JOURNAL
OF THE PENNSYLVANIA ACADEMY OF SCIENCE

Founded on April 18, 1924

ISSN: 1044-6753

January 1998
Volume 71, Number 2

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Easton, PA 18042-1778

Contents

Journal Information 54

BIOLOGY: Localization of Lysozyme Using Immunohistochemistry In Rat Salivary Glands 55
Charlotte Zalewsky, Kathleen Dobrosielski-Vergona and Lynn Zombeck

BIOLOGY: Relationship Between Food Distribution, Aggression and Sexual Behavior in Sib and Non-Sib Male Mice (*Mus musculus*) 61
Fred J. Brenner

BIOLOGY: Avian Food-Storing Behavior: A Review 66
Jennifer C. Isherwood-Iobst

BIOLOGY: Relationship Between Forest Fragmentation and Woodlot Characteristics on Breeding Birds 73
Fred J. Brenner and Rebecca Berad

BIOLOGY: A Morphological and Allozymal Study of a *Cystopteris tennesseensis* (Dryopteridaceae) Population: First Report of the Species in Lancaster County, PA 78
James C. Parks and Karissa A. Mosser

GEOGRAPHY: Pennsylvania Labor Market Area Migration, 1985 to 1990: Responses to Changing Employment Opportunities 84
Mark E. Reisinger and James P. Lewandowski

GEOLOGY: Geologic and Hydrologic Problems Encountered in the Construction and Operation of the Union Canal in Pennsylvania 90
John R. Ousey Jr., Ruth Egan and William Maun

DARBAKER PRIZE 96

ACADEMY OFFICERS 1997–1998 96

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(Continued to inside back cover)

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A Publication of
The Pennsylvania Academy of Science

Journal of the Pennsylvania Academy of Science 71(2):55-60, 1998

LOCALIZATION OF LYSOZYME USING IMMUNOHISTOCHEMISTRY IN RAT SALIVARY GLANDS

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ABSTRACT

Lysozyme is an important biological molecule secreted in saliva and other biological fluids of humans and other mammals and characterized by its antimicrobial properties. Renewed interest in this ubiquitous molecule has occurred as a variety of research studies show correlations between the presence of lysozyme and the progression of certain carcinomas and changes in the concentration of lysozyme in inflammatory bowel disease, dedifferentiation of tissues and intraabdominal abscesses. This study describes the localization of lysozyme using immunohistochemical techniques in rat salivary glands, since rats are a common experimental model and little work has been done describing the cellular location of lysozyme in rat salivary glands. Lysozyme was detected by immunohistochemical staining in both major and minor salivary glands. The ducts of these glands were strongly positive for lysozyme including intercalated, striated, granular and interlobular ducts suggesting that the entire length of the ductal system and its various, differentiated areas are involved in the storage of lysozyme and perhaps its synthesis. In contrast, the seromucous and mucous acinar cells whose secretions drain into the ductal system were negative for lysozyme with the exception of the seromucous acini of the minor salivary gland. These results suggest future studies examining the role of the salivary gland ductal system in determining the composition of saliva.
[J PA Acad Sci 71 (2) 55-60, 1998]

[1]Received for publication 7 September 1996; accepted 18 July 1997.

INTRODUCTION

Since the discovery of lysozyme by Fleming in 1922, this bacteriolytic enzyme has been the focus of attention in research and academic communities. Lysozyme is produced by animal tissues and can be found in several secretions including saliva, tears, gastric secretions and respiratory mucus. Lysozyme may be a valuable marker for various disease states and in following the course of these diseases. Several pathological conditions have been associated with an increase in lysozyme concentrations including various carcinomas, the dedifferentiation of tissues, inflammatory bowel disease and intraabdomi-

nal abscesses (3, 16, 18). By following the presence of lysozyme in saliva, a non-invasive method for monitoring disease states could be established. Lysozyme may also have therapeutic potential for oncology patients, since the enzyme may inhibit murine ascitic tumors (4). The widespread presence of lysozyme among organisms leads to inquiry about where the enzyme is located.

Lysozyme has been localized in salivary glands of humans (6,8,9,10,11,12,13,15), rats (17) and other vertebrates (14). Sites of localization vary depending on the organism, type of salivary gland and cell type. The results have been conflicting due to the differences in histochemical localization techniques and in antibody specificity of immunocytochemical techniques. The purpose of this study was to describe the localization of lysozyme in rat salivary glands, since rats are a common experimental model, and to compare these results with human studies.

MATERIALS AND METHODS

Salivary glands were removed from five metafane anesthetized white male rats (Sprague Dawley) under the direction of the staff at the University of Pittsburgh's Animal Care Center and in accordance with the University's policy on care and treatment of laboratory animals. Parotid glands and submandibular glands with attached sublingual glands were isolated, removed and fixed in either 2% paraformaldehyde in Van Muralt's phosphate buffer, pH 7.4 or Histochoice fixative (AMRESCO; Solon, Ohio) to which 95% ethanol is added at a 20% concentration. Glands of each rat were fixed and dehydrated, cleared in xylene, embedded in paraffin and sectioned at 5-8u onto sialinized Probe-on Plus slides for immunocytochemistry (2).

Immunocytochemical staining kits (Lymph-scan) from Biomeda (Foster City, CA) were used for the detection of lysozyme. The specificity of the primary antiserum, rabbit anti-lysozyme, has been determined by Biomeda Corp. (Foster City, CA) and is recommended for the detection of lysozyme in rat tissues. Rabbit polyclonal antiserum to human ly sozyme was used in conjunction with streptavidin Biotin Complex (S-ABC), a peroxidase tracer system and chromogen stain. The staining sequence consisted of 1. exposure to primary antibody (30 minutes at room temperature or overnight at 4 C); 2. secondary antibody conjugated to Biotin (30 minutes room temperature); 3. streptavidin-peroxidase conjugate (30 minutes at room temperature): 4. chromogen reagent including H_2O_2 and chromogen (30amino, 9 ethyl carbazole; 15 minutes at room temperature); 5. aqueous hematoxylin. All reagents were pre-diluted and ready to use. Endogenous peroxidase activity was blocked using 2% H_2O_2 in methanol prior to incubation in the primary antibody. A Micro Probe Staining System from Fisher Scientific (Pittsburgh, PA) which uses reagent conserving capillary technology and allowed the staining of up to 20 slides simultaneously was used. Negative controls were run in which the primary antibody to lysozyme was omitted and replaced with buffer.

RESULTS

The following immunohistochemical findings were based on multiple samples of tissues taken from five rats. Histochemical results were similar among the five rats. Figure 1 shows elements of the structure and organization of the submandibular salivary gland.

SUBMANDIBULAR GLAND

Figure 2 illustrates the intense positive staining for lysozyme as indicated by the red chromagen stain. Seromucous acini show no immunostaining for lysozyme (Arrows). Striated ducts lined with cuboidal epithelium are positive and stain red with intense staining of these cells at their apical surface facing the lumen. Figure 3 shows the absence of staining in a negative control. Figure 4 shows that the granular ducts of submandibular glands also stain positively (Arrows); the flocculent content of the granule area is apparent (non-granular cells of these ducts do not stain). Surrounding serous acini are negative. Larger interlobular excretory ducts also stain positively.

PAROTID GLAND

Seromucous acini stain negative. Intercalated ducts lined with flattened and cuboidal epithelium stain red and are positive. Figure 5 shows a larger excretory duct composed of stratified cells which stain red and are also strongly positive for lysozyme.

SUBLINGUAL GLAND

Mucous tubules stain negatively for lysozyme; however, seromucous demilunes are distinctly positive and stain red (Arrows in Fig. 6). Striated ducts and excretory ducts stain red and more intensely than the striated ducts of submandibular and parotid glands.

MINOR SALIVARY GLAND

This gland lies adjacent to the sublingual and consists of serous or seromucous acini. Serous acini stain intensely red and intracellular granules are visible. Striated ducts are positive and staining is more intense than in the major salivary glands. (Fig. 7)

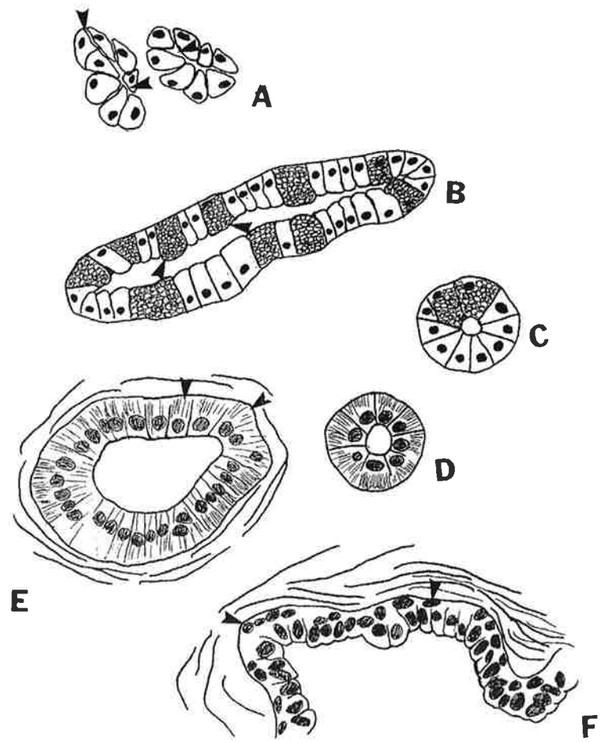

Figure 1. Microscopic Anatomy of the Submandibular Salivary Gland. These drawings illustrate the seromucous acini and cross-sectional profiles of the ductal system of the submandibular gland. (Granular ducts (b&c) are found only in rat submandibular gland.) The other ducts are common to rat and human. a. Seromucous acinar cells with basal nuclei have apical surfaces which face the intercalated ducts (arrows) that mark the beginning of the ductal system. (In sublingual gland, mucous cells replace seromucous cells in mucous tubules. Also, seromucous demilunes cap the tubules.) b. & c. Granular ducts follow the intercalated ducts and are approximately, 50 u in diameter, and consist of 8-10 cells in cross-section which are composed of scattered granular cells and nongranular cells whose nuclei are basal. Granules are apparent (arrows). d. & e. Striated ducts are continuations of the granular ducts and are characterized by distinct, basal striations (arrows) due to longitudinal arrays of mitochondria and by nuclei near the apical surface. Two populations of ducts include the small diameter ducts, 45-70 u, and the larger diameter ducts, approximately, 100u, in diameter. f. Interlobular ducts form the distal end of the ductal system and empty into the excretory ducts of the gland. These ducts are stratified in contrast to the other ducts and consist of columnar and basal (arrows) cells. These ducts are composed of 50-80 or more cells and are 200-330u in diameter.

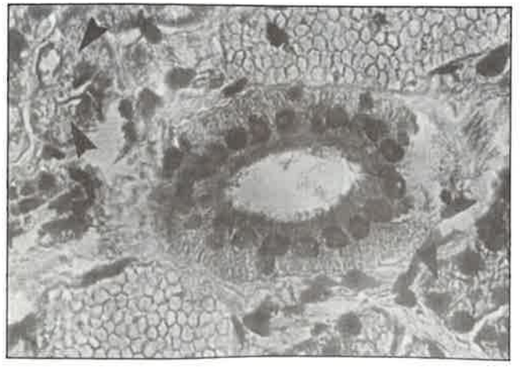

FIGURE 2. Submandibular Gland. A striated duct composed of cuboidal cells is shown in the center; nuclei stain blue. The apical surface of the ductal cells exhibits the red chromagen stain indicating the presence of lysozyme. Seromucous cells of the surrounding acini are negative (arrows), as are the red blood cells contained in capillaries above and below the duct. (X 400).

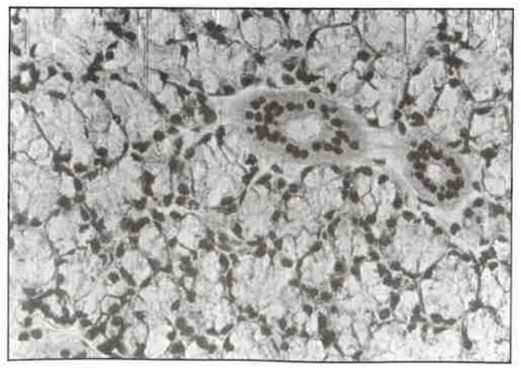

FIGURE 3. Negative Control. Seromucous cells of the submandibular gland as well as all associated ducts show an absence of chromagen stain, and are therefore negative for lysozyme, when the primary antibody is omitted in the staining procedure. The other salivary glands showed similar results. (X 200).

FIGURE 4. Submandibular Gland. Granular ducts stain intensely and positively for lysozyme (arrows). Surrounding seromucous acinar cells are negative. (X 200).

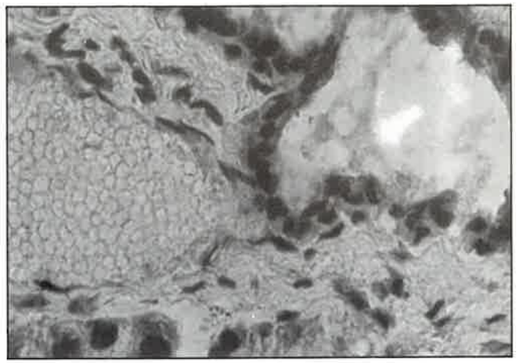

FIGURE 5. Parotid Gland. A large excretory duct is seen in the upper right hand corner. It's cells are positive for lysozyme. Surrounding seromucous acinar cells and the adjacent capillary with red blood cells on the left, stain negatively for lysozyme. (X 400).

FIGURE 6. Sublingual Gland. Serous demilunes which cap acinar cells stain darkly and are positive for lysozyme (arrows). Striated ducts also stain red. (X200)

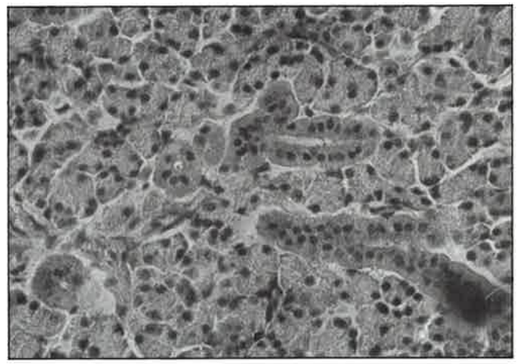

FIGURE 7. Minor Salivary Gland. Seromucous acini stain faintly with the chromagen stain.

Table 1 summarizes the results of the immunohistochemical staining for lysozyme in rat salivary glands. Serous/seromucous acini stain negatively in all major salivary glands including parotid, submandibular and sublingual. In contrast, acini of minor salivary glands are distinctly positive. Serous demilunes of sublingual glands are positive. Mucous cells of sublingual are clearly negative. The ductal systems of both major and minor salivary glands are predominately positive. The smallest caliber ducts, intercalated ducts, are positive in parotid gland. Striated ducts of all glands are strongly positive. Large interlobular ducts of specific glands also stain positively.

TABLE 1. Immunocytochemical Localization of Lysozyme in Rat Salivary Glands (a).

	Parotid Gland	Submandibular Gland	Sublingual Gland	Minor Salivary Gland
Serous/ Seromucous Acini	—	—	—	+
Serous Demilunes	NA	NA	+	NA
Mucous Cells (b)	NA	NA	—	NA
Intercalated Ducts	+	—	—	—
Granular Ducts (c)	NA	+	NA	NA
Striated Ducts	+	+	+	+
Interlobular Ducts	+	+	+	NF
Larger Ducts	+	+	+	NA

a. (+) indicates positive staining reaction
(-) indicates negative staining reaction
NA indicates non-applicable
NF indicates structures were not found
b. found only in sublingual gland
c. found only in submandibular gland

DISCUSSION

Few studies on lysozyme localization have been conducted in rat tissues. Klockars et al. (5) in 1974 using immunohistochemical staining methods did not find lysozyme in rat parotid gland which was the only salivary gland studied by the authors among other rat tissues. This lack of staining might be explained by the extensive fixation time used in the study that could have caused a loss of antigenic activity (15). In a second study, Spicer et al. (17) localized lysozyme in rat sublingual serous demilunes which agrees with the present study, although we also found lysozyme in the striated ducts of this gland. Three findings regarding the location of lysozyme have been consistently observed in a number of studies that used immunocytochemical methods as the primary investigative tool. The first is that lysozyme is rarely localized in mucous cells, although a few studies suggest its presence in select areas (10). Secondly, lysozyme is not found in association with serous and seromucous acini of major salivary glands (8,10,12,13), but is found in acinar cells of minor salivary glands and in fetal salivary glands (1,9,10,12). Thirdly, lysozyme is commonly found in cells comprising the ductal system of major and minor glands (1,7,10,11,12,13,15).

The results of this study show that in the rat, lysozyme is predominately found in the ductal system of all salivary glands and rarely in secretory acinar cells of the glands themselves. The small intercalated ducts of parotid gland are distinctly positive for lysozyme. Larger ducts such as the striated ducts of all glands are always strongly positive at the lumenal end of the cells that make up the ductal wall. These ducts are unique because their striations represent longitudinal arrays of mitochondria, whose energy production is thought to drive ion transport at the lumenal side of the ductal cell (16). It has been suggested that these ducts alter the composition of the fluid secreted from the acinar cells, and thus affect the composition of saliva. With regards to lysozyme, it has been suggested that the ductal system is a site for lysozyme storage which is available for secretion into the presumptive saliva as it passes through the extensive ductal system (5). A third type of duct found only in rat glands is the granular duct, and it too is strongly positive for lysozyme. Finally, the largest caliber ducts which are multilayered interlobular ducts whose secretions enter the excretory duct of glands also indicate the presence of lysozyme. Our results strongly support the presence of lysozyme in rat salivary gland ducts.

In contrast, the secretory acinar cells of all major glands, and the mucous cells of the sublingual gland were negative for lysozyme. The exception, to this finding in our study involved a minor salivary gland associated with the sublingual which showed lysozyme present in both the acinar cells and its ductal system.

Konttinen (7) using *in situ* hybridization identified cDNA lysozyme in the serous acinar cells of minor labial glands indicating synthesis of the protein in these cells based on the presence of lysozyme m-RNA. cDNA lysozyme was not found in the ductal system cells, although immunohistochemically, lysozyme itself was found in duct cells. These findings as interpreted by the authors, bring up the intriguing possibility that lysozyme might be synthesized in acinar cells but stored in ductal cells. Such a system may also be present in the rat tissue of this study, whereby a group of cells (acinar cells) synthesizes a product (lysozyme), and another group of cells (ductal cells) stores the product and perhaps regulates its secretion.

Our findings are in agreement with several research studies (5,6,12). However, some investigators have localized lysozyme in acinar cells and/or mucous cells (1,10,17). This may in part be explained by the fact that in one study (1), staining was not strongly positive. In addition, two of the studies were done on neonatal (1) or prenatal (10) salivary glands which may not be comparable to adult tissue.

The distribution of lysozyme in salivary glands is complex. An understanding of the evolutionary development of salivary glands with respect to lysozyme might explain the specific patterns of lysozyme presence. Interestingly, salivary glands in several species of bats have been studied, and the localization of lysozyme interpreted along evolutionary lines with regards to their diet. The parotid glands of frugivorous bats whose diet consist of fruits high in sugars have exceedingly long, intercalated ducts as compared to insectivorous and carnivorous bats. Phillips et al. (1993) suggests that a correlation between the elongated ductal system and an increased amount of lysozyme in their saliva might provide a potent antibacterial component to reduce tooth decay in bats at risk because of their sugary diet.

Enough similarity exists between rat and human salivary glands that rat glands could be used as a model to study human salivary gland secretory pathways. Lysozyme is a potential marker for several pathological states, and saliva, within which it is secreted, might serve as the basis for an excellent non invasive method to predict disease or follow the course of a disease. Understanding lysozyme secretion with regards to clinical applications is worthy of further investigation.

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Journal of the Pennsylvania Academy of Science 71(2):61-65, 1998

RELATIONSHIP BETWEEN FOOD DISTRIBUTION, AGGRESSION AND SEXUAL BEHAVIOR IN SIB AND NON-SIB MALE MICE (MUS MUSCULUS)[1]

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ABSTRACT

Aggressive and sexual behavior indices were determined for individuals from 32 populations containing four adult male mice from the same (sib) or different litters (non-sib) and subjected to random or central feeding. Twenty isolated males of the same age were used as a control population. In all groups, as well as in isolate males, body weight was a significant factor in determining the aggressive index (CAF) of male mice. In both sib and non-sibs, food distribution was a factor in determining the CAF index and the reproductive success of individual males. When offered a choice, females preferred high ranking non-sib males and low ranking sib males.
[J PA Acad Sci 71 (2) 61-65, 1998]

INTRODUCTION

Wynne-Edwards (1962), in summarizing the available literature, hypothesized that in a polygamist mating system, the social status of individual males will determine their reproductive success. This theory was expanded by Wilson (1980) and other sociobiologists who suggested that the majority of the offspring in polygamist species are sired by dominant males, thereby increasing the overall fitness of the species. Brenner (1989) found that in both C-57 and CF-1 strains of house mice (*Mus musculus*), there was a significant correlation between the aggressive and sexual behavior of males, with females indicating a preference for higher ranking males. Dewsberry (1991) and Drickamer (1992) indicated that female mice usually choose dominant males and/or the son of a dominant male for mating. Females also appear to react to odor, since they show a preference for the bedding of dominant males. Likewise, in freely growing mice populations, several studies have demonstrated a relationship between aggression and sexual behavior (Christian 1963, 1978, Lloyd 1975, Delong 1978 and Brenner et al. 1981).

Among the numerous factors related to dominance in vertebrate organisms, body weight has been shown to be a key in determining dominance in mammalian populations (Brenner 1973, 1989, Brenner et. al 1981, Brenner and Christian 1986, Brenner and Wirtz 1984, Brenner and Flemming 1974, Drickamer et al. 1973, and Vandenberg 1971). In the majority of these studies, food availability was not considered to be a factor in determining dominance since it was always provided *ad lib.* However, Brenner et al. (1978) reported that in captive chipmunk (*Tamis striatus*) populations, the majority of the social interactions occurred in the vicinity of the feeding stations. The current study analyzed the relationship between food location, aggressive activity and sexual behavior in sib and non-sib male mice maintained in populations of four individuals.

METHODS

All C-57 mice used in this study were obtained from breeding colonies maintained at Grove City College. Thirty-two populations of four males 21 days of age that were either from the same (S) or different litters (D) litters were housed in 27 X 30 X 16 cm polyethylene cages. All populations were fed *ad lib.* from either a central location (C) or distributed randomly (R) throughout the cage. Each cage was then assigned a number according to the litter association or type of feeding (i.e. DR, DC, SR, SC). In addition, two groups of 10 males from different litters, housed individually and subjected to random and central feeding, served as controls.

An aggressive and reproductive behavior index was determined for each individual in all populations, as well

[1]Received for publication 13 February 1997; accepted 18 September 1997.

as the control mice. The aggressive index was calculated by a series of round-robin encounters, according to the procedures described by Vandenberg (1971), Brenner (1989), Brenner and Wirtz (1984), Brenner and Christian (1985) and Brenner et al. (1978). Two randomly selected males were removed from their home cage and placed simultaneously in a strange 27 X 30 X 16 cm arena for 15 minutes. Each mouse was scored according to the number of times it initiated a contact (C): body contact initiated by one mouse upon another (one point); an attack (A): biting of one mouse by another (two points); or fight (F) (three points). An overt aggressive index (CAF) was then computed by a summation of the points for each individual. The container was cleaned with 70 percent alcohol after each trial. This procedure was repeated until an aggressive index was calculated for each individual in all populations, as well as the control animals.

An index of sexual (AMP) activity was then determined for individual males in each group, as well as the control males. A male was placed with a randomly selected estrous female from a different litter for 15 minutes and scored according to the number of times that the male approaches (A) the female (1 point); mounts (M) (2 points) or initiates a pelvic trust (P) (3 points). Each male was paired with three different estrous females and the summation of the scores was used as the AMP index for that male. As with the aggressive index, the arena was cleaned with 70 percent alcohol after each trial.

In addition to the AMP index, female preference for either a dominant (high CAF score) or subordinate (low CAF score) male was determined for each population. Two males of different ranks were restrained at each end of a 30 cm runway divided into three equal sections with the center 10 cm being the neutral zone. A randomly chosen estrous female from a different litter was released in the center of the runway and the amount of time the female associated with each male was recorded over a 10 minute period. Three different estrous females were used for each trial and the total time spent with each male was used as the index of female preference for either dominant or subordinate males. These trials were repeated with high and low ranking CAF males from each group and for high and low scoring isolated males.

Prior to each trail, males were weighed to within ±0.1gm and allowed a 48 hr adjustment period between each trial. Moreover, males were not subjected to an aggressive and reproductive encounter during the same 48 hr period. All statistical procedures used for data analysis were based on the criteria described by Brown and Downhower (1988) and an alpha level of $P < 0.05$ or less was used as significant for all procedures.

RESULTS AND DISCUSSION

According to a 2 x 2 Analysis of Variance (ANOV), there was not a significant difference in the body weights among the different groups of mice; hence, food distribution did not affect weight gain among the different populations or isolated males (Table 1). Food distribution did, however, affect the aggression and sexual behavior in grouped mice but not in isolated controls (random feed: $\bar{X}$ = 32.9, central feeding: $\bar{X}$ = 29.6; t = 1.11, $P < 0.1$). As in a previous study by Brenner (1989), there was a significant correlation between body weight and aggression (CAF index) according to a Pearson Correlation Analysis (r = 0.909), $P < 0.001$). With the exception of SC males (t = 1.25, $P > 0.1$), group males had higher CAF scores than isolated males (DR: t = 2.69, $P < 0.05$; DC: t = 4.3, $P < 0.001$; Sr: t = 5.1, $P < 0.001$) (Table 2, Figure 1). The CAF index, however varied among the different groups, depending on whether males were from the same (sibs) or different litters (non-sibs), and on the method of food distribution. Sibs with random food distribution (SR) had significantly higher CAF scores than sibs with central feeding (SC) (t = 23.9, $P < 0.001$). Likewise, SR males had significantly higher CAF scores than non-sibs, regardless of the type of food distribution (DR; t = 11.1, $P < 0.001$); DC: t = 3.6, $P < 0.01$) (Table 1, Fig 1). The type of food distribution also appeared to be a factor in the aggressive behavior of males since non-sibs with central feeding (DC) had significantly higher CAF scores that non-sibs with random feeding (DR)

TABLE 1: Comparison of Body Weights of grouped (4/group) and isolated male mice subjected to random and Central feeding. DR: non-sib with random feeding), DC: non-sib with central feeding, SR: sibs with random feeding, SC: sibs with central feeding.

		Body Weight gm.		
Population	N	Mean	S.E.	Range
DR	8	28.8	1.7	26.1-31.5
DC	8	28.5	1.4	27.3-32.1
SR	8	28.1	1.6	27.7-31.4
SC	8	28.2	1.5	27.9-29.6
Isolated R	10	29.6	1.3	27.3-32.9
Isolated C	10	28.8	1.4	26.1-32.3

TABLE 2. Comparison of the CAF and AMP Scores among the different groups and isolated controls (DR: non-sib with random feeding; DC: non-sib with central feeding; SR: sibs with random feeding; SC: sibs with central feeding).

	Population					
Parameter	DR	DC	SR	SC	Isolate R	Isolate C
N	8	8	8	8	10	10
CAF Score						
Mean	42	55	62	34	33	30
S.E.	1.7	2.0	1.7	2.4	1.3	1.2
Range	38-46	53-62	61-69	34-36	30-37	27-33
Per g body wt.	1.5	1.9	2.2	1.2	1.1	1.0
AMP Score						
Mean	26	53	74	13	22	20
S.E.	1.8	1.7	2.1	2.2	1.4	1.2
Range	24-28	50-58	73-83	13-14	20-24	18-22
Per g body wt.	0.9	1.9	2.6	0.5	0.7	0.8

(t = 6.8, $P < 0.001$). On the other hand, DR males had significantly higher CAF scores than sibs with central feeding (SC) (t = 4.2, $P < 0.001$). Likewise, the same relationship occurs when the CAF index is calculated as per unit body weight (Table 2), further suggesting an interaction between the genetic factors and food distribution. However, when analyzed individually, there was not a significant difference between food distribution and sib and non-sib males (Fig 1).; hence, there was an apparent interaction between food distribution and the genetic relationships of the males. In other words, sibs subjected to random feeding elicited a higher number of aggressive encounters than did sibs with central feeding. Moreover, according to a 2 X 2 Analysis of Variance, there was a significant interaction between the genetic relationship, food distribution and CAF scores among the different groups (F = 4.61, $P < 0.05$).

As with aggression, food distribution affected sexual behavior in grouped but not in isolated male mice (random feeding: $\bar{X}$ = 21.6, central feeding: $\bar{X}$ = 19.9; t = 1.17, $P > 0.1$). In all groups, except SC, males had significantly higher sexual scores than isolated males (DR: t = 9.2, $P < 0.001$); DC: t = 7.8, $P < 0.001$, SC: t = 9.5, $P < 0.001$). In contrast, SC males had significantly lower AMP scores than isolated males (t = 4.3, P 0.001). Among the different groups, sib males subjected to random food distribution (SR) had significantly higher sexual (AMP) scores than males from all groups (DR: t = 6.5, $P < 0.001$; DC: t = 11.0, $P < 0.001$; SC: 29.0, $P < 0.001$) regardless of whether the AMP index is based on total score or on a per unit weight basis (Table 2). Likewise, DC males had a higher sexual activity index (AMP) than either DR (t = 15.0, $P < 0.01$) or SC males (t = 21.0, $P < 0.001$). As with CAF scores, there was a significant interaction between the genetic relationship and food distribution (F = 10.61, $P < 0.01$). It appears, therefore that food distribution and group composition were factors in determining the level of aggressive and sexual behavior in male mice with high CAF scores also possessed higher sexual behavior indices (Fig 1).

In all groups, as well as in isolated males, there was a significant linear relationship between aggression (CAF) and sexual behavior AMP (Figure 2). The pattern of this

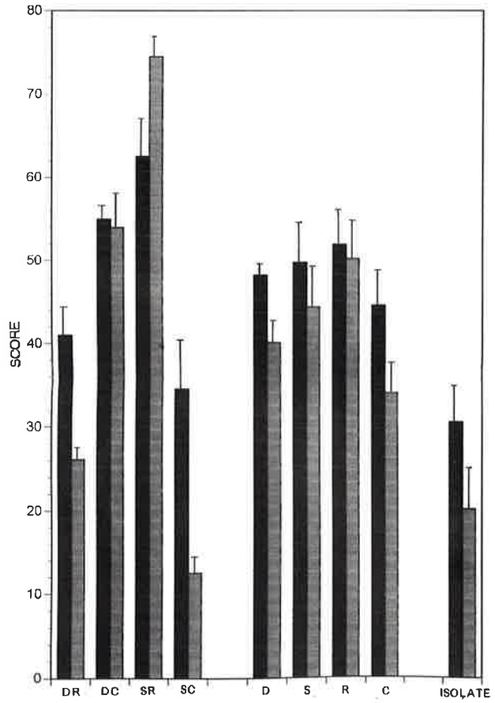

FIGURE 1. The aggressive (CAF) and sexual (AMP) indices exhibited by sib (S) and non-sib (D) male house mice and subjected ro random (R) or central feeding (C). The CAF score was calculated from the number of contacts (1 point) + attacks (2 points) + fights (3 points). The AMP score was calculated from the number of approaches (1 point) + mounts (2 Points) + pelvid trust (3 points). The bar represents the mean and 95% conf. intervald for each group.

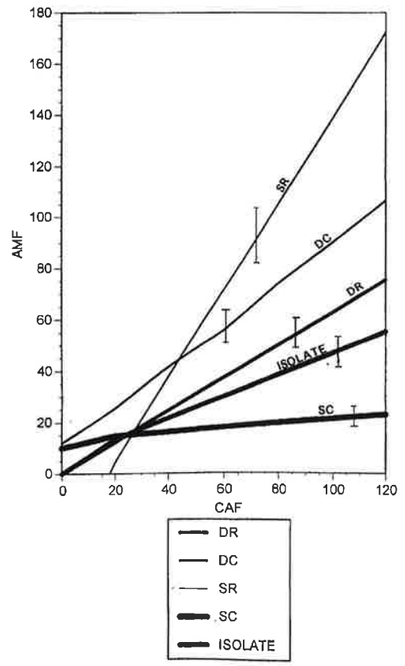

FIGURE 2. The calculated regression line and 95% conf. intervals indicating the relationship regression (CAF) and sexual activity (AMP) of sib (S) and non-sib (D) of male house mice subjected to random (R) or central (C) feeding.

relationship, however, varied among the different groups, as well as between grouped and isolated males. A comparison of the slopes of the calculated regression equations using a t distribution analysis indicated that the rate of increase was significantly greater in SR males than in the other groups, while being significantly less in SC males (t= $P < 0.05$). All other groups exhibited a similar relationship between the CAF and AMP indices.

Based on a partial correlation analysis of the relationship between body weight, aggression (CAF) and male sexual activity (AMP), body weight and aggression were determining factors in male sexual behavior with aggression being the most important parameter. In grouped mice, the partial correlation coefficient (R) was 0.906 ($P < 0.001$) and 0.304 ($P < 0.05$) for body weight and the CAF index and body weight and AMP, respectively. Overall, based on the coefficient of determination, 91.2 percent of the sexual behavior among the different groups could be accounted for by variations in their CAF scores and body weight. Although in isolated males the individual partial correlations were not significant (wt. vs CAF, $r = 0.184$; wt. vs AMP, $r = 0.085$, $P < 0.10$), but overall 82.3 percent of their sexual scores could also be accounted for by variations in CAF scores and body weight. It appears, therefore, that body weight or possibility other factors affect the aggressive behavior and may also affect sexual behavior in male mice.

In addition to the significant correlation between aggression and male sexual activity in both grouped and isolated males, females tested in an open field situation as to their preference for either high or low CAF scoring males, showed considerable variation among the different groups. Based on a 2 X 2 contingency table analysis, estrous females offered a choice between high or low CAF non-sib males spent significantly more time the high CAF male ($x^2 = 193.4$, $P < 0.001$). Whereas with sib males, the reverse occurred with females spending significantly more time with low CAF males ($x^2 = 141.3$ $P < 0.001$). Overall, when all groups were compared, females spent approximately 57 percent of the time in the neutral zone and 43 percent in association males (Figure 3).

When estrous females were tested as to their choice between males from the different groups and isolates, they also indicated a preference for high CAF non-sib ($x2 = 190.1$, $P < 0.001$). On the other hand, when females were offered a choice between isolated and sib males, they preferred low CAF males over isolates ($x^2 = 140.3$, $P < 0.001$) (Fig 3). The pattern of food distribution did not appear to be a factor in the choice of males by females.

In conclusion, body weight appears to be an important factor in determining the social status of male house mice. The interaction between food availability (random or central feeding) and the genetic relationships of the males (sibs or non-sibs) also were factors in determining the aggressive index of male house mice. In all four groups, as well as isolated males, and the results of

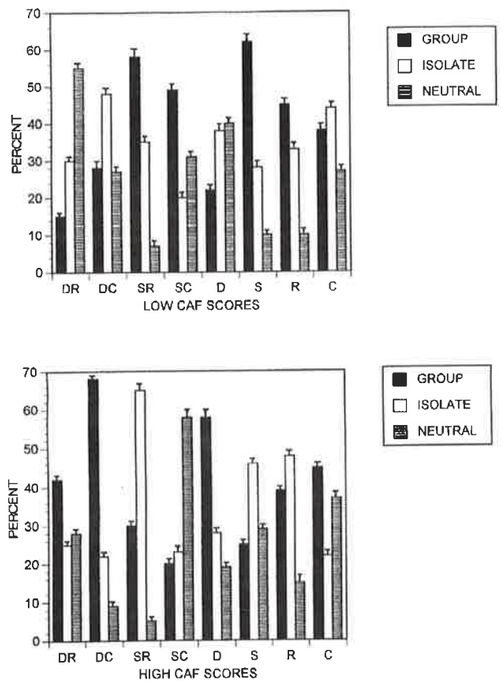

FIGURE 3. The proportion of time that estrous females spent with high or low CAF scores of sib (S) and non-sib (D) male house subjected to random ® or central (C) feeding.

previous studies (Brenner and Ketterer 1978, Brenner et al. 1981, Lagerspetz and Worenen 1995) suggest that dominance may be an important factor in determining the reproductive success of male house mice. However, the relationship between female choice and dominance varies among the different groups. In all cases, females preferred grouped over isolated males, but females preferred higher CAF non-sib males while the reverse occurred with sib males. Overall, dominance appears to be an important factor in the mating success of males, but other factors may be involved in mate selection and the corresponding breeding success of females.

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Journal of the Pennsylvania Academy of Science 71(2):66-72, 1998

AVIAN FOOD-STORING BEHAVIOR: A REVIEW[1]

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ABSTRACT

Certain avian species are known to seasonally store food in caches and retrieve it for later use. Some species, such as the black-capped chickadee (*Parus atricapillus*) store hundreds of items per day—all in different locations—and successfully retrieve most of them (Sherry 1989). To achieve this complex behavior, food-storing birds use memory of their spatial environment, such as landmarks, and specific behavioral strategies, such as cache clumping. The function of the avian hippocampus—like the mammalian hippocampus—is to encode such spatial memories and store working memory. Evidence of seasonal adult neurogenesis in the hippocampal complex (HC) of certain food-storing avian species and the larger size of their HC — in comparison to non-storing species — suggest that this forebrain structure is crucial in the acquisition of spatial memories (Paton and Nottebohm 1984; Sherry et al. 1989). My interpretation of current findings suggest that (1) cache retrieval is not accomplished by an exhaustive random search, a mnemonic device, or the senses of sight or smell, but is a complex process achieved by specific strategies and the use of significant spatial memory, and (2) the comparatively larger size of the HC of food-storing avian species indicates that this forebrain structure is crucial in the acquisition of spatial memories. However (3), neuronal birth and replacement in adult food-storing birds appears to be necessary for the acquisition of new spatial memories; therefore, neurogenesis is necessary to the behavior of seasonal avian food storing.
[J PA Acad Sci 71 (2) 66-72, 1998]

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INTRODUCTION

Avian food-storing behavior occurs mainly in a few avian families: parids, corvids, sittidae and picidae (Roberts 1979). However, it also occurs in a variety of raptors, shrikes and bellmagpies, in some muscicapid flycatchers, and in bowerbirds (Sherry 1989). Of interest is the fact that within a single family some birds store food while others do not, such as the storing marsh tit and the nonstoring blue tit.

There are several reasons why some birds store food and others do not. It has been theorized that many food-storing species live year-round in temperate winter environments where there is a seasonal fluctation in the abundance of food items, creating the need to cache food for later use (Roberts 1979). Food storers' diets are generalized, allowing them to eat invertebrate prey in the warmer months and to eat storable food items (i.e., hard and non-perishable) such as seeds, nuts and acorns in the colder months (Roberts 1979). Most birds that store food show behavior (such as territoriality and spatial memory strategies) in the warmer food-rich months that allows them to retain possession of stored items (Roberts 1979; Sherry 1989). Therefore, if a bird's food resources fluctuate seasonally, if its food is storable, and if it can defend its storage sites, then food storing is to its benefit.

The reasons why some species do not store follow from these criteria. Some species live in climates where food abundance does not seasonally fluctuate. Some species eat mostly insects and soft plant materials that are not conducive to storage. Other species may eat potentially storable food, but are unable to defend their caches (Roberts 1979). The birds—living in climates of seasonal food fluctuation—that either eat non-storable food or are unable to defend caches are likely the species that seasonally migrate to more food-rich climates.

This paper will analyze avian food-storing from the "outside in," that is, from the general observable behavior down to its specific neurological basis. Firstly, it will establish the complexity of avian food-storing behavior by describing exactly how it is achieved through behavioral strategies and spatial memory. Next, the theoretical organization of memory will be discussed as it relates to avian food storing. Lastly, this paper will describe how the comparatively larger size of the food-storer's hippocampus and the unusual process of adult neurogenesis function in the acquisition of memories.

AVIAN FOOD STORING:
Accurate retrieval of hundreds of food items daily through spatial memory

Of all the families that store food, the parids (chickadees and tits) have been studied the most. Consequently, the following description of food-storing behavior is gained from observations of this family. In a study of black-capped chickadees, it was found that their food storing begins in the late summer to fall and continues until spring (Barnea and Nottebohm 1994). Chickadees and tits store seeds, nuts, and sometimes insects and other invertebrate prey in a scattered distribution within their home range. Typical cache sites are tree bark, moss, lichen, conifer needles, hollow stems and buds. They may store several hundreds of food items per day, with only one or a few at each site. Amazingly, caches sites are not reused in the wild. The food is usually stored at sites a few meters away from where it was obtained (Sherry 1989).

Most studies conclude that in the wild the majority of food is retrieved after a period of hours or days (Sherry 1989). However, in captivity, memory of cache sites lasts for several weeks (Barnea and Nottebohm 1994). Cache pilfering by other animals does occur. Most pilfering is accomplished by rodents overnight, but it can be done by other birds.

Most laboratory studies on avian food storing compare birds' accuracy in retrieving stored food to their accuracy expected by chance or other methods of cache recovery, such as seeing or smelling the food (Sherry 1984; Sherry and Vaccarino 1989; Baker et al. 1988). In most of these experiments in indoor aviaries, the birds cache seeds in appropriate sites set up by experimenters, such as beds of moss or small holes in the tree branches. After a delay of several hours to several days, the birds are allowed to search for their caches. Usually the stored food is removed in the interim by the experimenter, so that the bird cannot find the cache simply by seeing or smelling the food itself. The birds' behavior is observed to determine how accurately they return to search at sites where they had stored food. Comparisons are made between how often the birds visit sites where food was stored to how often they visit sites where no food was stored (Sherry 1989).

Both marsh tits and black-capped chickadees have demonstrated their adeptness at finding stored food in laboratory conditions by returning to their cache sites more accurately than would be expected from control data. (One method to obtain control data is to allow the birds to search the empty aviary just prior to the experiment in order to observe any bias or preference to search in particular places, whether food has been stored there or not. Comparison between the control data and the actual cache recovery data can be used as a measure of whether the bird is using memory to retrieve its cache.) Since the appearance of cache sites does not vary much in laboratory conditions, birds are not using any kind of rule to perform well in cache recovery. Furthermore, birds are not using the order in which seeds were stored as a mnemonic device to assist in returning to cache sites because there is no evidence that seeds are recovered in either the same or reverse order of storage (Sherry 1984, Baker et al. 1988). However, if a landmark close to the cache site is altered then retrieval success decreases. This result is considered evidence for food-storing birds remembering the spatial locations of their caches. There is a significant amount of compelling evidence that the use of spatial memory is the major means of cache recovery.

FOOD-STORING STRATEGIES:
The use of landmarks, cues and features to aid in cache retrieval

There is evidence that birds remember landmarks, cues and features in the environment to find what has been stored. In one study, Vander Wall (1982) demonstrated the use of landmarks by Clark's nutcracker. A layout of objects was scattered in an open arena in an aviary in which nutcrackers could store pine seeds. After the birds had stored seeds, the object layout on one side of the arena was displaced by 20 cm, leaving the layout on the other side of the arena unmoved. When birds returned to search for their caches they probed in the ground at sites that were correct with respect to the objects' layout, missing their caches by 20 cm on the manipulated side of the aviary and retrieving them accurately on the undisturbed side. These results illustrate that the birds remembered a particular spatial arrangement of the arena and associated that arrangement with where they had hidden their seeds.

Another experiment (Herz et al. 1994), using distal cues (such as colored posters) placed on aviary walls and local cues (such as colored shapes) right next to cache sites, revealed that the birds were more influenced by the distal cues than the local ones. Additionally, rotation of these cues around the walls of the aviary prior to cache recovery invoked a rotation of the birds' search patterns.

In a revealing experiment, Brodbeck (1984) trained birds to find food hidden in one feeder amond an array of four and then return after 30 minutes to obtain another piece of food at the same feeder. One test trials with no food present in any feeder, the entire array of feeders was shifted 44 cm to one side and the formerly baited feeder was switched in position with another one

in the array. Birds searched first at the feeder that was in the correct location in the aviary, that is, the feeder nearest the place that the baited feeder had routinely occupied. Next, the birds searched at the feeder in the correct position in the array, and lastly searched at the feeder that matched the baited one in color and pattern. These studies suggest the birds memorize information about the food site, but then classify the use of this information into a ranking. They will search first at the site that is in the correct location in relation to surrounding landmarks (spatial location), then at the site correct in the local array (relative spatial location), and finally at the site correct in its features (Sherry and Duff 1996).

FOOD-STORING EFFICIENCY:
The use of directional clumping and memory of cache contents

Given the amount of information that food-storing birds must retain, birds must remember efficiently, without expending too much energy or putting their caches at risk for pilfering. The amount of information about external events that a brain can acquire may not only be limited by the brain's size, but also by the way in which memories are ordered and linked (Barnea and Nottebohm 1995). Information organized into groups, or "chunks" as some call them, is more easily remembered than random collections of data, and food-storing birds appear to have found a way to efficiently "chunk" food-storing information.

In food-storing behavior, there is an evolutionarily-stable strategy to the behavior that produces the best results. The best results of food-storing would be to retrieve the highest percentage of the food that was stored. However, the situation becomes more complex when other issues, besides simply the successfully retrieved food, are analyzed. Other issues such as energy cost and the biotic and abiotic interactions of the surrounding environment need to be dealt with by the food-storing animal. Considering all the influencing factors, the bird's strategy should: (1) minimize the energetic cost of caching, (2) minimize the chances of spoilage,(3) minimize the risk of losing access to stored food (via snow, ice, floods, etc.), (4) minimize the risk of pilfering, and (5) maximize the animal's ability to remember where each food item is stored (Barnea and Nottebohm 1995).

An experiment by Barnea and Nottebohm (1995) determined whether caches are deployed to reduce the overall memory load required for cache recovery. By setting up feeders and observing flocks of chickadees, they noted that within the flock home range there was no single preferred place for storing. However, most of the storing occurred within a radius of 15 m from where the seeds were offered. Additionally, they found that on any one day a chickadee flock used only a limited portion of a 360 degree arc around a food source, but the individual birds still distributed their caches in a scattered pattern within that limited area.

Barnea and Nottebohm (1995) theorize that the use of directionality in food-storing away from a food source may be related to neural mechanisms underlying cache memorization. This "directional clumping" still requires memory for the location of each item, but it does not require reference to the entire home range. A bird that directionally clumps also strengthens its memory of that particular sector and its landmarks. In fact, the physical act of traveling to a cache site clearly aids a bird's memory: birds have a better recovery performance when searching for seeds *they stored* than when searching for seeds *they saw* being stored by a conspecific (Baker et al. 1988). Thus, it is in birds' best energetic interest to search for seeds they stored themselves. All considered, the food cache distribution strategy of black-capped chickadees shows a compromise between the need to minimize the energetic cost of food storing (store close to source), minimize the risk of thievery (store in a scattered distribution), and optimize the memorization of caching sites (site-clumping) (Barnea and Nottebohm 1995).

In addition to their choice of location for caches, food-storing birds need to keep track of the status of their caches: Does that cache still have a seed in it? If not, is it due to pilfering? In a series of experiments to find out more about how black-capped chickadees' memory is organized, Sherry (1984) concluded that, indeed, these birds do remember very specific facts about their caches that improve the birds' overall food-storing efficiency. For example, black-capped chickadees cannot only accurately relocate cache sites, but they can recall which caches they have previously emptied, recall which sites they have discovered empty (due to pilfering), and recall what type of food is stored at each cache site (Sherry 1984). Chickadees store only one item per site and they do not leave any marks or discarded hulls at sites where a seed has already been recovered. Sherry (1984) concluded that chickadees must be able to remember information on the full/empty status of storage sites. In regard to cache pilfering, Sherry indicates that at sites where food items are lost at random and the chickadees have the opportunity to discover the loss, less time is spent searching in that area. The chickadees must remember, then, that they did store a seed at that site. To test the chickadees' ability to remember food type, Sherry (1984) set out a feeder with shelled sunflower seeds and safflower seeds with the shells still on them (chickadees prefer sunflower seeds). The birds were allowed to store the seeds and in an interval the seeds were removed by the experimenter. Then the birds were allowed to search for the seeds. More time was spent searching at sunflower sites and sunflower sites were visited more often than the safflower sites. These results illustrate that the type of item stored at a site influences the amount of searching effort directed to that site and that this effect is due to memory for the contents of cache, rather than the presence of the

FUNCTION OF AVIAN HIPPOCAMPUS:
Encoding spatial memory and retaining working memory

The findings described above should be sufficient evidence to establish at least that the cache recovery of certain food-storing birds is spatially precise, and not based on an exhaustive search. The processing of spatial information is one theoretical function of the hippocampus in mammals. This theory, called the cognitive mapping view, is supported by the theory of "place cells"(specific neurons receptive to visual stimuli) in the brain. The pattern of place cell-firing programs an animal's location within each environment, providing a kind of cognitive map for the animal. A cell may be active when the animal is in a particular place, for example in a specific tree, and inactive when an animal leaves that place (Sherry 1989; O'Keefe and Dostrovsky 1971). This theory is further supported by the fact that when the hippocampus' function is disrupted by lesioning, the spatial orientation and navigation of the animal is subsequently disrupted (Sherry and Vaccarino 1989; Bingman et al. 1990; Bingman and Yates 1992).

The working memory hypothesis is another theory on the function of the hippocampus. This theory proposes that memory can be divided into two functions: reference memory and working memory. *Working memory* is defined as memory for information that is applicable to only the current task, such as the most recent choices of cache location. Whereas *reference memory* retains information that is relevant to all performances of the same task, such as which trees are within a home range. The working memory theory is supported by the observation that memory for the recent performance of a task (spatial or otherwise) is disrupted by hippocampal lesioning, but memory for the nature of the task (reference memory) stays unimpaired (Sherry and Vaccarino 1989). In experiments with black-capped chickadees, birds with hippocampal lesions performed very poorly at cache recovery, compared to the control birds without lesions. The lesioned birds continued to search as intensely, but the cache recovery rate was no greater than that expected by chance. Thus, the birds were still able to remember how to search for food they had hidden (reference memory), but they were unable to relocate the food accurately in space (working memory and spatial memory) (Sherry 1989; Sherry and Vaccarino 1989).

These studies suggest that both theories serve a purpose in better understanding the function of the hippocampus. The cognitive mapping view provides a hypothesis on the cellular mechanisms that code for spatial memory and the working memory theory a hypothesis on the different kinds of memory. In addition, the results of lesioning experiments confirm that the avian hippocampus' function—playing an important role in spatial memory tasks—is generally similar to that of the mammalian hippocampus.

COMPARATIVE STUDIES OF HIPPOCAMPUS:
The relatively large size of the food-storer's hippocampus

Comparative studies of the avian hippocampus have resulted in some revealing facts about this particular dorsomedial forebrain structure. The area studied is often referred to as the *hippocampal complex* (HC) because it includes the closely interconnected hippocampal and parahippocampal areas. In a uniquely large and broad study involving 52 individuals belonging to 35 species of passerine birds, it was shown that the volume of the HC relative to brain and body size is significantly larger in species that store food than in non-storing species (Krebs et al. 1989). For example, this study revealed that the food-storing 11-g marsh tit (*Parus palustris*) has a hippocampal volume of 14.26 mm^3, while the non-storing 20-g great tit (*Parus major*) has a hippocampal volume of 11.2 mm^3. Thus, the hippocampus of the marsh tit is 31% larger than that of the great tit. Another study compared the HC of 3 food-storing families (chickadees, nuthatches, and jays) to the HC of 10 non-storing families and determined that the volume of the storing birds was, again, significantly higher (Sherry et al. 1989).

Food storing places special demands on spatial memory. In addition, the hippocampus plays a key role in spatial memory; but hippocampal lesions do not interfere with all aspects of food-storing behavior (lesioned birds still search for caches). Therefore, the correlation observed in the above studies appears to be more associated with memory than with other aspects of food storing.

To further test the hypothesis that the relative size of the avian hippocampus is directly connected to food storing, Healy et al. (1994) compared the development of the HC in storing and non-storing species. A previous study of two species in the Corvidae family reported that the difference in HC volume between storing and non-storing species is not evident in a comparison of nestling birds, but appears only after birds have left the nest (Healy and Krebs 1993). The onset of food-storing is generally thought to occur within the first few months of life. It is possible, then, that the changes in the hippocampal volume of storing species after fledging are associated with the onset of food storing (Healy et al. 1994).

In the 1994 experiment, Healy and colleagues compared the food-storing marsh tit to the non-storing blue tit. As expected, the experiment determined that the adult (older than 1 year) marsh tit has a larger relative HC than the adult blue tit. However, the prefledging marsh tits (7-17 days old) and prefledging blue tits (2-17 days old) do not differ in relative hippocampal volume. In addition, adult marsh tits have a larger relative HC than prefledging conspecifics, whereas adult blue tits do not differ in relative hippocampal volume from prefledging conspecifics. This last result suggests further that food storing and HC size are related. If the prefledging marsh tits have a smaller HC than adult marsh tits, perhaps the food-storing experience of the adult causes the increase

in the size of the hippocampus. In fact, if young post-fledging marsh tits are deprived of the opportunity to store and retrieve food, their hippocampus is smaller than in similarly aged, but more experienced, conspecifics (Healy et al. 1994). This study also determined that the younger birds of *both* species had more densely-packed HC neurons than their respective elders. However, the authors did not draw a conclusion regarding what the significance of this finding could be for the food-storing species.

NEUROGENESIS:
The necessity of new neurons in the adult avian hippocampus

The development and size of the hippocampus is correlated with a behavior that requires the use of spatial memory. As mentioned in the description of food storing, some birds, such as the black-capped chickadee, may store several hundreds of food items per day, all in different locations and they do not reuse the cache sites. Food-storing birds use certain strategies (such as directional clumping) to reduce their memory load, but it has not been clear how the neurons of the animal brain encode such memories or if the neurons are ever used up and replaced.

The majority of neurons in the central nervous system of endothermic vertebrates are born in prenatal or early postnatal development (Paton and Nottebohm 1984). This results in the adult brain being limited in its plasticity and ability to recover from damage. Therefore, neurons lost through disease or damage are not replaced and learning takes place not through adding more neurons, but through rearranging the connections of the existing ones. Understandably, any examples of neurons generated in adulthood have been of tremendous interest since Altman's claim of neurogenesis in rats and cats in the 1960's (Paton and Nottebohm 1984). However, Altman's results were not incontrovertible and neurogenesis in the adult mammalian brain is still not generally accepted (Paton and Nottebohm 1984; Nottebohm 1989).

In 1984, however, neurogenesis was demonstrated by Paton and Nottebohm in the brains of adult canaries through the [^{3}H] thymidine labeling of neurons in the telencephalon. Thirty days after receiving injections of [^{3}H] thymidine, a marker of DNA synthesis, intracellular potentials were recorded from telencephalic neurons and some of the neurons responded to auditory stimuli. This experiment provided direct evidence that central nervous system cells are generated in adulthood, adopt a neuronal morphology, show synaptic and action potentials, and are recruited into functional brain circuits (Paton and Nottebohm 1984).

The avian forebrain, which includes the telencephalon, is thought to be the part of the brain most involved with the control of complex learned behaviors, such as food storing or singing. These behaviors may depend on the availability of young neurons that can be used to build new circuits. New neurons are born in the ventricular walls, and from there they migrate to their final destination where differentiation occurs (Alvarez-Buylla and Nottebohm 1988). Cells in the ventricular walls of the adult canary brain are capable of dividing and some of the daughter cells may become young migrating neurons (Alvarez-Buylla et al. 1990). Recently, neuronal "chain migration" was discovered to be the process by which neuronal precursors born in the ventricular walls of adult rodents migrate long distances to the olfactory bulbs (Lois et al. 1996). In chain migration, cells attach to each other in long chains and migrate inside tunnels assembled by glial cell bodies and processes. When they reach the olfactory bulb, these migrating cells separate from the chains and travel radially for a short distance to differentiate into new neurons. Concerning exactly how a young neuron decides what type of neuron it will become, preliminary evidence suggests that the kinds of input a neuron receives may determine which of its genes are expressed, thus controlling the identity of the cell and what functions it performs (Nottebohm 1989).

In some cases, such genetic changes may be causing a kind of irreversible learning. In other words, the specific neurons involved with learning complex behaviors may be permanently changed by the long term memories they hold. The amount of learning that a bird can do may be limited by the number of its available neurons (Nottebohm 1989; Kim and Nottebohm 1993). An experiment with adult male canaries found that 41-49% of certain forebrain neurons involved with new song learning were lost in April and then replaced in October (Kim and Nottebohm 1993). This time period encompasses the yearly transition from stable song to song learning in canaries. These results suggest that the learning of new information may require that the old information—along with the cells that contain it—be discarded (Kim and Nottebohm 1993).

It is not certain if this pattern of neuronal loss and replacement is prominent in birds; nevertheless, there is speculation on why birds would be good candidates for this pattern. It may be related to their airborne lifestyle and relatively long lifespan: The weight of a canary and mouse are similar but a canary lives 10 times longer (Nottebohm 1989). If a bird had to carry all the brain cells necessary to gather, process and store all the information for its entire lifespan, its brain would have to be substantially larger and heavier. The additional brain weight would obviously interfere with flight.

The last piece of evidence that demonstrates the relationship between food storing, spatial memory, and neurogenesis is the seasonality of neurogenesis. As mentioned previously, food-storing birds begin storing in the late summer and early fall, when the abundant food sources of summer begin to diminish. In addition, changes in the landscape and territory boundaries occur in the fall. Newly

formed neurons appear in the hippocampal complex (HC) of birds during all times of the year, but they show a significant peak in the fall (Barnea and Nottebohm 1994). In Barnea and Nottebohm's study (1994) with wild black-capped chickadees, it was found that there is an increase in neuronal replacement of hippocampal neurons just prior to the time when the birds' spatial memory load will increase due to food-storing and environmental changes. In a subsequent study that compared the late summer HC size and volume of wild juvenile and wild adult black-capped chickadees, Barnea and Nottebohm (1996) found that the HC of 4.5-month-old juveniles was as large or larger than that of adults (at least a year old). In addition, they found that the packing density of HC neurons was 31% higher in juveniles than in adults. As mentioned earlier, a similar result regarding HC packing density was found by Healy et al. (1994) in their study with marsh and blue tits. Whereas no conclusion was drawn by Healy et al., Barnea and Nottebohm suggest that the HC of juvenile black-capped chickadees recruits (draws into its region) more neurons and contains more neurons than that of adults because juveniles encounter greater environmental novelty than adults; thus, the greater number of neurons found in juveniles allows them to learn more than adults. The conclusion of Healy et al. (1994) was the opposite: the food-storing adult marsh tit had a larger HC than the juvenile marsh tit. The larger HC in the *adult* marsh tit was reasoned to be the result of more food-storing experience. Whereas, for the black-capped chickadees, Barnea and Nottebohm (1996) reasoned that the larger size of the *juvenile* chickadee HC was due to an increased need for acquiring new spatial information.

There appears to be a sharp contradiction between the conclusions of these two studies; yet, in fact, they may be similar. The juvenile marsh tits in the Healy et al. (1994) study were 7-17 *days old*. The juvenile chickadees in the Barnea and Nottebohm (1996) study were 4.5 *months old*. The difference in ages of the juveniles in the two studies may explain why the 4.5-month-old chickadees' HC would have increased in size enough to reach or surpass that of the adult chickadees': The chickadees would have gained more food-storing experience than the younger marsh tits. In addition, if it is assumed (as Barnea and Nottebohm (1996) did) that the neuron and not the synapse is the unit of learning and that long-term memories irreversibly alter neurons, then as an adult bird becomes older it gains more long-term memories and has less space (i.e., fewer neurons) to dedicate to new learning. These assumptions help explain why adult chickadees sometimes have a similar HC than younger conspecifics: Long-term memory—or reference memory, not located in the HC as working memory appears to be—is more established in adults and the need for learning new tasks is diminished. If the need for learning new tasks is diminished, then the size (and neuron packing density) of the HC could be diminished also.

CONCLUSION

The research on avian food-storing behavior establishes that food-storing birds can not only remember such things as what kind of food they have hidden, but by using environmental landmarks, cues and features they can also distinctly remember—to within one centimeter—where they stored their food. Food-storing birds also employ such strategies as caching food in a reduced portion of their home range to improve upon their success in retrieving food. That the spatial memory abilities of birds should be located in the hippocampus is not too surprising; the hippocampus of the closely-related mammals is also the location of spatial memory. Recent research (Lois et al. 1996) regarding neurogenesis in adult rodents points to another parallel in the brains of birds and mammals. As for neurogenesis in birds, the research appears to show that this (presently) unusual process of neuronal birth and replacement is what allows food-storing birds to integrate the new memories necessary for seasonal food storing.

Further research on the development of food-storing behavior in young birds would lead to a better understanding of exactly how this behavior is formed and perfected. Is there a gene that is activated for good spatial memory or a gene for adult neurogenesis? In what other animals does adult neurogenesis occur? More research on the rare process of adult neurogenesis will likely lead to discovering how an animal's damaged neurons are repaired or replaced. If the mechanisms of neurogenesis are learned and able to be successfully reproduced, the prospects for curing or relieving degenerative diseases of the brain would obviously be very promising.

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Journal of the Pennsylvania Academy of Science 71(2):73-77, 1998

RELATIONSHIP BETWEEN FOREST FRAGMENTATION AND WOODLOT CHARACTERISTICS ON BREEDING BIRDS[1]

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ABSTRACT

During a 4 month period (May-August), 10 woodlots varying in size from 6 to 200 ha, located in Raccoon State Park in Beaver County, PA, were surveyed to determine the number and species richness of neotropical migrants. The number (N) and species richness (SR) of breeding birds were correlated with woodlot area ($P < 0.001$), tree species diversity (N, $P < 0.01$; SR, $P < 0.05$) and basal area (N, $P < 0.001$; SP $P < 0.05$). There was also a significant interaction among area, diversity of tree species and the number of breeding birds ($P < .001$), but not species richness ($P > 0.10$). The interaction between woodlot size and basal area and between basal area and tree species diversity were significantly correlated with species richness ($P < 0.001$), but not the number of breeding birds ($P > 0.10$). The results of this study indicate that not only size, but the maturity and diversity, of woodlots are important determinants in use by neotropical migrants.
[J PA Acad Sci 71 (2) 73-77, 1998]

INTRODUCTION

For over five decades, a variety of reports have expressed concern over the apparent decline of Neotropical migrant species in North America (Askins et al. 1990, Robbins 1979, Robbins et al. 1989, Whitcomb et al. 1981). Hill and Hagan (1991) analyzed population trends of 26 migratory species over a 53-year period and concluded that many species were declining. But, such declines in the past 20 years may be due in part to normal, shorter term population fluctuations, the fragmentation of forest habitats (Bollinger and Linder 1994), changes in habitat characteristics (Crawford et al. 1981, DeGraaf and Chadwick 1987, Yahner 1986), and low reproductive success due to predation and brood parasitism by brown-headed cowbirds (*Molorhrus ater*) along forest edges (Bollinger and Linder 1994, Gates and Gysel 1978, 1991, Hoover and Brittingham 1993). However, Hanski et al. (1996) reported that nest predation among ground-nesting species was significantly higher in forests than in clear cuts or regenerating aspen (*Populus spp.*) habitats. Kroodsma (1984) indicated that the response of avian species to forest fragmentation may depend primarily on forest island size, island isolation, and other factors rather than on the effects of edge. Whereas, Turchi et al. (1995) reported that the isolation of aspen stands had no significant effect on bird species richness. The current study was designed to investigate the impact of the size and structure of woodlots on the diversity and size of breeding bird populations.

STUDY AREA

The study area was located in Raccoon Creek State Park, Beaver County, in southwestern Pennsylvania and was comprised of scattered woodlots varying from less than a hectare to over 200 hectares in size. The forest community was dominated by maples (*Acer rubrum, A. saccharinum, A. saccharum*), oaks (*Quercus alba, Q. rubra*), hickories (*Carya ovata, C. glabra*), elms (*Ulmus americana, U. ruba*), black cherry (*Prunus serotina*), and white pine (*Pinus strobus*) (Table 1). The dominant understory species included Washington hawthorn (*Crataegus phaenopyrum*), American crabapple. (*Pyrus coronaria*), flowering dogwood (*Cornus florida*), and sassafras (*Sassafras albidum*), along with saplings of the canopy species.

[1]Received for publication 13 February 1997; accepted 14 June 1997.

METHODS

Ten woodlots varying in size from 6 to 200 hectares were selected as study areas and the number of breeding bird species were determined along predetermined transects according to the protocol described by Wallace (1994). In order to standardize the breeding birds surveys, one transect line was established for each 10 hectares or fraction thereof with a minimum of two transects per woodlot. Census points were established in the center of 0.1 hectare quadrats at 100-m intervals with the initial census point being located 100 meters from the edge of the woodlot. Beginning on 1 May until 30 August, an observer walked each transect from 0600 to 1200 hr, stopping in the center of each census point for 15 minutes, and recording all birds observed or identified by vocalizations. The relative densities of breeding neotropical species was estimated using the strip transect developed by Emlen (1972, 1977) and used by Jarvinen and Vaisanen (1975), Cox (1985) and Wakeley (1987) to estimate the size and density of bird populations. The presence of a female in association with a territorial male or the presence of a nesting female was recorded as a breeding pair. At each census quadrat, the frequency, density and dominance (basal area) of all canopy trees were recorded at each 0.1 hectare census point and these values were used to calculate an importance value for each species as described by Curtis and McIntosh (1951). Species richness of the breeding bird was calculated as $s / \sqrt{N}$, where s is the number of species and N is the number of individuals. The diversity of the forest communities was calculated as the $D = \sum (ni / N)^2$; where ni = the importance value of each species and N = the total importance value for the stand (Odum 1971). The relationships between woodlot characteristics and bird communities were analyzed using a least square regression analysis, correlation analysis and a partial correlation analysis (Brown and Downhower 1987). For all statistical procedures, an alpha level of $P < 0.05$ or less was accepted as significant.

RESULTS AND DISCUSSION

A total of 75 breeding pairs of neotropical migrants representing 10 species was confirmed using these woodlots and of these, 19 and 18 pairs were red-eyed vireos (*Vireo olivaceus*) and scarlet tanagers (*Piranga olicacea*), respectively (Table 2). These two species were observed on 8 of the 10 woodlots, which varied in size from 6 to 200 hectares (Table 2). The estimated density of the breeding bird populations varied from 10 to 33 pairs/100 ha with an average density of 21 pairs/100 ha (Table 3) Although these estimated breeding birds densities are lower than those calculated by Udvardy (1957), these studies were undertaken in isolated woodlots subjected to human disturbance which may have adversely impacted breeding bird communities. However, Udvardy (1957) also reported densities of less than 3 pair/100 acres and further indicated that species of low (3-10 pair/100 acres) and moderate (10-40 pair/100 acres) density constitute about 75 percent of the species evaluated in his study. It appears, therefore, that several factors may affect the size of breeding bird communities, including forest fragmentation and human disturbance.

In addition, several authors have suggested that nest parasitism by brown-headed cowbirds is a factor in the low reproductive success of breeding birds, especially along forest edges (Bollinger and Linder 1994, Gates and Gysel 1978, 1991, Hoover and Brittingham 1993). In the current study, 7 brown-headed cowbirds (*Molthrus ater*) were observed on 2 of the 10 woodlots, both of which were being inhabited by scarlet tanagers, while brown-headed cowbirds were only observed on one woodlot inhabited by red-eyed vireos. But observations of the 11 nests of the two species did not reveal evidence of nest parasitism by brown-headed cowbirds. The other Neotropical species were distributed throughout the different woodlots. In addition to ten species of Neotropical migrants, breeding pairs of red-tailed hawks (*Buteo jamaicensis*), common crows (*Corvus brachyrhynchos*) and red-headed woodpeckers (*Melanerpes erythrocephalus*) were observed using woodlots that were 10 hectares or larger.

The number (N) of breeding pairs and the species richness (SR) of Neotropical migrant species using these woodlots during the breeding season was significantly correlated with the size (N, $r = 0.625$, $P < 0.001$; SR, $r = 0.511$, $P < 0.05$) and the tree species diversity of the forest communities (N, $r = 0.588$, $P < 0.01$; SR, $r = 0.496$, $P < 0.05$). Likewise, the size of the bird community ($r = 0.649$, $P < 0.001$) (Figure 1), as well as

TABLE 1. Importance Values of Trees Species found in Woodlots in Raccoon State Park, Beaver County, PA.

Species	Site 1	2	3	4	5	6	7	8	9	10
Red Maple	181.0	155.9	149.1	120.0	80.0	139.99	98.0	88.6	171.1	125.2
Hawthorn	5.0	5.7	8.3	5.0	9.0	5.0	6.5	12.7	5.0	5.0
Crab Apple	1.0	1.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
White Pine	18.0	12.4	12.0	20.0	20.0	20.0	20.0	20.0	20.0	13.3
American Elm	7.0	8.6	10.8	11.1	9.7	9.0	7.0	14.9	10.1	7.0
White Oak	16.0	60.0	74.8	60.0	109.2	76.7	113.4	86.1	13.8	83.7
Silver Maple	10.0	10.0	10.0	16.9	10.0	10.0	10.0	10.0	26.0	10.0
Black Cherry	20.0	12.0	20.0	24.0	33.6	20.0	20.0	31.3	20.0	20.0
Flowering Dogwood	2.0	2.0	2.0	2.0	3.3	2.0	2.0	3.5	2.0	2.0
Slippery Elm	10.0	10.0	1.0	10.0	3.5	10.0	1.0	10.0	10.0	10.0
Shagbark Hickory	20.0	20.0	10.0	20.0	20.0	5.5	20.0	20.0	20.0	21.9
Sassafras	10.0	2.0	1.0	10.0	1.0	1.0	1.1	1.9	1.0	1.0
Total	300.0	300.2	300.0	300.0	300.3	300.1	300.0	300.0	300.0	300.1

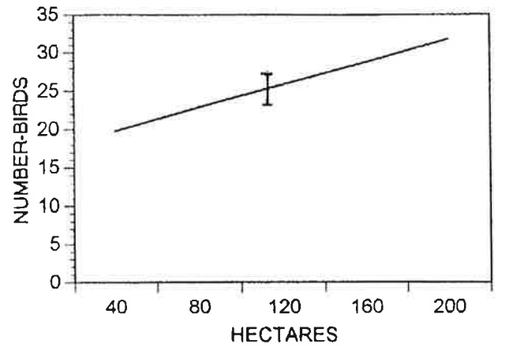
TABLE 2. Bird Species Observed using Woodlots at Raccoon Creek State Park, Beaver County, Pennsylvania

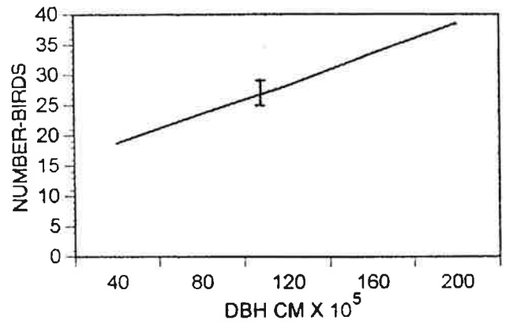
Species	Number Woodlots	Total Pairs Observed
Red-eyed Vireo *(Vireo olivaceus)*	8	19
Scarlet Tanager *(Pireanga olivacea)*	8	18
American Crow* *(Corvus brachyrhynchos)*	4	8
American Redstart *(Selophaga ruticilla)*	3	7
Brown-headed Cowbird** *(Molothrkus ater)*	2	7
Rufus-sided Towhee *(Pipilo erythrophthalmus)*	3	6
Kentucky Warber *(Oporornis formosus)*	5	5
American Robin *(Turdus migratorius)*	4	4
Red-headed Woodpecker* *(Melanerpes erythrocephalus)*	4	4
Wood Thrush *(Hylocichla mustelina)*	2	2
Eastern Pewee *(Contopus virens)*	1	1
Oven Bird *(Sciurus aurocapillus)*	1	1
Red-tailed Hawk* *(Buteo jamaicensis)*	1	1

* Permanent Resident
** Not considered in Neotropical Breeding Bird Index

TABLE 3. Comparison of the Size and Characteristics of Forest Communities and Breeding Bird Communities in Raccoon State Park, Beaver County, PA.

Woodlot	Area-Ha.	Trees Species	Trees Basal area (cm x 10^5)	Breeding Birds N Species	Breeding Birds Est. N./ 100 ha
1	10.8	11	17.5	5	30
2	6.0	15	72.3	4	15
3	21.2	14	67.3	6	18
4	30.0	12	66.5	3	10
5	54.0	16	133.3	5	28
6	120.0	13	39.8	3	20
7	40.0	14	80.3	4	33
8	78.0	16	20.3	3	10
9	200.0	13	143.8	4	18
10	164.8	13	49.0	4	25





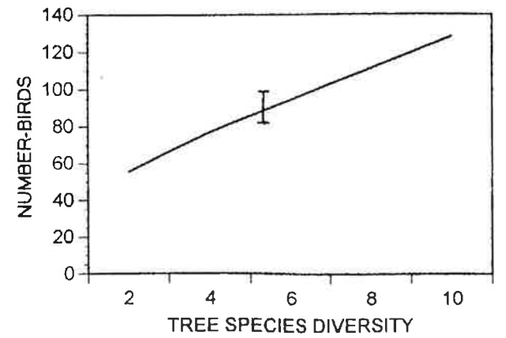


FIGURE 1. Calculated least square regression equations illustrating the relationships between woodlot size and characteristics and the size of breeding bird communities at Raccoon State Park, Beaver County, PA.

species richness (r = 0.443, P <0.05) (Figure 2) of the Neotropical migrants were significantly correlated with the basal area of the woodlot. Moreover, based on a partial correlation analysis, there was a significant interaction between woodlot size and the structure of the forest communities. The interaction between woodlot size and tree species diversity was significantly correlated with the size (r = 0.674, P < 0.001), but not with the species richness (r = 0.238, P > 0.10) of Neotropical migrants. There were significant interactions between the size and basal area of the woodlot (r = 0.681, P < 0.001) and between basal area and tree species diversity (r = 0.761, P < 0.001) and species richness (r = 0.681, P < 0.001), but not between the size of the breeding populations and these parameters: woodlot size and basal area (r = 0.422, P > 0.10); basal area and tree species diversity (r = 0.397, P > 0.10).

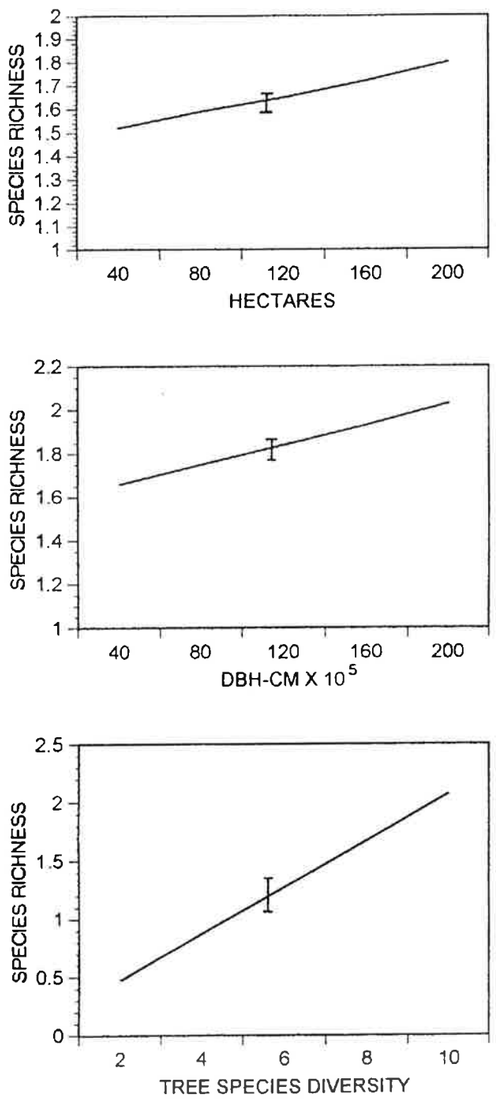


FIGURE 2. Calculated least square regression equations illustrating the relationships between woodlot size, characteristics and the species richness of breeding bird communities at Raccoon State Park, Beaver County, PA.

Previous studies have indicated that the age and structure of woodlots affect size and diversity of breeding bird communities (Whitcomb et al. 1981). In central Pennsylvania, Yahner and Smith (1990) indicated that avian abundance was greater in mesic sites at lower elevations than on xeric sites at middle and upper elevations and that vegetative structure and forest floor features (e.g. fallen logs) were important in providing habitats for breeding birds. Corbat et al. (1988) reported that gray catbirds (*Dumetella carolinensis*) did not use 1-year-old aspen (*Populus spp*) stands, but used 5-year-old and mature stands in proportions to their availability. However, eastern towhees (*Pipilo erythropthalmus*) showed no age-class preference in aspen habitats. But in mixed oak habitats, both catbirds and towhees used 5 year old mixed oak stands more and mature stands less than expected. In a 1993 study, Yahner suggested that the creation of a mosaic of small (1 ha) even-aged stands for the management of ruffed grouse (*Bonasa umbellus*) did not have a detrimental long-term effect on most species of breeding and wintering birds on a localized basis. Based on these, as well as the current study, the size and structure of forest communities should be given consideration in forest management practices and mature stands should be included in development of mosaic forest successional stages in order to maintain a diversity of breeding neotropical bird communities.

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Journal of the Pennsylvania Academy of Science 71(2):78-83, 1998

A MORPHOLOGICAL AND ALLOZYMAL STUDY OF A *CYSTOPTERIS TENNESSEENSIS* (DRYOPTERIDACEAE) POPULATION: FIRST REPORT OF THE SPECIES IN LANCASTER COUNTY, PA[1]

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ABSTRACT

Two populations of the fern genus *Cystopteris* (Dryopteridaceae) in Lancaster County, PA were examined. One mixed population consisted of *C. bulbifera* and plants morphologically like *C. tennesseensis* while the other was of *C. protrusa*. The mean spore size of the *C. tennesseensis* plants was found to be statistically significantly larger than spores of *C. bulbifera* and *C. protusa*. As *C. tennesseensis* is known to be an allotetraploid hybrid of *C. bulbifera* and *C. protusa,* an allozymal study was undertaken of these populations to determine if marker alleles in the diploids also would be found in the putative allotetraploid. Marker alleles for *C.protrusa* were found at the *phosphoglucomutase-2* (pgm-2), *glucosephosphate isomerase-2* (gpi-2), and *malate dehydrogenase-2* (mdh-2) loci and for *C. bulbifera* at the pgm-2 and mdh-2 loci that were combined in the pulative *C. tennessensis* plants. One triploid backcross of *C. tennessensis* to *C. protrusa* was analyzed allozymally. This study confirms the first report of *C. tennessensis* in Lancaster County, PA and provides specific allozymal evidence of its origin as an allotetraploid hybrid of *C. bulbifera* and *C. protrusa.*
[J PA Acad Sci 71 (2) 78-83, 1998]

INTRODUCTION

Cystopteris, the fragile fern, is a genus of 17-20 morphologically similar species. The Cystopteris species are noted for forming a polyploid complex in which the species have high chromosome numbers and are biologically distinct but are often difficult to distinguish morphologically (Haufler et al, 1993). The *C. fragilis* polyploid complex is centered in Eastern North America (Haufler et al, 1993). In this study, populations of *C. protrusa* (Weath.) Blasdell, *C. bulbifera* (L.) Bernhardi and their allotetraploid hybrid derivative *C. tennesseensis* Shaver were analyzed allozymally and by a spore study.

Cystopteris species are either epipetric or terrestrial although all grow in mesic to moist habitats. Of the species in this study, *C. protrusa* is terrestrial, growing in humus-rich, moist loam soils of woodlands. *C. bulbifera* and *C. tennesseensis* are both epipetric, most often found on shaded limestone, shale or dolomite. The habitats of *C. bulbifera* and *C. tennesseensis* overlap more than those of *C. tennesseensis* and *C. protrusa* but the hybrid can survive in areas intermediate of both progenitors (Lellinger, 1985; Taylor, 1984). Hybrids have been located growing with both presumptive parents, with but one, or remote from either (Wherry, 1961).

The two diploid species have distinct morphological characteristics which enable confident determination. *Cystopteris protrusa* is characterized by a protruding, copiously hairy rhizome, straw colored stipe, and a non-bulbiferous ovate to lanceolate blade (Haufler and Windham, 1991). Unfortunately, the plant must be sacrificed to examine the most definitive trait, its rhizome; so specimens exhibiting this trait are usually absent from herbarium specimens and only the fronds are present. *C. bulbifera,* with the rhizome never protruding beyond the current season's fronds, glandular fronds, a reddish brown to purplish stipe, distinctive spherical bulblets on the underside, sparse scales at the base, and a deltoid-attenuate blade (Lellinger, 1985) is also a fairly distinct species. *C. tennesseensis* displays variations of the parental characteristics such as rhizomes rarely protruding beyond the current season's growth, sparsely glandular fronds, abortive bulblets, and deltoid-ovate to lanceolate blade (Haufler et al, 1993). While these morphological characteristics usually permit preliminary determination of a *C. tennesseensis* specimen, more analysis is needed for confirmation, as it frequently resembles *C. bulbifera.* The possibility of back-crosses also exists.

[1]Received for publication 12 April 1997; accepted 15 July 1997.

C. tennesseensis has a single report in each of just five Pennsylvania Counties, including eastern Chester County (Rhoads and Klein, 1993). Both *Cystopteris protrusa* and *C. bulbifera* are common in Lancaster County and it is reasonable to expect that their allotetraploid hybrid would also be present, but as yet it has not been reported (Rhoads and Klein, 1993). Parks has discovered a site that contains plants with characteristic *C. bulbifera* and *C. tennesseensis* and another site within 7.0 km which contains *C. protrusa.* Both a spore study and an allozymal analysis of the plants in these populations have been undertaken.

An examination of relative spore size would indicate if a tetraploid species e.g. *Cystopteris tennesseensis* and diploid species e.g. *C. protrusa* and *C. bulbifera* were both present as the average spore size of the diploid progenitors is smaller than that of the tetraploid. Previous studies report the average spore size of *C. protrusa* and *C. bulbifera* ranges from 29 to 35 µm and *C. tennesseensis* ranges from 38 to 41 µm (Haufler et al. 1990; Moran, 1982a).However, while there is a correlation between spore size and ploidy level, other analyses should also be used (Haufler et al. 1990).

Because it is a hybrid of *Cystopteris bulbifera* and *C. protrusa, C. tennesseensis* is expected to show a combination of alleles from the two species (Haufler et al. 1990). An allozymal study should yield protein markers for *Cystopteris protrusa* and *C. bulbifera* permitting the determination of which alleles in *C. tennesseensis* were given to the allotetraploid from each progenitor. Furthermore, where enzyme polymorphisms are found within the putative diploid parents, evidence for multiple hybridization events having produced the various *C. tennesseensis* plants in the population is possible.

The primary goal of this study is to provide conclusive evidence for the first report of *Cystopteris tennesseensis* in Lancaster County, PA. The secondary goal is to gain insight into the origin of the *C. tennesseensis* plants in the mixed population; specifically to seek evidence of multiple origins for the various plants present.

METHODS

Study Sites

The first study site is 5 kilometers northwest of Marticville in Lancaster County, PA. It is a shaded northeast facing cliff along a roadside approximately 15 meters above the Pequea Creek in an isolated outcrop of the Conestoga limestone formation surrounded by the Wissahickon mica-schist formation. The cliff side with the mixed *Cystopteris* population runs approximately 20 meters along the roadside and at least 4 meters high. The two species were not evenly distributed throughout the site. The putative *C. tennesseensis* were commonly found growing on the cliff whereas the *C. bulbifera* plants were concentrated lower in the bank along the roadside. Each of the populations was sampled at intervals of approximately 30 centimeters. A few plants of *C. tenuis* (Michx.) Desv., a tetraploid hybrid of *C. protrusa* and an unknown diploid (Moran, 1983a; Haufler and Windham, 1991), were scattered about the periphery but no *C. protrusa* was found. *C. protrusa* plants were collected at site 2, approximately 7.0 kilometers away. This site is near the Conestoga River in the vicinity of Safe Harbor. It is a wooded area on a hillside and exhibits typical woodland alluvial soil. The specimens sampled from this population were from individuals or small clusters of individuals spaced approximately 2 meters apart. One specimen of *C. protrusa* included the protruding tip which extends beyond the new fronds, confirming its identification.

Spore Study

Thirty fronds of the putative *Cystopteris tennesseensis,* twenty-five fronds of *C. bulbifera* and twenty-nine fronds of *C. protrusa* were collected for analysis. Thirty of these fronds were selected for herbarium specimens. Fronds were collected late in August of 1995 and during the following spring. Both the sterile autumn fronds and the fertile fronds of *C. protrusa* were collected. Vouchers are on deposit at the Millersville University Herbarium (MVSC).

Specimens from each of the three populations were examined and the spores collected from 5 to 10 fertile specimens of each species to form a pooled sample. Twenty-five spores from each species were measured to the nearest 0.1 micron with a light microscope at 200X using a calibrated optical micrometer. One or two spores were also measured at 430X for greater precision. The average spore size and standard deviation for each of the species were calculated. The recorded data were analyzed by the ANOVA Newman-Keuls and Duncan multiple range test to determine if there were significant differences among the three species.

Allozymal Study

Fresh fronds were used for electrophoretic analyses and were kept refrigerated for a maximum of 7 days before protein extraction. Protocols used in this study were those of Soltis et al. (1983) except for the morpholine system which followed the protocols of Werth (1991) and the histidine running buffer, which was modified to pH 7.0. The specimens were ground in the phosphate extraction buffer system (pH 7.0) using spot plate wells and a round bottom test tube as a pestle.

The enzyme systems examined were shikimate dehydrogenase (SKDH), phosphoglucomutase (PGM-1 & PGM-2), isocitrate dehydogenase (IDH), glucose 6-phosphate isomerase (GPI), hexokinase (HK), 6-phosphogluconate dehydrogenase (PGD), and malate dehydrogenase

(MDH-2). Resolved on the morpholine system were 6-PGD, SKDH, and MDH; on the histidine system were SKDH, IDH, and PGM; and on the lithium system were PGM, GPI, and HK.

Refrigerated, iced gels were run for an average of five hours. The lithium gels were run at constant current of 66 mA. The histidine and morpholine gels were run using constant power of 13 watts. The gels were sliced and stained using the schedules of Soltis et al. (1983) but employing the xymecycle method of Werth (1989b). Developed gels were scored, photographed and discarded. The most anodal locus was numbered 1, and within loci the most anodal band was numbered 1. Alignment between runs was maintained by running the same reference plant in lanes 8 and 16 of all gels. The reference plant was an individual of the species *Cystopteris bulbifera* but was not from the study population.

RESULTS

Spore Study

The average diameter of the putative *Cystopteris tennesseensis* spores was 50.8 microns, *C. bulbifera* 38.5 microns, and *C. protrusa* 41.9 microns (Table 1). The results from the ANOVA analysis showed a significant difference at the 0.01 level in spore size between the putative C. tennesseensis and each of the parents but not between the parents.

Allozymal Study

The results from the allozymal analysis are seen in Table 2. The enzyme producing consistently resolvable bands on histidine was PGM-2; on morpholine SKDH; and on lithium GPI. Useful data were also obtained for IDH from histidine and MDH-2 from morpholine.

The bands from SKDH consistently resolved well. Putative *Cystopteris tennesseensis* exhibited the 1-2 band pattern in 100% of the specimens. *C. protrusa* exhibited two genotypes, homozygous 2-2 and the 1-2 genotype. The 2-2 genotype exhibited the higher frequency (89%) while the 1-2 genotype was uncommon and only seen in 11% of the specimens. *C. bulbifera* also displayed 2 different genotypes, homozygous 1-1 and the 1-2 genotype with the latter occurring at a slightly higher frequency.

TABLE 1: The average spore size in microns of *Cystopteris tennesseensis, Cystopteris bulbifera*, and *Cystopteris protrusa*. The data were analyzed using the ANOVA multiple range test (CL=95%). There was no significant difference between *C. bulbifera* and *C. protrusa*. There was a significant difference between *C. tennesseensis* and its progenitors.

Species of Cystopteris	Average Spore Size	Ploidy (n value)
C. bulbifera	38.5 ± 3.2	2n
C. protrusa	41.9 ± 5.5	2n
C. tennesseensis	50.8 ± 6.4	4n

Good clarity was also shown by PGM-2 and the genotypes observed were numerous. Only two band patterns were observed in putative *Cystopteris tennesseensis*; the 1-4 pattern which was observed 96% of the time and the uncommon 1-3-4 pattern observed only once. *C. protrusa* displayed three different genotypes, 4-4, 3-4 and 4-5. The most common genotype among the *C. protrusa* specimens was 4-4 (78%) and both the genotypes 3-4 and 4-5 were relatively uncommon, only being observed 9% and 13% of the time respectively. Four different genotypes were observed in *C. bulbifera* with no one genotype being especially common along the specimens analyzed.

The enzyme system IDH resolved well enough to score on fewer gels but three alleles in various genotypes were observed in both *Cystopteris protrusa* and *C. bulbifera.* Only one banding pattern was observed in putative *C. tennesseensis*, 2-3 phenotype. Five different genotypes were observed in the sample of *C. protrusa*. The most frequently observed genotype was 3-3 and was observed in 50% of the individuals sampled. The other genotypes, observed within the *C. protrusa* individuals were 2-3, 1-2, 1-1 and 2-2. *C. bulbifera* exhibited four different genotypes within the test sample with the 1-1 genotype being most abundant (58%). The genotypes 1-4, 2-3 and 2-2 were also exhibited.

GPI-2 also had scorable bands though resolution was not consistently good, especially in *Cystopteris protrusa*. Putative *C. tennesseens*is exhibited three banding types; 2-3, 3-3, and 2-3-4. The 2-3 pattern was most common, observed in 63% of the test sample, 3-3 pattern has a frequency of 32%. The 2-3-4 pattern was observed in only one individual. *C. protrusa* shows much variation but only 9 specimens had scorable bands. The 3-5 genotype was most frequently observed and the 2-4 genotype and 4-4 also occurred within the test sample. *C. bulbifera* exhibited only two genotypes, 3-3 (94%) and the 3-5 genotype (6%).

The banding patterns of the enzymes GPI-1, PGM-1 and MDH-1 were observed but none were scorable. The results of PGD, MDH-2 and HK enzymes had readable bands but were only observed on a few of the gels. The bands that were scored were consistent with the hypothesis but were too few to be included in the analysis.

DISCUSSION

Spore Study

Our results are consistent with earlier studies by Haufler et al. (1990) and Moran (1982a). Both studies found no significant difference between the spore sizes of *Cystopteris protrusa* and *C. bulbifera,* and a significant difference between the spore size of the parentals and the putative allotetraploid hybrid *C. tennesseensis.* Our data indicate 26% bigger spores for *C. protrusa* and the presumed *C. tennesseensis* and a 6% larger spore size in *C. bulbifera* then reported in Haufler et al. (1990). The previous study had samples collected throughout a large geographic area from Missouri to Kentucky and North Carolina which may explain the differences in the average spore sizes they reported and those found in the present study. Our data indicate that the putative *C. tennesseensis* spores are significantly larger than those of the diploid species growing with it. This analysis supports the premise that the population discovered is *C. tennesseensis.* Like Moran (1982a), we found that relative spore size can be a reliable character when diploid and polyploid taxa cannot be distinguished reliably based on frond morphology.

TABLE 2. A comparison of the allozymal results of the three *Cystopteris* species; *C. protrusa, C. tennesseensis,* and *C. bulbifera*. The observed genotypes for the diploid species and the banding patterns for the allotetraploid are shown as well as gene frequency in the individuals sampled, the number of individuals analyzed with each banding pattern, and the total number of individuals sampled. The banding patterns observed shows the complementarity between the two diploid species and the allotetraploid.

	C. protrusa			*C. tennesseensis*		*C. bulbifera*		
	genotype observed	number of individuals	frequency	band pattern observed	number of individuals	genotype observed	number of individuals	frequency
SKDH	2 - 2	25	2=0.95	1 - 2	35	1 - 2	15	1=0.73
	1 - 2	3	1=0.05			1 - 1	13	2=0.27
total(n)		28			35		28	
PGM-2	4 - 4	18	4=0.89	1 - 4	27	1 - 3	9	3=0.59
	4 - 5	3	5=0.07	1 - 3 - 4	1	2 - 3	7	1=0.21
	3 - 4	2	3=0.04			3 - 3	5	2=0.21
						2 - 2	1	
						1 - 1	1	
total (n)		23			28		23	
IDH	3 - 3	10	3=0.60	2 - 3	26	1 - 1	11	1=0.61
	2 - 3	4	2=0.18			2 - 3	4	2=0.28
	1 - 1	4	1=0.12			2 - 2	3	3=0.11
	1 - 2	1				1 - 4	1	
	2 - 2	1						
total (n)		20			26		19	
GPI-2	3 - 5	5	3=0.28	2 - 3	12	3 - 3	16	3=1.00
	2 - 4	3	5=0.28	3 - 3	6	3 - 5	1	
	4 - 4	1	4=0.28	2 - 3 - 4	1			
total (n)		9	2=0.17		19		17	
MDH-2	1 - 1	10	1=1.00	1 - 2	9	2 - 2	10	2=1.00
total (n)		10			9		10	
6PGD-2	1 - 2	3	2=0.70	2 - 2	5	2 - 2	5	2=1.00
	2 - 2	3	1=0.30					
total (n)		5			5		5	
HK	1 - 2	6	1=0.50	1 - 2	7	1 - 1	6	1=1.00
total (n)		6	2=0.50		7		6	

Allozymal Study

Inferring the alleles present from the electromorph or band patterns of allozymes is fairly straightforward for diploids and has been widely accepted among botanists (Weeden and Wendel, 1989; Wendel and Weeden, 1989; Werth, 1989a). However, determining the genotype of polyploids from the gel electromorph is complicated by many variables (Barrett and Shore, 1989) and is virtually impossible when the diploid progenitors are uncertain or their genetic variation is not well characterized (Werth, 1989a). When the diploid progenitors are known and their relevant genetic variation is available, prudent inferences can be made about the origin of alleles visualized on polyploid hybrids (Werth, 1991). The band patterns of the presumed *Cystopteris tennesseensis* exhibited alleles seen in its diploid progenitors for all enzyme systems examined in this study. However, various combinations of alleles can produce the same band pattern in a tetraploid

whereas the identical pattern in a related diploid species can be interpreted genetically with confidence. In this treatment, we infer the genotypes of band patterns seen in the allotetraploid specimens cautiously in context to genotypes seen in the diploids.

The enzyme system PGM-2 resolved consistently well on the Histidine buffer system and produced the strongest allozymal evidence that the plants identified as *Cystopteris tennesseensis* were really this allotetraploid species. The putative *C. tennesseensis* exhibited a fixed 1-4 heterozygous band pattern in all but one of the specimens for the monomeric enzyme PGM. The 4 allele is found only in *C. protrusa* where it is common (f=0.89) and the 1 allele is found only in *C. bulbifera* (Table 2), so these alleles serve as markers for their respective diploid species. The presence of both alleles in the unusual plants we found indicates that they have *C. protusa* and *C. bulbifera* as their parents and are *C. tennesseensis* (Haufler, et al., 1990). The presence of the 1-2 complementary band pattern far in excess of the 0.18 frequency expected based on allele frequencies in the diploids is evidence that all the *C. tennesseensis* examined here are descended from one allotetraploid hybrid.

Allozymal analysis of other enzymes yielded data consistent with that of PGM-2. SKDH was fixed 1-2 in *Cystopteris tennesseensis* (Table 2). In *C. protrusa* the 2-2 homozygote was predominant but in *C. bulbifera* the 1-1 homozygote and the 1-2 heterozygote were equally common. The 1-2 band pattern could have arisen from several different crosses of the diploids but with different probabilities based on the diploids' allele frequencies. Also, *C. tennesseensis* could be expected to exhibit a 2-2 single band pattern 25.6% of the time and the 1-1 pattern 3.6% of the time. Recovery of only the 1-2 band pattern is best explained as an unlikely hybridization event occurring once between parents with common alleles followed by reproduction of the fertile allopolyploid. Both *C. protrusa* and *C. bulbifera* have the same three alleles for IDH and *C. tennesseensis* is fixed for the 2-3 band pattern. These results support the hypothesis that the presumed population identified as *C. tennesseensis* arose from *C. protrusa* and *C. bulbifera* and that one hybridization event occurred but the origin of the IDH alleles cannot be ascertained. Resolution of MDH-2, PGD-2, and HK was not consistent, so fewer individuals could be scored reliably. All three enzyme systems yielded data consistent with the hypothesis. The preliminary data for MDH-2 suggests that each diploid is fixed for a distinct marker allele and that putative *C. tennesseensis* has inherited both alleles. Future allozymal studies of these taxa should utilize the MDH-2 locus.

The GPI-2 locus is the only one yielding data indicating that multiple hybridication events produced the plants in this population. The presumptive *C. tennesseensis* shows two distinct band patterns, suggesting that two hybridization events produced the plants examined. However, based on allele frequencies of SKDH, PGM-2, and IDH, the probability of producing the one genotype recovered at all three loci twice by chance is 0.0005. As we had technical problems resolving GPI, we have less confidence in the data for this system.

One plant of supposed *Cystopteris tennesseensis* yielded an especially interesting series of band patterns (Table 2). The anomalous 1-3-4 pattern seen in the monomeric PGM is best explained as being produced by a triploid. The putative *C. tennesseensis* as the tetraploid parent would contribute the 1 and 4 alleles to a backcross; but which diploid would add the 3 allele? Since *C. bulbefera* is growing with *C. tennesseensis,* the 3 allele is much more frequent in *C. bulbifera,* and this backcross has been reported previously based on allozymal evidence by Haufler et al. (1990), we reasoned initially that *C. bulbifera* is the diploid parent. Examination of the same plant for the dimeric GPI-2 allozyme pattern, however, revealed an equally anomalous four band pattern (Table 2). This is scored 2-3-4, which we interpret as a 2-3 heterozygote plus 4 as one copy. As the 4 allele was found only in *C. protrusa,* a backcross to that diploid species is indicated. This lead us to examine the band pattern for the well resolved monomer SKDH. In this population, both *C. protrusa* and *C. bulbifera* share the same two alleles so only one fixed heterozygote band pattern would be possible: 1-2, the one recovered. Further, a triploid backcross could not produce a distinct band pattern but would produce differential intensity in the 1-2 band pattern present. Careful examination of the gel indicated that the 2 band was darker, indicating that this two band pattern should be interpreted as a 1-2 heterozygote plus a second copy of the 2 allele (1-2-2). This is consistent with the results seen in the other allozyme loci and with the high frequency (0.95) of the 2 allele in *C. protrusa.* The report of Haufler et al (1985) of triploid *C. protrusa* may include backcrosses of this type.

In conclusion, this is the first report of the allotetraploid fern species *Cystopteris tennesseensis* in Lancaster County, PA (Rhoads and Klein, 1993). Preliminary determination based upon morphology was supported by the larger relative spore size of the tetraploid compared to spores of its putative diploid progenitors *C. protrusa* and *C. bulbifera.* This analysis only indicated the presence of a tetraploid population of *Cystopteris.* A comparative allozymal analysis demonstrated that the putative allotetraploid exhibits allele complementarity; that is, it has fixed heterozygotes for marker alleles of PGM-2, GPI-2, and MDH-2 each of which are unique to one or the other of its diploid progenitors. The presence of one fixed heterozygote banding pattern at three loci where there's allelic variation in the diploids suggests that all of the *C. tennesseensis* plants examined are descendants of one hybrid parent. Only the GPI-2 locus yielded contradictory results. Allozymal analysis also revealed one plant, originally thought to be a *C. tennessensis*, that proved to be a triploid backcross of that species to *C. protrusa.* Future work should include allozymal characterization of *C. tenuis,* another tetraploid common in the area (Rhoads and Klein, 1993) that shares a *C. protrusa* genome (Haufler and Windham, 1991). Searches for additional unreported *Cystopteris* hybrids likely to be present in Southeastern Pennsylvania such as *C.* x *illinoensis* (sterile hybrid of *C. bulbifera* and *C. tenuis;* cf. Moran, 1982b) and *C. x wagneri* (sterile hybrid of *C. tennesseensis* and *C. tenuis;* cf. Moran, 1983b) also should be conducted.

ACKNOWLEDGMENTS

The senior author would like to thank the Millersville University Faculty Grants Committee and the junior author would thank the Millersville University Undergraduate Grants Committee for partial support of this study. We thank Dr.'s Susan Dibartolomeis, David Dobbins and three anonymous reviewers for helpful comments on an earlier version of this paper.

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Journal of the Pennsylvania Academy of Science 71(2):84-89, 1998

PENNSYLVANIA LABOR MARKET AREA MIGRATION, 1985 TO 1990: RESPONSES TO CHANGING EMPLOYMENT OPPORTUNITIES[1]

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ABSTRACT

Pennsylvania has experienced significant changes in its migration patterns after 1985. Earlier explanations of Pennsylvania's migration patterns have focused upon economic issues, particularly employment opportunities, associated with restructuring in the state's economy. Such explanations imply that the current patterns are related to shifts in Labor Market Areas' economic circumstances.

This paper first briefly describes recent migration patterns and employment trends in Pennsylvania. It then estimates the responsiveness of Pennsylvania's migrants to changes in employment opportunities of the state's fourteen major Labor Market Areas between 1985 and 1990. Results indicate that migrants were, indeed, responsive to changes in Labor Market Areas' employment opportunities.
[J PA Acad Sci 71 (2) 84-89, 1998]

INTRODUCTION

From 1970 through the early 1980s two-thirds of Pennsylvania's counties registered net internal out-migration. Those migration patterns have been characterized as movements from western counties with poor economic opportunity, particularly in terms of employment prospects, to eastern counties with greater employment possibilities (DeJong and Cornwell, 1989). However, between 1985 and 1990 Pennsylvania's migration pattern changed, as roughly half of Pennsylvania's counties experienced net internal out-migration. Even more striking, between 1990 and 1995 only fourteen counties registered net internal out-migration.

Earlier explanations for Pennsylvania's migration patterns have focused on changes in the Commonwealth's economic landscape (DeJong and Cornwell, 1989). Specifically, they have focused on the spatial bias in the changes of employment opportunities as the state struggled through the restructuring of its economy. The restructuring involved the movement away from a predominately manufacturing-based economy to a service-based economy. Eastern counties, with more diverse economies than their western counterparts, continued to experience job growth through the restructuring period. Many western counties, on the other hand, endured significant employment losses. Therefore, it is tempting to explain Pennsylvania's current migration patterns in terms of recent employment opportunities for migrants.

There are many ways employment opportunities can be measured for use in migration analysis (Blanco, 1962, 1963, and 1964; Greenwood, 1975; Plane and Rogerson, 1994), and their role in provoking migration has been established (Lowry, 1966; Greenwood, 1975 and 1981; Weeks, 1996). Indeed, Lewis (1982) contends that little new has emerged over the past several decades despite ever more sophisticated models and approaches to analyzing employment opportunities as a cause of migration. That is, the direction and flow of migration is determined by basic territorial shifts in the regional balance of the economy. Hence, Pennsylvania's shifting internal migration patterns can be understood in terms of the response by migrants to changes in the spatial arrangement of the state's employment opportunities.

Employment opportunities are best measured at the scale of the Labor Market Area (LMA) because LMAs are aggregations of contiguous counties having similar sectoral economic structures. The LMA, then, is a county or group of counties having a central city without regard to population size and is considered to be an

[1]Received for publication 28 April 1997; accepted 13 June 1997.

economically integrated unit within which workers may readily change jobs without changing place of residence (PA Dept. of Labor and Industry). The U.S. Bureau of Census has identified fourteen major Labor Market Areas in Pennsylvania.

This paper estimates the responsiveness of Pennsylvania's migrants to changes in the fourteen major LMAs' economic performances between 1985 and 1990 in terms of employment opportunities. The 1985 to 1990 period is particularly interesting to study because it is during this time that significant changes in both Pennsylvania's migration and employment patterns occurred.

Following is a brief discussion of recent changes in the Commonwealth's migration flows after which employment trends are discussed. Migration data for 1985 to 1990 was obtained from the U.S. Census Bureau which provides county-to-county migration flows on CD-ROM. Labor Market Areas' employment data were aggregated from data prepared by the Pennsylvania Department of Labor and Industry, Bureau of Research and Statistics publications for 1985 to 1995. The publications provide employment and wage data by industry for those employers covered under the state's Unemployment Compensation Law, and accounts for about 95 percent of all non-agricultural wage and salary employment.

RECENT MIGRATION FLOWS IN PENNSYLVANIA

Table 1 shows LMAs' net migration values and net migration rates for 1985 to 1990 and for 1990 to 1995. Net migration is the difference between those who moved into and those who moved out of an LMA. The net migration rate adjusts for population differences in the LMAs and is the net number of migrants during the five years per 1,000 people in the population at the beginning of the period.

Five of the western LMAs recorded net internal out-migration for 1985 to 1990. Some of these net internal out-migration values and rates are relatively significant. For example, the Pittsburgh LMA registered net internal out-migration of 88,869 persons or a net migration rate of -37.1 persons per 1,000. The Johnstown LMA also experienced a relatively high rate of out-migration, -45,98 per 1,000, with 11,092 more persons moving out of the LMA than moving in.

Meanwhile, in the east for this period, the majority of LMAs were experiencing net internal in-migration. Indeed, the Lancaster LMA recorded a net migration rate of 50.82 per 1,000. Other eastern LMAs registering relatively high rates of net internal migration during the period include, York (29.3) Reading (27.88), Harrisburg-Lebanon-Carlisle (27.46), and Allentown-Bethlehem-Easton (25.24).

After 1990 the migration patterns changed. Two of the western LMAs, Altoona and Sharon, reversed their net internal migration rate signs from negative to positive and the rates of the other three improve significantly. For instance, the Pittsburgh LMA had 21,702 net internal out-migrants for this period compared to almost 89,000 for the earlier time. Its net internal migration rate increased from -37.1 per 1,000 to -9.06 per 1,000. The Johnstown LMA also had a significant decrease in its

TABLE 1. Net Migration and Net Migration Rates 1985 to 1990 and 1990 to 1995 for Pennsylvania's Fourteen Major Labor Market Areas

Labor Market (Counties)	Region	1985 to 1990 Net Migration	1985 to 1990 Net Migration Rate	1990 to 1995 Net Migration	1990 to 1995 Net Migration Rate
Allentown-Bethlehem (Carbon, Northampton, Lehigh)	East	15,022	25.24	5,134	8.37
Altoona (Blair)	West	-4,235	-32.44	490	3.72
Erie (Erie)	West	-4,955	-17.98	-3,811	-13.59
Harrisburg, Lebanon, Carlisle (Cumberland, Dauphin, Lebanon, Perry)	East	16,146	27.46	7,743	12.64
Johnstown (Cambria, Somerset)	West	-11,092	-45.98	-864	-3.59
Lancaster (Lancaster)	East	21,488	50.82	6,065	13.55
Philadelphia (Bucks, Chester, Delaware, Montgomery, Philadelphia)	East	-61,420	-16.47	-140,220	-37.57
Pittsburgh (Allegheny, Beaver, Butler, Fayette, Washington, Westmoreland)	West	-88,869	-37.1	-21,702	-9.06
Reading (Berks)	East	9,383	27.88	4,878	13.95
Scranton-Wilkes Barre- Hazleton (Columbia, Lackawanna, Luzerne, Wyoming)	East	2,013	3.15	-397	-0.624
Sharon (Mercer)	West	-474	-3.92	864	7.07
State College (Centre)	West	13,828	111.63	2,724	20.64
Williamsport (Lycoming)	East	-155	-1.31	-932	-7.75
York (York)	East	9,950	29.3	13,421	36.99

Source: Calculated by authors from 1990 U.S. Bureau of Census data and 1996 Population Estimates.

number of internal out-migrants from over 11,000 in 1985 to 1990 to 864 in 1990 to 1995. Its net internal migration rate increased from -45.98 to just -3.59.

Meanwhile, in the east, several of the LMAs saw rather significant declines in their net internal migration rates and a few even saw their positive signs reverse to negative. For example, the Scranton-Wilkes Barre-Hazleton LMA had net internal in-migration of over 2,000 persons (3.15 per 1,000) in 1985 to 1990. For the 1990 to 1995 period, this LMA had net internal out-migration of 397 persons (-0.62 per 1,000). The Philadelphia LMA experienced a huge increase in the number of net internal out-migrants 61,240 in 1985 to 1990 versus 140,220 in the latter period. Its rate of net internal migration rose from -16.47 per 1,000 to -37.57 per 1,000 over the two time periods.

RECENT EMPLOYMENT TRENDS IN PENNSYLVANIA

Earlier explanations for Pennsylvania's migration patterns have focused on changes in employment opportunities for migrants (DeJong and Cornwell, 1989). Following is a brief discussion of the recent changes that have occurred in employment opportunities for migrants in Pennsylvania. Between 1980 and 1990, Pennsylvania experienced a 23.5 percent decrease in manufacturing employment, far outpacing the 6.6 percent drop nationwide. Other sectors that registered employment declines in the decade were agriculture (5.1 percent) and public administration (4.1 percent). On the positive side, construction jobs rose 37.9 percent, employment in the financial sector jumped 36.9 percent, and professional services employment increased 32.6 percent (PA. State Data Center, 1996).

There was however, a spatial bias to the loss of manufacturing jobs and to the gains of employment in other sectors. Table 2 shows the total non-agricultural employment, employment change, and average annual rate of employment change for Pennsylvania's fourteen major LMAs for three time periods: 1980 to 1985, 1985 to 1990, and 1990 to 1995. Table 2 indicates that, for the 1980 to 1985 period, seven of the fourteen LMAs recorded employment growth. Six of these were located in the eastern part of the state. Meanwhile, five of the seven LMAs showing employment declines during the period were located in the western part of the state. Many of the job losses experienced by the western LMAs were in the manufacturing sector and were not being replaced with new employment in other sectors.

Meanwhile, eastern LMAs were also losing jobs in the manufacturing sector. However, unlike the western LMAs, there was significant employment growth in other industry sectors. Many of the newly created jobs were in the service sector and this allowed six of the eastern LMAs, as Table 2 indicates, to record overall employment growth for the

TABLE 2. Total Non-Agricultural Employment, Employment Change, Percent Employment Change, and Average Annual Rate of Employment Change, 1980 to 1995, for Pennsylvania's Fourteen Major Labor Market Areas

Labor Market (Counties)	Region	1980 Employment	Employment Change 1980 to 1985	Percent Employment Change 1980-1985	Average Annual Rate of Change	1985 Employment	Employment Change 1985 to 1990	Percent Employment Change 1985-1990	Average Annual Rate of Change	1990 Employment	Employment Change 1990 to 1995	Percent Employment Change 1990-1995	Average Annual Rate of Change	1995 Empolyment
Allentown-Bethlehem (Carbon, Northampton, Lehigh)	East	205,550	-3,584	-1.74	-0.35	201,966	14,035	6.95	1.39	216,001	993	0.46	0.09	216,994
Altoona (Blair)	West	38,879	-203	-0.52	-0.10	38,676	4,727	12.22	2.44	43,403	2,660	6.13	1.23	46,063
Erie (Erie)	West	97,416	-3,433	-3.52	-0.70	93,983	9,335	9.93	1.99	103,318	4,184	4.05	0.81	107,502
Harrisburg, Lebanon, Carlisle (Cumberland, Dauphin, Lebanon, Perry)	East	188,883	15,893	8.41	1.68	204,776	31,402	15.33	3.07	236,178	16,745	7.09	1.42	252,923
Johnstown (Cambria, Somerset)	West	67,398	-7,599	-11.27	-2.25	59,799	7,511	12.56	2.51	67,310	2,580	3.83	0.77	69,890
Lancaster (Lancaster)	East	135,475	11,944	8.82	1.76	147,419	23,466	15.92	3.18	170,885	6,373	3.73	0.755	177,258
Philadelphia (Bucks,Chester, Delaware, Montgomery, Philadelphia)	East	1,290,918	69,941	5.42	1.08	1,360,859	106,174	7.80	1.56	1,467,033	-33,773	-2.30	-0.46	1,433,260
Pittsburgh (Allegheny,Beaver, Butler,Fayette,Washington,Westmoreland)	West	855,252	-68,635	-8.03	-1.61	786,617,	73,867	9.39	1.88	860,484	26,387	3.07	0.61	886,871
Reading (Berks)	East	118,235	5,591	4.73	0.95	123,826	9,624	7.77	1.55	133,450	1,943	1.46	0.29	135,393
Scranton-Wilkes Barre-Hazleton, (Columbia, Lackawana, Luzerne, Wyoming)	East	199,230	1,156	0.58	0.12	200,386	24,436	12.19	2.44	224,822	1,095	0.49	0.10	225,917
Sharon (Mercer)	West	39,497 -	6,336	-16.04	-3.21	33,161	4,456	13.44	2.69	37,617	38,724	1,107	2.94	0.59
State College (Centre)	West	26,118	2,560	9.80	1.96	28,678	7,675	26.76	5.35	36,353	38,612	2,259	6.21	1.24
Williamsport (Lycoming)	East	38,654 -	1,150	-2.98	-0.60	37,504	5,320	14.19	2.84	42,824	43,809	985	2.30	0.46
York (York)	East	118,922	2,087	1.75	0.35	121,009	15,489	12.80	2.56	136,498	140,614	4,116,	3.02	0.60

Source: Calculated by the authors from Pennsylvania Department of Labor and Industry, Bureau of Research and Statistics data.

period. Clearly, it appears that the eastern LMAs were making the transition from a manufacturing-based economy to a service-based economy with greater success than the western LMAs during the 1980 to 1985 period.

After 1985 employment patterns in Pennsylvania changed. Table 2 shows that from 1985 to 1990 all fourteen LMAs recorded employment growth. The spatial bias in manufacturing job losses and gains in service sector employment also began to disappear. For instance, the average annual rate of service sector employment growth was greater in Pittsburgh than in Philadelphia for the period and the Johnstown LMA actually gained over 1,800 manufacturing jobs. Thus, the spatial arrangement of employment opportunity became more balanced between 1985 and 1990.

Both the changes in the sectors' employment and their spatial expression became even more balanced after 1990 and, if anything western LMAs may be considered to have outperformed eastern LMAs from 1990 to 1995. Table 2 shows the western LMAs continued to show improvement in their average annual rate of employment growth during this period. Losses of manufacturing jobs slowed significantly in the western LMAs while new opportunities in the service sector were more than making up for the losses.

Meanwhile, as Table 2 shows, five of the eight eastern LMAs saw a reduction in their average annual rate of employment growth. During the period employment losses in the manufacturing sector continued at approximately the same pace across the eastern LMAs while employment growth in other sectors slowed.

The previous discussion shows how the spatial arrangement of employment opportunity in Pennsylvania's fourteen major Labor Market Areas has shifted over the past several years. From 1980 to 1985 western LMAs experienced rather dramatic declines in employment in the manufacturing sector. There was also spatial bias in job growth in other sectors as eastern LMAs outperformed their western counterparts during the period. After 1985 the spatial arrangement of employment decline, particularly in manufacturing, and of growth in other sectors became more balanced. Hence, the spatial arrangement of opportunity, in terms of employment opportunity have been changing along with Pennsylvania's migration patterns.

This paper estimates the response of Pennsylvania's migrants to the changes that occurred in LMAs' economic performance between 1985 and 1990 in terms of employment opportunities. The following section presents the methods for estimating migrants' responses to changes in LMAs' economic prospects, after which results are discussed.

RESULTS AND DISCUSSION

In order to estimate the responsiveness of the state's migrants to changes in LMAs' economic performance, the LMAs' net migration values are separated into component parts of in-migration and out-migration. This is consistent with Rogers (1990) who demonstrated that using a net migration rate model results in false predictions because it violates the demographer's principle of using an at risk population as the denominator. It is also consistent with the notion that areas with high in-migration rates also tend to have high out-migration rates (Miller, 1973). To measure the responsiveness, the numbers of in-migrants and out-migrants are log transformed, resulting in a growth model (Gujarati, 1994). In growth models, estimated coefficients are interpreted as the proportional change in the dependent variable resulting from a unit change in the independent variable (Intrilligator, 1978). Thus, the models are written as:

$$\sum_{i=1}^{n} \ln(m_{ij}) = \Delta e_j \qquad (1)$$

for a LMA's in-migration, and for a LMA's out-migration as:

$$\sum_{j=1}^{n} \ln(m_{ij}) = \Delta e_i \qquad (2)$$

where m is a LMA's migration flow, e is the LMA's employment, delta (Δ) denotes change from 1985 to 1990, and the subscript i represents the origin LMA and subscript j the destination LMA. The models are estimated via OLS.

The following results were produced from estimations of equations 1 and 2. Both estimations have good explanatory power and yield statistically significant coefficients with the expected signs. Together, they indicate that Pennsylvanians were responsive to changes in employment between 1985 and 1990. For in-migrants, the results are:

$$\sum_{i=1}^{n} \ln(m_{ij}) = 9.994 + 0.032(\Delta e_j) \qquad (3)$$

$$(54.526) \quad (6.86)$$

$$r^2 = .788$$

$$F = 44.696$$

The coefficient for the employment term is interpreted as the proportional change in a LMA's in-migrant flow that accompanies a unit change in the LMA's employment. In growth models, multiplying the coefficient by 100 produces the percentage change in the dependent variable accompanying a unit change in the independent variable (Gujarati, 1994). In this case, then, a change of 1,000 in a Pennsylvania LMA's employment levels produces on average a more than 3 percent change in its in-migrant flow.

For out-migrants, the results are:

$$\sum_{j=1}^{n} \ln(m_{ij}) = 9.930 + 0.034(\Delta e_i)$$

$$(70.713) \quad (9.128) \qquad (4)$$

$$r^2 = .874$$
$$F = 83.312$$

and are interpreted as meaning that a change of 1,000 in a Pennsylvania LMA's employment level yields on average an almost 3.5 percent change in its out-migrant flow. This responsiveness is what might be expected when considered in light of the striking turnaround that took place in Pennsylvania's economy during the period.

This turnaround took place after a period of tremendous employment decline in Pennsylvania. Between 1980 and 1985, more than one in twenty jobs were lost. Losses were not spatially uniform. Western LMAs bore the brunt of the employment decline losing approximately 90,000 jobs and averaging a job-rate loss 2.5 times higher than eastern LMAs. Indeed, employment in the eastern LMAs actually increased by 0.62 percent, with all but two of the eastern LMAs showing job growth during this period. Hence, two elements likely underpin migrants' responsiveness to changes in LMAs' economic performance. First, there was pent-up job demand. Second, there had already been an enormous displacement of Pennsylvanians from home counties (DeJong and Cornwell, 1989). Together, these elements represent a tremendous migration potential, one especially sensitive to changes in the state's economy .

These changes become evident between 1985 and 1990, when all of Pennsylvania's fourteen major Labor Market Areas recorded employment growth. The significance lies in the fact that all of the western LMAs reversed the negative signs on their average annual rate of employment growth. For instance, the Johnstown LMA had an average annual rate of employment growth of -2.25 percent for 1980 to 1985 and a 2.51 percent growth rate for 1985 to 1990.

The spatial imbalance in job growth, moreover, was much less pronounced during this period. Indeed, average annual rates of job growth are almost in balance with western LMAs recording a rate of 2.30 percent and eastern LMAs 2.32 percent. This indicates that the average annual rate of employment growth was increasing much faster in the western LMAs than in the eastern LMAs from 1985 to 1990. Thus, the sectoral shifts in the state's economy, their spatial bias, and the magnitude of the turnaround should impel large population movements. These sectoral shifts are not nearly so pronounced during the 1990 to 1995 period. However, data limitations do not allow for testing the average responsiveness of Pennsylvania's migrants during that period.

The geography of Pennsylvania's migration flows has changed as has that of its economy. During the period of the state's economic restructuring, migration flows followed shifting spatial arrangements of employment gains. During the 1980 to 1990 period, rates of out-migration were both strongly concentrated and especially high in western LMAs.

This has changed. After 1990, two of the five western LMAs reversed the signs of their net internal migration to positive and the rate of internal out-migration was significantly reduced in the other three.Meanwhile, in the east, the Scranton/Wilkes Barre/Hazleton LMA had the sign on its net internal migration rate reversed to negative for the 1990 to 1995 period, and the Philadelphia and Williamsport LMAs showed significant increases in their rates of out-migration. Many other eastern LMAs, such as Lancaster, Harrisburg-Lebanon-Carlisle, and Allentown/Bethlehem/Easton recorded notable decreases in the rate of internal in-migration.

This change is not unexpected. The nature of changes in Pennsylvania's economy is exactly those that determine the direction and volume of migration. Typically, migration is viewed as a response to spatial variations in net economic advantages, especially employment opportunities (Hicks, 1932; Weeks, 1996). Migration is expected to be oriented toward places in which employment is growing most rapidly (Muth, 1968 and 1971; Olvey, 1972; Greenwood, 1973 and 1975).

These characteristics are notable in Pennsylvania. Employment gains have been essentially concordant within LMAs, while being spatially unbalanced among them. So, too, were the sectoral shifts underlying them. These gains remain not only unbalanced but in flux as both new sectors assume dominance, and as increasing demand for related and/or traditional sectors accompanies a national economic resurgence. As the state's spatial arrangement of employment growth has changed, so too has the direction and volume of its migration flows.

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Journal of the Pennsylvania Academy of Science 71(2):90-95, 1998

GEOLOGIC AND HYDROLOGIC PROBLEMS ENCOUNTERED IN THE CONSTRUCTION AND OPERATION OF THE UNION CANAL IN PENNSYLVANIA[1]

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ABSTRACT

Among the first canal routes to be considered in North America was one to connect the Schuylkill River and Susquehanna River in Pennsylvania. Unfortunately, all feasible routes necessitated crossing highly fractured and solution-weathered carbonate rocks. The attempts and ultimate failure to deal with the engineering problems encountered in this type of terrain provided important but very expensive lessons to the engineers and investors of the pre-railroad canal era in Pennsylvania.
[J PA Acad Sci 71 (2) 90-95, 1998]

INTRODUCTION

The canal era in Pennsylvania was short-lived, but important to the growing industrial economy of the Keystone State in the middle third of the 19th Century. An early link in the network of canals developed in Pennsylvania was the Union Canal, which connected the Schuylkill and Susquehanna rivers. The geologic and hydrologic problems encountered during the development and operation of the Union Canal provided hard-learned lessons for engineers and geologists of that and later times.

Preliminary surveys for a canal connecting the Schuylkill and Susquehanna rivers were conducted in 1762 and 1770 but work never proceeded due to the uncertainties associated with the Revolutionary War (Livingood 1937). In the early 1790's, two canal companies were incorporated in Pennsylvania. One was the Schuylkill and Susquehanna Canal Company, which was authorized to construct a canal along the Swatara and Tulpehocken Creeks from Middletown to Reading and then south along the Schuylkill River to Norristown, Montgomery County. The Delaware and Schuylkill Canal Company was authorized to construct a canal along the Schuylkill River below Norristown, ending at a point on the Delaware River north of Philadelphia. Both companies commenced work in the early 1790's with a few miles of canal completed in the summit level, near the City of Lebanon, and about 13 miles along the Schuylkill River (Ryan 1930). Both efforts ground to a halt in 1794 due to severe financial problems. In 1811, the Pennsylvania legislature combined the two firms to form the Union Canal Company, the name being derived from the combining of the companies, not the connection of the two rivers. In 1819, another Act limited the operations of the Union Canal Company to the streams that provided a connection between Reading, on the Schuylkill River, and Middletown, on the Susquehanna River (Grimes 1989). In addition, the Pennsylvania Legislature offered financial assistance to the canal builders due to the impending economic threat of the Erie Canal in adjacent New York State (Shank 1981).

Figure 1 shows the route of the Union Canal along the courses of Tulpehocken and Swatara creeks. Tulpehocken Creek has its headwaters immediately east of the City of Lebanon, Lebanon County, and flows easterly to the Schuylkill River at Reading, Berks County. On the other side of a divide near Lebanon are tributaries of Swatara Creek including Quittapahilla Creek and Clarkes Run. Swatara Creek flows into the Susquehanna River at Middletown, Lancaster County. Over seventy miles of canal were required to connect the Susquehanna and Schuylkill Rivers and nearly ten percent of that crossed carbonate bedrock terrain. Unfortunately, most of the carbonate bedrock was at the summit level of the canal, the headwaters area near

[1]Submitted for publication 21 March 1997; accepted 11 June 1997.

Lebanon. This offered a severe test of engineering skills in both designing and operating a water-conveyance transportation system through an area where sinkholes are common and surface water is scarce.

The type of bedrock found in the six-mile summit level of the Union Canal greatly compounded the usual water-related problems of canals—floods and droughts. As shown on Figure 2, taken from Berg et al., most of the summit level is underlain by the Ontelaunee Formation. Geyer et al. (1958) described the lithology of the formation as a "...dark grayish-brown weathering dolomite..." that is "thick-bedded" and "finely-crystalline." McGlade et al. (1972) described the joints in the Ontelaunee Formation as having a blocky pattern, being moderately abundant, with both steeply and gently dipping sets. They characterized the weathering of this formation as resulting in blocky fragments in an overlying mantle, usually thicker than 5 feet, with bedrock pinnacles being quite characteristic. The typical topography consists of valleys of low relief.

Critical portions of the summit-level canal were constructed on the Annville, Myerstown and Hershey formations. The Annville Formation is described by Geyer et al. (1958) as a "...very thick bedded, finely crystalline, light blue-gray to light pinkish-gray, high-calcium limestone." It has been extensively quarried since the demise of the Union Canal, partially obliterating the former course of the canal East of Lebanon. At the conclusion of their description of the Annville Formation, Geyer et al. (1958) wrote: "Outcrops of the Annville limestone are few because of the relatively high solubility of the rock." This is critical in understanding the problems encountered in constructing and operating the canal. McGlade et al. (1972) described the overlying mantle of the Annville Formation as being moderately thick and usually pinnacled. They describe the drainage as being good in the subsurface and poor on the surface with sinkholes being common.

The Myerstown Formation is described by Geyer et al. (1958) as being typically a "...medium dark-gray to dark-gray, medium to finely crystalline, thin-bedded limestone." McGlade et al. (1972) described the weathering of the formation as resulting in fragments that range from "pencil-like and jagged" to elongate plates and moderate size blocks having a thin overlying mantle.

Gray et. al. (1958) described the Hershey Formation as "...a dark-gray, graphitic, shaly or silty limestone." It statigraphically overlies the Myerstown Formation but is poorly exposed in the area. It makes up much of the bedrock north of the canal up to the clastic rock.

Gray et al. (1958) showed the cross-section structure of their respective geologic maps to be quite complicated with the beds dipping at various angles and overturned in places. Figure 3 is a simplified cross-section taken from their plates. The line of the cross section is just west of Myerstown and is shown on Figure 2. Under the heading of foundation stability for all these formations, McGlade et al. (1972) suggested thorough investigations for sinkholes, underlying caves, and collapse areas.

It was over these formations that the Union Canal engineers made the connection between the headwaters of the Tulpehocken Creek and a tributary of Swatara Creek. Just west of Myerstown, the Tulpehocken Creek turns northwest to its headwater springs, a mile or so

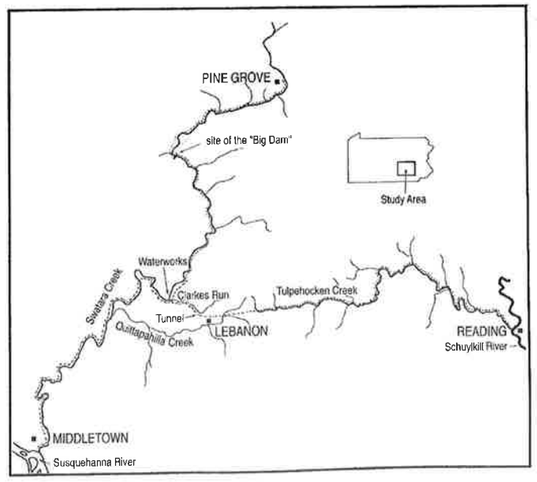

FIGURE 1. Map of study area showing the route (dashed lines) of the Union Canal between Middletown and Reading, and the Pine Grove Feeder Canal.

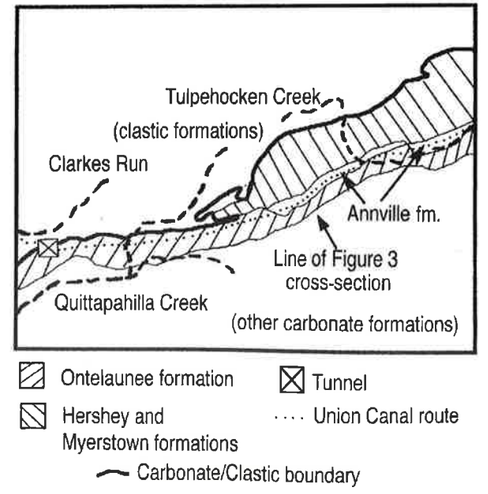

FIGURE 2. Geologic map of the summit area showing the route of the Union Canal (dotted line) over carbonate bedrock.

upstream. The Union Canal left the Tulpehocken Creek here with its route following the lowest topography that could be found on the broad divide between the Swatara Creek and Tulpehocken Creek drainage basins. Unfortunately, this meant that significant portions of the canal west of Myerstown were constructed on the Annville Formation bedrock.

Where the canal was either in, or immediately adjacent to, the Tulpehocken Creek, as it was from Myerstown East, there were fewer problems with sinkhole formation because the canal was at, or only slightly above, the water table. West of Myerstown, the surface elevation of the land is increasingly elevated above the natural water table level, up to the divide. On the Quittapihilla Creek side of the divide, for reasons described below, the canal engineer decided to forego the natural stream channel and constructed the canal parallel to a low ridge marking the boundary between carbonate and clastic rocks of the Hamburg Sequence to the North. Just west of Lebanon, a tunnel was constructed through the ridge to route the canal to another tributary of Swatara Creek, Clarkes Run, and onto bedrock that was not susceptible to solution and collapse. However, this left six miles of canal (from West of Myerstown to the tunnel) in a precarious position with respect to the nature of the underlying bedrock and construction techniques of the day. Further, the selection of this route ensured that there was an insufficient natural supply of water through the most critical segment of the entire canal.

This route proved disastrous to the profitability of the canal in two ways. First, auxiliary water was needed which required, at considerable expense, the construction and operation of a waterworks. Second, in an attempt to limit the amount of water needed to operate the canal through the summit region, the size of the canal (depth and width) was made smaller than the recommendation of the original engineer. This smaller size minimized the amount of water needed to operate the canal through the summit section, but it also had the effect of eliminating the most profitable traffic (heavy freight) and prevented the Union Canal from becoming an integral part of the Main Line of Transportation Works Canal that connected Philadelphia to Pittsburgh.

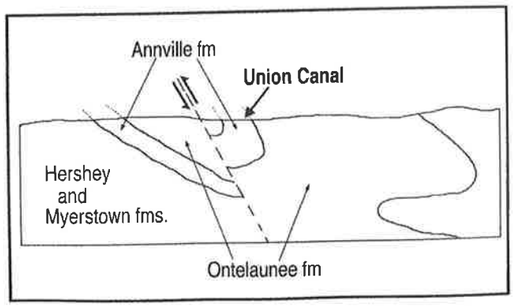

FIGURE 3. Geologic cross-section of carbonate bedrock just west of Myerstown. (see Figure 2 for line of cross-section).

The waterworks was constructed near the junction of Clarkes Run and Swatara Creek. The low summertime discharge in Swatara Creek made it necessary to construct a dam upstream to store sufficient water to operate the canal through the summit area during the peak traffic months of late summer and early autumn. The water wheels and steam powered pumps used to force the water up a pipeline to the summit area were always a problem to operate and the pipeline itself was a continuing maintenance headache. But these issues paled in comparison to the problems within the summit area that were a consequence of the carbonate bedrock.

The reaction of limestone and dolomite bedrock with the weakly-acidic natural precipitation enables the dissolution of carbonate minerals as bicarbonate ions. Left behind are the inert constituents of the original limey sediment such as sand or clay. This residue of inert minerals, plus weathering blocks of the carbonate rock, form the overlying mantle and well-known rich soils that the humid climate of Pennsylvania generates.

But the formations vary in their carbonate make-up and inert content. Even within the same formation, there will be considerable variation in weathering and solution susceptibility. Further complicating this is the distribution of joints and bedding planes that carry water into and through the rock. Thus, dissolution may occur more rapidly in a susceptible bed that lies under more resistant beds closer to the surface, creating caves. Sinkholes can form where joints and bedding planes intersect at the surface. Enhanced dissolution within these openings allows the residual inert materials to sag, creating a surface depression that accelerates the subsurface weathering processes by increasing the flow of water in one area. If the "plug" of inert materials in the sink get washed into the solution-enhanced openings below, a swallow-hole is formed. The swallow holes are more likely to form where the water table is under the bedrock-mantle interface and the buoyancy effect of the groundwater is lacking. The principal author has seen instances in the nearby Lancaster County where high rates of pumping in carbonate aquifers artifically lowered the water table below the mantle with resultant development of swallow-holes. The formation of the swallow-holes is exacerbated by the presence of water on the surface. This is because the saturated residual materials have decreased viscosity and increased mass—both increasing the likelihood of the material flowing downward.

It is across this complicated terrain that the engineers of the Union Canal Company placed a water-filled trough constructed by excavation of the soil. Excavation thins the spacing between the water in the canal and any openings in the underlying rock. Problems with swallow-hole development and resultant loss of water were seen almost immediately, and continued to plague operations throughout the life of the canal.

ENGINEERING

In 1821, a Chief Engineer was hired by the Union Canal Company. Loami Baldwin's initial task was to resurvey the proposed route which resulted in a recommendation to reroute the canal through a limestone divide in the City of Lebanon. Here Baldwin found two springs on opposite sides of the divide and at the same level, one a tributary of Tulpehocken Creek and the other of Quitapahilla creek (Francis 1939). A cut through the low ridge would have required rock excavation of up to three miles in length and a maximum depth of 50 feet—a formidable and expensive task in those days of hand labor and black powder explosives. Based on his experiences in Europe, Baldwin also recommended a change in the size of the canal, to support canal boats with 40- to 50-ton capacities, over twice the capacities initially planned for. Unable to resolve the differences between his ideas and those of the Board of Commissioners of the canal, Baldwin resigned in 1822.

Baldwin was replaced by Canvass White, an experienced engineer who was working on the nearly completed Erie Canal. The major problem he faced was to find a relatively inexpensive solution to the problem of water supply at the summit. White's solution was to route the canal to Clarkes Run, which empties into Swatara Creek upstream from Quittapahilla Creek. Clarkes Run had its headwaters northwest of Lebanon, but there was a significant obstacle in the way—a low ridge that marks the northern boundary of the carbonate terrain. Thus, White's route did not parallel the Quittapahilla Creek but cut perpendicular across some north-south tributaries while skirting the south side of the ridge. To connect to Clarkes Run, White proposed the construction of a tunnel through the ridge. The tunnel would enable canal boats to connect from the Swatara Creek watershed to the Tulpehocken Creek watershed. It would also convey water from waterworks that were to be constructed along the Swatara Creek, to the summit section on the south side of the ridge. This plan was approved by the Board, with construction to start in late 1824 and expected completion in 1827. In addition to the admission that external sources of water were required to operate the summit level, another fateful decision was made in regard to the summit-level construction that would haunt the canal for decades.

CONSTRUCTION

It was recognized that it would be necessary to line the summit level of the canal to prevent excessive leakage because the canal was situated above the local water table in a permeable-rock area. The alternatives available were "puddling" (packing low-permeability clay on the bottom and sides), and "planking" (lining the sides and bottom of the canal with wood planks). Planking was chosen and the six-mile long summit level was "sealed" with close to 2,000,000 board-feet of lumber.

The tunnel proved to be more difficult than originally anticipated due to a layer of quartzite encountered. The techniques were standard for the time. Holes were driven into the working face of the tunnel using hammers and hardened-steel chisels. Once an appropriate number of holes were driven, they were packed with black powder and detonated. After debris removal and "dressing" the face, more holes were driven. This process was repeated over and over with two 12-hour shifts and 25 pounds of black powder per day (Grimes 1989).

Two reservoirs were constructed by placing dams on streams that rose on the clastic rocks north of the canal on both sides of the summit level. In conjunction with the water pumped from the waterworks, the reservoirs were supposed to be capable of operating the lockage requirements through the summit level of the canal.

OPERATION (AND ADDITIONAL CONSTRUCTION)

The canal opened for business between Reading and Middletown in the Spring of 1828, over a year later than originally expected due to construction difficulties. As built, the 77-mile canal was 24 feet wide at the bottom, 36 feet wide at the surface and had a depth of 4 feet. Locks were 8.5 feet wide by 75 feet long. There were 135 bridges, 93 lift locks, 2 weigh locks, 2 guard locks, 47 culverts, 43 waste weirs, 12 aqueducts, and the tunnel (Shank 1981).

Water supply problems struck during the first year of operations. A summer drought forced the closing of the canal in late August except for a few days later in the Fall. The following few years were good ones for the canal and work proceeded on a feeder canal up the Swatara Creek to Pine Grove. This was a 22-mile navigable canal that led to the "Big Dam" constructed on the Swatara Creek at the gap through Blue Mountain. The reservoir behind the dam was six miles long and navigation was extended to Pine Grove. A few miles north of Pine Grove were reserves of anthracite coal that developed into an important source of revenue for the canal.

The waterworks were located at the junction of Clarkes Run and Swatara Creek. There, four pumps had the capacity to lift about 15,000 gallons per minute through 3.3 miles of wooden and brick pipes to the summit level, 95 feet above the pumps (Klein 1901). Two of the pumps were powered by water wheels, turned by water dropping from the feeder canal to the Swatara Creek, and

two were powered by steam engines.

The leakage of water through the planked areas became an increasing problem, especially in the Myerstown area where planks were collapsing into swallow holes that formed under and adjacent to the canal. Large portions of the canal were re-planked in 1832-33. Likewise the wooden portions of the feeder pipes from the waterworks to the summit had to be replaced (Annual Reports for 1831 and 1832).

The canal had its best year, in terms of revenue collected, in 1835 (Klein). By 1836, it was recognized that Baldwin was right. The canal needed to be larger to compete successfully, but first, the planks needed to be replaced again. The actual decision to rebuild was put off through the 1840's, although engineering work and surveys to that end were finished earlier. The use of water continued to rise for the usual reason of loss in the planked area, and another complete replanking was done in 1847. During this time, as locks were upgraded or replaced, they were made larger to be suitable for the larger boats that would be coming. Also, a new reservoir above Pine Grove was purchased to provide for water for the enlargement.

Work on the enlargement commenced in 1851 on the Western Division from Middletown, on the Susquehanna River, to the summit level and including the Pine Grove feeder canal. The locks were lengthened to 90 feet and widened to 17 feet. The canal bed was increased to 28 feet wide at the bottom, 43 feet at the surface and the depth was increased to 4.5 feet (Grimes, 52). With this completed in 1853, attention was turned to the Eastern Division and the problem of the summit level.

Consideration was given to the route suggested by Baldwin over 30 years before, but it was finally felt that keeping the existing level and using new technology of leak-proofing was the best strategy (Grimes 1989). The technique chosen was to "puddle" the canal bed and line it with the relatively new material—hydraulic cement. As described by Grimes:

> "The limestone, the cause of all the leaking, was laid bare wherever it appeared to be the cause of the leakage, and the fissures and apertures thus exposed were filled in with hand packed stone until the openings were filled to the top. Then earth was puddled by teams of mules used just for that purpose."

The Annual Report of 1856 contained the fateful boast: "There is now no canal in any part of the world that leaks less or is less likely to leak than the Summit Level of the Union Canal."

Finally, in the Spring of 1857 the newly widened canal, capable of handling any size boat from the Main Line of Transportation Works Canal or the Schuylkill Navigation Canal, opened for business. Within days, two miles of the newly puddled canal near Myerstown leaked so badly that the canal was shut down for repairs. The inaugural year of the newly renovated canal had only four months (instead of the expected eight) of operation and many of the same old water problems as the old canal (Managers' Minutes 1858).

DEMISE

Bankruptcy of the Union Canal Company in 1857 resulted in a board of trustees being appointed to run the operation. A disastrous flood in 1862 destroyed the Big Dam on the Swatara Creek and much of the Eastern Division works (Managers' Minutes 1863). This terminated the lucrative coal transportation trade from the Pine Grove area as rail transport was substituted. Within a year the trustees were being asked to approve the purchase of a new Cornish engine to pump water from the waterworks for the purpose of "...guarding against deficiencies of water at the summit" (Managers' Minutes 1864). The problem had come full circle.

The Union Canal limped along through the rest of the 1860's and 1870's. Recurrent problems with drought and insufficient supply of water at the summit, due to leakage, and even ice damage during the winter continued to take a toll. Near the end, in the early 1880's, the summit level was abandoned and, for a short while, the two remaining divisions continued conveying local agricultural products and lumber as feeders to the more successful Schuylkill Navigation Canal and remnants of the Susquehanna Canal. By the middle of the decade the Union Canal was out of business, unable to cope with the railroads, poor planning, and the carbonate bedrock of Lebanon County, Pennsylvania.

ACKNOWLEDGMENTS

We thank the librarians and staff of both the Berks County Historical Society, Reading, PA, and the Hagley Museum and Library, Wilmington, DE for their assistance in providing access to the historical documents that were the basis for much of the information in this paper.

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