esculenta ♀×*lessonae* ♂. Table II summarizes the morphological phenotypes, the genotypes for the B subunit, and the isozyme patterns of the parental female and male frogs, and those of the F_1 offspring in each of these crosses. The 5 *lessonae* ♀×*ridibunda* ♂ crosses provide the most clear-cut evidence for the hybrid nature of *esculenta*, since the experimental data agree perfectly with the prediction. However, a closer examination of the results of those crosses involving *esculenta* females or males showed that not all genotypes and isozyme patterns which were expected on the basis that *esculenta* consist of both *lessonae* and *ridibunda* genomes were observed. This can be explained by our finding that, in contrast to *lessonae* and *ridibunda*, eggs of the LDB^a/LDB^c *esculenta* female with pattern II showed only isozymes of pattern III, suggesting the presence of only the B^c subunit in the egg cytoplasm. Similarly, eggs of those LDB^a/LDB^b *esculenta* females with pattern VI showed only pattern V, indicating the occurrence only of the B^b subunit. The consistent absence of the B^a subunit, which originates from *lessonae*, demonstrates clearly that in the *esculenta* eggs only the *ridibunda* genome is retained. Analyses of the LDH isozymes in ovaries and testes suggest that the same event takes place during both oogenesis and spermatogenesis. In other words, in the female and male germ cells of *esculenta*, the genetic information from *lessonae* is always eliminated. With such a hypothesis, all observed isozyme patterns of the F_1 *esculenta* individuals can be explained.

It should be pointed out that the isozyme pattern VI in *esculenta* with the genotype LDB^a/LDB^b seems to be an exception, since inspite of the presence of 3 kinds of subunits (A, B^a, B^b) the zymograms showed only 5 bands (Figure 1). One line of evidence for the heterozygous condition of the B subunit in these frogs is that their most anodal isozyme occurs as 2 closely located subbands with an intermediate mobility between the most anodal band of pattern I (LDB^a/LDB^a) in *lessonae* and that of pattern V (LDB^b/LDB^b) in *ridibunda*. Owing to the small charge difference between the B^a and B^b subunits, it may be that the 15 isozyme bands expected were not resolved by our electrophoretic procedure. More extensive evidence is needed to clarify this point.

As to the morphogenetic changes of the LDH isozymes, our results can be summarized as follows: When eggs with the homozygous pattern I, III or V developed into larvae with the heterozygous pattern II or IV, the isozyme bands of such a heterozygous pattern became first detectable in embryos showing heart beat and at hatching (Shumway stage 19–20). This means that the paternal gene is activated only about 4–5 days after the beginning of development. Alternatively, when eggs showing heterozygous patterns developed into larvae with homozygous patterns, the maternal LDH isozymes persisted until about 14–20 days after hatching. This could be due either to a low turnover of the enzyme protein, or to the occurrence of stable mRNA. The same results have been reported by WRIGHT and MOYER[16,17] from their studies of parental influences on the LDH isozymes in various hybrid frogs.

Chromosomal Variation and Heterochromatin Polymorphisms in *Peromyscus maniculatus*

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Summary. Evidence is presented that chromosomal variation in *Peromyscus* results from 1. addition of heterochromatic short arms to acrocentric chromosomes, and 2. pericentric inversions. Constitutive heterochromatin polymorphisms contribute to variation in the amount of heterochromatin in *Peromyscus* populations.

Chromosome studies on *Peromyscus* have shown that although the diploid chromosome number is always 48, the total number of chromosome arms, or fundamental number (FN)[4], may vary greatly both between and within different subspecies populations[5–8]. Variation in the FN in *Peromyscus* originally was attributed to pericentric inversions[5,6]. However, based upon recent studies HSU has proposed that variation in the FN in *Peromyscus* results from the addition of heterochromatic short arms to acrocentric chromosomes[7,9]. The present study used both Giemsa banding and heterochromatin staining techniques to determine the basis for variation in both the FN and the amount of heterochromatin within a population of *Peromyscus maniculatus*.

Materials and methods. All *Peromyscus maniculatus* ssp. *nebrascensis* were trapped within 12 miles of Laramie, Wyoming. Animals were colchicinized 2 h prior to sacrifice. Bone marrow was flushed from the femur and tibia, incubated 20 min in 0.075 *M* KCl at 37 °C, and fixed 45 min in 3:1 methanol-glacial acetic acid. Cells were placed onto cold, wet slides and air dried. Chromosomes were stained with a urea Giemsa banding technique[10], photographed and destained. Constitutive heterochromatin was identified in the same cells from 10 individuals by the C-banding technique[11]. Homologous chromosomes were identified by the Giemsa banding pattern of their long arms. Chromosomes were classified according to the Giemsa banding pattern described for *P. m. nebrascensis*[12].

Results and discussion. The diploid chromosome number in *Peromyscus maniculatus nebrascensis* was 48 and the FN varied from 86 to 89 in 10 individuals studied

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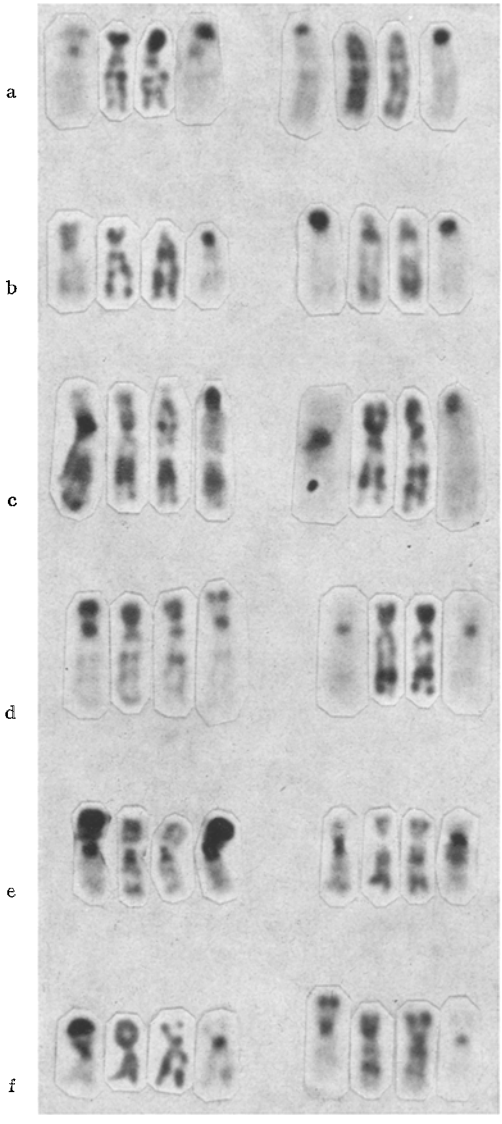
Chromosome constitution of 10 *Peromyscus maniculatus nebrascensis* for chromosome pairs showing structural or heterochromatin polymorphisms

Animal Number	Sex	2*n*	Number of biarmed chromosomes	FN	Number of heterochromatic short arms	Polymorphic chromosomes									
						6	7	8	9	10	12	13	16	18	*X*
L421	♂	48	38	86	10	b/A	B/A	b/b	A/A	B/B	B/B	B/B	B/B	B/b	b
L194	♂	48	38	86	11	b/b	A/A	B/B	A/A	B/B	B/B	B/B	B/B	B/b	b
L407	♂	48	39	87	10	b/b	A/A	b/b	B/A	B/B	B/B	B/B	B/B	B/b	b
L428	♀	48	39	87	11	b/A	B/b	b/b	B/A	B/B	B/B	B/B	B/B	B/b	b/A
L196	♂	48	40	88	10	b/b	b/b	b/b	B/A	B/B	B/B	B/B	B/B	B/b	A
L415	♂	48	40	88	10	b/b	B/B	b/b	A/A	B/B	B/B	B/B	B/B	b/b	b
L195	♂	48	41	89	10	b/b	b/b	b/b	B/B	B/b	B/B	B/B	B/B	B/b	A
L422	♂	48	41	89	10	b/b	B/B	b/b	B/A	b/b	B/B	B/B	B/B	B/b	b
L193	♂	48	41	89	10	b/A	B/B	b/b	A/A	B/B	B/B	B/B	B/B	b/b	b
L427	♀	48	41	89	11	b/b	B/B	b/b	B/A	B/B	B/B	B/B	B/B	b/b	b/b

A, acrocentric chromosome; B, biarmed chromosome with heterochromatic short arm; b, biarmed chromosome with euchromatic short arm.



Giemsa banding and C-banding patterns of polymorphic chromosomes in *Peromyscus maniculatus nebrascensis*. a) Chromosome 7 from L193 and L194; b) chromosome 9 from L196 and L194; c) chromosome 6 from L421 and L193; d) chromosome 8 from L194 and L193; e) chromosome 10 from L421 and L422; f) chromosome 18 from L195 and L194. × 3000 . For a further explanation of the figure see the text and Table.

(Table). Autosome pairs 6, 7, and 9 and the *X*-chromosomes were polymorphic in this population. The submetacentric and acrocentric forms of chromosome 7 and chromosome 9 had identical Giemsa banding patterns in their long arms; the short arm of the submetacentric forms of chromosome 7 (Figure a) and chromosome 9 (Figure b) were heterochromatic. These observations provide direct support for HSU's hypothesis that karyotypic changes can occur in *Peromyscus* by the addition of heterochromatic short arms to acrocentric chromosomes. However, the short arm of the submetacentric form of chromosome 6 lacked heterochromatin (Figure c), and has been identified on the basis of its Giemsa banding pattern as a pericentric inversion[12,13]. An *X*-chromosome pericentric inversion has also been described in this population[12,13]. Thus, variation in the FN in this population is attributable to two different mechanisms: 1. addition of heterochromatic short arms to acrocentric autosome pairs 7 and 9, and 2. pericentric inversions in chromosome 6 and the *X*-chromosome.

This population was homozygous for biarmed autosome pairs 12, 13 and 16 which had heterochromatic short arms (Table). Polymorphic autosome 9 always had a heterochromatic short arm whenever it was submetacentric (Figure b). The chromosome 9 polymorphism may be a transient polymorphism as the acrocentric chromosome 9 is replaced by its submetacentric form.

The amount of heterochromatin varied from individual to individual in this population (Table). If submetacentric autosomes arise in *Peromyscus* only through the addition of heterochromatic short arms to acrocentric chromosomes, then the number of heterochromatic short arms should be equal to the number of biarmed autosomes for each individual. This was not observed. For example, both L421 and L194 had an FN of 86 but had 10 and 11 heterochromatic short arms, respectively (Table). The lack of a direct correlation between the number of heterochromatic short arms and the number of biarmed autosomes indicates that other factors contribute to variation in the amount of heterochromatin in this population.

The biarmed autosome pairs 7, 8, 10 and 18 exhibited constitutive heterochromatin polymorphisms. L194 was homozygous for a submetacentric chromosome 8 with heterochromatic short arms, while in the other 9 individuals chromosome 8 was submetacentric and had

[13] J. D. MURRAY and R. M. KITCHIN, in preparation.

euchromatic short arms (Figure d). 8 *Peromyscus* were homozygous for a biarmed pair 10 with heterochromatic short arms, L422 was homozygous for a biarmed pair 10 with euchromatic short arms (Figure e), and L195 was heterozygous. 3 individuals were homozygous for a biarmed pair 18 with euchromatic short arms and 3 individuals were heterozygous and had 1 chromosome 18 with a heterochromatic short arm (Figure f). The structurally polymorphic autosome 7 was heterozygous for a heterochromatin polymorphism in L428 but had euchromatic short arms in L195 and L196. Heterochromatin polymorphisms could arise either by saltatory replication in the short arm of a submetacentric chromosome or by a translocation between the short arm of a submetacentric and the heterochromatic short arm of another chromosome. There was no evidence of translocation pairing configurations during spermatogenesis in any individual studied.

The present study demonstrates that variation in the FN in *Peromyscus* results from both the addition of heterochromatic short arms to acrocentric chromosomes and pericentric inversions. The amount of heterochromatin varied within this population due to changes in the number of biarmed chromosomes present in the karyotype and constitutive heterochromatin polymorphisms. The evolutionary significance of variation in the fundamental number and amount of heterochromatin in *Peromyscus* is unknown.

Frequency-Dependent Mating Success Among Mutant Ebony of *Drosophila melanogaster*[1]

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Summary. Measurements of the mating success of wild and ebony strains of *D. melanogaster* with different degrees of competition have shown a frequency-dependent effect in both cases, but with a negative correlation for the wild and a positive correlation for the ebony strain.

Frequency-dependent mating success, mainly among males of flies of the genus *Drosophila*, is a phenomenon broadly demonstrated at least under certain experimental conditions. The experiments developed by Petit[2–4] were more recently followed by several others with different species[5–8]. Nevertheless, the explanation of such behaviour is not yet well understood. Certain hypotheses have been advanced by Spiess[9], Ehrman[10], Ehrman and Spiess[11] in order to explain the experimental results without reaching general agreement.

Even with an apparent homogenity in the results it is possible that different causes are opperating in different genetical constitutions. On the other hand, different experimental designs may mask differences in the factors producing the frequency-dependent mating success. Different strains have different mating speeds which can be controlled either by the male, the female or both, depending of the strain. Sexual preference among certain strains has been suspected in some cases. The interaction between these components has not yet been completely studied. The scope of the present communication is to show a case in which the mating success of males of the wild genotype is more successful when rare, and the other genotype, on the contrary, is more successful when more frequent.

The experiment was done with wild type and ebony mutant strains of *Drosophila melanogaster*. The wild type stock was obtained from a mixture of 3 different strains: Oregon-R, Samarkand and Canton-S, and maintained in laboratory for about 3 years in large number. The ebony

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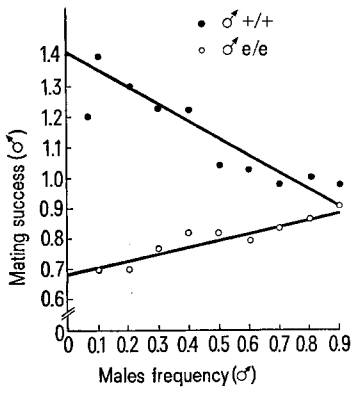

Regression lines of the mating success.

Table I. Mating success of wild and ebony males of *Drosophila melanogaster* in population cages with different compositions

Cage	Composition of male population		Total number of mating and the mating success of each genotype in 5 runs			
Cage	+/+	e/e	+/+		e/e	
1	18	2	88	0.98	7	0.70
2	16	4	80	1.00	14	0.70
3	14	6	68	0.97	23	0.77
4	12	8	62	1.03	33	0.82
5	10	10	52	1.04	41	0.82
6	8	12	49	1.22	48	0.80
7	6	14	37	1.23	59	0.84
8	4	16	26	1.30	69	0.86
9	2	18	14	1.40	82	0.91