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Effects of Marked Chromosome Sections on Quantitative Traits in the Mouse*

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Summary. An investigation of the influences of marked chromosome sections on quantitative traits in a backcross-generation with 2321 mice (C57BL/6JHan × (AKR/NHan × C57BL/6JHan)) is described. In the animals the chromosomes 1, 4, 7, and 8 were marked by the gene loci Idh-1, Gpd-1, Gpi-1s, Es-1, resp. Within the backcross-generation, for Idh-1 and Es-1, more heterozygous genotypes were found than expected under random conditions. By comparing animal-groups with different homologous sections of the marked chromosomes, effects were observed on quantitative traits (body length and weight, dry weight and matter, fat weight and content). The results indicate that a few chromosome sections influence to a major extent the genetic variation of some quantitative traits.

Key words: Inbred mice – Chromosomes – Quantitative traits – Marker genes

Introduction

The transfer of single homologous chromosome sections from parents to their offspring can be traced by suitable experiments. Afterwards one can test whether single chromosomes or chromosome combinations lead to an influence on the values of quantitative traits (Geldermann 1975). In the investigation described here inbred mice were used because they are defined for a great number of monogenic criteria (review paper by Staats 1976).

Materials and Methods

Mice of the inbred strains AKR/NHan and C57BL/6JHan were used. These strains differed in some of their quantitative traits (e.g. body weight, body fat content) and in the alleles of genotypes displayed after electrophoresis. After producing a F_1-generation from AKR ♂♂ × C57BL ♀♀, the F_1 ♀♀ were backcrossed with C57BL ♂♂ (Fig. 1). 2321 mice (1166 male, 1155 female) were obtained as backcross-generation and included this study.

The mice were kept at the Zentralinstitut für Versuchstiere (Hanover) under the following conditions: macrolone cages type II; SPF status; room temperature: 22±1 °C; relative humidity: 55±5%; light-dark sequence 12:12 h; diet containing 0.2% total fat (type 1006, Altromin GmbH, Lage/Lippe); food and water ad libitum. – The animals were mated permanently monogamously. Immediately after birth the litters were standardized to 10 animals. Added young were marked, so that their origin could be recorded later. At 3 weeks of age the young were weaned and separated according to sex.

The mice were measured at an age of exactly 11 weeks. They were first weighed. After killing they were bled and body length was determined. Head, one fore-leg, some adipose tis-

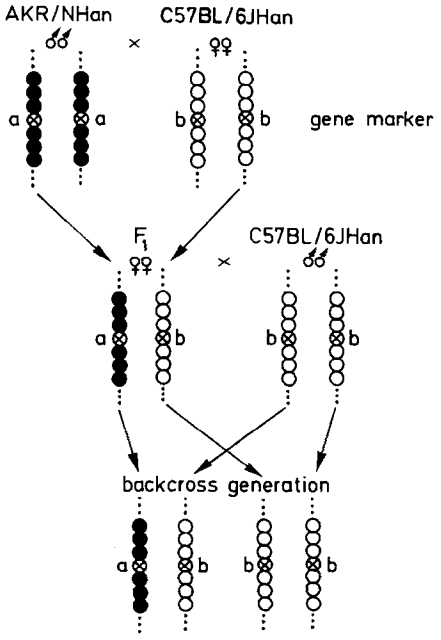

Fig. 1. Principle of the experiment

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Table 1. Genotype frequencies in the backcross generation

Gene loci		Genotypes aa	ab	bb	Significance (χ^2-test)
Idh-1	observed	1,104	1,217	0	$P<0.05$
	expected	1,160.5	1,160.5	0	
Gpd-1	observed	1,127	1,194	0	n.s.
	expected	1,160.5	1,160.5	0	
Gpi-1s	observed	0	1,154	1,167	n.s.
	expected	0	1,160.5	1,160.5	
Es-1	observed	1,071	1,250	0	$P<0.01$
	expected	1,160.5	1,160.5	0	

sue, and one kidney were then removed. The remaining parts were dried for 48 h at 105 °C and afterwards fat was measured using a Fosslet apparatus (Montag 1973; Eslami-Matin et al. 1974). The adipose tissue was diluted 1:2 with distilled water, homogenized by ultrasonification, cooled with ice water, and centrifuged (1 h, 5,600 g, 8 °C). In the watery supernatant, the following enzymes were investigated by way of horizontal starch gel electrophoresis:

- Isocitrate dehydrogenase (Idh-1, positioned on chromosome 1, method according to Henderson 1965 and Fox and Crockett 1977)
- Glucose-6-phosphate dehydrogenase (Gpd-1, pos. on chromosome 4, method according to Ruddle et al. 1968 and Shows et al. 1964)
- Glucosephosphate isomerase (Gpi-1s, pos. on chromosome 7, method according to DeLorenzo and Ruddle 1969 and Carter and Parr 1967)
- Esterase (Es-1, pos. on chromosome 8, method according to Birdsall et al. 1970 and Shaw and Prasad 1970).

The methods of the cited authors were modified as given by Kluge (1980).

Genotypes were determined by a qualitative analysis of the electrophoretically-represented isoenzymes. In the backcross-generation, the genotype frequencies were compared with the expected values presuming random formation of gametes and zygotes.

Analyses of the quantitative traits (weights, lengths, dry substance, and fat content) were carried out separately for male and female mice because of great sex-influences were found (Kluge et al. in prep.). First all identifiable influences on

Table 2. Effects of marked chromosome sections on the quantitative traits. Differences between heterozygous and homozygous combinations are given and significant differences are shown in percentage of the standard deviations

Traits	Sexes	Effects of the chromosome sections marked by Idh-1	Gpd-1	Gpi-1s	Es-1
Body length (without tail) (cm)	♂♂	0.00±0.00	0.01±0.01	0.01±0.01	0.03±0.01** (11.1%)
	♀♀	0.00±0.00	0.02±0.00** (8.7%)	0.00±0.00	0.01±0.02
Body weight (g)	♂♂	0.15±0.07	0.18±0.07 (7.1%)	0.09±0.10	0.25±0.07** (9.8%)
	♀♀	0.00±0.05	0.11±0.05* (6.4%)	0.00±0.05	0.03±0.14
Dry weight (g)	♂♂	0.06±0.03	0.11±0.03** (9.5%)	0.05±0.04	0.12±0.03** (10.4%)
	♀♀	0.01±0.02	0.01±0.02	0.00±0.02	0.12±0.07** (15.0%)
Dry matter (%)	♂♂	0.01±0.08	0.26±0.08** (9.6%)	0.10±0.10	0.13±0.08
	♀♀	0.15±0.08	0.11±0.08	0.02±0.08	0.57±0.23** (20.1%)
Fat weight (g)	♂♂	0.03±0.02	0.06±0.02* (7.5%)	0.06±0.03* (7.5%)	0.05±0.02
	♀♀	0.02±0.01	0.01±0.01	0.02±0.01	0.13±0.05** (21.0%)
Fat content in dry mater (%)	♂♂	0.14±0.18	0.21±0.18	0.52±0.24** (8.6%)	0.14±0.18
	♀♀	0.20±0.18	0.30±0.18	0.44±0.18* (7.0%)	1.30±0.50** (20.7%)
Fat content in fresh matter (%)	♂♂	0.05±0.09	0.17±0.09	0.24±0.12** (7.9%)	0.09±0.09
	♀♀	0.10±0.09	0.16±0.09	0.16±0.09	0.72±0.26** (22.1%)

* $P<0.05$; ** $P<0.01$

the quantitative traits were individually tested with the help of analyses of variance followed by F-tests. Then a linear model was used considering only the significant interactions:

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \tau_m + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\alpha\tau)_{im} + (\beta\gamma)_{ik} + (\beta\delta)_{jl} + (\beta\tau)_{jm} + (\alpha\delta\tau)_{ilm} + \varepsilon_{ijklmn}$$

In this model the symbols have the following meanings:

- μ mean
- α_i effect of allele i at the locus Idh-1 (i = 1,2)
- β_j effect of allele j at the locus Gpd-1 (j = 1,2)
- γ_k effect of allele k at the locus Gpi-1s (k = 1,2)
- δ_l effect of allele l at the locus Es-1 (l = 1,2)
- (. . .) the symbols in brackets represent the significant interactions ($P < 0.05$)
- τ_m effect of the number of mice per cage after separating the sexes (m = 2, . . . , 9)
  The classes with 1 and 2 animals per cage and the classes with 9 and 10 animals per cage were summarized respectively
- ε_{ijklmn} random effects
- Y_{ijklmn} value of the quantitative trait Y of the animal n with the allele i at the locus Idh-1, the allele j at the locus Gpd-1, the allele k at the locus Gpi-1s, the allele l at the locus Es-1, and m animals per cage

The influences given in the model were estimated in a manner such that they became zero in the mean of the backcross-generation. The F-test was used to prove the significance of the influences considered. The calculations were carried out at the Regionale Rechenzentrum (Hanover) by means of the MANOVA-procedure of the SPSS-programs.

Results

The genotype frequencies of the backcross-generation are shown in Table 1. For the gene loci Idh-1 and Es-1 significantly more heterozygous animals were observed than expected.

Table 2 gives the calculated effects of the homologous chromosome sections on quantitative traits. The chromosome section marked by Idh-1 influenced none of the considered quantitative traits in a significant amount. In case of the chromosome section marked by Gpd-1, effects were obtained on body length and weight, whereas effects on the fat-connected traits (dry matter, dry and fat weight) were significant only within males. However, from the Gpi-1s-marked chromosome, only the variation of fat-connected traits (fat weight and content) were influenced significantly. For the chromosome section with the locus Es-1 effects could be established on body length and weight in the males, whereas the fat-connected traits (dry weight and matter, fat weight and content) were influenced considerably only in the females (except that dry weight was also effected significantly in the males).

Discussion

In the backcross-generation more mice with heterozygous genotypes at the loci Idh-1 and Es-1 were observed than expected. There is no information in the literature about an advantage of heterozygosity at these loci. In the backcross-animals, the heterozygous genotypes were combined simultaneously with different chromosome sections because one homologous chromosome came from the inbred line AKR and the other from C57BL. Therefore, heterosis effects on fitness during the embryonic and juvenile development can be assumed, which arise from the chromosome actually marked.

The main aim of the experiment was to estimate the effects of different homologous chromosomes on the quantitative traits, when they are transferred to the backcross-generation. In the inbred strains AKR and C57BL, different alleles are fixed at several loci, so that the origin of certain homologous chromosome sections can be determined in the backcross-generation (Fig. 1). So, when animal groups with different alleles at the marked loci are compared in the backcross-generation, possible differences in their quantitative traits are caused by the possession of different homologous chromosome sections. However, for the results it is important that in the F_1-females one meiosis occured before the gametes are formed for the zygotes of the backcross-generation. Therefore crossing-over could take place between the homologous chromosomes of the two inbred strains, so that one allele, used in the experiment, does not mark a whole chromosome but a section which can differ in size among the individuals. Effects of genes placed on such chromosomes can be expected to be lower estimated the more recombination units they are distant from the marker gene (Geldermann 1975).

If the homologous chromosome section, originating from the C57BL-parent, is transferred by the F_1-females to the backcross-generation, homozygous chromosome combinations will occur. When the other homologous chromosome section, originating from the AKR-parent, is inherited by the F_1-females, however, the backcross-mouse will receive a heterozygous chromosome combination. Therefore the effects shown in Table 2 also represent the heterosis of the respective chromosome sections. In reciprocal backcrosses, now being prepared, such effects will be analysed.

The relations found are true in detail merely under the conditions of this experiment, particularly only for the inbred strains used. There are several examples in the literature about genetic variation of quantitative traits which differ according to strain and sex (for the strains used in this study see Kluge et al. in prep.). For instance, the fat metabolism in mice can be completely changed by mutations at single gene loci (Coleman 1978).

The results in Table 2 show that the genetic variation of some quantitative traits is determined to a major extent by a few chromosome sections. Therefore,

other experiments which show the importance of single chromosome sections in the development of quantitative traits, for instance the investigations about the major histocompatibility complex in mice (Benacerraf and Germain 1978) and about the scutellum bristles of Drosophila (Rendel 1979) are complemented by our findings.

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