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# Studies of the Effects of Variation at the Transferrin Locus on Reproductive Processes in Deer Mice

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The pattern of inheritance and function of variation at the transferrin locus of deer mice, *Peromyscus maniculatus* (Hoy et Kennicott, 1857), were assessed in several studies. Despite substantial evidence of such function in other species, consequences of variation at this locus in deer mice were minimal. There was an underrepresentation of animals homozygous for the fast-migrating allele in both homozygote-heterozygote and double heterozygote crosses. There were no significant differences attributable to genotype in breeding performance. With respect to copulatory behavior, there was some evidence of a small difference attributable to male genotype and several differences related to female genotype. There was no effect on representation in litters resulting from tests in a situation of sperm competition. However, effects of a variety of experimental treatments were consistent with previous results. The transferrin locus of deer mice appears to be useful as a marker gene in studies relating to reproduction and reproductive competition.

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## INTRODUCTION

The purpose of this research was to analyze the pattern of inheritance and the functions in reproduction of variation in the transferrin allele in deer mice, *Peromyscus maniculatus* (Hoy et Kennicott, 1857). Transferrin is an iron-binding protein in blood serum that functions in iron transport. Genetically distinctive variants are recognizable by different electrophoretic mobilities and appear governed by an autosomal locus with codominant alleles. Transferrin has been used as a marker gene in many field studies (e.g., Krebs *et al.*, 1973; Dobrowolska *et al.*, 1983). In addition, laboratory studies have suggested functional consequences of variation in transferrin for reproduction and development (e.g., Smith & Small, 1982). Because of its use as a marker gene and of its apparent functional role, it is important that the consequences of electrophoretic variation in transferrin be understood. However, there has been little detailed study of functional consequences for reproductive behavior and differential reproduction. The present research addressed that need in the context of an ongoing research program in which the transferrin

locus is being used as a marker for paternity determination in studies of sperm competition, behavioral dominance, differential reproduction, and related processes (e.g., Dewsbury, 1984).

It should be fully recognized throughout this paper and others in the field that it is difficult to separate direct effects of the transferrin locus from effects of loci linked to transferrin. Any effects attributed to transferrin must be treated as possibly due to a linked locus unless direct evidence to the contrary is provided.

There are two classes of previous studies that are directly relevant. In one, the ratios of numbers of individuals bearing different genotypes are studied in progeny analyses where segregation can occur; in others functional differences are correlated with electrophoretic variation (Boettcher, 1974). An example of the former is the study in rhesus monkeys of Smith (1982a), in which segregation distortion at the transferrin locus was reported. Although inheritance of transferrin alleles in both *P. maniculatus* and the closely-related *P. polionotus* have been reported to approximate Mendelian ratios (Griswold & Dawson, 1971; Rasmussen & Koehn, 1966), there are some suggestions of segregation distortion in the former (Canham, 1969; Fairbairn, 1976).

Functional correlates of transferrin variation have been reported in a variety of vertebrate species and for a variety of aspects of reproduction and development. Such correlates include differential fertility in skipjack tuna, cattle, and house mice (Ashton, 1961; 1965; Berry & Peters, 1977; Fujino & Kang, 1968), differential growth rates in cattle, rhesus monkeys, and voles (Fowle *et al.*, 1967; Gaines & Krebs, 1971; Gaines *et al.*, 1978; Smith & Small, 1982), differential mortality and viability in voles, rhesus monkeys, and tuna (Fujino & Kang, 1968; Gaines *et al.*, 1971; 1978; Smith & Small, 1982), and differential aggression in yellow-bellied marmots (Schwartz & Armitage, 1981). Among microtine rodents, allele frequencies have been found to vary with gender (Dobrowolska & Zajączkowski, 1983), population density, and season (Birdsall, 1974; Dobrowolska, 1983; Gaines & Krebs, 1971; Kohn & Tamarin, 1978, Mihok *et al.*, 1983; Tamarin & Krebs, 1968) and dispersing animals have been found to be a nonrandom sample of the populations with respect to transferrin alleles (Baird & Birney, 1982; Keith & Tamarin, 1981; Myers & Krebs, 1971).

In deer mice, there are reports of seasonal fluctuations in allele frequencies (Canham, 1969; Fairbairn, 1976) differences in growth rate, and fluctuations with population density (Canham, 1969). Linkage relationships have been developed (Dawson, 1982; 1984). In the present research the pattern of inheritance was examined with a sample size larger than in previous studies. Detailed analyses of patterns of cop-

ulatory activity were conducted. In addition, a study of sperm competition modelled after previous work with coat-color markers (Dewsbury & Baumgardner, 1981), was completed. Such studies often reveal differential fertilizing ability as a function of genotype (see Lanier *et al.*, 1979).

## MATERIAL AND METHODS

Animals: Animals were deer mice, *Peromyscus maniculatus bairdi*, from the colony maintained at the University of Florida. The colony is derived from progenitors trapped in the vicinity of East Lansing, Michigan in the early 1970's; wild stock have been bred into the colony on several occasions. Mice were maintained in a windowless, air-conditioned room on a rewersed 16L:8D photoperiod. They were housed in polycarbonate cages measuring either 48×27×13 cm or 29×19×13 cm and had continuous access to Purina laboratory rodent chow and water. Wood shavings were used as bedding. Animals were weaned at 21 days of age and housed as litters until they were approximately 60 days of age, at which time the sex ratio was determined and they were housed in groups of like-sexed littermates until separated for use in experiments.

Breeding: The colony was maintained by breeding approximately 18 breeding pairs at any time. At pairing all animals were at least 80 days of age. Individuals that were mated were selected so as to be non-siblings, of selected genotypes for transferrin, and to maintain as much genetic diversity in the colony as possible. The dates of pairing and of litter births were available and analyzed for a period for approximately three years. In addition, for litters born from November, 1982 until July, 1984, the numbers of pups at birth and weaning were recorded. It should be noted when interpreting data on breeding performance (e.g., Table 2) that the data bases for different measures include different samples and thus certain apparent inconsistencies can result.

Electrophoresis: The genotypes for transferrin of animals from the breeding colony were determined at an age of approximately 60—90 days. This was done with horizontal starch-gel electrophoresis using a modification of the methods described by Selander *et al.* (1971). Blood was drawn from the suborbital canthal sinus of ether-anesthetized animals. Where, as in the study of sperm competition, the genotypes of newborn animals were to be determined, pups had to be sacrificed in order to obtain blood. A representative gel is shown in Figure 1. In this population there are two alleles with codominance. These are labelled, for simplicity, slow (S) and fast (F), to refer to the differential rates at which they migrate along the gel. In cases where the gel was ambiguous or the genotype discrepant from that of the parents, a second or third sample was run.

Copulatory Behavior: Copulatory behavior was analyzed as a function of genotype in three studies. Animals from this colony were used in a number of other studies of sperm competition and related phenomena. Before serving in such studies, all males were pre-tested for copulatory behavior to ensure that they would copulate and had some copulatory experience. Animals were of all three genotypes; data from these pretests were analyzed as a function of genotype.

The data from pretests were suggestive of an effect of genotype. However, results of a male's first test tend to be especially variable and uncontrolled factors can affect parameters when just one test is given. Therefore, a study was done in which males of varying genotypes received three tests spaced at least two

weeks apart (see Dewsbury, 1983). To minimize environmental effects, as well as some effects of loci other than transferrin, the behavior of like-sexed littermates of different genotypes was compared, each in tests with one male and one female present at a time. Because like-sexed homozygotes of opposite genotypes were relatively rare, both homozygous slow and homozygous fast males were compared to heterozygous littermates. There were 16 matched littermate pairs of males of each type.

The third set of data on copulatory behavior was from the sperm competition study described below.

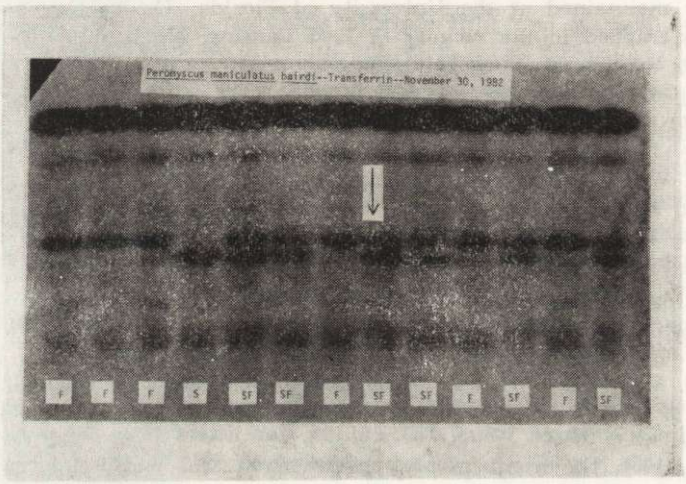


Fig. 1. Sample transferrin gel run in our laboratory.

Males to be tested for copulatory behavior were at least 90 days of age and housed individually in the larger-sized cages. Tests occurred in the males' home cages. Standard procedures were used (e.g., Dewsbury, 1979a). Females were of the mutant blonde line of deer mice (Pratt & Robbins, 1982). Females were made behaviorally receptive with injections of 0.06 mg estradiol benzoate approximately 72 h before testing and 0.6 mg of progesterone approximately 6 h before testing. Tests were conducted during the dark phase of the photoperiod. Females were introduced into the male's home cage and at least 1 h allowed for copulation to begin. If there was no copulation, the test was scored as negative. Animals were permitted four negative tests before being scored as "noncopulators". If copulation was initiated, animals were permitted to continue mating for three ejaculations or until it appeared that a third ejaculation would not occur.

Behavioral patterns were recorded on an Esterline-Angus operations recorder. Copulatory behavior in deer mice includes three primary events: mounts (with no vaginal insertion), intromissions (with vaginal insertion but no ejaculation) and ejaculations (with mounting, insertion, and ejaculation of sperm). These occur in organized ejaculatory series, with each series ending with an ejaculation and separated from other series by a postejaculatory refractory period. The following standard measures (see Dewsbury, 1979a) were taken: mount latency (ML) — time in sec from start of a test until the first mount or intromission, intromission latency (IL) — time in sec from start of a test until the first intromission, ejaculation latency (EL) — time in sec from the first intromission of a series

until ejaculation, intromission frequency (IF) — the number of intromissions in a series, mount frequency (MF) — the number of mounts in a series, mean interintromission interval (MIII) — the mean interval in sec separating the intromissions of a series, and postejaculatory interval (PEI) — the time in sec from an ejaculation until the next intromission. When an abbreviation for a measure is followed by a hyphen and a number, it refers to a particular series (e.g., IF-2). In addition, the number of negative tests preceding the first positive test (no. of passes) was determined.

Sperm Competition: Methods in the study of sperm competition were patterned after those of Dewsbury and Baumgardner (1981). Basically, this entails a female copulating successively with two different males, each for two ejaculations. All animals were homozygous for the transferrin allele. Twenty females with the slow allele and 18 with the fast allele completed the study. Each female copulated in two valid tests, one in each order. In one (S-F order) the slow male copulated first and the fast male second; in the other (F-S order) the fast male copulated before the slow. Approximately half of the females received their tests in each order (i.e. S-F/F-S vs. F-S/S-F).

Females were in naturally-occurring estrus, determined by following cycles by monitoring vaginal smears. Smears were taken each morning using tap water and a thin wire loop and females were tested on the first day of the virtual disappearance of leukocytes from the smear. The female was introduced into the cage of the first male and permitted to mate for two ejaculatory series. Immediately after the second ejaculation, the female was removed and placed in the cage of a male of the opposite genotype to mate for two series. If copulation did not occur, the female was usually tried with a second male of the appropriate genotype. Temporal variation in the range used has little effect on litter composition in this species (Dewsbury & Baumgardner, 1981). Litters resulting from these matings were sacrificed and transferrin genotypes determined — typically within one week of birth.

In order to complete a valid test, a female had to mate for two ejaculations with both males and deliver a litter that survived until electrophoresis. Further, she had to complete tests in both mating orders. A total of 448 tests were initiated in order to complete the 76 valid tests.

## RESULTS

Pattern or Inheritance: The genotypes of 1,774 animals born in the breeding colony and whose transferrin genotypes were determined are summarized in Table 1. The genotypes of all but four of these animals were concordant with the genotypes of their parents. As the genotypes of these four animals were checked at least twice, these exceptions probably resulted from mixtures among litters in the handling of the animals. As can be seen in the table, the ratios approximate Mendelian ratios. The largest exception is in the F×SF cross, where the chi-square value just exceeded the level required for statistical significance (3.84). In that cross there was an underrepresentation of homozygous fast animals. A similar underrepresentation, though not significant, can be seen in the SF×SF cross.

Table 1

Genotype of offspring as a function of parental genotype

| Parental genotype | No. of pairs | No. of litters | No. of pups | Percent of Pups | | | $\chi^2$ | df | $p$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | S | F | SF | | | |
| S×S | 14 | 85 | 353 | 99.7 | 0.3 | 0.0 | | | |
| F×F | 13 | 88 | 405 | 0.0 | 100.0 | 0.0 | | | |
| S×F | 2 | 8 | 28 | 3.6 | 0.0 | 96.4 | | | |
| SF×SF | 17 | 98 | 451 | 23.9 | 21.5 | 54.5 | 4.27 | 2 | ns |
| S×SF | 7 | 54 | 219 | 50.2 | 0.4 | 49.3 | 0.18 | 1 | ns |
| F×SF | 9 | 78 | 318 | 0.3 | 44.3 | 55.3 | 3.86 | 1 | .05 |

Breeding Performance: Data on the breeding performance of 71 breeding pairs of various genotypes are summarized in Table 2. Analyses of variance revealed no statistically significant variation as a function of parental genotype in any of the six measures presented.

Table 2

Breeding performance as a function of parental genotype.

| Parental genotype | No. of pairs | No. of litters | Mean litter size, at | | | Percent female | Litter latency | Litter interval |
|---|---|---|---|---|---|---|---|---|
| | | | Birth | Wean | Trf | | | |
| S×S | 16 | 130 | 5.2 | 3.4 | 4.2 | 43.7 | 32.3 | 28.0 |
| F×F | 18 | 148 | 5.4 | 3.2 | 4.8 | 51.1 | 31.2 | 25.9 |
| S×F | 2 | 10 | 5.5 | 3.4 | 3.5 | 35.5 | 28.5 | 28.2 |
| SF×SF | 19 | 138 | 5.6 | 4.1 | 4.7 | 50.2 | 28.3 | 27.7 |
| S×SF | 7 | 69 | 5.2 | 3.8 | 4.2 | 46.6 | 31.1 | 27.6 |
| F×SF | 9 | 102 | 4.8 | 4.0 | 4.1 | 47.3 | 27.3 | 26.7 |

Copulatory Behavior in Pretests: The copulatory behavior of 93 males of varying genotypes was evaluated in pretests of copulatory behavior; results for selected measures are presented in Table 3. Although none were statistically significant at the .05 level, three

Table 3

Selected measures of copulatory behavior in pretests.
ML — Mount latency, IL — Intromission latency, MF — Mount frequency.

| Measure | Male genotype | | | $F$ | $p$ |
|---|---|---|---|---|---|
| | Slow | Fast | Heterozygote | | |
| N | 33 | 39 | 21 | | |
| No. Passes | 0.4 | 0.8 | 0.7 | 2.57 | .08 |
| ML | 1005.3 | 1516.1 | 1364.9 | 2.39 | .10 |
| MF-1 | 4.8 | 5.3 | 3.3 | 0.37 | — |
| MF-2 | 4.4 | 3.5 | 3.2 | 0.16 | — |
| MF-3 | 3.2 | 3.7 | 7.4 | 2.34 | 10 |

differences reached the .10 level. Given the inherent variability in first-test data, this provided impetus for further study.

Copulatory Behavior of Matched Littermates: The copulatory behavior of 16 littermate pairs of S and SF males and 16 pairs of F and SF males were compared on three tests each. Analyses of variance with repeated measures were used to determine the significant differences. Significant differences are presented in Table 4. For both comparisons there was a significant effect of test on mount and intromission latency. Animals initiated copulatory activity more quickly with copulatory experience. The only significant strain difference was for the mount frequency in the first series for slow versus heterozygote brothers. There was thus one significant difference in 36 comparisons (2.8%).

Table 4

Selected measures of copulatory behavior in study of matched littermate male pairs. ML — Mount latency, IL — Intromission latency, MF — Mount frequency.

| Measure | Male Genotype Mean | | Genotype | | Test | |
|---|---|---|---|---|---|---|
| | Homozygote | Heterozygote | *F* | *p* | *F* | *p* |
| Slow vs. Heterozygote: | | | | | | |
| ML | 1104.5 | 863.4 | 1.66 | ns | 9.06 | .001 |
| IL | 1121.9 | 907.5 | 1.32 | ns | 9.02 | .001 |
| MF-1 | 3.6 | 1.8 | 4.96 | .05 | 0.28 | ns |
| Fast vs. Heterozygote: | | | | | | |
| ML | 765.4 | 893.1 | 0.41 | ns | 5.21 | .02 |
| IL | 789.2 | 950.5 | 0.58 | ns | 5.76 | .01 |

Sperm Competition: Valid tests were completed in just 76 of 448 tests initiated (17%). A summary of the reasons can be found in Table 5. The major cause for failure was the complete failure of cop-

Table 5

Outcomes of 448 tests of sperm competition in deer mice.

| Measure | Slow females | | Fast females | | Total |
|---|---|---|---|---|---|
| | SF | FS | SF | FS | |
| No. valid tests | 20 | 20 | 18 | 18 | 76 |
| No. females with litters in one condition only | 0 | 2 | 0 | 2 | 4 |
| Tests with litter lost or killed | 2 | 3 | 6 | 4 | 15 |
| No. complete tests with no litter | 21 | 8 | 22 | 19 | 70 |
| No. tests one male mating only | 12 | 3 | 6 | 6 | 27 |
| No. tests 1st male incomplete | 4 | 2 | 4 | 7 | 17 |
| No. tests 2nd male incomplete | 2 | 0 | 0 | 1 | 3 |
| No. tests negative | 79 | 42 | 56 | 59 | 236 |
| Total | 140 | 80 | 112 | 116 | 448 |

ulation (53% of tests). There was no obvious relationship between male or female genotype and success or failure in these tests.

Results regarding litter composition from the 76 valid litters in this study are summarized in Table 6. Results are presented in relation to female genotype and male order. They are summarized in terms of the percent sired by the first male, the percent homozygous, and the percent sired by the slow male. Neither mating order nor homozygosity, nor sire genotype had a major effect on litter composition; overall all percentages were between 42 and 58. Wilcoxon matched-pairs signed-ranks tests revealed no significant effect of male mating order on litter composition in either slow females ($T=56.5$) or fast females ($T=63.5$). Mann-Whitney $U$-tests revealed no significant effect of female genotype on litter composition in either the S-F ($U=172.5$) or the F-S ($U=176$) mating order.

**Table 6**

**Genotypes of offspring from study of sperm competition in deer mice.**

| Female genotype | Male order | No. of pups by genotype | | | % Sired 1st male | Percent homozygous | % Sired slow male |
|---|---|---|---|---|---|---|---|
| | | Slow | Fast | Heterozygote | | | |
| Slow | SF | 53 | 0 | 59 | 47 | 47 | 47 |
| | FS | 44 | 0 | 61 | 58 | 42 | 42 |
| | Total | 97 | 0 | 120 | 52 | 45 | 45 |
| Fast | SF | 0 | 38 | 40 | 51 | 49 | 51 |
| | FS | 0 | 39 | 43 | 48 | 48 | 52 |
| | Total | 0 | 77 | 83 | 49 | 48 | 52 |
| Grand total | | 97 | 77 | 203 | 51 | 46 | 48 |

In order to analyze the copulatory behavior in the sperm competition study as a function of male and female genotype, data were used for the 47 males (24S and 23F) that completed at least one test mating first and one second mating with females of each genotype (4 tests per male). Only a male's first test in each f the four eonditions was used. These data were analyzed using repeated-measures analyses of variance with male genotype, mating order, and female genotype as main factors. Another set of analyses was done based on the first tests with complete behavioral data for the 23 S females and the 25 F females providing complete behavioral data for at least one test in each mating order. As the results were substantially the same as with the male-based analyses, only those are presented in Table 7. The order of mating had a major effect on copulatory behavior, with males mating second having shorter mount and intromission latencies, but requiring more intromis-

sions before ejaculating and having longer ejaculation latencies. There were no significant effects of male genotype. However, there were three significant effects of female genotype, Males mating with S. females had shorter first-series ejaculation latencies and fewer mount in that series, as well as shorter postejaculatory intervals following the first series.

Table 7

Measures of copulatory behavior in study of sperm competition.
ML — Mount latency, IL — Intromission latency, EL — Ejaculation latency, IF — Intromission frequency, MF — Mount frequency, MIII — Mean intromission interval, PEI — Postejaculatory interval.

| Measure | Mean by male | | Mean by female | | Male strain | Female strain | F:Male |
|---|---|---|---|---|---|---|---|
| | Slow | Fast | Slow | Fast | F | F | order |
| ML | 757.4 | 626.7 | 631.3 | 752.6 | 1.03 | 3.08 | 11.06 ** |
| IL | 805.1 | 701.3 | 682.4 | 826.2 | 0.61 | 4.00 | 11.23 ** |
| EL-1 | 475.4 | 448.4 | 424.2 | 500.1 | 0.11 | 4.81 * | 16.21 *** |
| IF-1 | 12.5 | 10.1 | 11.0 | 11.6 | 1.23 | 0.52 | 26.74 *** |
| MF-1 | 4.5 | 4.2 | 3.6 | 5.1 | 0.14 | 4.60 * | 1.72 |
| MIII-1 | 55.4 | 62.1 | 55.2 | 62.2 | 0.29 | 1.20 | 1.74 |
| PEI-1 | 382.0 | 405.6 | 381.6 | 405.5 | 1.39 | 4.42 * | 0.02 |
| EL-2 | 191.2 | 156.3 | 172.4 | 175.8 | 1.44 | 0.04 | 1.94 |
| IF-2 | 10.6 | 9.1 | 9.5 | 10.2 | 0.83 | 0.53 | 1.78 |
| MF-2 | 2.6 | 1.7 | 1.8 | 2.6 | 2.78 | 3.63 | 0.54 |
| MIII-2 | 21.5 | 22.1 | 22.1 | 21.4 | 0.03 | 0.06 | 6.68 * |

* $p<.05$; ** $p<.01$; *** $p<.001$

A series of $t$-tests was run to determine whether the parameters of copulatory behavior differed between complete tests after which females delivered a litter (95) versus those in which they continued cycling normally (43) or became pseudopregnant (27). The major result was a difference in the total number of copulations received by females. Although all females received four complete series with ejaculations, those delivering litters received significantly more intromissions than those continuing to cycle (45.3 vs. 32.5, $t=3.24$, $p<.01$) or becoming pseudopregnant (45.3 vs. 33.7, $t=2.43$, $p<.02$).

## DISCUSSION

As noted in the introduction, there is good evidence for effects of variation in transferrin genotype on reproductive and developmental processes in a variety of species (e.g., Ashton, 1961; 1965; Smith & Small, 1982). There is some evidence suggestive of such relationships in deer mice (Canham, 1969; Fairbairn, 1976). The overall picture obtained from the present studies, however, is one of minimal effects on the processes

measured. There is evidence of a) underrepresentation of animals of the F genotype in segregating generations, b) some small differences in male copulatory behavior, and c) some differences in copulatory behavior attributable to the female. For most measures, however, animals bearing different transferrin genotypes did not differ. The data from the sperm competition study are particularly notable, as this method provides a rather subtle and sensitive analysis of reproductive processes. In our only other study with animals of these genotypes (Dewsbury, 1985), there were also no major differences in reproductive processes as a function of genotype. In that study, an S and an F male had simultaneous access to a receptive female. Because the transferrin locus appears basically neutral with respect to most of the processes under study, it would appear to be an appropriate marker gene in studies of paternity, sperm competition, and related processes.

The reason for the underrepresentation of F animals in the litters resulting from F×SF and SF×SF matings is not clear. Some possible mechanisms have been discussed by Boettcher (1974) and Smith (1982b). Data from the sperm competition reveal no apparent underrepresentation. In that study transferrin was analyzed shortly after birth rather than at 60 days of age or more. It is possible that there is differential mortality in the interim. The survival rate of the offspring from the breeding colony of F×F matings (66%) (including all litters with complete data for numbers at birth and weaning) was somewhat lower than that for S×S matings (74%). However, the survival rate of offspring of the F×SF matings was the highest of all (82%), while that of SF×SF), matings was 79 percent. With relatively low mortality rates, the argument for differential mortality, though possible, seems strained. Canham (1969) found a significant underrepresentation of heterozygotes in a double heterozygote cross with deer mice (38% rather than 50%). Fairbairn (1976), by contrast, obtained an overrepresentation of heterozygotes in such a cross that fell between the .10 and .05 significance levels. Rasmussen and Koehn (1966) obtained no such differences. Thus, available data on deer mice are inconsistent. It is notable that the sample size in the present study is substantially higher than that in the three previous studies combined.

The data from both the breeding colony and the sperm competition study are consistent in revealing no subtle effects of transferrin variation on reproductive processes. There was only one significant difference in male copulatory behavior as a function of transferrin genotype. This effect, on MF-1 in the study of matched slow and heterozygote siblings, was not replicated in other studies. However, in another study using animals with these genotypes, S males with simultaneous access with

F males to a female displayed significantly higher mount frequencies in the first series (Dewsbury, 1985). Thus, although the effect appears to be weak and not always obtained, it may be a real one. Mount frequency tends to be a relatively unreliable measure in the study of copulatory behavior.

The effect of female genotype on copulatory behavior appears more substantial, as there was just one study of such an effect and this revealed three significant differences in 11 comparisons. For two of these measures, EL-1 and MF-1, there is previous evidence that the female may play a particularly important role in affecting the measures (Dewsbury, 1978b, table 8). In general, males tended to copulate faster with S than with F females. Differences among females that might generate such differences were not apparent to the observer.

When obtaining negative or small effects, one must be cautious in interpretation as problems of design or procedure may be responsible. One must also remember possible effects of multiple statistical tests. However, the data in the present studies are consistent with a variety of significant effects obtained in previous studies, thus increasing confidence in conclusions drawn from this work. The parameters of copulatory behavior were well within the range generally reported for this species under these conditions (e.g., Dewsbury, 1979a). As in previous studies with both deer mice and laboratory rats, mount and intromission latencies have been found to change more substantially with repeated tests than do other measures (Dewsbury, 1969; 1979a). That males should have reduced mount and intromission latencies and increased numbers of intromissions before ejaculating when mating with mated, rather than unmated, females also has been found previously (e.g., Dewsbury, 1979b; Dewsbury & Baumgardner, 1981). This is consistent with the proposed role of multiple intromissions in dislodging copulatory plugs (Dewsbury, 1981). Such effects were especially strong in the present study (see Table 7). Significant differences between pregnant and nonpregnant females with respect to the number of intromissions received were found in both the present sperm competition study and that of Dewsbury and Baumgardner (1981).

Patterns of sperm competition vary among rodent species with some species showing a first male advantage, others a second-male advantage, and others no order effects (Levine, 1967; Lanier *et al.*, 1979; Oglesby *et al.*, 1981; Dewsbury & Baumgardner, 1981). In three previous studies of deer mice, done using different coat-color genes as markers, there were no order effects in sperm competition (Dewsbury & Baumgardner, 1981). The present data reveal a similar lack of effect of mating order on litter composition. The presence of significant effects that are generally

consistent with those previously reported gives one increased confidence in the validity of the present results and the sensitivity of the measures used.

In many studies of sperm competition, there are between-strain differences in differential fertilizing capacity, the ability of males of different genotypes to gain representation in litters resulting from multiple-male matings when the timing, order, and number of ejaculations is controlled. In other cases there may be behavioral differences correlated with a proposed marker gene that render it problematical for use. The lack of such effects in the present data make the transferrin locus in deer mice very appealing for use as a marker gene.

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## ZMIENNOŚĆ W LOCUS TRANSFERYNY A ROZRÓD U *PEROMYSCUS MANICULATUS*

Streszczenie

Celem pracy było zbadanie, czy zmienność w locus transferyny u myszaka *Peromyscus maniculatus* (Hoy et Kennicott, 1857) wpływa na procesy związane z rozrodem — przebieg kopulacji (Tabele 3, 4). oraz liczbę urodzonego potomstwa (Tabele 2, 3) w warunkach konkurencji plemników pochodzących od dwóch samców (Tabele 5, 6, 7). Mimo faktu, iż u innych gatunków wykazano zależność między parametrami rozrodu a zmiennością w badanym locus to wyniki pracy na *P. maniculatus* dały zasadniczo odpowiedź negatywną. Wykazano zaledwie drobne różnice w przebiegu poszczególnych faz kopulacji, które można przypisywać genotypowi samca lub samicy. Stwierdzono niedobór fenotypu „fast" w segregacji po skrzyżowaniu osobników o fenotypie F×SF (F=fast, S=slow; Ryc. 1), co sugeruje różnice w śmiertelności. Ponieważ więc zmienność transferyny u *Peromyscus maniculatus* zachowuje się jak cecha neutralna to może stać się ona dogodnym wskaźnikiem do testowania parametrów rozrodu.