Inheritance and Mapping of *Compact* (*Cmpt*), a New Mutation Causing Hypermuscularity in Mice

László Varga,* Gyula Szabó,* Ariel Darvasi,†,[1] Géza Müller,‡ Miklós Sass§ and Morris Soller†

**Institute for Molecular Genetics, Agricultural Biotechnology Center, H-2101 Gödöllő*, Hungary, *†Department of Genetics, The Alexander Silberman Life Science Institute, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel, ‡Egis Pharmaceuticals Ltd., H-1475 Budapest, Hungary and §Department of General Zoology, Eötvös University, H-1088 Budapest, Hungary*

Manuscript received March 14, 1997
Accepted for publication June 25, 1997

ABSTRACT

During selection for protein content in mice at the Technical University of Berlin, individuals showing high protein content and a compact exterior were noted. Animals showing this "Compact" phenotype were separated to form a new line. The present investigations were carried out on a Hungarian subpopulation of this line, selected for maximum expression of the Compact phenotype, and apparently at fixation for the relevant genes. Fertility and viability of the Compact subpopulation was normal. As compared to normal mice, carcass percentage values for male and female Compact mice were 9.4 and 6.8% greater, respectively; and the muscle:bone weight ratio in males was 1.61-fold greater. The Compact phenotype showed variable expressivity and was of intermediate dominance in males, but almost fully recessive in females. The hypothesis that a single gene is solely responsible for the Compact phenotype was rejected by maximum likelihood analysis. Linkage mapping using selective DNA pooling located a single locus (denoted *Cmpt*) strongly associated with the Compact phenotype on mouse chromosome *1*. Fine mapping, using individual selective genotyping and haplotype analysis, located *Cmpt* to the region between *D1Mit375* and *D1Mit21*, approximately one third of the way to *D1Mit21*.

HEAVY muscularity of the entire body is a desired phenotype in domestic animals bred for meat production. A genetic "double-muscled" phenotype, determined by a partially recessive autosomal major gene (*mh*) was mapped recently to chromosome *2* in Belgian Blue Cattle (GEORGES 1990; CHARLIER *et al.* 1995). It is at present unknown whether the *mh* gene determines the double-muscled phenotype in other cattle breeds as well. The callipyge phenotype of sheep, which also results in muscular hypertrophy, is determined by an autosomal gene showing polar overdominance (*CLPG*) mapped to chromosome *18* (COCKETT *et al.* 1994). These genes do not appear to be homologous (COCKETT *et al.* 1994). Transgenic mice (SUTRAVE *et al.* 1990), pigs (PURSEL *et al.* 1992) and a calf (BOWEN *et al.* 1994), carrying chicken c-ski cDNA, also show a muscular phenotype. However, muscle degeneration was observed in some pigs and in the calf. Recently, MCPHERRON *et al.* (1997) reported a muscular phenotype for knock out mice for the GDF-8 growth / differentiation factor.

We here report on the mode of inheritance and chromosomal map location of a mouse mutant designated "*Compact*" (*Cmpt*). The Compact phenotype was first noted in a line of mice selected for high body weight and high protein content at the Technical University of Berlin (F. MAJOR and W. SCHLOTE, personal communication). Mice showing the Compact phenotype are much more muscular than the conventional laboratory mouse and appear phenotypically similar to the muscular phenotypes of domestic animals noted above. Animals showing the Compact phenotype were separated from the main selection line and a new COMPACT line, based on these individuals was originated. In this article we focus primarily on the genetics of the mutant, giving only a preliminary characterization of its salient phenotypic characteristics. A comprehensive description of the mutation will be published elsewhere (F. MAJOR and W. SCHLOTE, personal communication).

Corresponding author: László Varga, Institute for Molecular Genetics, Agricultural Biotechnology Center, P.O. Box 411, H-2101 Gödöllő, Hungary. E-mail: varga@hubi.abc.hu

[1] *Present address:* The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609-1500.

MATERIALS AND METHODS

Stocks: *HCR (Hungarian randombred compact line):* This is a randombred subline of the original German COMPACT line. It was initiated by G.M. in 1989 with five males and seven females (generously provided by W. SCHLOTE) showing strong expression of the Compact phenotype. The HCR subline was perpetuated through random mating of males and females selected as having strong expression of the Compact phenotype.

HCI (Hungarian inbred compact line): Starting from generation 16 of the HCR subline, a brother × sister inbreeding program was initiated by G.M. Single brother × sister mating was carried out for a further 16 generations, to form the HCI line (Figure 1A).

Animals were kept in an animal room with controlled temperature (22° ± 1°), humidity (55 ± 10%) and lighting (12 hr light and 12 hr dark). They were fed a proprietary laboratory

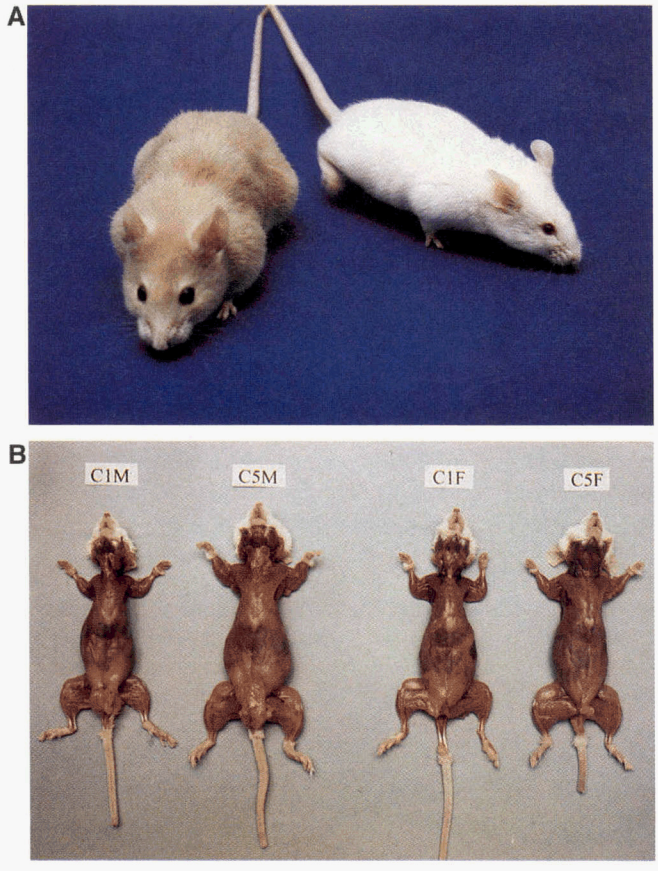

FIGURE 1.—Mice were classified into five phenotypic categories, ranging from extremely muscular (category C5) to normal phenotype (category C1). (A) Male mice showing the normal phenotype (right) and Compact phenotype (from the HCI inbred subline; left). (B) Muscularity of males (M) and females (F) showing normal (C1) and Compact (C5) phenotype (ventral view).

mouse chow (LATI, Gödöllő, Hungary), and supplied with tap water *ad libitum*.

Characterization of the Compact phenotype: The individuals of the HCR and HCI lines and their crosses show various degrees of the Compact phenotype. Gradations between the types are smooth and continuous. For descriptive purposes, however, the animals were classified into five phenotypic categories, ranging from extremely muscular (category C5) to normal phenotype (category C1). Animals classified as C5 are characterized by a short but broad body, broad head with short snout and short thick tail. The body, neck and limbs are extremely muscular as compared to a normal mouse. The increased muscularity of the forelegs and the back are especially striking, with particular hypertrophy of biceps and Longissimus dorsi muscles. Detailed quantitative characterization of C2 to C4 categories has not yet been done. (Figure 1B; Note, the truncated tails are not part of the Compact phenotype; tail tips were taken for DNA extraction).

Carcass percentage was calculated as carcass weight × 100 divided by total body weight, where carcass includes all body parts except for skin, internal organs, head, tail and lower part of the legs. This measure was obtained on 10 C1 and C5 males and 10 C1 and C4 females, taken from among the Cross-2 F_2 animals (see later for description of Cross-2). For measuring the mass of individual muscles, six 4-mo-old C5 male mice from the HCI subline, and six 4-mo-old BALB/c male mice were skinned and eviscerated and the remaining carcass fixed in 4% formaldehyde for 7 days. Several large body muscles (m. gluteus magnus., m. peroneus, m. plantarius longus, m. pectoralis and m. deltoideus) and several large bones (femur, tibia and scapula) were individually dissected and weighed, and average ratio of summed muscle weight:summed bone weight was then calculated separately for each animal. Formaldehyde fixation was necessary because of difficulties in quantitative dissection of individual muscles in fresh carcasses.

For investigations with the light microscope, the m. deltoidus, m. pectoralis and m. plantarius longus of six C1 and six C5 animals of both sexes were fixed in Bouin's fixative for 6 hr at room temperature. The fixed materials were washed in 80% ethanol, embedded in paraplast (Dulbecco) and 5 μm longitudinal and cross sections were cut. After rehydration the sections were stained by the conventional hematoxylin eosin method or impregnated by osmium tetroxide and investigated under an Axioscope (Carl Zeiss Co.). Ten pictures were taken of each section, originating from five C1 and five C5 mice. The diameter of the muscle fibers, the number of nuclei per muscle fiber and the ratio of the contractile elements, connective tissues and vascular components of the muscle fibers were estimated by simple planimetry or counting.

Crosses: Two series of crosses, designated Cross-1 and Cross-2, were carried out.

Cross-1 was initiated in 1992. For this purpose, 10 C5 males of the HCR subline were individually mated with 10 females of the BALB/c inbred mouse line (LATI). From the resulting 10 litters, a total of 73 F_1 animals were reared and used to produce an F_2 population (2508 offspring). Six F_1 females and 12 F_1 males were used to produce backcross populations to the HCR and BALB/c parental lines, denoted BC:HCR (207 offspring) and BC:BALB (189 offspring), respectively. All offspring were classified once for the Compact phenotype at age 6–7 wk. Classification was by T.S., as described above. The F_2 and BC individuals were produced and classified during the same time period.

Cross-2 was initiated in 1994. For this purpose 13 C5 males from generation 16 of the HCI subline were individually mated to 28 BALB/c females. From the resulting 40 F_1 litters, 262 animals were intermated to generate the F_2 population (8285 offspring). Each of the F_2 animals was classified three times independently and the average score was computed.

Pattern of inheritance: A two-step procedure (ELSTON 1981) was used to test the hypothesis that three genotypes provisionally denoted: *CC*, *Cc* and *cc* determines the Compact phenotype. Rejection of the hypothesis would imply that more than one gene is involved. In the first step, maximum likelihood methods were used to obtain the probability on the one gene hypothesis, that an individual having any particular genotype develops a particular Compact category. These probabilities were calculated separately for each sex. In the second step, the maximum likelihood probabilities were used to calculate the expected distribution of Compact categories among the F_2 and the two backcrosses, and these were compared to the observed distribution using a chi-square goodness-of-fit test. To avoid cells with zero or a small number of members, a number of adjacent Compact categories were merged. In particular, for males, categories C1 and C2 were combined and redefined as category rC1; category C3 was redefined as category rC2; and categories C4 and C5 were redefined as category rC3. Merging cells with small expected number of observations is standard procedure when working with chi-square contingency tests and does not bias the analysis. For females, categories C1 and C2 were redefined un-

changed as categories rC1 and rC2, respectively; while categories C3, C4 and C5 were redefined as category rC3.

Under the one gene assumption, three genotypes, provisionally denoted *CC*, *Cc* and *cc*, can be present. A priori, we assume the possibility of incomplete penetrance and variable expressivity. This means that an individual of any of the assumed genotypes (*CC*, *Cc* or *cc*) has some probability of being assigned to any of the three redefined Compact categories, but the probability of being assigned to a specific category will depend on the genotype and sex. On this basis, and assuming Mendelian segregation at the postulated *C* locus and consistent assignment of individuals to Compact categories in the various generations and crosses, we can write the following general expression for the probability, P_{kj}, within each sex separately, that an individual of cross type k falls into redefined Compact phenotypic category rC_j

$$P_{kj} = \sum_i q_{ki} p_{ij}$$

where $k = 1, 2, 3, 4$ for F_1, F_2, BC:HCR and BC:BALB, respectively; $j = 1, 2, 3$ for rC1, rC2, and rC3, respectively; $i = 1, 2, 3$ for genotypes *cc*, *Cc* and *CC*, respectively; p_{ij} is the sex-specific probability that an individual of genotype i falls into redefined Compact phenotypic category j, and q_{ki} is the probability that an individual of cross type k has genotype i. The q_{ki} will depend on the frequency P of the postulated *C* allele in the HCR subline. For reasons detailed in RESULTS, we believe that it is plausible that the later generations of the HCR subline were at fixation for the loci involved in determining Compact phenotype. In this case, the q_{ki} will have the following values: $q_{11} = 0$, $q_{12} = 1$, $q_{13} = 0$; $q_{21} = 1/4$, $q_{22} = 1/2$, $q_{23} = 1/4$; $q_{31} = 0$, $q_{32} = 1/2$, $q_{33} = 1/2$; $q_{41} = 1/2$, $q_{42} = 1/2$, and $q_{43} = 0$.

The p_{ij} were estimated separately for each sex, from the observed distributions of the three redefined Compact categories among the population types by means of a maximum likelihood procedure (MOOD *et al.* 1974). The likelihood function had the form:

$$L = \prod_{k=1}^{4} \prod_{j=1}^{3} (P_{kj})^{N_{kj}}$$

where, N_{kj} is the number of individuals of the kth population type that are classified as redefined Compact phenotypic category j. Note that the probabilities of the three redefined Compact categories for each of the three genotypes must sum to zero. Hence,

$$p_{i3} = 1 - p_{i1} - p_{i2}$$

so that the p_{i3} are not additional unknown parameters. Consequently, the likelihood function consists of only six unknown parameters.

The likelihood function was maximized for each sex separately, using a Simplex search method (DIXON 1972). Using the estimated sex-specific probabilities of the p_{ij}, the expected numbers of the three redefined Compact categories were calculated separately for the four genetic populations within each sex (F_1, F_2, BC:HCR and BC:BALB). A chi-square contingency test was carried out over all of the populations, separately for each sex, to test for goodness-of-fit between expected and observed values. Since six parameters were estimated for each sex, degrees of freedom (d.f.) equal the number of phenotypic categories across the four cross types (=12), less 4 d.f. for cross types and 6 d.f. for estimated parameters. This left 2 d.f. for testing the hypothesis. In the females, however, the rC3 category of the F_1 cross turned out to have zero expected individuals, leaving only 1 d.f. for testing the hypothesis. Similarly, in the males, the rC1 category of the F_1 cross turned out to have only a small number of expected individuals, so that it was pooled with the rC2 category, again leaving only 1 d.f. for testing the hypothesis.

Linkage mapping: Linkage mapping was carried out in three stages. In the first stage, selective DNA pooling (DARVASI and SOLLER 1994) was used with a total genome scan, to obtain approximate indications of the chromosomal location of the locus (or loci) determining the Compact phenotype. In the second stage, individual selective genotyping (DARVASI and SOLLER 1992) was used to obtain more precise intrachromosomal localization of these loci. In the third stage, haplotype analysis was used to localize the locus to a specific marker interval and to determine its approximate location within the interval.

In the selective DNA pooling study, DNA samples of the extreme individuals in the Cross-1 and Cross-2 F_2 populations were pooled separately by crosses, and linkage analysis was based on the relative intensity of alternative allelic bands derived from the two parental lines of the crosses. Because the Compact phenotype is of intermediate dominance in males, but recessive in females, three pools were formed, denoted C1M, C5M and C5F; and consisting of C1 males, C5 males and C4 + C5 females, respectively. In Cross-1, there were 64, 108 and 78 animals in each group, respectively; in Cross-2 there were 100 animals in each group. Linkage between a marker and a locus determining the Compact phenotype was accepted in cases where the allele corresponding to that shown by the HCR or HCI male-parent pool was stronger in the C5M and C5F pools; while the alternative allele, corresponding to that shown by the BALB/c female-parent pool was stronger in the C1M pool. Male parental pools and female parental pools were formed separately for each cross, by collecting the DNA of all parental males and of all parental females, respectively.

In the fine mapping study, selective individual genotyping was carried out using Cross-2 F_2 animals from the C1M, C5M and C5F groups.

Microsatellite markers: A total of 270 microsatellite markers (Research Genetics) were evaluated for ability to distinguish unequivocally between the HCR or HCI and BALB/c parental individuals and for clarity of discrimination between alternative parental alleles in pooled samples. This required that the alternative parental alleles be well separated, with minimum or zero overlapping of shadow bands and main bands. At the end of the marker evaluation process, a total of 56 microsatellites were chosen on the basis of these criteria and also on the basis of map position (Figure 2). The number of microsatellites tested per chromosome varied widely. Twenty-three chromosome *10* microsatellites and 47 chromosome *X* microsatellites were tested to uncover two and three suitable markers, respectively. In contrast, of five chromosome *6* microsatellites that were tested, four were found to be suitable. The low polymorphism rate of chromosome *10* and chromosome *X* markers is consistent with previous reports (DIETRICH *et al.* 1996).

The chosen markers span 96.6% of the mouse genome, with a 20 cM sweep radius. All internal marker intervals were <40 cM, and all terminal marker intervals were <30 cM.

DNA preparation and genotyping: One centimeter from the tail was cut and kept at −70° until DNA preparation (LAIRD *et al.* 1991). After measuring the concentration of the individual samples, a 100 ng/μl concentration dilution was prepared from each sample. A pool was made from equal quantities of the 100 ng/μl dilution of the appropriate samples. For genotyping, C1M, C5M and C5F pools and parental pools were always run on the same gel.

PCR and allele separation: Microsatellite detection was carried out using radioactive label for densitoetry and silver staining for individual genotyping. The same thermocycling proto-

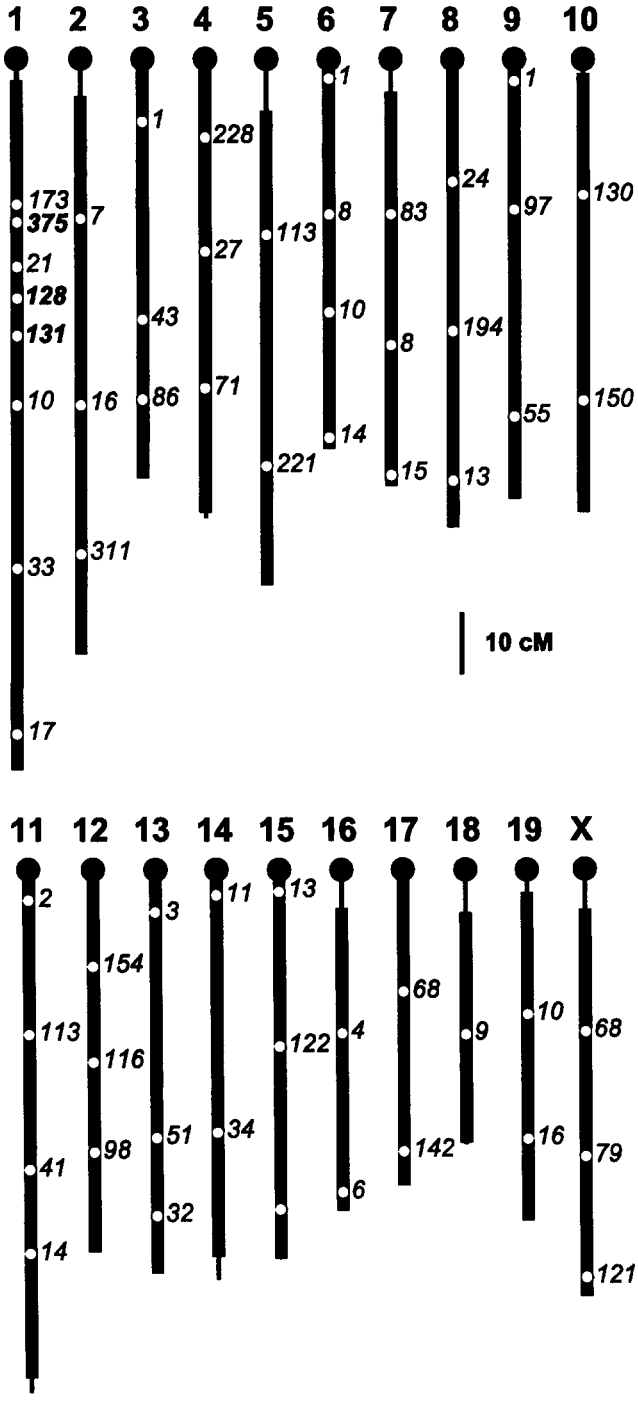

FIGURE 2.—Genome coverage of the 56 selected microsatellites used for selective DNA pooling experiments. Formal locus names have been abbreviated. Thinned lines represent chromosomal regions that are >20 cM from the nearest marker. The microsatellites printed in boldface are those that were added after the initial mapping phase in the *Cmpt* region.

col was used in both cases: initial denaturation at 94° for 3 min, and then 32 cycles at 94° for 1 min, 55° for 1 min, 72° for 1 min and the final elongation step at 72° for 5 min. Reaction volumes were overlaid with light mineral oil (Sigma).

Radioactive method: PCR reaction was carried out in 10 μl volume from 100 ng of pooled DNA samples. Reagent concentrations were 250 nM of each primer; 200 μM of dATP, dGTP and dTTP; 10 μM of cold dCTP; 0.1 μCi/μl alpha-[^{32}P]dCTP; 2 mM $MgCl_2$ and 0.05 units/μl Taq DNA polymerase (Promega). Microsatellite alleles were separated on 6% denaturing polyacrylamide sequencing gel. The gel was exposed overnight to Agfa Curix X-ray film at room temperature for autoradiography. Densitometry was by Soft-Imagine Software (GmBH, Germany).

Silver staining method: Amplifications of 100 ng of each sample were performed in a reduced reaction volume of 5 μl containing 200 μM of each dNTP, 120 nM of each primer and the same concentration of both $MgCl_2$ and Taq DNA polymerase as above. After separation of PCR products on 6% denaturing polyacrylamide sequencing gel, a special plexiglass frame was fixed on the outer glass of the sequencing apparatus, which converted it into a tray, having the outer plate as the bottom and the plexiglass frame as the walls (G. GYAPAY, personal communication). The gel was then silver stained as described elsewhere (BUDOWLE *et al.* 1991).

RESULTS

Preliminary description of Compact phenotype: Carcass percentage values in the Cross-2 F_2 animals were as follows: for males, 40.4 and 49.8% for C1 and C5 animals, respectively; for females, 40.3 and 47.1%, for C1 and C4 animals, respectively. The pooled standard deviation within Compact categories was 2.08%. Thus, both sexes show a marked increase in carcass percentage relative to the normal phenotype. The difference between the C1 and C5 categories was equal to 3.9 phenotypic standard deviations.

In a separate study of several large muscles and bones of C5 males from the HCI subline and BALB/c normal males, the average ratio of summed muscle weight: summed bone weight was 3.79 for the BALB/c animals and 1.61-fold greater (6.10) for the C5 animals. The difference was highly significant and equal to 8.8 within-group phenotypic standard deviations.

The morphometric survey of the histological structure of six C1 and six C5 animals of both sexes did not uncover any significant differences between the two groups. Vascularization, ratio of connective and contractile elements, ratio of the sarcoplasmic reticulum/myofibrils, number of nuclei in the fibers and the diameter of the individual muscle fibers did not differ between the two groups. It therefore appears that the increase of the size and mass of the muscle system in the Compact phenotype is due to an increase in the number of muscle fibers, but to support this conclusion a more detailed histological and morphometric analysis is needed.

Inheritance of the Compact phenotype: Mean Compact phenotype of selected parents and unselected offspring was followed over 16 generations of the HCR subline (data not shown). In the first seven generations an increase in Compact phenotype with generation number was observed in both male and female offspring. From generation 7 or 8, however, there was a clear plateau in male and female mean Compact pheno-

type. In generations 13 to 16, only females of Compact category C4 or C5 were chosen as parents. In these generations, the mean Compact phenotype of the offspring of C4 and C5 parent females were nearly identical (for male offspring, 4.78 and 4.79, respectively; for female offspring, 4.10 and 4.07, respectively). Except for generation 2, there was a slight excess of male offspring. The overall sex ratio at weaning was 1.16:1.00 (males:females), which differs significantly from equality. However, overall fertility of the HCR subline was quite satisfactory, and there are no indications that the homozygosity at the loci determining the Compact phenotype results in lethality or semilethality. Thus, taken in all, the results are consistent with the assumption that, by generation 13, the HCR subline had essentially reached fixation for the genotype responsible for the Compact phenotype.

Figure 3 shows the distribution of the five Compact categories in the later generations of the HCR subline and in the F_1 (HCR × BALB/c), F_2 (F_1 × F_1) and the BC:HCR (F_1 × HCR) and BC:BALB (F_1 × BALB/c) backcross populations, separately for males and females of Cross-1. In all cross types, the proportion of C4 and C5 females was lower than the proportion of C4 and C5 males. This confirms that the Compact phenotype is strongly sex influenced. Modal values were at the C3 category for F_1 and F_2 males but at the C1 category for F_1 and F_2 females. This indicates that the Compact phenotype, when visually scored, is of intermediate dominance in males but almost fully recessive in females. It is possible that a more quantitative measure (*e.g.*, carcass percentage or muscle weight) may have revealed a different dominance pattern. The F_2 distribution is broader than that of the F_1; and the BC:HCR and BC:BALB distributions are skewed to the right and left, respectively, as expected for a trait with variable expressivity under the control of one or more genes having a major effect on trait value. The relative paucity of C5 males and females in the F_2 generation argues for the involvement of more than one gene in determining full expression of the Compact phenotype.

Table 1 presents, for each putative genotype *CC*, *Cc* or *cc*, the maximum likelihood estimates of the probabilities p_{ij} that a particular redefined Compact phenotypic category j is expressed; where i and j are defined as above. Values are given separately for males and females. Estimates were calculated on the assumption that a single Mendelian locus is responsible for the Compact phenotype and that the HCR subline was at fixation for the genotype determining the Compact phenotype, at the time it provided parental males for Cross-1. Also shown in Table 1 are the distribution of redefined Compact categories over generations 13–16 of the HCR subline for comparison to the maximum likelihood estimates for the *CC* genotype. The correspondence is good for males. For females, however, the maximum likelihood estimates for the rC3 category are somewhat less than observed in the HCR subline.

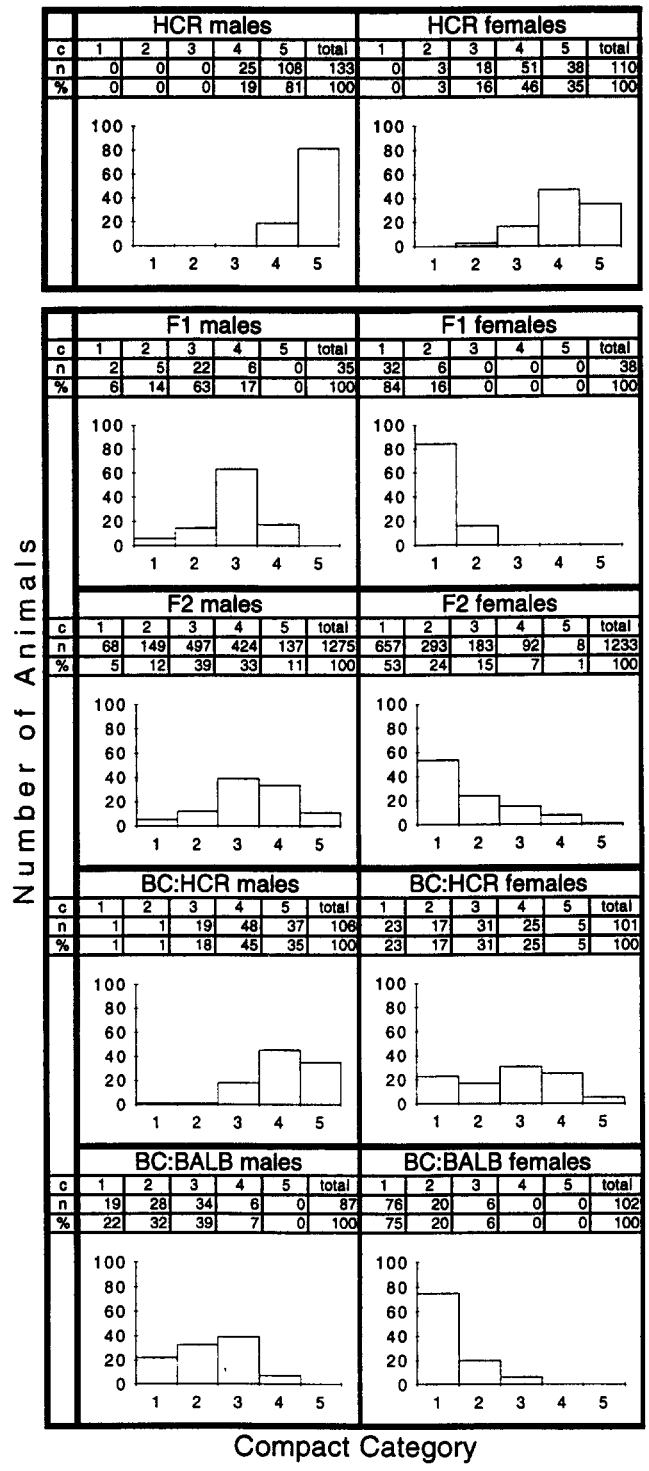

FIGURE 3.—Distribution of Compact phenotypes in HCR subline pooled over generations 13–16, F_1 (HCR × BALB/c), F_2 and backcrosses to HCR (BC:HCR) and BALB/c (BC:BALB) parental lines, separately for males and females. In the histograms shown here, the *x* axis represents the compact category and the *y* axis represents the number of animals. c, Compact categories; n, number of animals; %, frequency distribution of Compact phenotypes.

Using the probabilities of Table 1, expected propor-

TABLE 1

Maximum likelihood estimators of the expected frequencies for each postulated *Compact* genotype at each of the redefined categories for males and females

Compact genotype	Males redefined categories			Females redefined categories		
	rC1	rC2	rC3	rC1	rC2	rC3
CC	0.00	0.00	1.00	0.00	0.12	0.88
Cc	0.08	0.56	0.36	0.70	0.30	0.00
cc	0.59	0.41	0.00	0.73	0.18	0.09
HCR	0.00	0.00	1.00	0.00	0.03	0.97

Observed redefined Compact category frequencies in the HCR subline are also shown.

tions and numbers of the three redefined Compact categories were calculated for each of the four population types, separately for males and females, and compared to the observed values (Table 2). A chi-square value of 33.66 with 1 d.f. was obtained for the males; and a value of 19.73 with 1 d.f. was obtained for the females. Thus, the hypothesis that one gene only is responsible for the Compact phenotype was rejected both for males and females with $P < 0.001$. The significant chi-square values are due to an excess of C4 and C5 animals in the BC:HCR backcross for both males and females and an excess of C1 and C2 animals in the BC:BALB backcross for males (data not shown).

The two backcross populations and the F_2 population were produced and scored for Compact category at the same time, and at any given scoring session the F_2 animals were 85% of the total. Thus, subjective categories were determined primarily by the distribution of category types among the F_2 animals, and there is no reason to expect that they might have differed between the two BC populations. For this reason, we believe it is unlikely that the opposite excesses observed in the two BC populations were due to a change in definition of category boundaries. Consequently, the simplest explanation for these results is the possible presence of a recessive modifier increasing *Compact* expressivity in the HCR subline; although more complex explanations, such as segregation distortion or segregation of background muscularity QTL, are also possible; although we have not found a reference to segregation distortion in the *Cmpt* region. The presence of a modifier is also supported by the very high proportion of C5 phenotypes among males and females of the HCR subline, relative to the proportion of C5 phenotypes observed in the F_2 generation. It is not possible, however, to test specific alternative hypotheses, involving two or more genes, since there are not sufficient degrees of freedom to allow estimates of the additional parameters that such hypotheses would require.

Linkage mapping: *Selective DNA pooling:* Genotyping of the C1M, C5M and C5F pools of Cross-1 revealed clear indications of linkage for a set of linked chromosome *1* markers spanning the region defined distally by *D1Mit173* and proximally by *D1Mit33* (Figure 4). These results were confirmed on the corresponding pools of Cross-2. *D1Mit17*, which is 26.7 cM distal to *D1Mit33*, did not show linkage. Densitometric intensity ratios of the HCR male-parent allele in the C5M and C5F pools and the BALB/c female-parent allele in C1M (data not shown) revealed strong indications of linkage for chromosome *1* markers in the 32.5 cM region between *D1Mit173* and *D1Mit10*. Weaker linkage was detected at *D1Mit33;* this marker is ~50 cM (corresponding to proportion of recombination of 0.30) from *D1Mit21*, which showed tight linkage to *Cmpt* (see later). Examination of additional markers in this region (*D1Mit375*, *D1Mit131* and *D1Mit128*) revealed a high association with the Compact phenotype in the 19.6 cM region between *D1Mit173* and *D1Mit131*. These results were confirmed on pooled DNA samples of 100 C5M, 100 C5F and 100 C1M F_2 individuals taken from Cross-2 (data not shown). The locus in this region associated

TABLE 2

Observed and expected numbers of redefined Compact phenotypes in F_1, F_2 and backcrosses to HCR and BALB/c parental lines

Cross	O/E	Males				Females			
		rC1	rC2	rC3	χ^2	rC1	rC2	rC3	χ^2
F_1	O	(7	22)[a]	6		32	6	0	
	E	(2.8	19.6)[a]	12.6	5.40	26.6	11.4	0	5.21
F_2	O	217	497	561		657	293	283	
	E	239.1	487.7	548.3	2.51	656.6	277.5	299.0	1.72
BC:COMP	O	2	19	85		23	17	61	
	E	4.2	29.7	72.1	7.32	35.4	21.3	44.4	11.42
BC:BALB	O	47	34	6		76	20	6	
	E	29.2	42.2	15.7	18.43	72.9	24.5	4.6	1.38
Total χ^2					33.66*				19.73*

O, observed; E, expected; F_1 backcross, HCR × BALB/c; BC:HRC, HRC backcross; BC:BALB, BALB/c backcross.
*$P < 0.001$.

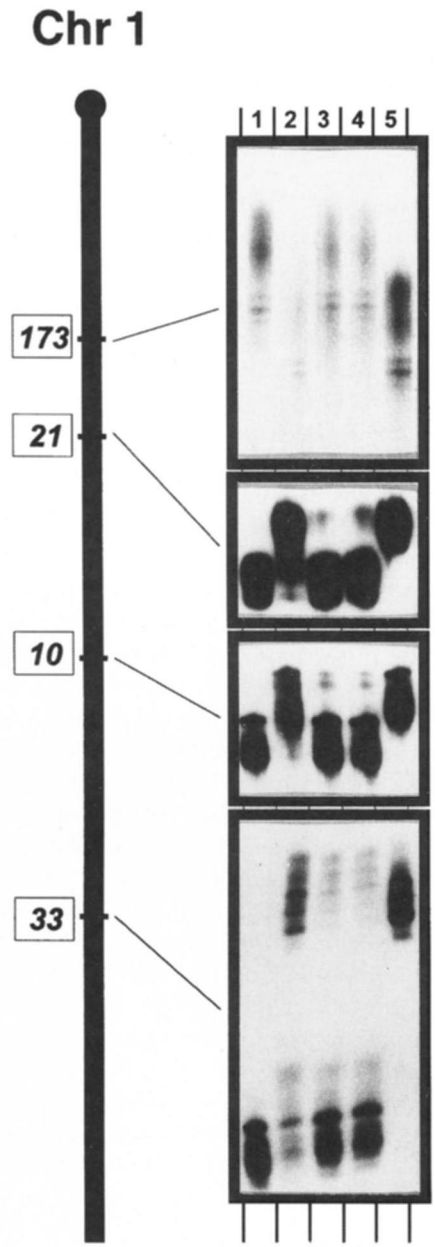

FIGURE 4.—Detection of linkage with four *D1Mit* microsatellites by selective DNA pooling in the Cross-1 F_2 population (formal locus names have been abbreviated). The F_2 pools were always genotyped together with the parental pools, as "pool-sets" in the following arrangement of pools: lane 1, C5 male parents of the F_1; lane 2, C1M selected group; lane 3, C5M selected group; lane 4, C5F selected group; lane 5, BALB/c female parents of the F_1.

with the Compact phenotype will henceforth be termed the *Compact* gene, denoted *Cmpt*.

Genotyping of the Cross-1 and Cross-2 pools for markers on the other chromosomes gave indications of linkage for two other markers: *D3Mit217* on chromosome *3* and *D12Mit63* on chromosome *12*. However, *D3Mit42* and *D3Mit43*, which map 2 cM proximal to *D3Mit217*, did not confirm the linkage. Similarly, *D12Mit109*, *D12Mit172* and *D12Mit154*, which map, respectively, at the same location, 1.2 cM distal and 2 cM proximal to *D12Mit63*, also did not confirm linkage. For this reason, we did not attribute effects on the Compact phenotype to these chromosomal regions.

Fine mapping: To determine the location of *Cmpt* more precisely, selective individual genotyping was carried out on Cross-2 F_2 animals from the C1M, C5M and C5F groups. Genotyping results showed that the C1M group was heavily contaminated with *Cmpt* heterozygotes. Consequently, further work concentrated on the C5M and C5F groups of Cross-2. In all, the C5M group comprised 444 males having individual mean Compact phenotypes of 5.0; the C5F group comprised 511 females having individual mean Compact phenotypes of $\geq$4.0. All animals in these groups were individually genotyped with respect to a battery of four markers spanning the 19.6 cM region between *D1Mit173* and *D1Mit131*. Frequencies in the C5M and C5F groups did not differ appreciably, so that results were pooled over both groups. The pooled frequencies of HCI male-parent alleles were as follows: *D1Mit173*, 0.940; *D1Mit375*, 0.970; *D1Mit21*, 0.948 and *D1Mit131*, 0.905. The two highest frequencies of HCI male-parent alleles were obtained for *D1Mit375* and *D1Mit21*, placing *Cmpt* between these two markers. Elimination of the region distal to *D1Mit131* was confirmed by genotyping 305 C5F individuals for the distal marker *D1Mit10*. The proportion of male parent alleles for *D1Mit131* and *D1Mit10* in this group were 91.0 and 86.2, respectively.

To further explore the location of the *Cmpt* gene, genotypes were constructed for 284 C5F and 370 C5M animals for which complete data on the above four markers was available. Of these, four C5F and 11 C5M animals were found to be heterozygous or homozygous for BALB/c female-parent alleles across the entire region from *D1Mit173* to *D1Mit131*. This indicates that these animals were heterozygous *Cmpt* or homozygous normal. Thus, the proportion of contamination of these genotypic groups by heterozygous or homozygous normals was 0.02. When these animals were eliminated, 280 C5F and 359 C5M genotypes remained. Since the haplotypes of the original parental animals were known, it was possible to decompose these genotypes into recombinant haplotypes, as shown in Figure 5.

Examination of the haplotypes shows clearly that there are haplotypes in which BALB/c alleles are present at both ends of the two marker brackets *D1Mit173* to *D1Mit375* and *D1Mit21* to *D1Mit131*. Hence, *Cmpt* is almost certainly not located in the chromosomal regions bracketed by these two marker pairs. In contrast, there were no haplotypes in which BALB/c alleles were found at both ends of the marker bracket *D1Mit375* to *D1Mit21*. This indicates that *Cmpt* is almost certainly found within the chromosomal region bracketed by these two markers. There were 39 recombinant haplotypes carrying the BALB/c allele at *D1Mit375* and the HCI allele at *D1Mit21* and 66 recombinants having the opposite arrangement. This indicates that the *Compact*

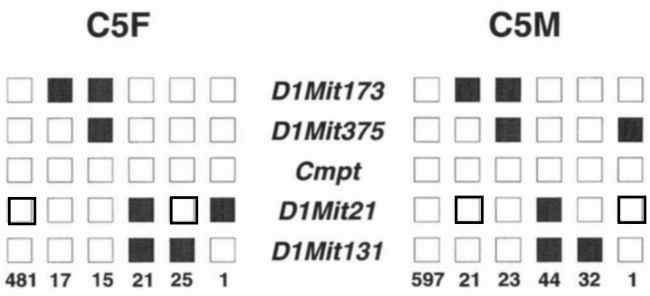

FIGURE 5.—Linkage mapping of the *Cmpt* gene through individual selective genotyping and haplotype analysis in a HCI × BALB/c Cross-2 F_2 population. □, the presence of the HCI male-parent allele; ■, the presence of the BALB/c female-parent allele. At the bottom of each column is the number of F_2 animals inheriting that particular chromosomal haplotype.

gene is closer to *D1Mit375*, approximately one third of the way to *D1Mit21*. Although one or two of these recombinants may represent misclassifications, this cannot change the overall tendency of the data.

Genetic distances (cM), calculated separately for females and males were as follows: Females: *D1Mit173*–3.0–*D1Mit375*–6.6–*D1Mit21*–4.6–*D1Mit131*; Males: *D1Mit173*–3.1–*D1Mit375*–9.5–*D1Mit21*–4.5–*D1Mit131*; Average: *D1Mit173*–3.0–*D1Mit375*–8.2–*D1Mit21*–4.5–*D1Mit131*. Although different in some details, the overall distance across the *D1Mit375*–*D1Mit21* interval corresponds closely to that found in the MIT map (DIETRICH *et al.* 1996).

DISCUSSION

In this study the mode of inheritance of a new hypermuscular mutant in mice, designated "*Compact,*" was characterized. Carcass percentage of the Compact phenotype was 3.9 phenotypic standard deviations greater than that of normals; the ratio of summed muscle weight:summed bone weight was 8.9 phenotypic standard deviations greater than that of normals. Thus, this represents a major mutation affecting body composition. It was shown that a simple monogenic hypothesis did not fully explain the distribution of Compact phenotypic categories among the F_1, F_2 and backcross populations. This implies that at least two genes, possibly a major gene and one or more modifiers, are involved in determining the degree of expression of the Compact phenotype. Nevertheless, the original COMPACT line and the HCR subline have been maintained for a very long period. During this time only individuals showing strong expression of the Compact phenotype were selected as parents. Deleterious pleiotropic effects of the mutation on fertility or litter size were not observed. Thus, it seems reasonable to conclude that the HCR subline, on which the inheritance studies was carried out, is at fixation for the genotype responsible for determining the Compact phenotype. On this assumption, the variety of Compact categories found in the parental line shows that the homozygous *Compact* genotype, although fully penetrant in males and females, is characterized by variable expressivity.

The appearance in a selection line of a major mutant affecting a trait under selection has been documented many times in Drosophila (BATEMAN 1957; CLAYTON and ROBERTSON 1957; HOLLINGDALE 1971; FRANKHAM *et al.* 1978; FRANKHAM 1980; YOO 1980; CABALLERO *et al.* 1991), in rats (CASTLE, cited in MULLER 1914) and in mice (MACARTHUR 1949; ROBERTS and SMITH 1982; BRADFORD and FAMULA 1984). Such mutational events are ordinarily attributed to the occurrence of a random mutation, which is then retained in the population by selection, and whose expression may be enhanced by presence of positive modifiers in the selection line. This may have been the case in the present instance. Alternatively, because of the quantitative nature of the trait and its incomplete dominance and variable expressivity, it is possible that the *Cmpt* allele was present at low frequency in the founder population of the original selection line but was not noticed until brought to sufficient frequency for the appearance of homozygotes.

The specific nature of the "more than one gene" involved in the Compact phenotype is unknown. It is well known, however, that the degree of expression of newly arisen mutations is affected by numerous hitherto unrecognized "modifier" loci (THOMPSON 1975). On this basis, we would speculate that the Compact phenotype derives primarily from a major mutation, which arose *de novo* or was already present in the selection line, and that the selection line was also segregating at one or more modifier loci, which came to phenotypic expression only in conjunction with the major *Compact* mutation. Major mutant and modifiers would all have been brought to fixation in the course of selection for extreme expression of the Compact phenotype in the original COMPACT line and in the HCR subline derived from it. The presence of modifier loci affecting hypermuscularity in double muscle animals is also suggested by anecdotal evidence, namely: present day double-muscled animals of the Belgian Blue Cattle breed are said to show a much more pronounced muscular development than double-muscled animals of 20 years ago (CHARLIER *et al.* 1995). This is attributed to continued selection for increased muscular development in this population after fixation for the *mh* allele. The nature of the presumptive "modifier" gene implied by the genetic analysis is intriguing. It is plausible that these modifier loci are also involved in some manner in normal trait physiology, without in themselves being a source of genetic variation in trait expression. In this case, in the absence of the major mutant, the modifier loci would not be uncovered by linkage or candidate gene analysis. In the presence of the major mutant, however, phenotypic effects of the modifier loci would come to expression, enabling the modifiers to be identified and mapped in turn.

The distribution of the male and female phenotypic categories in the parental line show that the degree of expression of the Compact phenotype is strongly sex influenced, being fully recessive in females and partially recessive in males, and with a higher expressivity in males than in females, even in homozygous *Cmpt* animals. In this the Compact phenotype differs somewhat from the other known major genes affecting muscularity and body composition. In its genetic behavior *Cmpt* generally resembles *mh*, except the *mh* is partially recessive (but closer to +/+ animals) in both sexes. *Cmpt* also resembles *mh* in that the muscular hypertrophy is primarily a histological hyperplasia (HANSET *et al.* 1982). In contrast to the *hg* gene of mice (BRADFORD and FAMULA 1984), *Cmpt* does not appear to have adverse effects on overall fitness or fertility.

The results of this study provide strong support for the efficacy of selective DNA pooling (DARVASI and SOLLER 1994; TAYLOR and PHILLIPS 1996) as an efficient means for rapid total genome scanning and for the efficacy of individual selective genotyping (DARVASI and SOLLER 1992) as an efficient means of fine mapping. The locus designated *Cmpt* in the chromosome *1* interval bracketed by *D1Mit375* and *D1Mit21* obviously has a powerful effect on the Compact phenotype. Despite the fact that the genetic analysis suggested the presence of additional genes affecting the Compact phenotype, we did not uncover any other chromosomal regions unequivocally associated with the Compact trait. This is in accord with the hypothesis that the other loci involved are modifier gene(s). These would be expected to have much smaller effects than the major gene and hence might not have been amenable to detection using the selective DNA pooling technique at the marker spacing used.

Mylf (myosin light chain fast skeletal muscle) locus (ROBERT *et al.* 1984) and its enhancer (DONOGHUE *et al.* 1988) located 24 kilobases downstream are found in the general chromosomal region to which *Cmpt* was mapped and hence were initially considered as plausible candidate genes. However, since *Cmpt* was mapped by individual genotyping to a location 4–6 cM centromerically from *D1Mit21*, which itself is a microsatellite derived from *Mylf* (DIETRICH *et al.* 1994), this eliminated the gene and its enhancer as candidates.

The mouse chromosomal region containing the *Cmpt* gene belongs to an extensive region of mouse chromosome *1*, which has syntenic homology to the 2q32-qter region in humans (NAYLOR 1996). We note that the *mh* gene of cattle has also been mapped to a region of bovine chromosome *2*, which has syntenic homology with the human 2q22–2q37 region (CHARLIER *et al.* 1995; SOLINAS-TOLDO *et al.* 1995). This raises the possibility that *Cmpt* might be homologous to the *mh* gene of cattle.

It is hoped that the studies reported here will eventually lead to positional cloning of the locus involved. This will require high resolution genetic and physical mapping of *Cmpt* (DARVASI and SOLLER 1995; DARVASI 1997). Once fine mapping leads to a potential candidate gene or genes, the question of gene verification will become acute; in particular the problem of distinguishing functional candidate molecular variation from neutral candidate polymorphisms distinguishing the original parental strains. In addition to its basic biological interest, *Cmpt* is of particular interest from a pharmaceutical and agricultural point of view, since it represents a gene having powerful effects on body composition. Once cloned, the *Cmpt* could serve to identify and clone homologous genes in other vertebrate species. Such genes are of interest as potential candidate genes for segregating QTL determining normal genetic variation in body composition in man and domestic animals. They may also be attractive candidates for manipulation of carcass composition through transgenic methodologies and may provide leads for pharmaceutical intervention in body composition.

We thank T. SÁGHY for his help with phenotypic classification and L. SZABÓ for computer analysis of DNA pools. We are grateful to Z. VARGA and F. OLASZ for helpful discussions as well as to L. OROSZ for support. We also thank K. SÓVÁRI for her skillful technical assistance. This research was supported by grant no. T5432 from Hungarian National Scientific Foundation (OTKA).

LITERATURE CITED

BATEMAN, G., 1957 Ph.D. Thesis, pp. 178–182 in *The Strategy of the Genes*, edited by C. H. WADDINGTON. George Allen and Unwin, Edinburgh University, London.

BOWEN, R. A., M. L. REED, A. SCHNIEKE, G. E. SEIDEL, A. STACEY *et al.*, 1994 Transgenic cattle resulting from biopsied embryos: expression of c-ski in a transgenic calf. Biol. Reprod. **50:** 664–668.

BRADFORD, G. E., and T. R. FAMULA, 1984 Evidence for a major gene for rapid postweaning growth in mice. Genet. Res. **44:** 293–208.

BUDOWLE, B., R. CHAKRABORTY, A. M. GIUSTI, A. J. EISENBERG and R. C. ALLEN, 1991 Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am. J. Hum. Genet. **48:** 137–144.

CABALLERO, A., M. A. TORO and C. LOPEZ-FANJUL, 1991 The response to artificial selection from new mutations in Drosophila melanogaster. Genetics **127:** 89–102.

CASTLE, W. E., 1914 Pure lines and selection. American Breeders' Magazine, 1914 Cited in MULLER, H. J., 1914 The bearing of the selection experiments of Castle and Phillips on the variability of venes. Am. Nat. **48:** 567–576.

CHARLIER, C., W. COPPIETERS, F. FARNIR, L. GROBET, P. L. LEROY, *et al.*, 1995 The mh gene causing double-muscling in cattle maps to bovine chromosome 2. Mamm. Genome **6:** 788–792.

CLAYTON, G. A., and A. ROBERTSON, 1957 An experimental check on quantitative genetical theory. II. The long-term responses to selection. J. Genet. **55:** 152–170.

COCKETT, N. E., S. P. JACKSON, T. L. SHAY, D. NIELSEN, A. MOORE, *et al.*, 1994 Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. Proc. Natl. Acad. Sci. USA **91:** 3019–3023.

DARVASI, A., 1997 Interval specific congenic strains (ISCS): an experimental design for mapping a QTL into a one centimorgan interval. Mamm. Genome **8:** 163–167.

DARVASI, A., and M. SOLLER, 1992 Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theor. Appl. Genet. **85:** 353–359.

DARVASI, A., and M. SOLLER, 1994 Selective DNA pooling for deter-

mination of linkage between a molecular marker and a quantitative trait locus. Genetics **138:** 1365–1373.

DARVASI, A., and M. SOLLER, 1995 Advanced intercross lines, an experimental population for fine genetic mapping. Genetics **141:** 1199–1207.

DIETRICH, W. F., J. C. MILLER, R. G. STEEN, M. MERCHANT, D. DAMRON-BOLES *et al.*, 1994 A genetic map of the mouse with 4,006 simple sequence length polymorphisms. Nat. Genet. **7:** 220–245.

DIETRICH, W. F., J. C. MILLER, R. G. STEEN, M. MERCHANT, D. DAMRON-BOLES *et al.*, 1996 A comprehensive genetic map of the mouse genome. Nature **380:** 149–152.

DIXON, L. C., 1972 *Nonlinear Optimization.* The English University Press, London.

DONOGHUE, M., H. ERNST, B. WENTWORTH, B. NADAL-GINARD and N. ROSENTHAL, 1988 A muscle-specific enhancer is located at the 3′ end of the myosin light-chain 1/3 gene locus. Genes Dev. **2:** 1779–1790.

ELSTON, R.C., 1981 Testing one- and two-locus hypotheses for the genetic difference of a quantitative trait between two homozygous lines, pp. 283–293 in *Genetic Research Strategies for Psychology and Psychiatry*, edited by E. S. GERSHON, S. MATTHYSSE, X. O. BREAKEFIELD and R. D. CIARANELLO. The Boxwood Press, Pacific Grove, CA.

FRANKHAM, R., 1980 Origin of genetic variation in selection lines, pp. 56–68 in *Selection Experiments in Laboratory and Domestic Animals.* Commonwealth Agricultural Bureaus, Slough, UK.

FRANKHAM, R., D. A. BRISCOE and R. K. NURTHEN, 1978 Unequal crossing over at the rRNA locus as a source of quantitative genetic variation. Nature **272:** 80–81.

GEORGES, M., 1990 A reverse genetic approach towards the bovine "Muscular Hypertrophy" gene based on the use of DNA fingerprints, pp. 227–233 in *Genome Analysis in Domestic Animals*, edited by H. GELDERMAN and F. ELLENDORFF. VCH. Verlagsgesellschaft mbH. Winheim, Germany.

HANSET, R., C. MICHAUX, C. DESSY-DOIZE and G. BURTONBOY, 1982 Studies on the 7th rib in the double muscled and conventional cattle, pp. 341–349 in *Muscle Hypertrophy of Genetic Origin and Its Use to Improve Beef Production,* edited by J. W. B. KING, and F. MÉNISSIER. Martinus Nijhoff, The Hague.

HOLLINGDALE, B., 1971 Analyses of some genes from abdominal bristle number selection lines in Drosophila melanogaster. Theor. Appl. Genet. **41:** 292–301.

LAIRD, P. W., A. ZIJDERVELD, K. LINDERS, M. A. RUDNICKI, R. JAENISCH *et al.*, 1991 Simplified mammalian DNA isolation procedure. Nucleic Acids Res. **19:** 4293.

MACARTHUR, J. W., 1949 Selection for small and large body size in the house mouse. Genetics **34:** 194–209.

MCPHERRON, A. C., A. M. LAWLER and S.-J. LEE, 1997 Regulation of sceletal muscle mass in mice by a new TGF-β superfamily member. Nature **387:** 83–90.

MOOD, A. M., F. A. GRAYBILL and D. C. BOES, 1974 *Introduction to the Theory of Statistics.* McGraw-Hill Book Co., New York.

NAYLOR, S. L., 1996 Comparison of human chromosome 2 to the mouse map. Cytogenet. Cell Genet. **73:** 271–272.

PURSEL, V. G., P. SUTRAVE, R. J. WAII, A. M. KELLY and S. H. HUGHES, 1992 Transfer of c-ski gene into swine to enhance muscle development. Theriogenology **37:** 278.

ROBERT, B., P. DAUBAS, M. A. AKIMENKO, A. COHEN, I. GARNER *et al.*, 1984 A single locus in the mouse encodes both myosin light chains 1 and 3, a second locus corresponds to a related pseudogene. Cell **39:** 129–140.

ROBERTS, R. C., and C. SMITH, 1982 Genes with large effects. Theoretical aspects in livestock breeding. pp. 420–438 in *Second World Congress on Genetics Applied to Livestock Production,* Vol. 6, Editorial Garsi, Madrid.

SOLINAS-TOLDO, S., C. LENGAUER and R. FRIES, 1995 Comparative genome map of human and cattle. Genomics **27:** 489–496.

SHAPIRO, J. A., 1995 Adaptive mutation. Who's really in the garden? Science **268:** 373–374.

SUTRAVE, P., A. M. KELLY and S. H. HUGHES, 1990 ski can cause selective growth of skeletal muscle in transgenic mice. Genes Dev. **4:** 1462–1472.

TAYLOR, B. A., and S. J. PHILLIPS, 1996 Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics **34:** 389–398.

THOMPSON. J. N., 1975 Quantitative variation and gene number. Nature **258:** 665–668.

YOO, B. H., 1980 Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster.* II. Lethals and visible mutants with large effects. Genet. Res. **35:** 19–31.

Communicating editor: P. D. KEIGHTLEY