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'*Midget*', a new dwarfing gene in the house mouse dependant on a genetic background of small body size for its expression

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INTRODUCTION

During the course of selection for large and small body size in mice of strain *N*, Falconer (1953, 1955) found that the phenotypic variability in the small line, as measured by the coefficient of variation, was doubled between generations seven and nine. He suggested that the increased variance was due to a mutation at a single locus causing a large reduction in body size, a dwarfing gene (*pygmy*) having previously been found in MacArthur's (1944*a*, *b*) small selected line (King, 1950). But the existence of a mutant gene could not be verified experimentally (Falconer, 1953, 1955). After twenty-two generations of selection, the offspring of some matings in the small line were again observed to be noticeably smaller than their litter-mates. Some of these smaller mice were obtained for study, and the present paper describes aspects of their genetics and physiology. The mice were named '*midget*'.

OBSERVATIONS

Description

Midget mice were found in the small line (*NS*) of Falconer's strain *N*. They could first be distinguished from their normal *NS* litter-mates between 12 and 14 days of age by an abnormally short and rounded head, wide-set eyes, and ears which were more horizontal than normal. These characteristics which persisted into adult life (Plate 1) made them easily distinguishable from any 'runts' in the litter. A considerable variation was found between matings in the severity of the expression of the mutant, though the characteristic phenotype was unlike that of *pituitary dwarf* (Snell, 1929) or *pygmy* (King, 1950).

Genetics

When phenotypic variability increased in line *NS* between the seventh and the ninth generations of selection, attempts to establish the existence of a single

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Fig. 1. A *midget* male.

Fig. 2. An *NS* male.

Fig. 3. A *midget* male (bottom) and a normal *NS* male litter-mate at 6 weeks of age.

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mutant gene were made by backcrossing the smaller *NS* mice to those of the line selected for large body size (*NL*) (Falconer, unpublished). However, no noticeably smaller mice were found among the backcross progeny, or among intercrosses between the progeny. Moreover, the decrease in body size in line *NS* was not due to a heterozygous effect, for back selection only reduced the variance in body weight half-way towards the original level (Falconer, 1955). The retarded growth of *midget* mice in generation 22 may have been due to the same gene(s) that had increased the variance in body size in earlier generations.

Table 1. *The segregation of* midget *and normal offspring in crosses within line* NS

	Phenotype of offspring		
Type of cross	*Midget*	Normal	Total
Midget × *midget*	96	0	96
Backcrosses	70	77	147
Heterozygous *NS* matings	49	145	194

A stock of *midget* mice was isolated for further study by setting up full sib pairs. All of these matings produced only *midget* offspring (Table 1), the proportion of males to females being 42:54. Tests for a single gene segregation were accordingly made by crossing *midgets* with normal *NS* mice, and then backcrossing the normal female offspring to *midget* males. Results are shown in Table 1. The ratio *midget*:normal progeny was very close to the expected 1:1 ratio. No significant

Table 2. *The segregation of* midget *and normal offspring in backcrosses to lines* MS *and* CS

	Phenotype of offspring			
Type of backcross	*Midget*	Normal	Total	Post-natal mortality
1. *MS*				
Series 1	24	75	99	2
Series 2	54	93	147	10
Total	78	168	246	12
2. *CS*	6	36	42	3

Each backcross ratio differed significantly from the expected 1:1 ratio ($P < 0{\cdot}01$).

heterogeneity was detected between matings in the ratio of *midget*:normal offspring. In a further experiment, the offspring were classified from six normal *NS* pairs which were giving *midget* and normal offspring. These *NS* mice, which were presumably heterozygous for *midget*, gave *midget*:normal offspring in a ratio close to the expected 1:3 (Table 1). The *midget* phenotype thus appeared to be due to a single gene.

The backcrosses were repeated using two other unrelated small strains. The two strains were also the result of selection: MacArthur's (1944*a*, *b*) small line *MS*, and another of Falconer's: line *CS* (Falconer, 1960). The ratio of *midget*:normal offspring in these backcrosses is given in Table 2, the two series of backcrosses to

line *MS* being made at an interval of approximately one year. Classification of many of the *midget* offspring was much more difficult in these backcrosses than in those to *NS* mice, the phenotype appearing to be much less extreme. The ratio of *midget*: normal offspring was significantly lower than the expected 1:1 (Table 2). The lack of *midgets* could not be attributed to foetal mortality before birth, for the mean litter size of the heterozygous females was similar whether mated to *MS* or *midget* males (5·5 and 6·2 respectively, no significant difference between means). Moreover, death of offspring after birth was insufficient to account for the lack of *midgets* (Table 2). More *midgets* occurred in the second than in the first series of *MS* backcrosses, although the ratios in the two series did not differ significantly ($F^1_{13} = 3{\cdot}5$, $P > 0{\cdot}05$). The above results indicated that the *midget* phenotype was a product of a single gene difference in the *NS* background only, and perhaps in a few of the *MS* matings in which certain modifying factors (genes ?) were present.

Five pairs of *midgets* from the first *MS* backcrosses produced more than 100 offspring, all of which were *midget*.

Growth of midgets

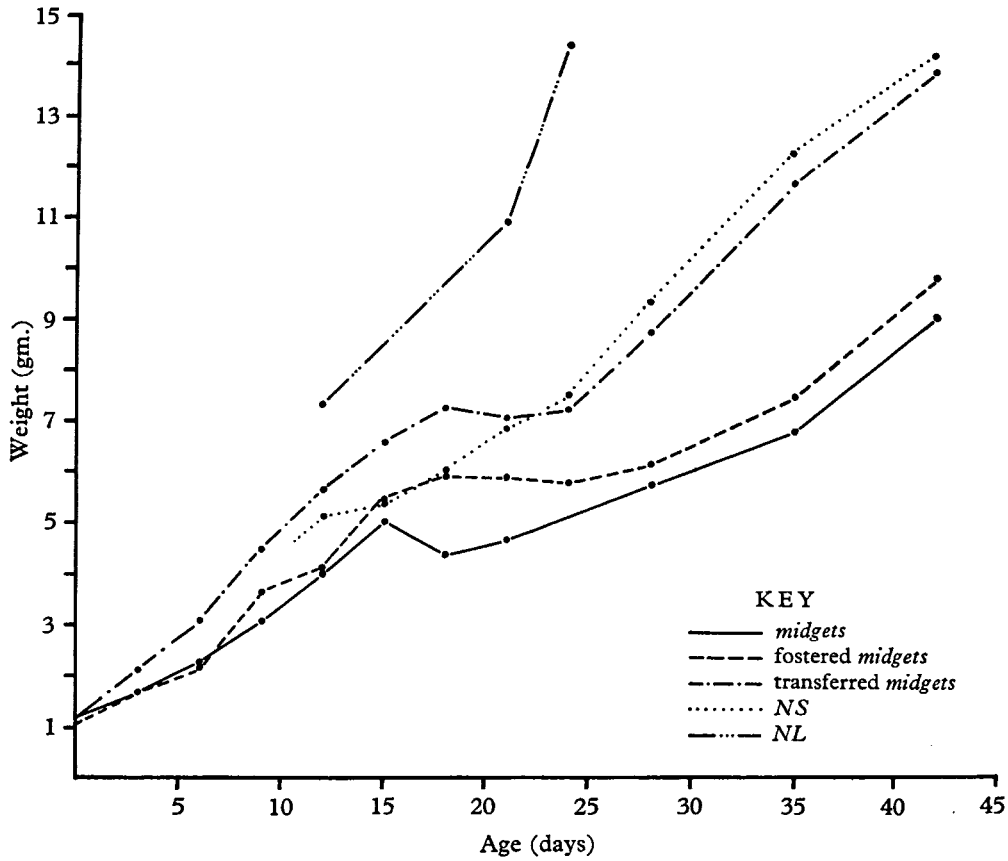
The growth-rates of *midgets* from homozygous matings are given in Table 3 and Text-fig. 1, corresponding data on the growth-rates of normal *NS* males being given for comparison. The mean body weights of the non-fostered *midgets* are probably overestimates, and those of the fostered *midgets* underestimates,

Table 3. *Mean body weights of* midget *mice between birth and* 84 *days of age*

Age (days)	1. Not fostered		2. Fostered	
	Males	Females	Males	Females
Birth	1·2	1·0	1·1	1·0
6	2·3	2·6	2·2	2·1
12	4·0 ± 0·3	4·1 ± 0·2	4·6 ± 0·2	4·1 ± 0·3
15	4·6	4·8	5·5	4·9
18	4·4	4·8	5·9	5·0
21	4·6 ± 0·2	4·7 ± 0·2	5·9 ± 0·2	5·0 ± 0·2
24	—	5·3	5·8	5·3
28	5·7	5·9	6·1	5·5
35	—	7·5	7·4	6·4
42	9·0 ± 0·7	8·4 ± 0·4	9·8 ± 0·4	7·2 ± 0·5
84	13·0	11·6	—	—

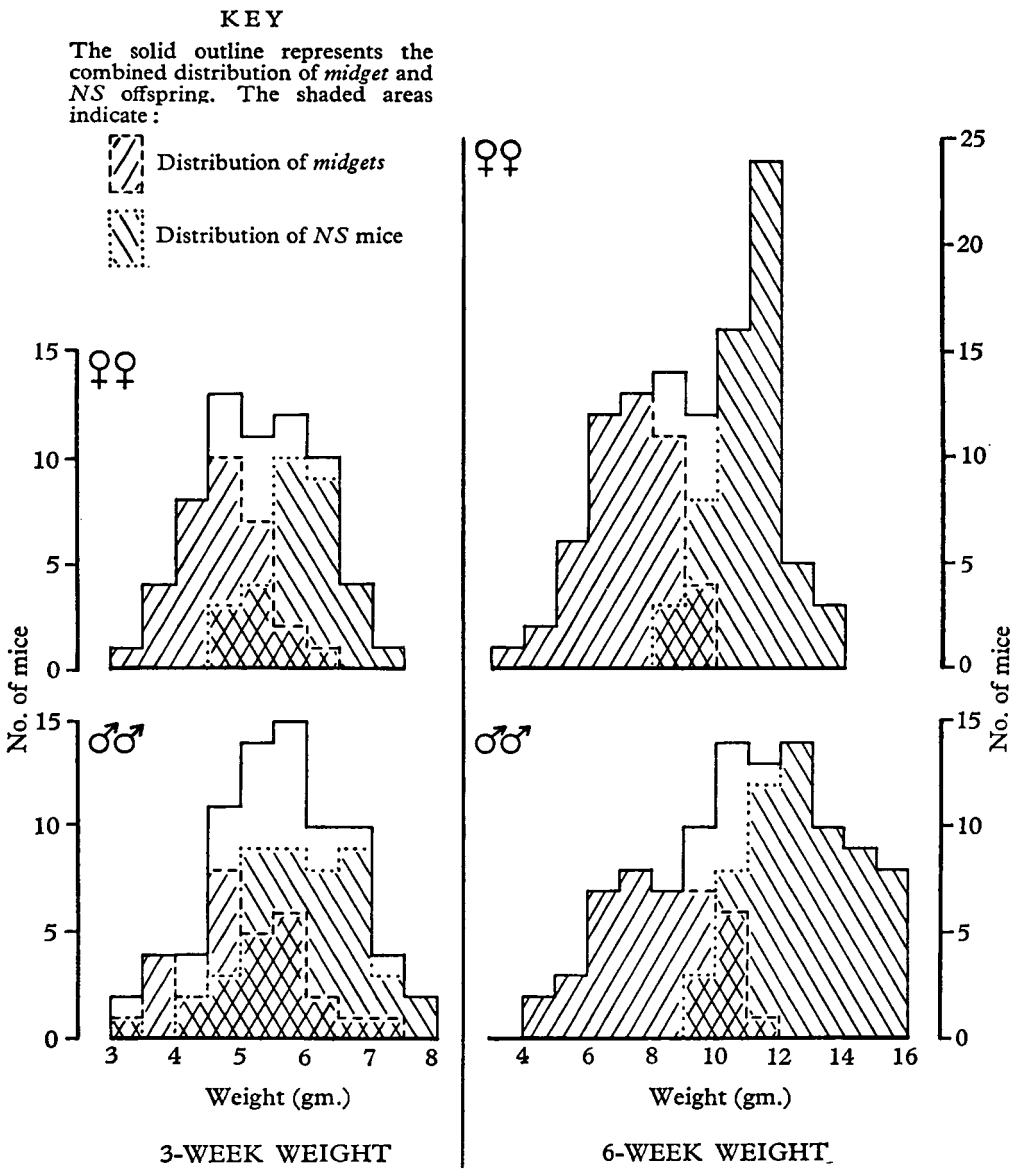
because the smallest *midget* mothers were poor sucklers and their offspring had to be fostered. *Midget* mice were smaller than *NS* mice at all stages of growth, and seldom exceeded 10 g. in weight at 6 weeks of age. Their growth-rate was especially retarded between 15 and 21–24 days of age, when many of them actually lost weight. Due to their small size, weaning of many *midgets* had to be delayed from 3 until 5–6 weeks of age.

A detailed comparison was made between the 3- and 6-week weights of midgets and their normal litter-mates in the *NS* backcrosses and heterozygous matings. In these matings and backcrosses, the *midgets* were competing for milk with their *NS* litter-mates, and were weaned at 3 weeks of age. In litters containing both *midgets* and normal *NS* mice, a mean weight was obtained for each type, the difference between these means was found for each litter, and the mean difference over all litters was then calculated. Data on the two sexes were analysed separately.



Text-fig. 1. The growth curves of *Midget* and *NS* males between birth and 42 days of age.

Results are given in Table 4. The difference in weight between *midgets* and *NS* mice was more marked at 6 weeks than at 3 weeks. Despite the considerable difference in body weights, however, a clear bimodal distribution in 3- or 6-week weight was not apparent when the frequency distribution of the weights of individual mice was plotted, due to an overlap in the distribution of the two types (Text-fig. 2). Classification of *midgets* on weight alone was obviously difficult, which could explain why Falconer (unpublished), in earlier generations, was unable to separate *midgets* from normal litter-mates in the absence of morphological differences.



Text-fig. 2. Frequency distribution of the 3- and 6-week weights of offspring of *midget-NS* backcrosses and heterozygous matings.

Data were taken from litters containing *midgets* and normal *NS* mice, the numbers of animals contributing to the data being as follows:

3 weeks, females: 33 *midgets*, 31 *NS*
3 weeks, males: 30 *midgets*, 46 *NS*
6 weeks, females, 49 *midgets*, 59 *NS*
6 weeks, males, 41 *midgets*, 64 *NS*

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Thirty-nine female and thirty-one male *midgets* from *MS* backcrosses were weighed at 6 weeks of age. They were heavier than *midgets* on an *NS* background (Table 4).

Table 4. *Weight differences between* midgets *and their normal litter-mates at* 3 *and* 6 *weeks of age*

	Females			Males		
	Mean weight			Mean weight		
Age (weeks)	*Midgets*	Normal litter-mates	Difference between means	*Midgets*	Normal litter-mates	Difference between means
			1. *Midget* × *NS*			
3	4·4	5·7	1·2 ± 0·2	4·7	5·8	1·3 ± 0·2
6	7·2	11·1	3·6 ± 0·3	7·9	12·4	4·4 ± 0·4
			2. *Midget* × *MS*			
6	9·4	12·3	2·8 ± 0·4	10·1	14·8	4·1 ± 0·5

Does the uterine environment influence the midget phenotype?

The influence of the maternal environment on the *midget* phenotype was studied by transferring 3½-day-old blastocysts and morulae into recipient females. The embryos were taken from *midget* females which had mated to *midget* males; the recipients were *NL* females which had mated to *NL* males 2½ days previously. Eye-colour markers were used to identify the *NL* and *midget* offspring.

Three recipient females received a total of ten embryos. Two of these females gave birth to litters. Four offspring, of which one was dead, had eye colour identifying them as *midgets*, ten others being *NL*. The morphology of the *midgets* was unaffected by the uterine environment of the host. However, the growth-rate of the three *midgets*, which suckled the host *NL* female, was higher than that of *midget* offspring from *midget* matings although the characteristic check in growth-rate occurred at approximately 20 days of age (Text-fig. 1). Some *midget* offspring from *midget* mothers were fostered at birth on to *NL* or other large-type mothers. The growth-rate of these offspring was slightly increased, though their growth pattern was unchanged (Text-fig. 1).

The natural fertility of midgets

Though there was a delay in reaching maturity, most *midget* males were fertile and sexually active. There was a pronounced delay in the onset of sexual maturity in *midget* females, as judged by vaginal smears. Most females were non-cyclic until at least 4 or 5 months of age, and others never became fertile. The fertility of the cyclic mice was studied as follows.

Nine *midgets* were autopsied 24 hours after mating. Eight of them were found to have recently ovulated eggs (Table 5), the mean number ovulated being 5·3. Some *NS* mice also fail to ovulate during the oestrous cycle in which mating occurs (Fowler & Edwards, 1960) (see Table 5), but this happens only rarely in the other

lines. More than 95% of the eggs recovered from the *midgets* were fertilized when examined under a phase-contrast microscope.

Eleven *midgets* were autopsied 12 days after mating. Only four of them had any implanted embryos (Table 5), a similar proportion of *NS* females also having implanted embryos after mating. Seven other *midgets* were accordingly given daily supplements of progesterone (2 mg./day) from day 2 until day 12 after mating (for details of this technique see Smithberg & Runner, 1956, 1957). Six of these mice had implanted embryos (Table 5), the mean number of implanted embryos being similar to that in untreated mice. Progesterone also increased the proportion of *NS* mice with implanted embryos. More data on the influence of progesterone on the maintenance of pregnancy are given below.

Table 5. *Ovulation and pregnancy in* midget *and* NS *mice after mating during natural oestrus*

1. Ovulation 24 hours after mating

	No. that mated	No. with eggs	Mean no. of eggs
Midget	9	8	5·3 ± 0·5
NS	32	25	4·5 ± 0·2

2. Pregnancy 12 days after mating

	No. that mated	Treatment after mating	No. and % with implanted embryos	Mean no. of embryos
Midget	11	None	4 (36%)	3·8 ± 0·8
	7	Progesterone*	6 (86%)	3·5 ± 0·4
NS	34	None	14 (41%)	4·3 ± 0·3
	17	Progesterone*	14 (82%)	4·9 ± 0·3

* See text.

Treatment of midgets with exogenous gonadotrophins

The gonadotrophins pregnant mares' serum (PMS) and human chorionic gonadotrophin (HCG) can be used to induce oestrus and ovulation in immature (Runner & Gates, 1954) and adult mice (Fowler & Edwards, 1957). Previously, infertile non-*midget* mice of line *NS* had been brought into oestrus and induced to ovulate by use of these gonadotrophins, though the great majority of the resulting pregnancies were not maintained unless daily progesterone injections were given to the mice (Fowler & Edwards, 1960). A similar experiment was carried out on sterile *midget* mice. Injection of 3 i.u. PMS proved to be excessive, for only 7/10 *midgets* ovulated; 1 i.u. or less was therefore employed (Table 6). Oestrus and ovulation were readily induced in the sterile *midget* mice (Table 6). In the absence of further treatment, however, the majority of *midgets* possessed no implanted embryos 12 days after mating. Progesterone supplements given as described above were successful in all ten *midgets* treated, and the mean number

of implanted embryos was significantly higher than in naturally-mated *midgets* ($F^1_{19} = 15{\cdot}5, P < 0{\cdot}01$). These results were similar to those obtained with *NS* mice, except that more *NS* females could maintain pregnancy without progesterone supplements (Table 6). Clearly, both *midgets* and *NS* mice were deficient in follicle-stimulating and luteinizing hormone, and in the secretion of prolactin, progesterone, or both.

Table 6. *Ovulation, mating, and pregnancy in* midget *and* NS *mice after treatment with gonadotrophins*

1. Ovulation 24 hours after treatment of *midgets*

Amount of PMS	No. of mice treated	No. that mated	No. autopsied	No. with eggs	Mean no. of eggs
3 i.u.	24	17	10	7	$9{\cdot}3 \pm 2{\cdot}4$
1 i.u.	25	21	6*	5	$9{\cdot}4 \pm 2{\cdot}6$
½ i.u.	7	4	7*	7	$9{\cdot}0 \pm 2{\cdot}7$

* Each group includes 3 mice that did not mate.

2. Pregnancy 12 days after mating (1 i.u. PMS)

	No. that mated	Treatment after mating	No. and % with implanted embryos	Mean no. of embryos
Midget	9	None	1 (11%)	8
	10	Progesterone†	10 (100%)	$6{\cdot}5 \pm 0{\cdot}8$
NS	17	None	6 (35%)	$9{\cdot}2 \pm 1{\cdot}8$
	15	Progesterone†	13 (87%)	$7{\cdot}2 \pm 1{\cdot}1$

† See text.

DISCUSSION

The gene *midget* has arisen in a line of mice selected for small body size (*NS*), and requires the genetic background associated with this particular line for its full phenotypic expression. Within line *NS*, the *midget* phenotype was due to a single recessive gene. In backcrosses to other small lines, however, the frequency of *midget* offspring was much lower than expected, and the severity of the expression of the gene was reduced. The lack of *midgets* in these backcrosses could not be explained by pre- or post-natal mortality. It thus appeared that on these genetic backgrounds many homozygous *midgets* were no longer distinguishable from their non-*midget* litter mates. Moreover, the incidence of *midget* offspring was lower in backcrosses to the heavier *CS* mice than to the lighter *MS* mice, and Falconer (unpublished) found no undersized mice in backcrosses to large (*NL*) mice. The modifying factors necessary for the full phenotypic expression of *midget* were evidently absent from or ineffective in mice selected for large body size. Evidence of the genetic nature of these modifying factors was provided by the experiment on the transfer of 3½-day-old *midget* embryos into the uteri of large (*NL*) mice: after birth these *midgets* retained their typical phenotype and pattern of growth despite the changed uterine environment.

The presence of a mutant gene at a single locus having a large effect on body

size and dependent for its expression on a number of modifying genes would explain the increased variance in line *NS* from generation 7 onwards (Falconer, 1953, 1955). At that time classification could only be made on body weight, for the undersized mice were evidently otherwise indistinguishable from their normal litter-mates. The reappearance of undersized mice among the offspring of generation 22, with their characteristic *midget* phenotype, may have been due to the fixation of more modifying genes in the intervening generations. These modifiers may have been associated with small body size, since the 6-week weight of *NS* mice declined by approximately 5 g. between generations 7 and 22. The parallel between the discovery of *midget* in Falconer's small line and that of the gene *pygmy* in MacArthur's small line is most striking. Furthermore, a gene for large body size, *adipose*, has been found in a line selected for large body size (Falconer & Isaacson, 1959). Unlike *midget*, however, the emergence of *pygmy* and *adipose* was probably not due to the accumulation of modifiers during selection, for both of these genes segregate normally on a wide variety of genetic backgrounds. Either of these mutants could presumably have been found in any strain of mice.

Several comparisons are possible between the physiological traits associated with *midget* and those of other dwarfing genes. The lack of growth in *pituitary dwarf* mice has been related to the absence of typical eosinophil cells of the anterior pituitary associated with a deficiency of growth hormone (Smith & McDowell, 1930, 1931; Francis, 1944). Preliminary histological studies have shown no gross abnormality of the anterior pituitary of *midget* mice. Both *pituitary dwarf* and *midget* show a severe retardation in growth between 16 and 24 days of age, even when food is plentiful, though adult *midgets* are larger than *pituitary dwarfs*, which average 5–8 g. (Boettiger & Osborn, 1938). *Pygmy* mice lose weight between 12 and 21 days of age through lack of milk, and weigh approximately one-half of normal litter-mates at 6 weeks of age, averaging 5–6 g. in MacArthur's small line (King, 1950, 1955).

Each of the three genes is associated with sub-fertility. *Pituitary dwarf* males may possess near-normal testes and accessory glands (Smith & McDowell, 1931) but are usually sterile, whereas *midget* males are fertile though delayed in reaching maturity. Smith & McDowell also showed that though 'gonad-stimulating hormone' was not deficient in *pituitary dwarfs* (see Elftman & Wegelius, 1959) the reproductive organs of the females were infantile. The absence of an oestrous cycle in some *midgets*, and the failure of others to ovulate after natural mating, imply deficiencies of both follicle-stimulating and luteinizing hormones respectively, and the lack of progesterone secretion during pregnancy could be due to inadequate secretion of prolactin. Although less severe, similar deficiencies in pituitary secretion evidently occur in *NS* mice (Fowler & Edwards, 1960), and these hormonal defects are therefore probably associated with the reduced growth-rate arising from selection for small body size rather than with *midget*. Sterility in *pygmy* mice is also conditioned by the genetic background, though the cause of the sterility is not known (King, 1955).

SUMMARY

Midget, a new dwarfing gene in the house mouse, was found in a line (*NS*) selected for small body size. Backcrosses to, and heterozygous matings of *NS* mice gave midget offspring in the expected 1:1 and 1:3 ratios. Backcrosses to two other lines of mice selected for small body size resulted in fewer *midget* offspring than expected and in a reduced phenotypic expression of the gene, though matings between these *midget* offspring gave all *midget* progeny.

A characteristic check in growth occurred between 15 and 21–24 days of age, and *midgets* rarely exceeded 10 g. at 6 weeks of age. They were 1·2 g. lighter than *NS* litter-mates at 3 weeks, and 3·6–4·4 g. lighter at 6 weeks. Fostering or transferring $3\frac{1}{2}$-day-old *midget* embryos to the uteri of large mothers slightly increased the growth-rate, though the characteristic growth check still persisted. Transfer as embryos did not influence the *midget* phenotype.

Midget males were of near-normal fertility. The females were semi-sterile due to irregularity or absence of oestrous cycles, failure to ovulate, or the death of embryos before implantation. Each of these factors, which were also found in *NS* mice, could be remedied by the administration of exogenous hormones.

The genetic factors underlying the expression of *midget* are discussed, and the physiological traits associated with this and with other dwarfing genes are compared.

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