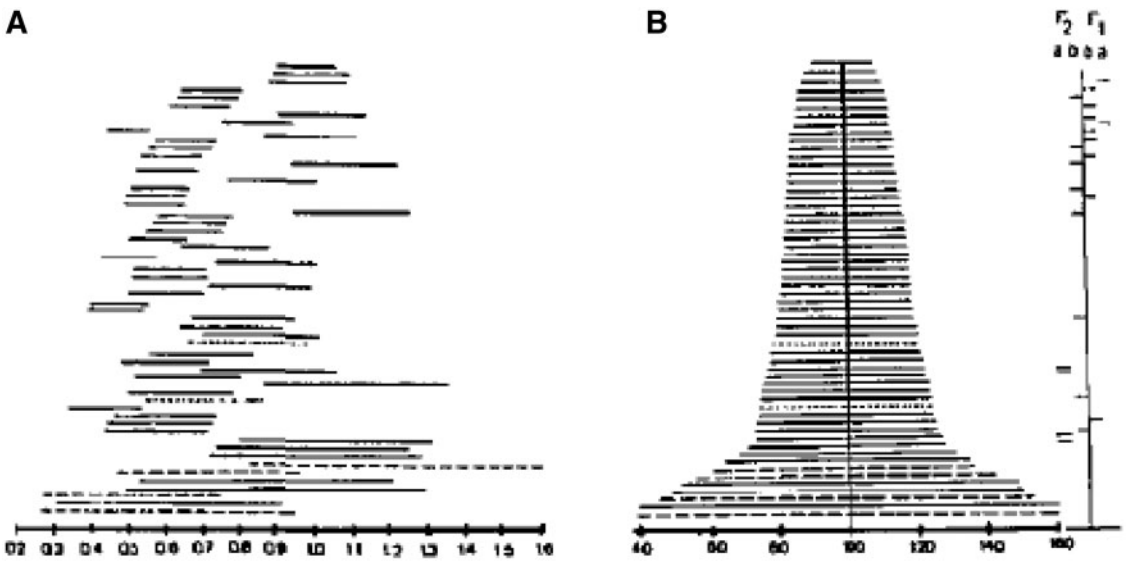

Figure 1 Ranges of the kidney weights in 58 groups of male or female rats, each group 25 animals, 81 ± 7 or 121 ± 9 days of age. Groups caged single or 4 rats together; animals belong to the inbred strains ACI/Ztm, DA/Ztm, LEW/Ztm, BH/Ztm, the outbred strain Wistar or the hybrids F_1(DA x LEW), F_1(BH x DA), F_2(BH x DA), F_2(DA x LEW); groups were living under highly standardized environmental condition in the animal house and were free from pathogen (SPF) (+ or contaminated by *M. pulmonis* (——) or living in the wild (. . . .). Left: ranges of the weights in gram. Right: coefficients of variation multiplied by 4. - The line on the right-hand side shows the positions of the F_2- or F_1-hybrids of (BH x DA) (a) and (LEW x DA) (b)

unfertilized oozytes and/or from different genomic modulations within the isogenic genome of the animals, as shown in the second part of this paper. The second component, the real environment, has only a small effect on the creation of random variability of quantitative traits in inbred animals.

For an improved study of random variability in, for example, kidney weights, the fixed effects can be omitted by calculating the coefficient of variation for each group. On the right-hand side of Fig. 1 the single lines represent all the coefficients of variation [(standard deviation/mean) × 100] multiplied by a factor of 4 for the 58 groups of examined rats. Multiplication of the coefficients of variation by 4 was performed to give a more realistic figure of the phenotypic range of the body weights. For a better comparison, all the lines are put in order of magnitude. The kidney weights within the more uniform groups ranged between 80–120% of the mean and in the most heterogeneous groups from 40–160% of the mean, a threefold range.

The influence of standardizing different genetic or environmental conditions on the range of random variability of more than 25 quantitative characteristics of these 1160 rats divided in 58 groups has been reported in detail elsewhere.[1,2,3] The two most important results of those experiments are shown also in Fig. 1.

(1) In 49 of the 58 groups shown in Fig. 1, the random variability of the kidney weights were quite similar. It ranged from 80–120% of the mean. In the other groups the ranges of kidney weights were significantly larger. In these groups, the animals were frequently infected with *Mycoplasma pulmonis*. In addition to kidney weights, we compared about 25 other traits in the same manner. The results were similar for all traits: an approximately trait-specific constant range of the random variability in the healthy groups, standardized in different ways and increased ranges in groups of ill animals. Therefore, an effective way to reduce random variability is to eliminate infections and spontaneous diseases.

(2) Various efforts of standardization of the genotypic differences or the environmental conditions hardly influenced the range of the random variability of a designated group: The reduction of the genetic variability by using inbred animals did not influence the random variability very obviously (Fig. 1, right). The coefficients of variation of F_1 and F_2 hybrids are marked in Fig. 1 (right). F_2 tend to larger dimension in comparison with F_1. By rank-sum tests the small differences could be verified ($P < 0.01$). These results agree with Festing[4] and Jay,[5] and show the same tendency as the reports of Dawson[6]; Oliverio et al,[7] Gärtner,[8] and Hagemann.[9]

The standardization of different environmental components, such as food, temperature, group size, bedding, humidity, etc., does not substantially reduce the trait-specific, relative random variability. The random variability of the kidney weight remains in the trait-specific range between 80–120% of the mean.

The last conclusion is supported by a very convincing result. The coefficients of variability were compared between groups of DA inbred rats-males or females-living in cages under highly standardized laboratory conditions in an animal house and groups of health DA-males or females which lived under wild conditions for over 5 months in a confined area of about 200 m^2 at an altitude of 500 m (Fig. 1, right). Very often the temperature was cold at night with occasional rain or snow. Food was provided from the litter of a burned-down general store or from pellets, twice a week. The environment was completely unstandardized. In the beginning of the study, 40 DA-females and 40 DA-males were placed in the area and at the end about 400 animals lived there. Many animals born during the observation period died during the suckling period or later. Only the males and females first introduced were investigated and compared with those living in the animal house. All four groups showed the same range of relative random variability of kidney weights (see Fig. 1, right). Similar results were estimated in other gravimetric and morphometric characteristics, and in such haematological and biochemical traits which are only slightly influenced by handling-stress or manipulation.[3]

These results were unexpected. Surprisingly, a tremendous increase of environmental variability did not influence the range of random distribution. These results strongly suggest that the postnatal physical environment does not play a major role as a source of random variability.

Twin experiments designed to estimate directly the environmentally caused components of random variability: evidence for a third component.

Usually, the environmental component is calculated indirectly by subtracting the genetically caused component from the total genotypic variability. As mentioned above, the term 'environment' refers to all variation that is not of genetic origin. These are all influences on the individual after fertilization. Due to the lack of genetic variability in inbred strains all the phenotypic variability should be environmentally induced. Frequently, prenatal influences are discussed (uterine position, sex of the neighbouring fetus, uterine blood supply, etc.) as reasons for prenatally acquired random variability in laboratory animals living under highly standardized conditions.

One way to measure in a direct manner the environmentally caused variability is by dividing 8 cell stage embryos and transferring each half to different

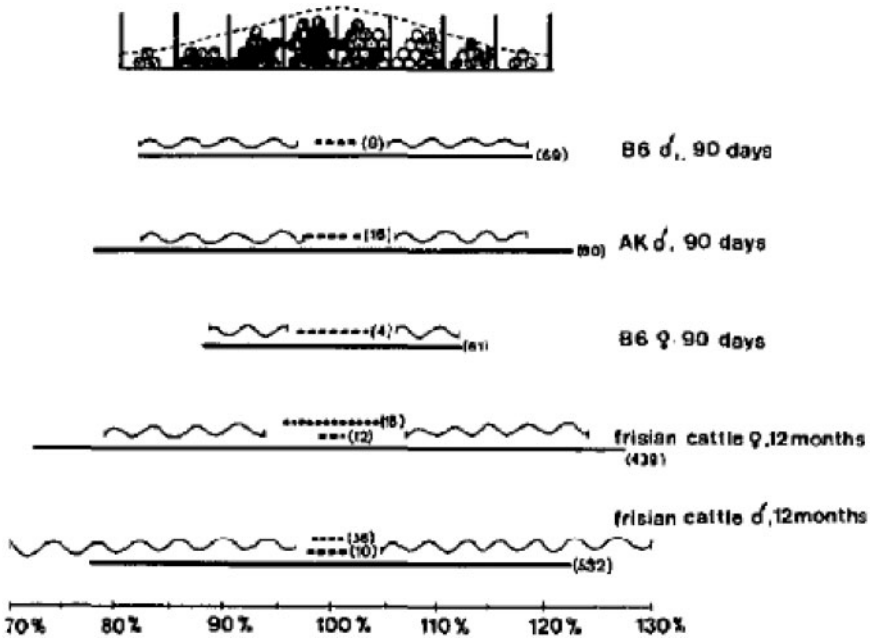

Figure 2 Comparison of the total range of phenotypic random variability of the body weight and its environmental component. *Upper:* schema of the Gaussian random distribution. *Lower:* -, ranges of the total random variability (4 x coefficient of variation) of the body weight in natural born adult male and female B6 and AK inbred mice or Friesian cattle (calculated from s_t^2 of Table 1); —, ranges of random variability of the body weights within the two monozygotic twin mates and acquired by influences of the environment which are effective at or after fertilization;. . .... each halved embryo transferred to and grown up in different uterine foster mothers; —— both halved embryos transferred to and grown up in one foster mother; ~ part of the random variability of the body weight which originates from influences which are effective at or before fertilization

foster mothers and estimating the phenotypic differences between these genetically identical twins. Each is born and raised in different litters. Until recently, the preparation has only been successfully carried out with cattle by a group working in cooperation with us. We have performed such experiments using AKR (AK) and C57BL/6 (B6) mice with the two twin mates raised by the same foster mothers. After birth the body weight and other characteristics were estimated in mice at 90 days of age and in cattle at 1 to 24 months. The results in mice are described elsewhere.[10,11] The results in cattle are now completed.[12]

The main results of the experiments are shown in Fig. 2. More detailed information on the analysis of variance type II is shown in Table 1 and reported elsewhere.[10,11,12] In groups of naturally born animals the coefficients of variation of the body weights were similar in the cattle at 1 year of age and in the two inbred mouse strains. The body weights ranged from about 70 to 130% of the mean in AK males and in Friesian cattle and from 90 to 110% in B6 female mice.

If all the random variability of the body weight in inbred mice is environmentally caused, the dissimilarities within the twin mates in inbred mice should be of the same dimension as the dissimilarities among naturally born sisters. The random variability within the homozygotic twin mates are also shown in Fig. 2 (after calculation of its range from results listed in Table 1). It is compared with the range of the total variability of the naturally born animals. In all 5 series the ranges within the twin pairs are remarkably smaller. Only 3–30% of the total random variability were environmentally caused (Fig. 2, Table 1).

Differences of the ranges within the monozygotic twin mates reared by the same uterine foster mother or by two different uterine foster mothers have only been compared in the cattle. The ranges differed to a small degree. The influence of different uterine environments are obvious but small, in comparison to the total range of the naturally born animals. The ranges within the different twin groups in cattle resemble those observed within the twin mates of inbred strains of mice.

A large range of about 70–97% of the random variability remains inexplicable. It is demonstrated in Fig. 2. It originates from influences which are effective at or before fertilization. In cattle there must be some genetic variability involved. However, it is very difficult to explain this remaining range for inbred mice. Is it caused by a residual heterozygocity in the body weight loci? By using a strongly divergent selective breeding design over four generations we tried to find out if residual heterozygocity exists in those gene loci in our inbred mouse strains B6 and

Table 1 Analysis of variance components of body weight in male and female adult monozygotic (MZT), dizygotic (DZT) twins and natural born (NBA) mice of the inbred strains CS7BL/6 and AKR/N and Friesian cattle

Strain	Prenatal preparation	N_1/N_2[d]	Mean body weight (g/kg)	s_t^2	s_w^2	(DF)	s_b^2
B6 ♂	MZT mono	8/4	27.8[a]	5.79	0.31	(3)	5.48
	DZT mono	4/2	24.5[a]	5.26	0.81 NS[c]	(1)	4.45
	NBA	69/20	25.9[a]	5.20	1.77*[c]	(19)	3.43
AK ♂	MZT mono	16/8	36.6[a]	8.63	1.10	(7)	7.53
	DZT mono	10/5	29.5[a]	19.45	10.49***[c]	(4)	8.96
	NBA	60/18	29.8[b]	11.21	3.47***[c]	(17)	7.78
B6 ♀	MZT mono	4/2	21.8[a]	0.90	0.28	(1)	0.62
	DZT mono	6/3	20.8[a]	1.67	2.07 NS[c]	(2)	−0.40
	NBA	61/14	21.2[b]	1.41	0.97 NS[c]	(13)	0.44
Friesian cattle ♀ 12 months of age	MZT mono	12/6	377	1452	6.3***[c]	(5)	1446
	DZT biut	16/8	345	1315	112.7	(7)	1202
	NBA	439	329	2231	–		–
Friesian cattle ♂ 12 months of age	MZT mono	10/5	410	5048	285**[c]	(4)	4763
	DZT biut	36/18	390	3323	119	(17)	3204
	NBA	532	427	2788	–		–

Twin mates transferred into the uterus of the same foster mother (mono) or into the uterus of two different foster mothers (biut). Components of variance s_w^2 (within litter or within twin mates); s_b^2 (between litters or between twin mates), and $s_t^2 (= s_w^2 + s_b^2)$
a individual body weight estimated from the mean of the weight on day 81, 91, 101;
b individual body weight estimated from the mean of the weight on day 80, 94, 101;
c F-test between s_w^2 of MZT and s_w^2 of DZT or NBA in mice; between s_w^2 of mono and s_w^2 biut in cattle;
d N_1 number of animals; N_2 number of twin groups or litters.

AK. This study took 3 years. The divergently selected lines were identical in body weight giving no evidence of residual heterozygocity.[13]

Our results in monozygotic twins correspond with the many studies on naturally born monozygotic twins in man[14,15,16] or cattle.[17] Monozygotic twin mates resemble each other more than one could expect from their genetic identity alone. The common uterine environment is suggested to be the reason for the astonishing similarity.[18] Our results in cattle twins do not support this suggestion. Neither do our results in experimentally prepared dizygotic twin mice support the widely accepted hypothesis. The dizygotic mice twin mates differ remarkably, in spite of identical genotypes and the same uterine environments (Table 1).

Therefore, we assume a third component that creates biological random variability, in addition to the genetic and the environmental component. It must be an inborn component and effective at or before fertilization. It has a very large dimension, i.e., in inbred mice it accounts for 70–80% of the total phenotypic variability of body weight. It seems to be the major component of the phenotypic variability which fixes the range of the random variability of quantitative traits which are related with body weight in inbred strains of rats and mice under highly standardized conditions.

We were unable to influence this type of fixed and unchallengeable variability by our efforts of standardization. This component may resemble the 'intangible variance' which Falconer[19] and other genetists have formerly described or with emergenesis, described by Lykken.[20]

In primitive plants, Mather and Jinks[21] have shown that ooplasmic differences sometimes have a large influence on the phenotypic variability. Similar results have been seen in *Drosophila*.[22] We obtained some hints for the ooplasmic origin of this component by investigating the range of body size in mouse litters born from females whose ovaries had been transplanted,[13,23] or by comparison of the body weight ranges between reciprocal F_1-hybrids and their parental strains in mice.[24]

The participation of the body weight's random distribution in processes of natural selection

The following consideration should help to explain the evolutionary significance of this third and inborn component of variability. The twin results suggest that each individual of an isogenic inbred strain has received information concerning its position in the random distribution of the adult phenotypes at

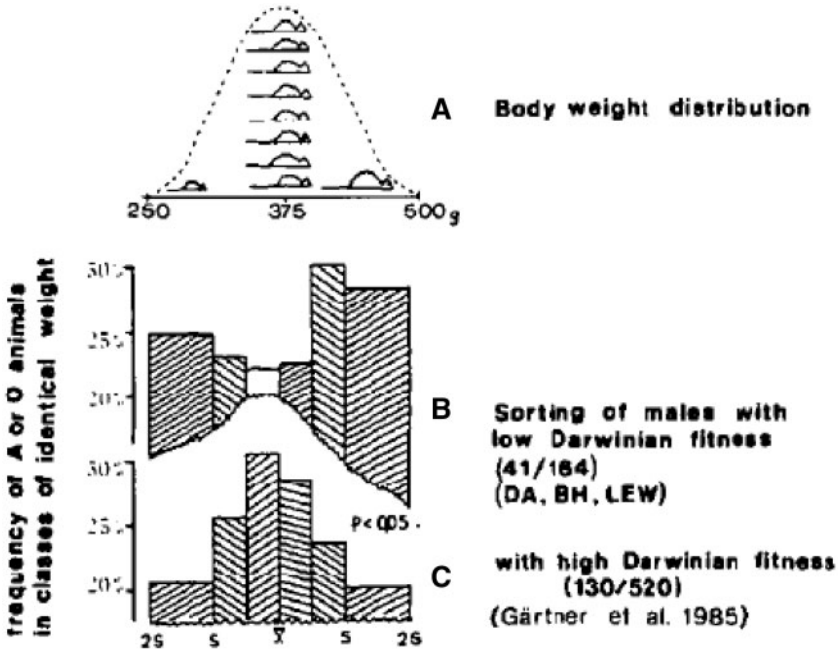

Figure 3 The bodyweight distribution of male inbred rats. (A) as an arrangement supporting natural selection. Males are more frequently found gathered in classes of identical body weights which have less (B) or more (C) capabilities supporting the reproduction process of the population

a very early ontogenetic stage. This results in a Gaussian distribution of the isogenic animals. From other fields of science[25] we know that such a prospective arrangement of a population is of high efficiency for natural selection.

Evolution is characterized by creation of variability and processes of natural selection. Therefore, we tested if the body weight's random distribution in inbred strains, mainly caused by the third component, also participates in processes of natural selection. Two processes essential in natural selection were investigated: (1) the males' capability of breeding under competitive conditions; and (2) the differences in the susceptibility to specific infections.[26] This paper summarizes only results concerning the former point. They are described in detail elsewhere.[8,27] Male adult rats of the same inbred strain and age were randomized and grouped in fours. After an adaptation period they were confronted with one oestric female for 2 h on 10 days and the frequencies of intromissions and ejaculations per male were counted. The frequencies differed remarkably between the 4 males. Animals with the highest and lowest frequencies were named A and O, respectively. Means and standard deviations of the body weights of the 4 animals per cage were calculated and the weight of each individual was expressed in units of that standard deviation. The frequencies of A and O-males within the 6 classes of the body weight distribution were compared (Fig. 3).

Males that have the highest chance to parent the next generation were found gathered in the central classes, around the mean of the body weight. In contrast, males that were not sexually accepted by oestric females were more frequently found at the two opposite extreme classes of the body weight and were absent in the middle. The groups exhibit signs of a centralizing selection programme. Similar correlations of male's mating success and intermediate body size is described repeatedly in *Drosophila*[28,29] in *Acrididae*[30], and in chickens.[31,32] The individual differences in sexual activity correlates with imbalances of the endocrine and vegetative nerve systems, i.e., differences in blood pressure, corticosterone reactivity following confrontation with a stressor and androgen levels in the plasma.[8,33,34]

The findings support the hypothesis that the variation in body weight in male inbred rats is not the result of heterogeneous environmental influences. On the contrary, it is determined at a very early ontogenetic stage in order to facilitate natural selection.

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