SELECTIVE REDUCTION OF GENETIC LETHALITY [1]

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In mice the dominant gene *Cd* produces phenotypically crooked-tailed individuals (1). Although normal-tailed mice were excluded from stock matings for several generations, no true-breeding lines of mice homozygous for the Crooked gene could be established in the parental albino strain (2). Subsequent mating of the heterozygous mutants to unrelated stocks produced lines in which the lethality of *Cd* was greatly reduced (3).

In the parental strain approximately two-thirds of the *CdCd* embryos died prior to parturition; only two of the survivors produced young (2). Outcrossing of the heterozygotes to unrelated stocks produced a pigmented line in which the degree of tail-crooking was reduced. This suggested that continual selection, within the hybrid line, might produce a genetic milieu in which the homozygotes would survive. The purpose of this paper is to report some of the results of this selection program.

MATERIALS AND METHODS

Seven of the labeled chromosomes in the mouse were tested against *Cd* in an attempt to locate that mutation. No special linkage-test stock, which carried the requisite marker genes, was available. Crooked stock which was used in the linkage tests was at F_5. Unrelated stocks which were used to provide test animals, because they carried known genetic markers, were not of a homogenous background. The proportions of mice which were classified as Crooked in the first outcross are recorded in Table I. Percentages in the last column of this table make it obvious that all of the stocks with which the Crookeds were hybridized contained dominant modifiers which decreased expressively of tail abnormalities.

The identification of proven overlaps in the albino stocks (2) and the presence of dominant modifiers in unrelated stocks (Table I) suggested the following hypothesis. If there are newly introduced modifiers in the foreign stocks which decrease the single identifiable abnormality of the heterozygotes (specifically to suppress the expression of crooked tails), then perhaps these same modifiers, or possibly even other hidden modifiers, could reduce the severity of the deleterious effects of the Crooked mutation in the homozygotes.

[1]This research was conducted while the author was a Research Associate at Columbia University at the Nevis Biological Station located at Irvington on Hudson, New York.

TABLE I

PHENOTYPIC EXPRESSION OF CROOKED-TAIL RESULTING FROM MATINGS OF ALBINO INBRED Cd+ WITH UNRELATED NORMAL-TAILED ++ MICE

Genetic marker	Chromosome location	F_1 progeny normal	crooked	% crooked
color	I	238	108	31.2
short-ear	II	165	46	21.8
black & tan	V	150	65	30.2
caracul	VI	176	78	30.7
black	VIII	167	60	26.4
ruby eye	XII	142	76	34.9
		1038	433	29.4

Only two of 65 observed "smalls" *(CdCd)* in the parental inbred stock sired progeny when they were continuously mated with attractive mature females; no "small" female bore young.

The original hybrids selected were those which were particularly prolific. Animals which were used in producing linkage data from chromosome VIII were employed. Brown, belt, pink-eye, normal-tailed *(bb btbt pp ++)* female No. 393 was mated to an inbred albino male which was heterozygous for Crooked. In the second and third generation following this mating three brown, pink-eyed males homozygous for *Cd* were reared. All had abnormal tail-fur and microphthalmia, as did the inbred "small" mice (2) but, unlike the inbred "smalls," they had erupted lower incisors, grew well, and produced offspring. Three pigmented F_2 descendants from one of these males had a marked deficiency of iris pigmentation (approx. 30% of normal) and proved to be *CdCd*.

One of the males, No. 2453, when mated to a black normal-tailed female, produced two litters with eleven normal-tailed offspring and no abnormals. Selection through this narrow bottleneck for viable homozygotes was indicated. Five of the normals which were saved from this pair-mating proved to be genetically heterozygous for *Cd*. The other seven were males and were skeletonized. By using the alizarin-red technique, as previously described (2) and examining individual vertebrae, it was ascertained that these seven males were also heterozygous for *Cd*. Further tests of presumed homozygotes proved their genetic identity (Table II) and also proved that

viability and reproductivity were greatly improved in the hybrids. Early classifications were least reliable; breeding classifications were best. Disregarding the questionables, and progeny from 2782 which had only one early classification, the percentages of mutants were 52 for "at birth," 54 for "at weaning," and 100 for those classified by breeding tests. Therefore, all of the adequately tested progeny from the males which were tentatively classified as phenotypic homozygotes proved to be heterozygous for *Cd*.

TABLE II

TAIL-TYPE CLASSIFICATIONS OF PROGENY FROM CROOKED-TAILED HYBRID MALES (WHICH WERE PHENOTYPICALLY CLASSIFIED AS HOMOZYGOTES) MATED TO ++, NORMAL-TAILED FEMALES

			Classification								
			At birth			At weaning			Breeding		
Sires	Litters	Progeny	*Cd	nt	?	Cd	nt	?	Cd	nt	No test
2453	4	24	9	13	2	10	14	0	15	0	9
2674	3	21	13	8	0	13	8	0	17	0	4
2676	4	20	11	9	0	12	8	0	17	0	3
2782	2	12	7	3	2	....	....	....	....	....	....
Totals	13	77	40	33	4	35	30	0	49	0	16

* Cd is crooked-tail; nt is normal-tail.

In the parental albino stock, phenotypically crooked-tailed mice could normally be identified at birth. However, unless the newborn mice were exencephalic or acraniate, the *CdCd* individuals could not be identified. When F_2 and F_3 progeny were born to the hybrid stock, it was realized that pigmentation of the iris could serve as a criterion for the immediate identity of homozygotes at birth. In Table III is recorded the appearance of the irides of newborn mice from three different mating-types.

Within the newly formed hybrid line, female and male homozygotes were both capable of breeding. However, amongst the segregating color genes were the pink-eye gene and the gene for albinism. Consequently, there were three genes which produced reduction or absence of pigmentation in homozygotes. "At birth" classification of mice from *CdCd* X *CdCd* are therefore not included in Table III.

TABLE III

INCIDENCE OF REDUCED IRIS PIGMENTATION IN NEWBORN MICE FROM MATING-TYPES INVOLVING GENE Cd

Mating-type	Normal eyes	Abnormal eyes	% normal
Cd + X *Cd Cd*	21	16	43.2
Cd + X *Cd* +	46	13	22.0
++ X *Cd Cd*	70	0	0.0

Three litters from this mating-type produced nine mice with no observed pigmentation, four with reduced pigmentation, and four with grossly normal pigmentation, as classified by the appearance of the eye-ring.

DISCUSSION

In a series of matings which involved successive backcrosses, Green (4) was able to lessen the influence of the Brachyury gene, which is another dominant tail mutation. The principal observable manifestations of that gene were (unlike *Cd*) to shorten the tail and (like *Cd*) to cause irregular tail crooks. When outcrossed to an unrelated inbred strain, the number of tail vertebrae was nearly doubled in the brachyuric F_1 hybrids. Thus, dominant modifiers reduced the severe action of that mutation just as dominant modifiers reduced the action of mutation *Cd*. However, in that study no mention was made of the influence of the dominant modifiers upon the lethality of Brachyury homozygotes.

Obviously the ultimate goal of producing viable, productive homozygotes cannot be accomplished by indiscrimately selecting against defects. From a hypothetical standpoint it might be possible to select for dominant modifiers which would mask all mutant deformities. Similar effects, which increased or decreased the expression of mutation *Tailshort* depending upon the source of the unrelated inbred stocks, have previously been described (5). The end result could effect a change which would be the opposite of that which has been proposed by Fisher (6) but argued against by Wright (7, 8) as it relates to the origin of dominance. The value of *Cd* would be greatly reduced if the mutants were not phenotypically identifiable. Indeed, if the phenotypic expression, or expressions, were only detectable in the homozygotes, it would be classified as a recessive rather than a dominant mutation. Line 2159 (9) provided a normal-tailed female source, with a minimum of dominant modifiers, for the testing of potential *CdCd* males.

Many of the related abnormalities described for the *CdCd* mice of the inbred albino strain have been observed in hybrid *CdCd* mice. As previously described, the hybrid homozygotes were not characteristically "small" (as were the inbred albinos) because their lower incisors erupted and grew apparently normal. It has been reported that a growth hormone from the pituitary gland and thyroxin from the thyroid were responsible for tooth eruption and growth (10). No assays were conducted to attempt to detect hormonal differences between the inbred albino and the hybrid *CdCd* mice. Improved dentition provided a means for eating the pelleted ration and malnutrition did not result. The extra work entailed in pulverizing the pellets so that the powdered diet could be licked-up, and the cutting of the curved upper incisors, both of which were necessary for the albino inbred "smalls," was no longer necessary with the hybrids.

One of the best criteria for identifying *CdCd* mice was to observe the tail fur. Fraser (11) has described in detail the growth of hair in mice. Actually, both qualitative and quantitative studies can be made. The hairs on the tail of *CdCd* mice were reduced in number, rather than being of an altered type. As the tail was passed between the thumb and the index finger, from tail-tip to tail-base, a rough irregular contour was evident. Some of the tail hairs were relatively stubby. As described by Fraser, coat patterns of this type are deficient in guard-hairs. Another observation was that the tail crooks of *CdCd* mice were sharper and more frequent than for *Cd* + mice. However, abnormal tail fur remained the most reliable criterion for correct classification of homozygotes. It has also been reported (12) that domesticated albino mice have less fur (approximately 46 hairs per unit square) than do wild mice (approximately 70 hairs per unit square).

Frequently, the mutant homozygotes had defective eyes (microphthalmia or anophthalmia) and occasionally had inclined ears. The relationship of abnormalities produced in *CdCd* mice from the inbred albinos to similar human abnormalities has been described (13). The relationship of ear and eye abnormalities has been further discussed by Goldenhar (14). Hence, it is evident that the terminal effects of two *Cd* genes are operative in the hybrids as well as in the original inbred line.

SUMMARY

A monofactorial dominant gene, which produced a lethal effect on most of the homozygotes in the parental inbred stock, has had its severity of expression greatly reduced by introducing modifiers from an unrelated stock. In the hybrid, there continued to be more

abnormalities in the homozygotes than in the heteroygotes, but the *CdCd* mice grew well and bred satisfactorily. It has been demonstrated that beneficial selection pressure could be applied to hybrid stock bearing a semi-lethal mutation. This selection resulted in altering the homozygote from: (1) a sterile, dwarf individual, of which less than 30% survived to birth in the parental albino stock to (2) a fertile, normal-growing, and normal-sized individual with little, if any, apparent mutant induced prenatal mortality.

All pigmented mice from the hybrid stock which had reduced pigmentation of the iris, proved to be *CdCd*. However, some of the *CdCd* mice did not show this pigmentation reduction. Prior to sexual maturity and subsequent breeding tests, the most valid criterion for identifying *CdCd* mice was by observing the tail fur. Homozygotes had irregular and stubby hairs which could best be identified by strokes directed from the tail tip to the tail base.

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