Genetic Variance in the Prairie Deer Mouse

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Donna Pritchard

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Prairie deer mice (Peromyscus maniculatus bairdii) were obtained from Lethbridge, Alberta, Canada (49° 35' N) and Emporia, Kansas (38° 24' N) in order to examine the effects of latitude on genetic variance. The mice were put into a photoperiod cycle which consisted first of 12L/12D, then constant light (LL), followed by a return to 12L/12D, and finally, constant darkness (DD). The free-running locomotor activity of the two groups of mice was then compared. A significant difference was found between the circadian activity patterns of the northern and southern mice during exposure to both LL and DD. Genetic variance also was investigated by employing enzyme studies. This was done by gel electrophoresis of the isoenzymes of esterase, lactic dehydrogenase, and xanthine dehydrogenase. Both esterase and xanthine dehydrogenase showed positive evidence of genetic variation in band appearance and migration. In conclusion, this research showed that the genetic components of these two populations of the prairie deer mice vary latitudinally. This was evidenced by statistically significant differences in the endogenous locomotor circadian rhythms, as well as distinctly different enzyme banding patterns.

John Parrish
Approved for Major Department

Harold E. Durst
Approved for Graduate Council

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INTRODUCTION

The environmental variables affecting a given species or population should be understood when working with wildlife. These variables may be limiting factors which are responsible for regulating the growth or decline of a particular species. Examples of these factors are what constitutes a particular animal's range, what the nutrient needs are for that animal, what keeps it in a specific area, and what controls its growth or decline. Often attempts at reestablishing an animal have failed or were not fully successful due to lack of understanding of the animal's limiting factors. One of these factors is latitudinal variance and its influences upon a species or a population. Leopold (1966) cited an example of the latitudinal variance problem in black-tail deer from California which, when kept in Vancouver, did not adjust their breeding season to fit the later spring.

The present study was done to determine what effects latitudinal variation had on the genetic polymorphism of prairie deer mice (Peromyscus maniculatus bairdii). Gunderson (1976) described the range of the Prairie deer mouse as from the lower portions of Alaska through Central America. These small mice prefer areas of low precipitation in open grasslands. According to Burt (1977), prairie deer mice are grayish-brown in color with white underbellies and feet, plus a distinctive bi-color tail. Gestation is usually 21 days with average litter sizes between four and six. The breeding season is from mid-March to mid-November, depending upon seasonal temperature variations.

It has only been recently that genetic variations in species have been demonstrated by photoperiodicity and circadian rhythms. A study by Desjardins (1981) reported that white-footed mice (Peromyscus leucopus) from 60°N and 30°N latitudes exhibited differences in

reproductive fitness in response to short-day photolengths. Lynch et al (1979) while conducting a similar study, found that P. leucopus exhibited considerable intraspecific variability in the photoperiodic regulation of seasonal reproduction. Lynch and co-workers further stated that the more northern populations regulated their breeding more closely in accordance with photoperiod than did the southern populations. Those results contrast with an earlier study that compared wild mice and domestic mice (Mus musculus), in which Bronson (1979) found no significant importance to photolength. Connolly and Lynch (1981), however, recently found evidence for genetic variations in four inbred strains of the house mouse (Mus musculus). This was shown by direct correlations between the circadian rhythms of body temperature and open-field locomotor activity.

With the supportive evidence given above for genetic variance in mice, it was decided to examine genetic variation in deer mice by investigating their circadian rhythm patterns and their banding patterns by electrophoresis. The circadian rhythms examined were the free-running locomotor activities in the mice under constant light and dark.

Gel electrophoresis was performed to examine enzyme polymorphism in the body tissues of the mice. There are ten enzymes which are essential for 90 % of glucose utilization and energy metabolism (Clifford et al 1979). The simplest process for examining the difference in these enzymes utilized in metabolism is that of electrophoresis. In a paper on genetic polymorphism in mink plasma, Juneja et al (1981a) used gel electrophoresis as a determining technique. Juneja concluded that electrophoresis was an effective technique for dealing with protein polymorphism. Juneja et al (1981b) also determined genetic polymorphism

in the plasma proteins in rabbits utilizing electrophoresis. In those studies, several different breeds of rabbits were tested and found to show considerable polymorphism in the serum post-albumin proteins. The work conducted by Piletz and Ganschow (1981) also used electrophoresis to determine variations in the milk proteins of 58 inbred strains of house mice. Two of the 58 strains of mice showed definite variance in the milk proteins, further demonstrating the sensitivity of this technique for studies of population genetics.

The work performed in this research examined the enzyme polymorphism of esterase (EST), lactic dehydrogenase (LDH) and xanthine dehydrogenase (XDH). LDH was chosen because it has shown polymorphic bands in the red blood cells (Hillman and Finch, 1979).

Esterase is a hydrolytic enzyme which acts as a catalyst for introducing water into a specific bond in the substrate. Esterase hydrolyzes the ester linkage of glycerides resulting in the products of fatty acids and glycerol (Lehniger, 1973).

Lactic dehydrogenase (LDH) acts as a catalyst in the reduction of L-lactate to pyruvate. This process results in nicotinamide-adenine dinucleotide (NAD) and L-lactate being reduced to pyruvate and NADH(H+) with LDH as the oxidation agent (Hawk, 1965).

Xanthine dehydrogenase, which was found to be polymorphic in nature by Ho et al (1978) in the liver and milk of animals was the last enzyme studied. Xanthine dehydrogenase's catalytic action involves the oxidation of hypoxanthine to xanthine and then into uric acid. The enzyme also may be responsible for a certain degree of catalytic oxidation of aldehydes (Hawk, 1965).

METHODS AND MATERIALS

Animals

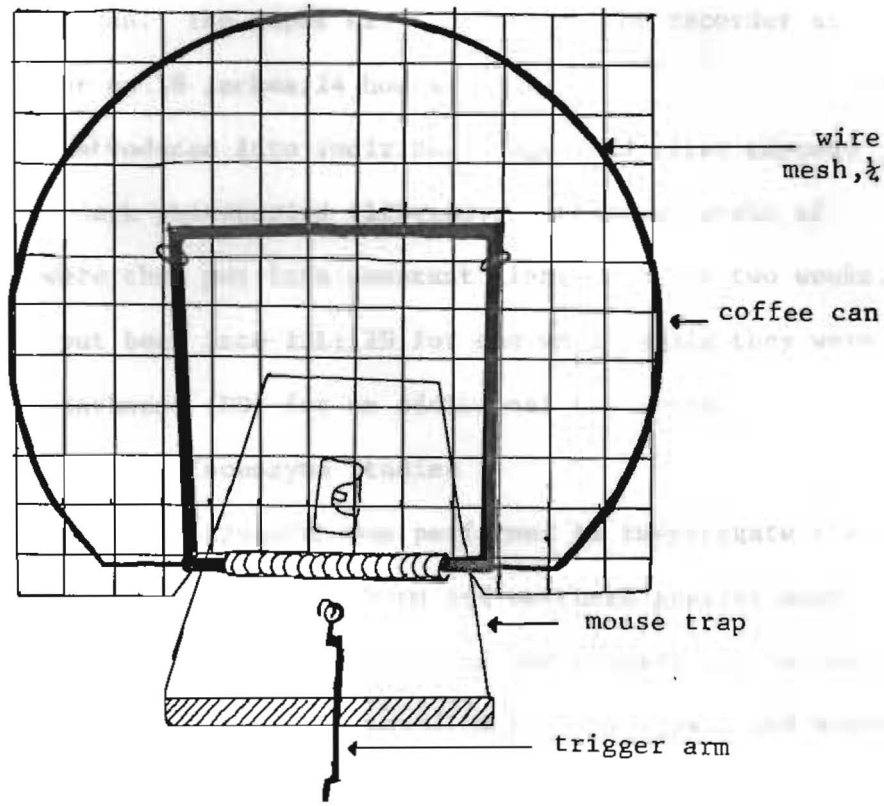
The specimens from the southern population of P. maniculatus bairdii were live-trapped outside of Emporia, Kansas, during late September and early October of 1981. The location was a pasture of native tallgrass prairie five miles west of Emporia. The mice were caught in live-traps (Figure 1) baited with peanut butter and milo. The traps were set 20 meters apart and checked both in the morning and at night. After the mice were caught and brought into the laboratory, there were two litters born. With the birth of six pups to each of those litters, it was decided to use both the wild adults and their young in the current research.

The northern mice were trapped by Dr. Bruce McMullin of the University of Lethbridge in Lethbridge, Alberta, Canada. They were then shipped via air from Canada to Kansas City, Kansas, in early June of 1982 and then transported to Emporia, Kansas. Fifteen adults and five young were received. Three weeks after arrival in Kansas, two of the northern females gave birth to six young apiece. Both the adults and the young mice were used in the photoperiod studies.

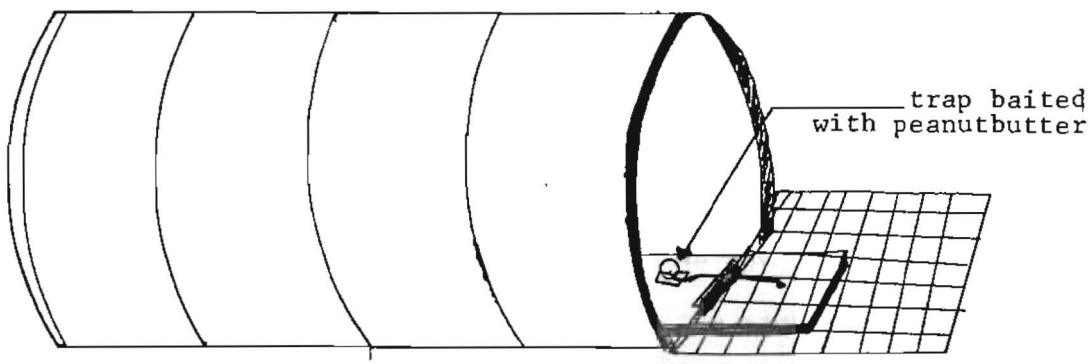
Finally a male from the northern litter was mated with a female from a southern litter of the same age. This was done to determine if they would successfully produce offspring.

Photoperiodic Studies

The photoperiodic responses were measured by the use of a free-running wheel attached to an Esterline-Angus event recorder. The recorder was triggered by a magnet on the wheel as it rotated past a reed switch. As the magnet became aligned with the reed switch, the switch closed and current was carried to a solenoid, which caused the



Trap closed, front view



Trap open, side view

Figure I. Design for livetrap.

pen on the recorder to deflect. The recorder measured when and how frequently the mouse ran. The paper was fed through the recorder at a rate of 3/4 inch/hour or 18 inches/24 hours.

The mice were introduced into individual cages and first exposed to a 12 light to 12 dark photoperiod (12L:12D). After two weeks of 12L:12D, the mice were then put into constant light (LL) for two weeks. The mice were next put back into 12L:12D for one week before they were subjected to total darkness (DD) for an additional two weeks.

Isoenzyme Studies

The second part of the research was performed to investigate the differences in isoenzymes between northern and southern prairie deer mice. Gel electrophoresis was used to examine and compare the isoenzymes of the mice. The technique consisted of placing tissue and serum samples on paper wicks and inserting the wicks into a gel. The gel was then placed into a tray with a buffer solution and an electrical current was run through it. The enzymes were then drawn across the gel to the positive or negative pole, opposite to their own charge. A more highly charged enzyme travelled a greater distance from the place of insertion. The isoenzyme bands were visualized when the gel was placed in the proper stain. The location and appearance of those bands was an indication of what type of isoenzyme was present in the tissue or serum smaple. The variations in the bands between the samples from the northern and southern mice were an indication of genetic variances between the mice.

Techniques for Sample Preparation from the Mice for Electrophoresis

Step 1. The mice were etherized and the chest cavity opened. A cardiac puncture was made to obtain the blood, resulting in the death of the

mice. The blood was immediately mixed with a 1 % solution of Ethylenediamine tetraacetic acid (EDTA) dissolved in distilled water, in order to prevent coagulation.

Step 2. The body cavity was further opened and the liver and kidneys were removed. Those were weighed to the nearest mg and placed on ice.

Step 3. The organs were rapidly homogenized in a solution of 1 ml of gel buffer to 100 mg of tissue. The samples were then divided into three vials per sample and labeled. All samples were frozen at -60°C to retard any enzyme breakdown until electrophoresis.

Procedure for Electrophoresis

Step 1. A starch gel was made by suspending 27.5 grams of Hydrolysis starch (Sigma Chemical Co., St. Louis, MO) in 220 ml of gel buffer and heating it to just before a boil. The gel solution was vacuumed as soon as air bubbles were observed coming off the bottom, to remove excess air. The gel was then poured on to electrophoresis plates, allowed to set up and then covered with Saran Wrap and refrigerated until use. Because the gels tended to dry out, they were prepared just prior to use.

Step 2. Just before usage, the gel was allowed to reach room temperature, and excess moisture was removed by blotting. The gel was then cut lengthwise, down the middle.

Step 3. The homogenized tissue samples were allowed to thaw out just prior to use. Paper wicks, cut to fit the depth of the gel, were soaked in the sample and then placed in the lengthwise cut, approximately one cm apart. The samples from Northern and Southern mice were placed on the gel alternately. A control wick was prepared by being soaked in a sample of homogenized fruit flies.

Step 4. The gel plate was then set in a Gelman electrophoresis apparatus and the appropriate pH control-buffer was added (See Appendix). Larger paper wicks were used to bridge the buffer solution to the gel, and were inserted in such a way as to form a complete circuit. The tray had two compartments; one negative, one positive. When the electrical current was applied, the enzymes were pulled across the gel and separation of the isoenzymes occurred.

Step 5. After a specific amount of time had passed, in accordance with the enzyme being tested, the gel was removed from the buffer. The gel was then stained according to the particular enzyme system (See Appendix). If more than one enzyme was being tested, the gel was sliced into thin layers and the appropriate stain applied.

Step 6. After the gel slices had been stained and the isoenzymes bands had appeared, they were fixed in 7 % acetic acid for 10 minutes. The fixing agent was necessary to discontinue further enzyme reactions and reactions and separation. The fixing agent was then rinsed off, and the gels wrapped in Saran Wrap for storage under refrigeration. The gel bands were measured and recorded.

A second procedure for analyzing the quantity of LDH in the liver samples was conducted by St. Mary's Hospital laboratory. The analysis was done by use of a DuPont Automatic Clinical Analyzer (ACA) on the already prepared samples, after they were diluted with a Tris buffer. The ACA measured the level of absorbance of NADH at 340 nm over a 17.07 second period, which was directly proportional to LDH activity in the sample. This was accomplished following a step by step process in which the LDH enzyme from the sample material was allowed to react with a substrate of L-lactate and NAD+, resulting in the oxidation of L-lactate

to pyruvate, and the reduction of NAD+ to NADH (H+).

RESULTS

Circadian Rhythms

Both the northern and southern deer mice were equally entrained by the 12L:12D photoperiod. The comparison of the results of the free-running locomotor activities between the two strains of mice during constant light, however, showed significant differences ($p<0.05$) in the free-running period (tau) (Figure 2). The southern mice's free-running period averaged 27.14 hours for two mice out of the five; the three remaining mice did not run at all. In contrast, the average free-running period for the five northern mice in constant light was 30.59 hours. The free-running period also was found to be significantly different ($p<0.05$) between the two strains of mice during the total dark period. The average free-running period for the southern mice during total darkness was 21.22 hours, while the average period for the northern mice was 24.24 hours (Figure 2). Significant differences were determined for these results by use of the Student t-test.

Enzyme Polymorphism

The first attempts at electrophoresis were done for the isoenzymes of LDH. Both a Paulik's Gel and JRP gel buffer system (Ayala et al 1972) were tried to determine which would give the best results. Paulik's Gel was the easiest to handle during staining and after fixing.

The esterase enzyme was tested next. Again both the Paulik's Gel and JRP gel systems were used (See Appendix). Both of the systems gave satisfactory results with regard to distinctive banding and ease of handling. The results of a six hour run gave bands that were visible, but not distinctive. Also, runs which were done with a voltage below 350V did not give the desired clarity in banding. After several attempts, it was found that they were clearer when one of two possible systems

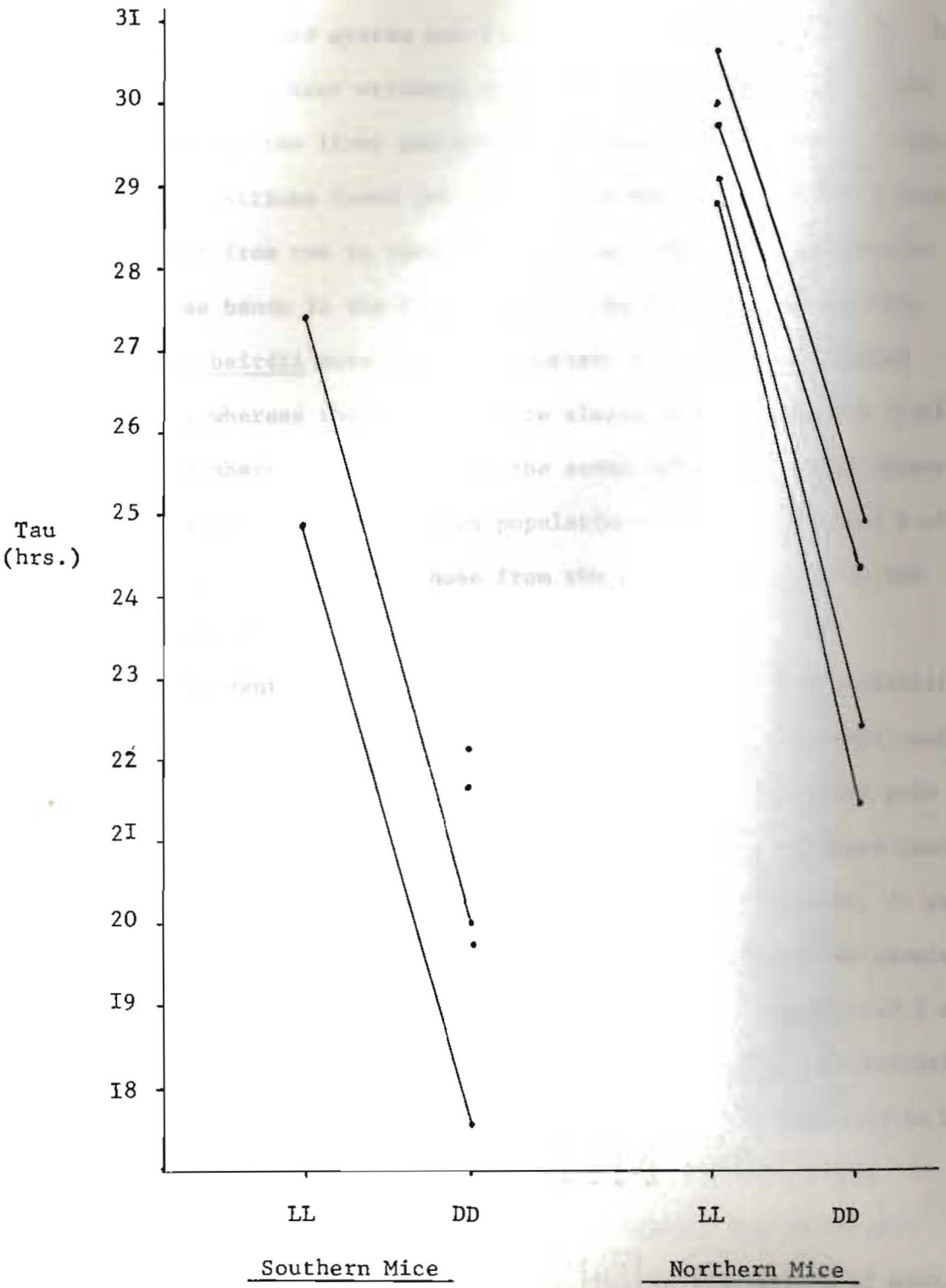


Figure 2. Circadian rhythms in the period (Tau) of free running locomotor activity in P. maniculatus from 38 N and 49 N latitudes in response to constant light (LL) and constant dark (DD).

were used. The first system used was an 18 hour run at a voltage of 350V/20 ma. The second system was a run for 9 hours at 500V/20 ma. In each case there was clear evidence of variation or polymophism in the esterase enzyme in the liver and kidneys of the mice (Figure 3). There were ten band positions found for esterase in the liver, of which individual mice had from two to five of the bands. The major differences in the esterase bands in the liver between the northern and southern P. maniculatus bairdii were that the southern mice always possessed bands A and C, whereas the northern mice always possessed band B (Table 1). They both shared a variation in the seven other bands that appeared. In contrast, mice from the northern population all exhibited band B of esterase in the kidney, while those from the southern population had either band B or C (Table 1).

Banding in xanthine dehydrogenase, however, showed less variability, but still distinctive differences were apparent. There were four bands that were detected, two of which moved to the positive (cathode) pole and two of which migrated to the negative (anion) pole. Of those bands, the liver of the mice from the northern population had bands B+, A- and B-, although the two anion bands were not shared throughout the sample population (Table 2). The southern population had A+, and only 25 % of the population had the anion band, B-. Additionally, the concentration of the liver xanthine dehydrogenase in the northern mice appeared to be higher, as evidenced by a much heavier band than occurred in the southern mice (Figure 4). The kidneys, in contrast, failed to show distinctive banding patterns in either of the two populations of mice.

Finally, the bands for lactic dehydrogenase were examined, and found to exhibit no noticable differences between the two populations (Figure 5). However, the enzyme activity of LDH in the liver was

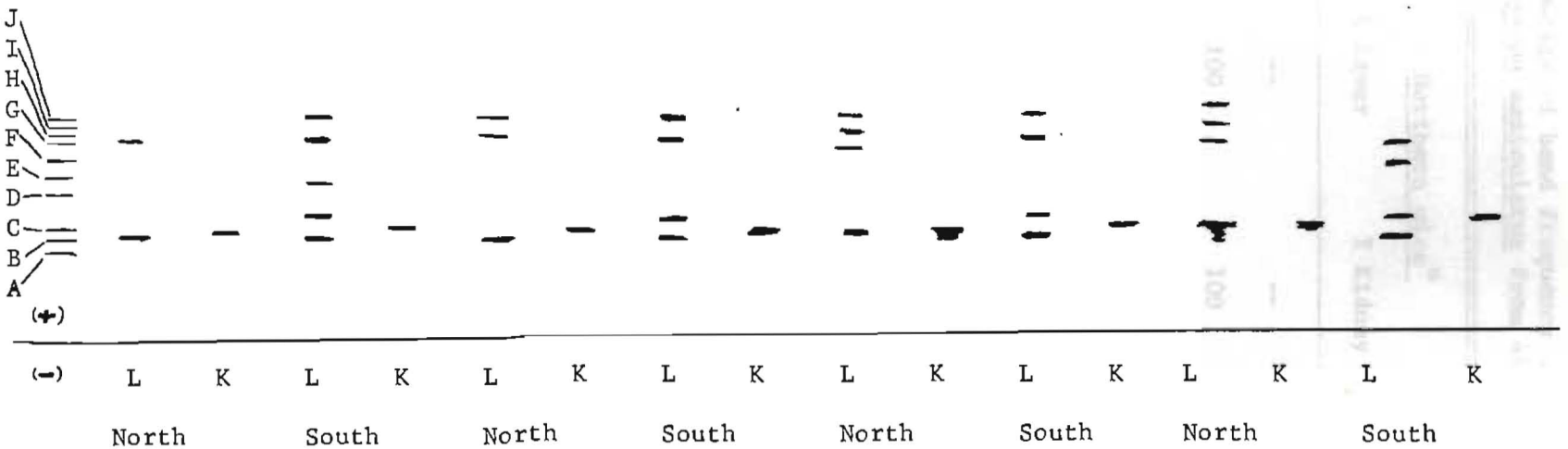


Figure 3. Isoenzyme bands for esterase in the liver and kidneys of P. maniculatus from 49°N and 38°N latitude.

TABLE 1. Percentage of band frequency of esterase in tissues of *Peromyscus maniculatus* from 49°N and 38°N latitude.

	Northern mice[a]		Southern mice[a]	
Bands	% Liver	% Kidney	% Liver	% Kidney
A	--	--	100	--
B	100	100	--	25
C	--	--	100	75
D	--	--	25	
E	--		25	
F	50		25	
G	50		50	
H	50		--	
I	50		25	
J	--		25	

[a] Sample size equal four mice in each group.

TABLE 2. Percentage of band frequency of xanthine dehydrogenase in the liver of Peromyscus maniculatus from 49°N and 38°N latitudes.

	Northern mice[a]	Southern mice[a]
Bands	% Liver	% Liver
A+	--	100
B+	100	--
A-	50	--
B-	25	25

[a] Sample size equals four mice in each group.

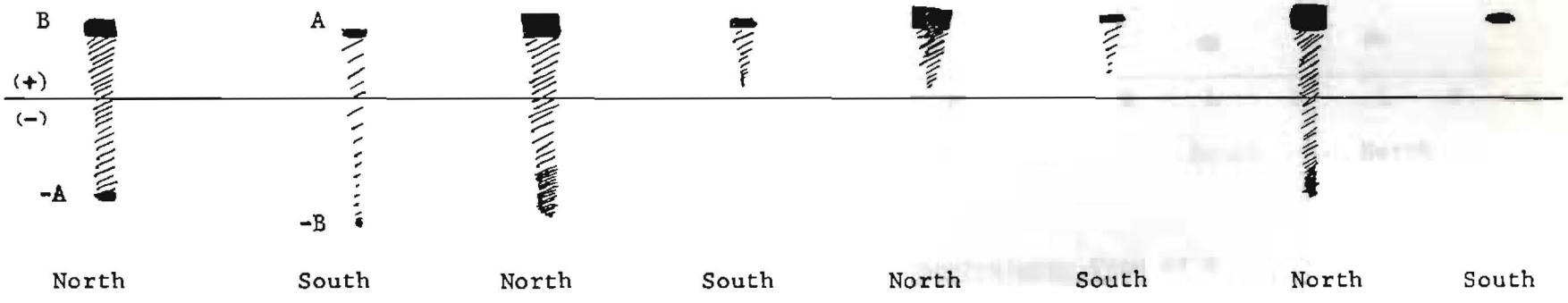


Figure 4. Isoenzyme bands for xanthine dehydrogenase in the liver of P. maniculatus from 49°N and 38°N latitude.

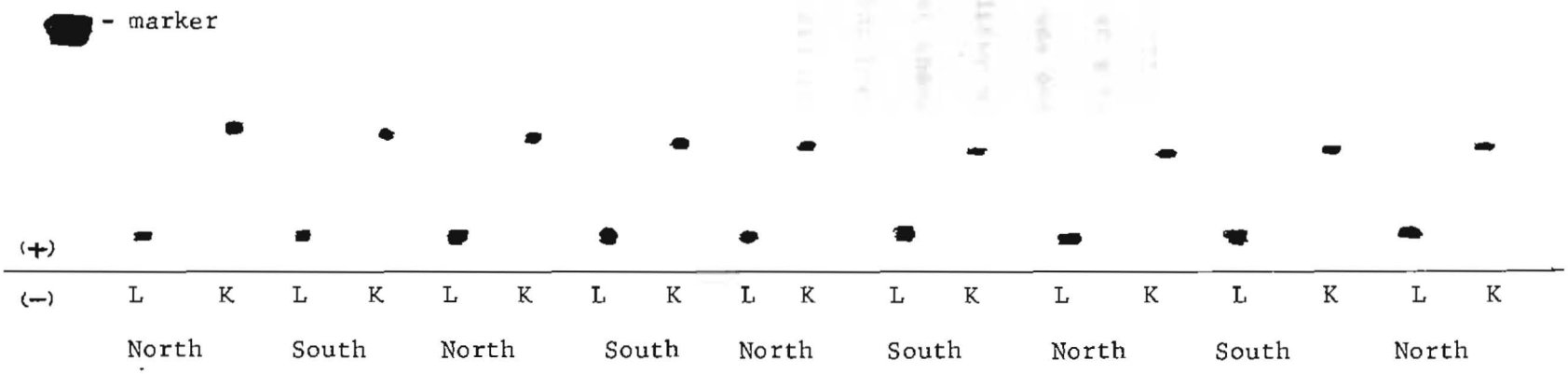


Figure 5. Isoenzyme bands for lactic dehydrogenase in P. maniculatus from 49°N and 38°N latitude. (L=liver, K=kidneys).

found to be somewhat greater in the mice from the northern population (4330 IU/L) than the level of LDH in the southern population of mice (3536 IU/L).

Besides the results obtained in the photoperiod and enzyme electrophoresis studies, there were several other interesting observations noted. In handling and caring for the mice, differences were observed in the physical characteristics of the northern mice compared to the southern mice. One of these was that the northern young appeared to grow and reach maturity at a faster rate than the southern young in a lab environment. This was noted when a litter of northern pups was born two days after a litter of southern pups, but were weaned first. Later it was noticed that these same northern juveniles quickly lost their soft gray coloring for the browner pelage, typical of the adults, while the southern young did not exhibit that change until approximately two weeks later. Another interesting observation which occurred was that as the days began to shorten in the subsequent fall, the northern mice began to gain weight and their coats became distinctively grayer than those of the southern population. The southern population did not seem to have a distinct color change with the onset of the winter photoperiod.

DISCUSSION

The responses of animals to photoperiod have been used as a measure of the genetic variation among many different species of the animal kingdom. An early report by Kennedy, et al (1973) presented evidence which clearly indicated a genetic control of the regulatory mechanism that permitted the constant shifting of the activity period of golden hamsters (Ochotomys nuttalli) to change in photoperiod. More recently, Possidente and Hegmann (1980) found that there was a positive genetic correlation between the phase angle and the period of endogenous circadian rhythms in five inbred strains of the house mouse. Those workers also suggested that the endogenous circadian rhythms would likely respond to natural selection as a single "complex", under a common gene control. In a later article Possidente and Hegmann (1981) suggested that the genetic differences that control phase shifts in relation to photoperiod may be found in either a circadian pacemaker or the visual system. Regardless of the site of the control system, both articles present convincing evidence that photoperiodic responses are controlled by genetic mechanisms.

Elliott and Goldman (1981) reported that while the Syrian hamster (Mesocricetus auratus) was found to be entrained by an extremely short photoperiod (1L:23D), testicular regression occurred because the photoperiodic hamster requires a photoperiod of at least 11.5 hours or more in order to maintain reproductive competence. During exposure to long photoperiods, pineal melatonin levels are low near activity onset in hamsters, while during exposure to short photoperiods, melatonin levels are high near activity onset. Similar results have been demonstrated in the pineal melatonin levels in white-footed mice (P. leucopus) from northern latitudes (Lynch et al 1982). In marked contrast, pineal

melatonin levels in a southern population of the same species were essentially the same, regardless of whether they were in a short or long photoperiod. Those results correlate with the fact the northern populations of white-footed mice are photoperiodic, while southern mice are year-round breeders. The fact that melatonin levels are low near activity onset in the southern mice prevents the antigonadal activity of melatonin that occurs in the northern mice, which have high melatonin levels near activity onset in short photoperiods.

Another important aspect of photoperiodic regulation of reproduction in hamsters was demonstrated by the finding that testicular regression did not occur in animals exposed to a photoperiod of 1L:22.34D (Elliott and Goldman, 1981). It also was found that as the light stimulation was shifted to the 23.34 hour days, the locomotor activity period also shifted. Under those conditions, melatonin would be predicted to be low near activity onset. Although data are not yet available to substantiate that idea, it is implied from the results of a study of the effects of a three-hour phase advance on pineal melatonin in the Djungarian hamster (Phodopus sungorus) by Yellon, et al (1982). Those workers found that pineal melatonin was reduced near activity onset when hamsters were exposed to the three-hour phase advance. The southern population of mice in the present experiment, which had a free-running activity period of 21.22 hours in DD (Figure 2), thus would be exhibiting the equivalent of a daily three-hour phase advance. One might assume that if melatonin levels were low near activity onset that the southern deer mice would not be photoperiodic and would remain reproductively active year-round. Further research is needed to confirm that such is the case.

The data obtained from the enzyme studies permit one to draw several conclusions. The first, and most important, is that the northern population is more heterogenous than the southern population (Figures 3 and 4). The mice from the northern population showed more individual variability in the banding in two of the three enzymes tested. Of the three enzymes, LDH was the least affected by latitude, as evidenced by the fact that there were no noticable differences in the banding patterns between the two populations (Figure 5). Xanthine dehydrogenase showed definite genetic variation between the two populations, however, very little individual variance occurred within each population. Eighty percent of the mice from the northern population had two XDH bands, while only 20 % of the mice exhibited two bands (Table 2).

The Esterase enzyme was the most variable or sensitive to latitudinal variance (Table 1). In the representatives from the northern population, 25 % of the mice demonstrated two bands, 25 % showed three bands, and 50 % had four bands. In comparison, 75 % of the mice in the southern population had four bands and 25 % exhibited five bands. Esterase was not only the most variable of the enzymes among the three proteins studied, but it also was the most variable in the northern population. A population-dependent variability in esterase also was demonstrated by the work of Tegelstrom and Ryttman (1981) in the house mouse. In that study it was found that esterase not only showed population and sexual differences in the mouse, but also that the organs with the greatest variability were the liver and kidneys. The present findings demonstrated a correspondingly high degree of variability of esterase in the liver in deer mice, as well (Figure 3). A possible reason for the high variability in esterase could be related to the

metabolic differences between northern and southern animals (Vaughan, 1978). Since esterase catalyzes the breakdown of fats and lipids into fatty acids and glycerol in the liver, it is possible that differences in the esterase isoenzymes between the northern and southern deer mice may be related to the importance of lipids to hibernation in the more northerly populations of the mice.

It can be concluded from the present research that photoperiod can be used as an indication for possible genetic variation in mice. It also can be concluded that the combination of the marked differences in both the photoperiod studies and enzyme studies imply that the northern population and the southern population of P. maniculatus are not genetically identical. However, since representatives of the two populations were capable of mating and producing offspring in the laboratory, it must be concluded that they are not two separate species, but different populations of the same species. It is possible, however, that with the prolongation of the physical separation of these two populations, further genetic variations will likely result, and that two distinct species might eventually be produced.

SUMMARY

Prairie deer mice were obtained from Lethbridge, Alberta, Canada (49° 35' N) and Emporia, Kansas (38° 24' N) in order to examine the effects of latitude on genetic variance between the two populations. Two methods were utilized to examine the mice for genetic variance. The first method was an examination of the effects of photoperiod on free-running locomotor activity. This procedure involved placing the mice into first 12L:12D then into constant light followed by a return 12L:12D and finally they were placed into total dark. In both constant light and total darkness, significant differences were found which implied that there was genetic variance due to latitude.

The second method involved a comparison of the isoenzyme patterns of esterase, lactic dehydrogenase and xanthine dehydrogenase between the northern and southern mice. Utilizing gel electrophoresis, it was determined that the enzyme esterase was the most influenced by latitude and lactic dehydrogenase was not affected at all.

In conclusion, it was found that northern mice are more heterogeneous and responsive to photolength than the southern mice. In addition, the southern mice appeared to exhibit greater enzyme heterogeneity than the mice from the northern population. These data present evidence that both endogenous circadian rhythms and enzyme polymorphism are useful in the evaluation of genetic variance in the gene pool of cosmopolitan species such as Peromyscus maniculatus.

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APPENDIX

APPENDIX

Buffers used for electrophoresis (Ayla et al, 1972)

Paulik's Gel Buffer pH 8.65

18.6 g Tris
2.3 g Citric acid (Monohydrate) or
2.1 g Citric acid (anhydrous)

Distill H_2O to 2 liters

Paulik's Bridge Buffer pH 8.1

38.2 g Boric Acid
5.04 g NaOH

Distill H_2O to 2.1 liters

JRP Gel Buffer

120 ml *New* Bridge Buffer
.82 g EDTA

H_2O to 1.8 liters

JRP Bridge Buffer pH 8.1

29.43 g Tris
14.87 g Citric Acid
.82 g EDTA

H_2O to 1.8 liter

New Bridge Buffer pH 7.1

35.97 g Tris
19.87 g Citric acid (monohydrate) or
18.17 g Citric acid (anhydrous)

H_2O to 2.2 liters

Enzyme Stains

Esterase

30 mg a naphtyl acetate to 3 ml acetone
.4 g of Fast blue RR Salts

H_2O - 195 ml
Incubate at 37°C

LDH (lactate dehydrogenase)

100 ml 0.5 M Tris - HCl pH 7.1
20 mg Nitro Blue Tetrazolium
25 mg B Nicotinamide Adenine
Dinucleotide (B-NAD) Grade III

To 10 ml of Substrate:

85 % DL-lactate 10.6 ml
1 M Na_2CO_3 - H_2O 49 ml

H_2O to 100 ml

Keep flask cool during mixing
Add PMS (Phenazine Methosulfate) after incubating at 37°C.

XDH (Xanthine dehydrogenase)

100 ml 0.05 M Tris - HCl pH 7.5
100 mg Hypoxanthine

Heat to boiling and cool to room temperature.

Add:

20 mg Nitro Blue Tetrazolium (NBT)
25 mg Nicotinamide Adenine Dinucleotide
15 mg KCl
5 mg Phenazine Methosulfate (PMS)

Incubate at 37°C