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Academy Editors

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Proceedings of the

**ARKANSAS ACADEMY
OF SCIENCE**

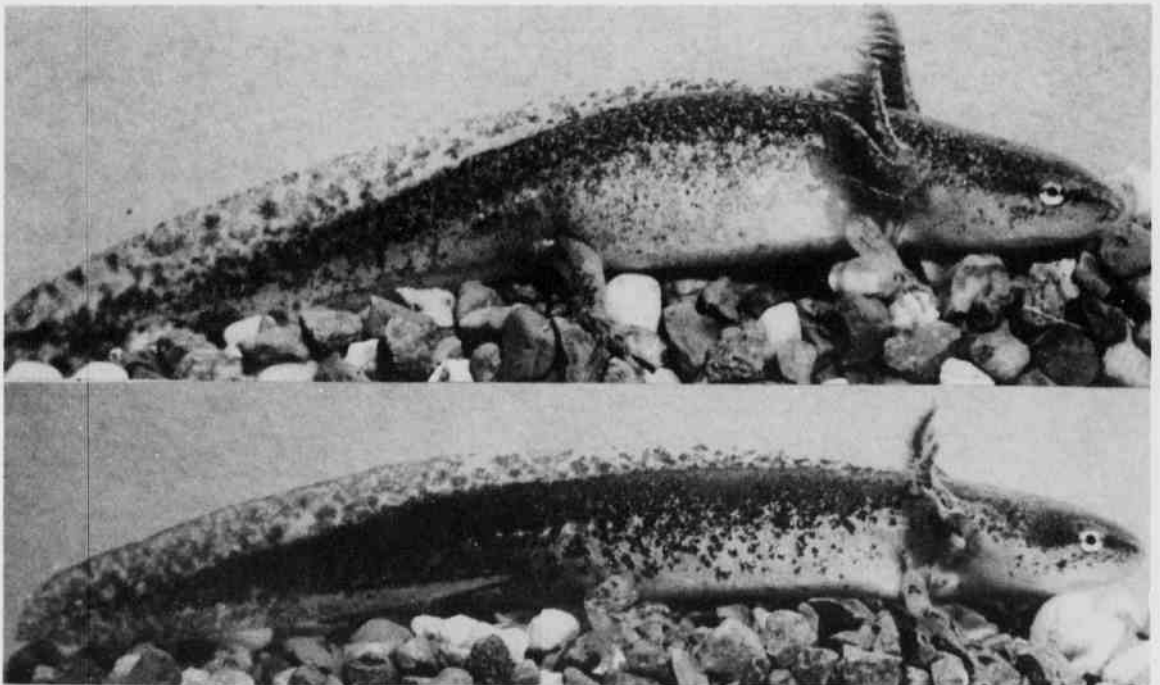
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1989**

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**ARKANSAS ACADEMY OF SCIENCE
DEPT. OF NATURAL SCIENCE
MONTICELLO, ARKANSAS 71655**

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PAST PRESIDENTS OF THE ARKANSAS ACADEMY OF SCIENCE

Charles Brookover, 1917	Delbert Swartz, 1953	George E. Templeton, 1972
Dwight M. Moore, 1932-33, 64	Z. V. Harvalik, 1954	E. B. Whitlake, 1973
Flora Haas, 1934	M. Ruth Armstrong, 1955	Clark McCarty, 1974
H. H. Hyman, 1935	W. W. Nedrow, 1956	Edward Dale, 1975
L. B. Ham, 1936	Jack W. Sears, 1957	Joe Guenter, 1976
W. C. Munn, 1937	J. R. Mundie, 1958	Jewel Moore, 1977
M. J. McHenry, 1938	C. E. Hoffman, 1959	Joe Nix, 1978
T. L. Smith, 1939	N. D. Buffaloe, 1960	P. Max Johnston, 1979
P. G. Horton, 1940	H. L. Bogan, 1961	E. Leon Richards, 1980
I. A. Willis, 1941-42	Trumann McEver, 1962	Henry W. Robison, 1981
L. B. Roberts, 1943-44	Robert Shideler, 1963	John K. Beadles, 1982
Jeff Banks, 1945	L. F. Bailey, 1965	Robbin C. Anderson, 1983
H. L. Winburn, 1946-47	James H. Fribourgh, 1966	Paul Sarrah, 1984
E. A. Provine, 1948	Howard Moore, 1967	William L. Evans, 1985
G. V. Robinette, 1949	John J. Chapman, 1968	Gary Heidt, 1986
John R. Totter, 1950	Arthur Fry, 1969	Edmond Bacon, 1987
R. H. Austin, 1951	M. L. Lawson, 1970	Gary Tucker, 1988
E. A. Spessard, 1952	R. T. Kirkwood, 1971	David Chittenden, 1989

INSTITUTIONAL MEMBERS

The Arkansas Academy of Science recognizes the support of the following institutions through their Institutional Membership in the Academy.

ARKANSAS COLLEGE, Batesville
ARKANSAS STATE UNIVERSITY, State University
ARKANSAS TECH UNIVERSITY, Russellville
COLLEGE OF THE OZARKS, Clarksville
HARDING COLLEGE, Searcy
HENDERSON STATE UNIVERSITY, Arkadelphia
HENDRIX COLLEGE, Conway
JOHN BROWN UNIVERSITY, Siloam Springs
MISSISSIPPI COUNTY COMMUNITY COLLEGE,
Blytheville

OUACHITA BAPTIST UNIVERSITY, Arkadelphia
PHILLIPS COUNTY COMMUNITY COLLEGE, Helena
SOUTHERN ARKANSAS UNIVERSITY, Magnolia
UNIVERSITY OF ARKANSAS AT FAYETTEVILLE
UNIVERSITY OF ARKANSAS AT LITTLE ROCK
UNIVERSITY OF ARKANSAS FOR MEDICAL
SCIENCES, Little Rock
UNIVERSITY OF ARKANSAS AT MONTICELLO
UNIVERSITY OF ARKANSAS AT PINE BLUFF
UNIVERSITY OF CENTRAL ARKANSAS, Conway

EDITORIAL STAFF

EDITOR: DR. HARVEY BARTON, Dept. of Biological Sciences, Arkansas State University, State University, AR 72467.

NEWSLETTER EDITOR: RICHARD A. KLUENDER, Dept. of Forest Resources, University of Arkansas at Monticello, Monticello, AR 71655

BIOTA EDITOR: LEO J. PAULISSEN, Botany and Bacteriology Department, University of Arkansas, Fayetteville, AR 72701.

ASSOCIATE EDITORS:

AGRONOMY/PLANT PATHOLOGY: George E. Templeton (UA)

AQUATIC/ENVIRONMENTAL: George L. Harp (ASU)

BIOMEDICAL/PHYSIOLOGY: W. Donald Newton (ASU)

BOTANY: Carol J. Peck (UCA)

GEOLOGY: John T. Thurmond (UALR)

PHYSICS: Mustafa Hemmati (UALR)

WILDLIFE MANAGEMENT: Gary A. Heidt (UALR)

In Memory of

DR. EUGENE B. WITTLAKE

and

MR. ROBERT T. KIRKWOOD

PROCEEDINGS ARKANSAS ACADEMY OF SCIENCE

Volume XXXXIII

1989

Dave Chittenden
President

Dick Spears
President-Elect

Walt Godwin
Secretary

Robert Wiley
Treasurer

James Fribourgh
NAAS Delegate

Henry Robison
Historian

Secretary's Report

MINUTES OF THE SEVENTY-THIRD ANNUAL MEETING, - APRIL 1989

FIRST BUSINESS MEETING

Dave Chittenden, President, called the meeting to order.

Jim Daly, Local Arrangements Chairman, welcomed the Academy to the Annual Meeting. Daly made several announcements including mentioning the availability of Dave Saugey's paper from last year's meeting. He asked the judges to meet for instructions after the business meeting. He also asked the section chairs to pick up copies of the abstracts to post. He introduced Dr. Harry Ward, Chancellor, who welcomed the group to the University of Arkansas for Medical Sciences campus. He stated that the campus was pleased to host the Academy. He summarized the campus and mentioned that a new research building has been approved.

Henry Robison, Historian, stated that this was the 73rd meeting and that the meeting had been held six times previously on the medical sciences campus. He stated that a history of the Academy is planned for the 75th meeting.

Walter Godwin, Secretary, presented the minutes of the Seventy-Second Annual Meeting. He stated that there were copies in the *Proceedings* and extras at the back of the room. He asked for any corrections to be presented in writing before the Second Business Meeting.

Bob Wiley, Treasurer, presented the Financial Report. He indicated that extra copies were available in the back. He briefly discussed the report and indicated that the Academy is in good financial shape. He indicated that expenses exceeded income since we paid for two volumes of the *Proceedings* during the past year. He stated that more money will be coming in to cover these expenses. An Audit Committee consisting of Dick Spears and Art Johnson will examine the report. A copy of the report follows.

ARKANSAS ACADEMY OF SCIENCE

ANNUAL FINANCIAL STATEMENT

SUMMARY

(17 MARCH 1988 TO 20 MARCH 1989)

Balance Approved by Audit on 2 April 1988		\$15,112.84
Total Income (Page 2)	10,783.45	
Total Expenses (Page 3)	15,915.81	
Balance for the Year	<5132.36>	-5132.36
TOTAL FUNDS AS OF 20 MARCH 1989		\$9980.48

DISTRIBUTION OF ACCOUNTS

Interest Bearing Checking Account (Union Bank and Trust Co., Monticello, AR)		3694.21
Certificates of Deposit		
Dwight Moore Endowment (1st National Bank of Conway - No. 78940 - 7% Interest)		1077.25
Life Membership Endowment (Heritage Federal Savings and Loan - Monticello - No. 502440 - 8% Interest)		5209.02
TOTAL		\$9980.48
INCOME: 17 March 1988 to 20 March 1989		
1. ANNUAL MEETING: ARKANSAS TECH UNIVERSITY, 1-2 APRIL 1988		371.19
2. INDIVIDUAL MEMBERSHIPS		
a. Regular (140)	2100.00	
b. Sustaining (12)	240.00	
c. Sponsoring (2)	60.00	
d. Life (10)	550.00	
e. Associate (11)	55.00	
Total	3005.00	3005.00
3. INSTITUTIONAL MEMBERSHIPS (6)		300.00
4. PROCEEDINGS, LIBRARY SUBSCRIPTIONS		478.00
5. PROCEEDINGS, MISC. SALES (UAF)		1318.17
6. PROCEEDINGS, PAGE CHARGES		3685.00
7. BIOTA RECEIPTS		48.00
8. INTEREST		
a. Interest Bearing Checking Account	184.22	
b. Dwight Moore Endowment	75.41	
c. Life Membership Endowment	353.46	
Total	613.09	613.09
7. ENDOWMENTS		965.00
TOTAL INCOME		\$10,783.45

EXPENSES: 17 March 1988 to 20 March 1989

1. AWARDS

a. Luke Eggering (#514)	25.00	
b. Yvette Randle (#515)	25.00	
c. J. G. Ross (#516)	50.00	
d. John D. Peck, Plaques - Arkansas Science Talent Search (#518)	103.01	
e. Arkansas Junior Academy of Science (#519)	250.00	
f. Arkansas Science Fair Association (#517)	200.00	
Total	653.01	653.01

Secretary's Report

2. MEETING EXPENCES (ARKANSAS TECH UNIV.)			
a. The Shirt Shop (Plaques) (#520)	74.20		
b. David Chittenden (Plaques) (#523)	42.67		

	116.87	116.87	
3. PROCEEDINGS			
a. Phillips Litho (Vol. 40) (#526)	6175.20		
b. Peggy Brown, Editorial Consultant Vol. 40 (#529)	500.00		
c. Phillips Litho (Vol. 41) (#522)	7122.79		
d. Robin Matthews, Editorial Consultant Vol. 41 (#511)	500.00		

Total	14,297.99	14,297.99	
4. OFFICE EXPENSES			
a. Secretary's Office (#513, #525, #527)	375.00		
b. Treasurer's Office (#521, #528)	51.02		
c. Editor's Office (#524)	189.46		

Total	614.48	614.48	
b. DUES - National Association of Academies of Science (#530)		39.00	
6. MISCELLANEOUS (#510)			
		194.46	

TOTAL EXPENSES		\$15,915.81	

ENDOWMENTS

NAME: DWIGHT MOORE ENDOWMENT
 PLACE: 1st National Bank of Conway
 NUMBER: 78940
 AMOUNT: \$1077.25
 DATE PURCHASED: 2 January 1987
 INTEREST RATE: 7% (Interest paid to AAS semiannually)
 DURATION: 30 Months
 MATURITY DATE: 2 July 1990

The funds for this CD are derived from money contributed by members and friends of the AAS in memory of Dwight Munson Moore 1891-1985. It is intended that the interest generated from this capital be used to give awards in the name of the honoree. There is no obligation to use all of the interest so that the fund can grow. No provision has as yet been made as to what the awards might be other than they would be in education and would honor outstanding work in the sciences.

NAME: LIFE MEMBERSHIP ENDOWMENT
 PLACE: Heritage Federal Savings and Loan Assn. (Monticello)
 NUMBER: 502440
 AMOUNT: \$5209.02
 DATE PURCHASED: 11 March 1989
 INTEREST RATE: 8% (Interest compounded quarterly)
 DURATION: 1 month
 MATURITY DATE: 11 April 1989

The funds for this CD are derived from money paid by members of the AAS for Life Memberships (\$200.00). It is thought that these monies should be kept separate from other monies and perhaps earmarked for some special purpose. As individuals become life members they no longer pay annual dues. Thus it may be desirable to annually remove from this endowment an amount equal to the number of life members x the annual dues and deposit it in the checking account. The additional interest earned would be used for other purposes (education and research). Life members would thus be contributing to the Arkansas Academy of Science long after they have become inactive. Life Memberships would have value not only for the life of the individual, but for the life of the Arkansas Academy of Science.

NAME: ARKANSAS ACADEMY OF SCIENCE ENDOWMENT
 PLACE:
 NUMBER:
 AMOUNT:
 DATE PURCHASED:
 INTEREST RATE:
 DURATION:
 MATURITY DATE:

The majority of funds in this CD are unrestricted as to their use. However some funds within the Arkansas Academy of Science Endowment were donated with restrictions as to their use within the following categories: Student Research Awards/Grants, Publications, and Other.

(NOTE: When CD's mature additional monies collected for that particular CD since investment are added to the maturity amount and reinvested in a new CD.)

* ARKANSAS ACADEMY OF SCIENCE *

- COST OF PROCEEDINGS -

VOL.	COPIES	PAGES	PRINTER CHG.	TOT. VOL. COST	COST/COPY	COST/PAGE
35 (1981)	450	96	\$3694.68	\$4620.99	\$10.27	\$48.14
36 (1982)	450	110	\$5233.28	\$5291.69	\$11.76	\$48.11
37 (1983)	450	103	\$5326.91	\$5944.44	\$13.21	\$57.71
38 (1984)	450	97	\$5562.97	\$6167.72	\$13.71	\$63.58
39 (1985)	450	150	\$7856.20	\$8463.51	\$18.81	\$56.42
40 (1986)	469	98	\$6175.20	\$6675.20	\$14.23	\$68.11
41 (1987)	450	116	\$7122.79	\$7811.25	\$17.36	\$67.34
42 (1988)		116				

The Total Volume Cost equals the printers charge (Phillips Litho) plus the editor, editorial assistant, and other miscellaneous charges.

Respectfully Submitted,


 Robert W. Wiley, AAS Treasurer

Annual Meeting: 7-8 April 1989
 University of Arkansas for Medical Sciences, Little Rock, AR

Dick Spears, Chairman of the Constitution Committee, reported on recommendations by the committee. The committee recommends that the Institutional Dues be raised from \$50 to \$100 and that the Life Membership be raised from \$200 to \$300 which may be paid in four installments of \$75 each. The recommendation was seconded.

Chittenden stated that a graduate student award has been underwritten by Sigma Xi and will be presented this year.

Chittenden reported for Jim Peck, Editor of the *Proceedings*, and indicated that Peck has resigned to become editor of a national journal in his field. He stated that Harvey Barton had been appointed to fill the unexpired term. Barton presented the following motion:

Mr. President, I move that the Academy appropriate \$500.00 for editorial assistance and \$120.00 for travel for preparation of Volume 43 of the *Proceedings* for the year 1989. The travel expenses will be used only if needed.

The motion was seconded and will be voted on at the Second Business Meeting. He also reminded section chairs to be sure to collect papers at the sessions and to turn in those papers.

Gary Tucker, Editor of the Newsletter, presented the following motion:

Mr. President, I move that the Arkansas Academy of Science appropriate \$250.00 in support of the Arkansas Academy of Science Newsletter for the academic year 1989-90.

The motion was seconded and will be voted on at the Second Business Meeting.

John Peck, reported for Mike Rapp, Director of the Arkansas Science Fair Association. He presented the following motion:

Mr. President, I move that the Arkansas Academy of Science provide \$200.00 support to the Arkansas Science Fair Association for distribution among the seven regional science fairs and the state science fair.

Arkansas Academy of Science

The motion was seconded and will be voted on at the Second Business Meeting.

John Peck reported for Paul Krause concerning the Arkansas Junior Academy of Science. He presented the following motion:

Mr. President, I move that the Arkansas Academy of Science provide \$250.00 support to the Arkansas Junior Academy of Science.

The motion was seconded and will be voted on at the Second Business Meeting.

John Peck, Director of the Arkansas Science Talent Search, presented the following report:

Following is the winner of the 38th annual Arkansas Science Talent Search, held in conjunction with the 48th Annual Westinghouse Science Talent Search.

First Place
Christopher McLean Skinner On the Diophantine
3724 Sierra Forest Drive Equation
Little Rock, AR 72212
Hall High School $ap^x + bq^y = c + dp^2q^w$
Sponsored by Mr. Dennis R. Brant

He presented the following motion:

Mr. President, I move that the Arkansas Academy of Science provide \$100.00 for the purpose of a plaque to be presented to Chris Skinner.

The motion was seconded and will be voted on at the Second Business Meeting.

Tom Palko, Director of the Arkansas Junior Science and Humanities Symposium, stated that he was not requesting any financial support but did want the blessing of the Academy. He stated that the Symposium was held the previous weekend involving 47 schools. Seventy-one papers were submitted of which 16 were read and six of those will be going to the national symposium.

Leon Richards reported for the Nominating Committee consisting of himself and Tom Lynch. The nominees for Vice President were Dick Cohoon, Arkansas Tech University, and Mike Rapp, University of Central Arkansas. The nominees for Secretary were Robert Wright, University of Central Arkansas, and John Rickett, University of Arkansas at Little Rock.

Leo Paulissen reported on the Biota Survey. Combined checklists are available for \$10. Several collected lists will be released in the future as supplements.

Robbin Anderson, Chairman of the Science Education Committee, presented a report concerning the reorganization and duties of the Committee. This report follows.

1. Make-up and duration of service.
The committee should consist of no more than 12 members, chosen to be representative of different regions, institutions, or fields in the Academy, but with membership open to members with industrial or agency backgrounds.

The term of service should be three years, but with the initial appointments staggered by lot so that one third of the group will rotate off each year. The continuing members will be responsible for submitting a list of suggestions for replacements each year, and additional suggestions may be made by other Academy members, the officers, or the Executive Committee.

2. Duties.
The general responsibilities of the committee on science education would seem to be covered well within the objectives of the Academy for "promotion and diffusion of knowledge of the fields of science." Perhaps some specific attention should be recommended also to the next objective on "unification of these interests in the state." This would seem to indicate that, in addition to planning activities that will take place at the annual meetings, the science education committee should also work actively to foster cooperative activities and programs with other educational groups in Arkansas.

He noted that this reorganization had been approved by the Executive Committee. He also discussed some general plans for the committee and invited suggestions from all members.

President Chittenden announced that the Resolutions Committee will consist of George Harp.

President Chittenden asked section chairmen to keep their sections on time.

President Chittenden mentioned that Banquet tickets were still available.

President Chittenden adjourned the First Business Meeting.

SECOND BUSINESS MEETING

Dave Chittenden, President, called the meeting to order.

Walter Godwin, Secretary, moved the approval of the minutes of the Seventy-Second Annual Meeting as printed with no corrections. The motion was seconded and passed.

Art Johnson presented the following report from the Audit Committee.

We have examined the financial records of the Arkansas Academy of Science. The records are kept by Dr. Robert Wiley, Treasurer of the Academy.

We find the books to be legible and logical. The posting is convenient for the auditors to follow as it is accurate and organized. Documentation of monies in certificates of deposit and checking accounts is excellent.

The Academy is justified in having confidence in the manner in which its financial affairs are handled. All the figures in the 1989 financial statement are in order. We commend Dr. Wiley on his care and precision.

Signed: The Audit Committee
Richard K. Spears, Jr.
Arthur A. Johnson

It was moved and seconded to accept the report of the Audit Committee. The motion passed. Robert Wiley, Treasurer, moved that the Treasurer's Report be approved. He indicated that copies were available if needed. The motion was seconded. The motion passed.

Chittenden stated that the nominees for Vice President were Dick Cohoon, Arkansas Tech University, and Mike Rapp, University of Central Arkansas and the nominees for Secretary were Robert Wright, University of Central Arkansas, and John Rickett, University of Arkansas at Little Rock. He called for additional nominations. It was moved, seconded and passed to cease nominations. Ballots were distributed and collected.

Secretary's Report

The motion presented at the First Business Meeting by Harvey Barton, Editor of the *Proceedings*, to allocate \$500 for editorial assistance and \$120 for travel for the *Proceedings* for next year was passed without additional comment.

The motion presented at the First Business Meeting by Gary Tucker, Editor of the Newsletter, to allocate \$250 for the Newsletter for next year was passed without additional comment.

The motion presented at the First Business Meeting by John Peck on behalf of Mike Rapp, Director of the Arkansas Science Fair Association, to provide \$200 support to the Arkansas Science Fair Association for next year was passed without additional comment.

The motion presented at the First Business Meeting by John Peck on behalf of Paul Krause, Director of the Arkansas Junior Academy of Science, to provide \$250 support to the Arkansas Junior Academy of Science for next year was passed without additional comment.

The motion presented at the First Business Meeting by John Peck, Director of the Arkansas Science Talent Search, to provide \$100 to the Arkansas Science Talent Search for a plaque for Chris Skinner was passed without additional comment.

Robbin Anderson, Chairman of the Science Education Committee, noted the acceptance of the reorganization of the Committee by the Executive Committee and again asked for comments and suggestions. He moved for the acceptance of the committee report from the first meeting. The motion was seconded and passed.

Leo Paulissen reported on the Biota Survey. He urged members to purchase the composite lists which are now available.

Robert Watson reported on the undergraduate awards. He reported that the awards had been won by:

- Physical Sciences: (\$50)
 Anders Amelin - UCA
 Research Octane Numbers by Nuclear Magnetic Resonance Spectroscopy.
- Life Sciences: tie (\$25 each)
 Bryant Turbeville - UALR
 The Potential for Nonglycolytic Energy Production in Diving and Nondiving Vertebrates.
 D. K. Cartwright - UAF
 Preliminary Evaluation of a Dodder Anthracnose Fungus from China as a Mycoherbicide for Dodder Control in the U.S.

John Sorenson reported on the graduate award. He reported that the award of \$50 and a plaque had been won by:

- George T. Blevins - UAMS
 Extradial (E2) Influences Cholecystokinin (CCK) Stimulated Amylase Release from Rat Pancreatic Acini.

George Harp, Chairman of the Resolutions Committee, moved the adoption of the following resolution.

Be it resolved:

The members of the Arkansas Academy of Science express their gratitude to University of Arkansas for Medical Sciences for hosting the Academy's 1989 meeting. In particular, the Academy thanks James Daly, who was in charge of local arrangements, and the entire Local Arrangements Committee. Appreciation is expressed to all who helped provide comfortable and convenient facilities and an excellent banquet. Dr. Joycelyn Elders'

outstanding banquet address concerning environmental health was a highlight of the meeting.

The Academy appreciates the work of the various section chairmen and recognizes the important role they played in the conduct of the meeting. They are as follows: Cliff Orr, John Sorenson and Parimal Chowdhury (Biomedical); Gary Tucker (Botany); Kim Fifer (Chemistry); Hal Berghel, Ron Goforth, Richard Rankin, Rama Reddy, David Sallach, Frederick Spiegel and John Talburt (Computer Science); Edmond Bacon and Forest Payne (Environmental and Aquatic); Wayne Gray and Dwight Talburt (Microbiology and Immunology); V. Rick McDaniel (Vertebrate Zoology); and Robbin Anderson (Physics, Geology and Science Education).

The Academy expresses gratitude to the various directors of the science youth activities supported by the Academy: Robbin Anderson, the Science Education Committee; Mike Rapp, Director of the Arkansas State Science Fair; Tom Palko, Director of the Junior Science and Humanities Symposium; Paul Krause, the Arkansas Junior Academy of Science; John Peck, Director of the Arkansas Science Talent Search; Leo Paulissen, the Biota Committee; and Ed Bacon, the Development Committee.

The Academy is only as successful as its leadership in planning, working, and directing the various activities. To David Chittenden, President; Richard Spears, President-Elect; Robert Watson, Vice President; Walter Godwin, Secretary; Robert Wiley, Treasurer; Gary Tucker, Past-President and Newsletter Editor; Jim Peck, *Proceedings* Editor; and Henry Robison, Historian, the Academy expresses gratitude and thanks for an excellent year.

The Academy expresses thanks and encouragement to continue to the exhibitors for their booths at the meeting.

The Academy recognizes with appreciation the work of Dennis Baeyens, Parimal Chowdhury, and John Sorenson for their efforts on the Awards Committee; Leon Richards and Tom Lynch for their work on the Nominating Committee and as tellers; George Harp, Resolutions Committee; and Dick Spears and Art Johnson, Audit Committee.

Finally, the Academy appreciates the work in designs of plaques and calligraphy by Robert Wiley and Calvin Cotton.

The motion was seconded and passed. Harp then moved the adoption of a second resolution.

Be it resolved:

The members of the Arkansas Academy of Science express their congratulations to Christopher Skinner, a senior at Hall High School, for his scientific accomplishments which resulted in several honors this year.

Mr. Skinner's research paper presented to the 23rd Arkansas Junior Science and Humanities Symposium was judged to be the top research paper and will represent Arkansas at the National Science and Humanities Symposium at West Point, New York in May.

Mr. Skinner was also chosen as first place winner of the Arkansas Talent Search. He culminated this year of achievement by being selected as a National Winner in the 48th Annual Westinghouse Science Talent Search and was awarded a \$20,000 unrestricted scholarship.

The motion was seconded and passed.

President Chittenden announced that the Academy will meet at Arkansas State University in 1990 on April 6 & 7, and at the Univer-

Arkansas Academy of Science

sity of Arkansas at Fayetteville under the sponsorship of Sigma Xi in 1991, probably the first weekend in April.

President Chittenden called for Old Business. None was forthcoming.

President Chittenden called for New Business. John Sorenson asked for an opinion on whether the Academy favored presenting awards in each discipline both at the graduate and undergraduate levels. Considerable discussion followed. It was suggested that the Executive Committee should discuss this at its next meeting. This should also be discussed with Sigma Xi. The possibility of support by other societies, such as ACS, etc., was mentioned. The possibility of additional awards such as first, second and third was also mentioned. It was suggested that this might generate more interest in some areas. Both agreement and disagreement in general were indicated.

The recommendation of the Constitution Committee that the Institutional Dues be raised from \$50 to \$100 and that the Life Membership be raised from \$200 to \$300 which may be paid in four installments of \$75 each was restated. The recommendation which was seconded at the First Business Meeting was passed with the change in Institutional Dues being effective immediately and the Life Membership dues change being effective January 1, 1990.

It was suggested that publicity for the Academy should be discussed by the Executive Committee.

The possibility of the inclusion of engineers in the Academy was also mentioned and referred to the Executive Committee.

President Chittenden discussed the establishment of liaison with SILO. Communications with a designated member have been established. SILO will notify the Academy of pending legislation. The Academy may furnish additional persons for advice on issues when SILO needs assistance. The possibility of revising the Directory of Scientific and

Research Personnel was mentioned. Chittenden will meet further with SILO later.

Leon Richards announced the results of the election. John Rickett was elected Secretary and Mike Rapp was elected Vice President.

President Chittenden thanked the Executive Committee and other members for help he has received in the past year.

President Chittenden called on President-Elect Dick Spears and passed the gavel to him.

President Spears recognized the services in the past of retiring Executive Committee members. He presented retiring Editor Jim Peck with a plaque in recognition of his years of service. He presented retiring Secretary Walter Godwin with a plaque in recognition of his five years of service. He presented Past-President Chittenden with a plaque in recognition of his year of service.

President Spears encouraged members to support the Academy. He also encouraged members to let retirees know that they were missed. He expressed hope that members would encourage younger scientists to participate. He encouraged increased participation by two-year institutions.

President Spears asked that a representative from each institution or organization pick up copies of the *Proceedings* for those not in attendance.

It was moved, seconded and passed to adjourn the meeting.

Respectfully submitted,

Walter E. Godwin
Secretary

REGULAR MEMBERS

Stephen R. Addison	University of Central Arkansas	Malsom K. Cleaveland	University of Arkansas
John W. Ahlen	Arkansas Science & Technology Authority	Richard R. Cohoon	Arkansas Tech University
Syed M. Aijaz	University of Arkansas at Pine Bluff	George W. Colton	Arkansas Geological Commission
Bob Allen	Arkansas Tech University	Janice L. Cooper	Arkansas State University
Robert T. Allen	University of Arkansas	Robert M. Cordova	Cordco Consulting
Silke Hufnagel Allen	Hendrix College	William Coutts	Univ. of Ark./Little Rock
Neil T. Allison	University of Arkansas	Bob W. Cowling	
Cynthia Annett	Univ. of Ark./Fayetteville	Randy T. Cox	University of Arkansas at Monticello
John T. Annulis	University of Arkansas at Monticello	James G. Culpepper	University of Arkansas at Monticello
Michael L. Armstrong	Arkansas Game & Fish Commission	Fred Dalske	University of Central Arkansas
Robert K. Bacon	University of Arkansas	James J. Daly	University of Arkansas for Medical Sciences
Gwen Barber	University of Central Arkansas	James T. Daniels	SAU - Tech
Sara Mills Barnett	Texas Education Agency & UT-Austin	Stanley N. David	Arkansas State University
Henry Barwood	Arkansas Mining Institute	Leo Carson Davis	Southern Arkansas University
Adelphia M. Basford	Henderson State University (Retired)	Don C. De Luca	University of Arkansas for Medical Sciences
John K. Beadles	Arkansas State University	Robert H. Dilday	Univ. of Ark. Cooperative Extension Service
Helen Benes	University of Arkansas for Medical Sciences	Ronald H. Doran	Harding University
Ann Marie Benson	University of Arkansas for Medical Sciences	Larry W. Dorman	Univ. of Ark. Cooperative Extension Service
Hal Berghel	Univ. Ark./Fayetteville	Peggy Rea Dorris	Henderson State University
C. Bhuvaneshwara	University of Arkansas for Medical Sciences	Marion Douglas	
Veryl V. Board	Arkansas College	Lee Lee Doyle	University of Arkansas for Medical Sciences
Marilyn D. Bocksnick	Arkansas Tech University	Benjamin T. Duhart	University of Arkansas at Pine Bluff
Laurence J. Boucher	Arkansas State University	Danny J. Ebert	U. S. Forest Service
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PROGRAM

Arkansas Academy of Science

Seventy-third Annual Meeting

UNIVERSITY OF ARKANSAS AT LITTLE ROCK

Little Rock, Arkansas

Meeting concurrently with sessions of

The Collegiate Academy of Science

Friday, 7 April

SENIOR & COLLEGIATE ACADEMIES -- Registration

SENIOR ACADEMY -- Executive Board Meeting

SENIOR ACADEMY -- First General Business Meeting

SENIOR AND COLLEGIATE ACADEMIES: Papers [Concurrent Sessions]:

Biomedical I
Botany
Chemistry
Computer Science, Track I
Computer Science, Track II
Environmental and Aquatic
Microbiology and Immunology
Vertebrate Zoology

SCIENCE EDUCATION COMMITTEE

SENIOR AND COLLEGIATE ACADEMIES -- Banquet

POST BANQUET SPEAKER -- Dr. Joycelyn Elders, Director
Arkansas State Health Department

Saturday, 8 April

SENIOR AND COLLEGIATE ACADEMIES -- Registration

SENIOR AND COLLEGIATE ACADEMIES: Papers [Concurrent Sessions]:

Biomedical II
Computer Science, Track I
Computer Science, Track II
Graduate Student Competition
Physics, Geology & Science Education

SENIOR ACADEMY -- Second General Business Meeting

SECTION PROGRAMS

[*Undergraduate Student Competition; **Graduate Student Competition]

BIOMEDICAL I

Session Chairpersons: Dr. Cliff Orr and Dr. John Sorenson

***STRUCTURE, FUNCTION, AND PURIFICATION OF G-PROTEINS ASSOCIATED WITH ADRENERGIC SECOND MESSENGER SYSTEMS.**

Patric Anderson, John Exton, and Janet Atkinson, Dept. Molecular Physiology, Vanderbilt University, Nashville, TN and Dept. of Biology, Hendrix College, Conway, AR.

***BIOLOGICAL EFFECTS OF OXAZOLIDINES DERIVED FROM EPHEDRINE.**

D. Mark Wood, M. Mateen Akmal, and Richard B. Walker, Chemistry Dept. UAPB, Pine Bluff, AR.

***THE POTENTIAL FOR NONGLYCOLYTIC ENERGY PRODUCTION IN DIVING AND NONDIVING VERTEBRATES.**

Bryant Turbeville, Dennis Baeyens, W. Grady Smith, C. Bhuvaneshwaran, Dept. of Biology, UALR, Little Rock, AR and Dept. of Biochemistry, UAMS, Little Rock, AR.

SUBUNIT AFFINITY OF HEMOGLOBIN: A DETERMINANT OF HEMOGLOBIN A2 LEVEL IN THALASSEMIA SYNDROMES.

A. Mansouri, Dept. Medicine, LRVA Hospital and UAMS, Little Rock, AR.

CHARACTERIZATION OF SECONDARY STRUCTURE IN HEN OVALBUMIN mRNA.

Darren K. McGuire, Charles D. Liarakos, and Randall A. Kopper, Dept. of Chemistry, Hendrix College, Conway, AR.

NEUROTRANSMITTER EFFLUX FROM THE SALAMANDER RETINA: EVIDENCE FOR HOMOEXCHANGE, BUT NOT HETEROEXCHANGE, OF GABA AND GLYCINE.

Chris D. Melton, Hendrix College, Conway, AR and Jeanne M. Frederick, Samuel Wu, and Joe G. Hollyfield, Baylor College of Medicine, Houston, TX.

METAL COFACTOR REPLACEMENT AND ACTIVITY OF ALKALINE PHOSPHATASE.

Rhonda L. Primm and Randall A. Kopper, Dept. of Chemistry, Hendrix College, Conway, AR.

PHOTOREACTIVATION OF THE EFFECT OF UV LIGHT ON GAMMA RAY INDUCED CHROMOSOME ABBERATION PRODUCTION IN G1 PHASE XENOPUS CELLS.

Rebecca A. Rowe and H. Gaston Griggs, Dept. of Biology, John Brown University, Siloam Springs, AR.

PHOTOREACTIVATION OF CHROMATID DELETIONS INDUCED BY UV-IRRADIATION OF G1 PHASE HAMSTER X *Xenopus* HYBRID CELLS.

Joel E. Stagers and H. Gaston Griggs, Dept. of Biology, John Brown University, Siloam Springs, AR.

****DIGESTION OF PARTIALLY PURIFIED ALPHA 1 - ADRENERGIC RECEPTOR BY *Staphylococcus aureus* V-8 PROTEUS.**

R. E. McGehee, Jr., A. A. Nash, J. S. Norris and L. E. Cornett, Dept. of Physiology and Biophysics, UAMS, Little Rock, AR.

THE EFFECTS OF SOLVENT POLARITY ON ENZYME ACTIVITY.

Giovanni Chiechi and Randall A. Kopper, Dept. of Chemistry, Hendrix College, Conway, AR.

BOTANY

Session Chairperson: Dr. Gary Tucker

SEM ANALYSIS OF VARIOUS WHEY-DERIVED ADHESIVE RESINS.

W. Bowen, T. Viswanathan, D. Prince, W. Coutts, G. Lindsey, and A. Toland, UALR, Little Rock, AR.

FLORISTIC NOTES ON THE ARKANSAS CRUCIFERAE (BRASSICACEAE).

Edwin B. Smith, Dept. of Botany and Microbiology, UAF, Fayetteville, AR.

INDICATORS OF MINIMALLY DISTURBED PRAIRIES IN NORTHWEST ARKANSAS.

Edward E. Dale, Jr., Dept. of Botany and Microbiology, UAF, Fayetteville, AR.

BLACKLAND PRAIRIES OF SOUTHWESTERN ARKANSAS.

Thomas L. Foti, Arkansas Natural Heritage Commission, Little Rock, AR.

STATUS OF *Halesia diptera* ELLIS (STYRACACEAE) IN ARKANSAS.

Raymond G. Erickson, Soil Conservation Service, Lewisville, AR.

ALLELOPATHIC ACTIVITY IN RICE (*Oryza*) GERMPLASM.

R. H. Dilday, P. Nastasi, and R. J. Smith, Jr. USDA/ARS Stuttgart, AR and Dept. of Agronomy, UAF, Fayetteville, AR.

***PRELIMINARY EVALUATION OF A DODDER ANTHRACNOSE FUNGUS FROM CHINA AS A MYCOHERBICIDE FOR DODDER CONTROL IN THE U.S.**

D. K. Cartwright and G. E. Templeton, Plant Pathology Dept., UAF, Fayetteville, AR.

*****In vitro* CULTURE OF ARKANSAS RICE CULTIVARS.**

A. F. Mirlohi, F. H. Huang, J. M. Ali-Khayri, and L. F. Thompson, Depts. of Agronomy, and Horticulture and Forestry, UAF, Fayetteville, AR.

****FURTHER STUDIES ON POPULATIONS AND SUCCESSION OF PROTOSTELIDS.**

S. W. Miller and F. W. Spiegel, Dept. of Botany and Microbiology, UAF, Fayetteville, AR.

A MODIFIED VACUUM EXTRACTION SYSTEM FOR PLANT TISSUE ETHYLENE.

Forrest E. Lane, Dept. of Botany and Microbiology, UAF, Fayetteville, AR.

THE ROOT PULLING RESISTANCE TECHNIQUE FOR THE SELECTION OF DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.) GENOTYPES.

M. Price, Dept. of Agriculture, UAPB, Pine Bluff, AR, R. H. Dilday, USDA/ARS Rice Research Station, Stuttgart, AR, and A. L. Allen, Agricultural Expt. Station, UAPB, Pine Bluff, AR.

GENETIC AND PLANT GROWTH REGULATOR MANIPULATION OF RICE (*Oryza sativa* L.) MESOCOTYL AND COLEOPTILE LENGTHS.

Ronnie S. Helms and Robert H. Dilday, University of Arkansas Southeast Research and Extension Center, Monticello, AR.

THE PROTOSTELIDS: A HISTORY OF TAXONOMIC POINTS OF

VIEW.

Frederick W. Spiegel, Dept. of Botany and Microbiology, UAF, Fayetteville, AR.

REPRODUCTION OF *Lindera melissifolia* IN ARKANSAS.

Robert D. Wright, UCA, Conway, AR.

CHEMISTRY

Session Chairperson: Dr. Kim Fifer

SYNTHESIS OF CARBOHYDRATE-DERIVED CHIRIONS FROM D-ARABINOSE.

Brock K. King, Heidi E. Mills and Thomas E. Goodwin, Dept. of Chemistry, Hendrix College, Conway, AR.

THE USE OF QUININE ACETATE IN ALDOL CONDENSATIONS.

Heidi E. Mills and Thomas E. Goodwin, Dept. of Chemistry, Hendrix College, Conway, AR.

SYNTHESIS OF DEAZAPURINE NUCLEOSIDE PROBES OF THE MECHANISM OF ADENOSINE DEAMINASE.

Luke J. Moix, Dept. of Chemistry, Hendrix College, Conway, AR and Linda C. Kurz, Washington University, St. Louis, MO.

*SYNTHESIS OF TETRAHYDROCANNABINOL CONGENERS. 5-ALKYLRESORCINOL SYNTHESIS.

Robert W. Allen and Kevin M. Diamond, ATU, Russellville, AR.

*RESEARCH OCTANE NUMBERS BY NUCLEAR MAGNETIC RESONANCE SPECTROMETRY.

Anders Amelin and Paul Krause, Dept. of Chemistry, UCA, Conway, AR.

*GAS CHROMATOGRAPHIC ANALYSIS OF A MIXTURE OF SEVERAL DIHALONICOTINIC ACIDS.

H. Koshaba, A. U. Shaikh and F. L. Setliff, Dept. of Chemistry, UALR, Little Rock, AR.

SYNTHESIS TRANSFORMATIONS OF AN ENONE DERIVED FROM D-GLUCOSE.

N. Michele Rothman and Thomas E. Goodwin, Dept. of Chemistry, Hendrix College, Conway, AR.

PREPARATION OF A SERIES OF PYRIDYL PHENYLUREAS OF POTENTIAL AGRICULTURAL INTEREST.

Frank L. Setliff, Steve H. Rankin, and Mark W. Milstead, Dept. of Chemistry, UALR, Little Rock, AR.

SYNTHESIS OF THE ⁶³CU AND ¹⁴C DOUBLE LABELED COMPLEX OF TETRAKIS-U-3,5-DIISOPROPYLSALICYLATO-DIAQUODICOPPER(II).

John R. J. Sorenson, M. V. Chidamaram, C. E. Epperson, S. Williams, and R. A. Gray, College of Pharmacy, UAMS, Little Rock, AR.

1,3-DIPOLAR ADDITION. AN INTERESTING REARRANGEMENT OF ISOXAZOLIDINE TOSYLATES.

Van Ahn Pham, Cheryl Lichti and Thomas E. Goodwin, Dept. of Chemistry, Hendrix College, Conway, AR.

ENANTIOSPECIFIC PREPARATION OF A COMPLEX DIKETONE FROM A CARBOHYDRATE TEMPLATE.

Angela K. Wilson and Thomas E. Goodwin, Dept. of Chemistry, Hendrix College, Conway, AR.

COMPUTER SCIENCE

Section Chairperson: Hal Berghel

**Track I, Session A
(Logic Programming)**

Chairperson: Richard Rankin

USING PROLOG FOR MEDICAL LOGIC.

Mark Shifman, University Hospital of Arkansas, Little Rock, AR.

LOGIC PROGRAMMING FOR INDUSTRIAL ENGINEERING.

Susan Johnsen, Industrial Engineering Dept., UAF, Fayetteville, AR.

APPROXIMATE STRING MATCHING IN PROLOG.

David Roach, Computer Science Dept., UAF, Fayetteville, AR.

EFFICIENT GENERATION OF LEXICAL PALINDROMES.

Tielin Xu, Taiyuan Institute of Technology, Fayetteville, AR.

Track I, Session B**(Knowledge Based Systems for Scientific Discovery)**

Chairperson: Dr. David Sallach

KNOWLEDGE BASE SYSTEMS AND THE SCIENCES OF COMPLEXITY.

David Sallach, Computer Science Dept., UAF, Fayetteville, AR.

CAUSAL INFERENCE USING A TAXONOMIC MODEL.

Roger Ghormley, Computer Science Dept., UAF, Fayetteville, AR.

CAUSAL INFERENCE AND PART-WHOLE AGGREGATION.

Girish Natarajan, Computer Science Dept., UAF, Fayetteville, AR.

QUERY LANGUAGE EXTENSIONS FOR SCIENTIFIC ANALYSIS.

Siongo Japit, Computer Science Dept., UAF, Fayetteville, AR.

Track II, Session A**(Simulation and Expert Systems)**

Chairperson: Dr. Ron Goforth

THE USE OF EXPERT SYSTEMS TECHNIQUES FOR SIMULATIONS WHICH ARE NOT CALCULATION INTENSIVE.

Ron Goforth, Computer Science Engineering Dept., UAF, Fayetteville, AR.

EMBEDDED EXPERT SYSTEMS FOR TECHNOLOGY TRANSFER.

Rodney Tolander, Energy Techniques Inc., Fayetteville, AR.

EMBEDDED EXPERT SYSTEMS IN CAD.

Ali Monash, Arkansas Center for Technology Transfer, UAF, Fayetteville, AR.

COMPUTER SIMULATIONS OF THE FUTURE INCIDENCE OF SEXUALLY TRANSMITTED DISEASES.

Michael Stephens, College of Engineering, UAF, Fayetteville, AR.

Track II, Session B**Steering Committee Meeting.****Arkansas Computer Science Conference**

Chairpersons: Hal Berghel, John Talburt

ENVIRONMENTAL AND AQUATIC

Session Chairperson: Dr. Edmond Bacon

AGE ASSESSMENT OF WHITE BASS FROM OTOLITHS, DORSAL SPINES, AND SCALES.

Raj V. Kilambi and Thoniot T. Prabhakaran, Dept. of Zoology, UAF, Fayetteville, AR.

MERISTIC VARIATIONS IN THE CALIFORNIA KILLIFISH *Fundulus parvipinnis*.

Alan William Newman, Dept. of Biology, Hendrix College, Conway, AR, and Stuart G. Poss, Gulf Coast Research Laboratory, Ocean Springs, MS.

DISTRIBUTION OF YELLOW GRUB (*Clinostomum marginatum*) ALONG CROOKED CREEK (MARION CO., AR) IN SMALLMOUTH AND LARGEMOUTH BASS POPULATIONS.

J. J. Daly, B. DeYoung, T. Hostetler, Dept. of Microbiology and Immunology, UAMS, Little Rock, AR, and E. Walker, Little Rock Veterans Administration Hospital, Little Rock, AR.

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Arkansas Academy of Science

COMPUTER SCIENCE

Track I, Session C (AI and Expert Systems)

Chairperson: Dr. John Talburt

CONCEPT DECOMPOSITION.

Walter Sedelow, Computer and Information Science Dept., UALR, Little Rock, AR.

CONCEPT ASSOCIATION.

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Donna Mooney, Computer and Information Science Dept., UALR, Little Rock, AR.

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(Computer Applications in Engineering and Industry)

Chairperson: Dr. Rama Reddy

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Mike Stewart, Engineering Technology Dept., UALR, Little Rock, AR.

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Bill Russell and Roger M. Hawk, UALR/GIT, Little Rock, AR.

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G. T. Blevins Jr., Y. S. Huang, D. W. McKay, P. Chowdhury, and P. L. Rayford, Dept. Physiology and Biophysics, UAMS, Little Rock, AR.

³¹LI NMR OF ERYTHROCYTES.

Rao P. Gullapalli and Roger M. Hawk, GIT/UALR, Little Rock, AR, and Richard A. Komoroski, UAMS, Little Rock, AR.

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Gary Fuller and Roger Hawk, GIT/UALR, Little Rock.

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Mitchell K. Marks and George L. Harp, Dept. of Biological Sciences, ASU, State University, AR.

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S. A. Tedder and R. T. Allen, Entomology Dept., UAF, Fayetteville, AR.

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Lance Raney, OBU, Arkadelphia, AR.

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Thomas J. Turner, OBU, Arkadelphia, AR and Don Avery, Henderson State University, Arkadelphia, AR.

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M. J. George and L. A. Mink, Dept. of Computer Science, Mathematics, Physics, ASU, State University, AR.

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Dianne Phillips and Peggy Guccione, Dept. of Geology, UAF, Fayetteville, AR.

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Rudolph J. Eichenberger, Southern Arkansas University, Magnolia, AR.

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Rudolph J. Eichenberger, SAU, Magnolia, AR, Marilyn Jones, and Joe Thomason, Magnolia Central School, Magnolia, AR.

COMPUTER ASSISTED INSTRUCTION IN INTRODUCTORY PHYSICAL SCIENCE.

Mattie M. Glover and William Willingham, Dept. of Chemistry, UAPB, Pine Bluff, AR.

PRELIMINARY EVALUATION OF A DODDER ANTHRACNOSE FUNGUS FROM CHINA AS A MYCOHERBICIDE FOR DODDER CONTROL IN THE U.S.

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ABSTRACT

Dodder (*Cuscuta* spp.) is a noxious, parasitic, annual weed throughout most of the United States. A fungus used to control it in China was imported under permit for studies with U.S. dodder species in containment. The fungus, *Colletotrichum gloeosporioides*, sporulated on liquid and solid media at room temperature. Conidia from 7-12 day old cultures were diluted to 3.5 to 7×10^6 spores ml^{-1} for host range inoculations. Germination on water agar at 24 hrs was higher at 28 than 30 or 24 C. Inoculated plants were exposed to dew periods of 12-14 hrs at 24 or 28 C, then transferred to growth chambers with 12-hr photoperiods at constant temperatures of 24, 28, and 32C. Dodder species were severely diseased but rarely killed. Symptoms were most severe on native collections of *Cuscuta campestris* after 4 to 5 days incubation when this species on periwinkle seedlings was inoculated with 3.5 to 7×10^6 spores ml^{-1} . *Cuscuta cuspidata*, *C. pentagona*, and *C. campestris* from a California seedlot were also tested under optimum conditions for disease. The *C. campestris* from California was the most susceptible. Inoculation of 16 species in eight plant families revealed no other host except sweet potato which developed a necrotic fleck. This research indicates a need for strain improvement prior to field tests.

INTRODUCTION

Dodder (*Cuscuta* spp.) is a parasitic weed problem on many important crops throughout the world (Ashton and Santana, 1976; Kuijt, 1969). The genus is in the family Convolvulaceae and contains over 100 species. It appears as a tiny, yellow, orange, or green vine that entwines and attaches itself to a host plant. It eventually loses contact with the soil and becomes dependent upon the host for nutrients (Musselman and Sand, 1984). The primary problem of dodder infestations in crops is loss of vigor, resulting in yield loss. It also causes problems by transmission of certain systemic diseases, and by spread of seed into uninfested areas resulting in denial of seed certification (Ashton and Santana, 1976; Musselman and Sand, 1984; Woodham and Krake, 1983).

Control of dodder is achieved primarily by chemical and cultural means. Chemical control usually utilizes soil-applied herbicides such as chlorpropham, DCPA, trifluralin, and fluometuron; while cultural control involves use of weed-free seed, roguing, and burning of infested areas (Ashton and Santana, 1976; Bewick *et al.*, 1988). These methods are often ineffective and chemicals may create environmental hazards from residues or contamination of food or groundwater.

Because of difficulties and risk of existing controls, alternative methods are needed. Some of the most promising means of alternative control are biocontrol; specifically, mycoherbicides. Mycoherbicides are fungal plant pathogens that are applied as inundative inoculum, as in standard herbicides, to control specific weeds (Templeton, 1985, 1986, 1987). In some cases, mycoherbicides have proven to be as effective or more effective than chemical herbicides (Daniel *et al.*, 1973). To date only two mycoherbicides have been commercialized: COLLEGOTM, a formulation of the fungus *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* for control of northern jointvetch (*Aeschynomene virginica* [L.] B.S.P.) in rice and soybeans, and DeVine[®], a fungus (*Phytophthora palmivora* Butler) for control of stranglervine (*Morrenia odorata* H. and A. Lindl.) in Florida citrus groves (Templeton, 1987).

There have been other attempts to control dodder with fungal pathogens in Russia and China. In the Soviet Union, an *Alternaria* species has been used to control dodder in certain crops (Ashton and Santana, 1976), and a *Colletotrichum* species has been used for control in China (Gao *et al.*, 1985; Li, 1985).

A *Colletotrichum* species, *C. gloeosporioides* (Penz.) Sacc. f. sp. *cuscutae* (Cgc), was obtained from China for use in this study. The purposes were: (a) to determine if the fungus would infect and cause disease

on dodder species of the U.S. in controlled environments, and (b) to examine the host range of this fungus as a prelude to further tests of it as a mycoherbicide for indigenous species of dodder in the U.S.

LITERATURE REVIEW

Musselman and Sand (1984) describe dodder as a highly specialized parasite stripped of all but the essential parts, and appearing as a tiny yellow vine that smothers its host. Dodder is reported worldwide, with the largest number of species occurring in the Western Hemisphere. Dodder has little or no host specificity. It is a significant pest worldwide on tomatoes, alfalfa, sugar beets, raspberries, cranberries, onions, asparagus, carrots, potatoes, and tobacco (Ashton and Santana, 1976; Bewick *et al.*, 1988; Musselman and Sand, 1984).

Colletotrichum gloeosporioides (Penz.) Sacc. is the conidial form of the ascomycete, *Glomerella cingulata* (Stonem) Spauld & v. Schr. This species is responsible for a number of important anthracnose disease of *Citrus* and many other plant genera (Bessey, 1950).

An *Alternaria* species has been used to control dodder in the Soviet Union. Although this species (*A. cuscutacidae* Rudak) was less effective for control of dodder on sugar beets, other genera of fungi, including (*Cladosporium*, *Fusarium*, and *Rhizoctonia*) improved control when they were added as secondary pathogens. A *Curvularia* species has also been used for dodder control in Russia (Ashton and Santana, 1976). A strain of *Colletotrichum gloeosporioides* specific to *Cuscuta* has traditionally been used for control in China (Li 1985; Gao *et al.*, 1985), and is reported to be very effective. The only problem reported was loss of virulence, which was promptly overcome by selection of isolates for improved virulence.

In this country, the mycoherbicide concept was initiated by researchers at the University of Arkansas in the early 1970's (Daniel *et al.*, 1973). A cooperative research and development effort led to commercialization of COLLEGO, a formulation of an indigenous strain of the fungus *Colletotrichum gloeosporioides*. It is marketed for the control of northern jointvetch (*Aeschynomene virginica* [L.] B.S.P.) in rice and soybeans in Arkansas by Ecogen Corporation, Langhorne, PA. Another mycoherbicide is DeVine, a formulation of *Phytophthora palmivora* (Butler) Butler for control of stranglervine (*Morrenia odorata* [H & A Lindl.] in Florida citrus groves. The persimmon wilt fungus used for control of persimmon, *Diospyros virginiana*, in rangeland in Oklahoma is *Acremonium diospyri* (Crandall) W. Gams and is provided free to ranchers by the Noble Foundation in Ardmore, Oklahoma.

MATERIALS AND METHODS

The dodder strain of *Colletotrichum gloeosporioides* (Penz.) Sacc. was obtained in the summer of 1987 from Dr. Yang Han Li, Nanjing University, Peoples Republic of China. It was grown and maintained on torula yeast, maltrin (M-100), potassium phosphate (dibasic), and magnesium sulfate agar (TA); cornmeal, glucose, and yeast agar (CGY); and homemade potato, dextrose, and streptomycin agar (H - PDA + S) (Tuite, 1969). Torula agar was the preferred medium because of lush colony growth and spore production. Cultures for inoculation were grown at room temperature. Stock cultures were made by inoculating TA slants with mycelial plugs and storing the slants with or without mineral oil at 5C.

Seeds of two species of dodder (*C. campestris* Yunker and *C. cuspidata* Englem) were collected from local sites and germinated two ways; either seeds were soaked for one or five minutes in concentrated sulfuric acid, rinsed in water, and placed on Whatman No. 1 filter paper in a petri dish at room temperature, or seeds were planted directly in soil (prepared by mixing 3 parts potting soil and 1 part fine grade vermiculite) contained in 7.6cm plastic or 10.8cm clay pots. Seeds of *C. pentagona* Englem and *C. campestris* were obtained from Florida and California, respectively.

The primary host plant used for dodder was tall periwinkle (*Vinca rosea* L.). Periwinkle plants were propagated either by cuttings or by seeds. Cuttings were made from mature periwinkle plants by stripping the leaves up to a node, severing the stem just below the node, and placing the cutting in a vial of water for root growth. After rooting, the cuttings were planted in pots and placed in a greenhouse. Periwinkle seeds were planted directly in pots and placed in a greenhouse. All host range plants were started and maintained utilizing the same methods and conditions as the periwinkle plants. All plants were fertilized weekly with a commercial fertilizer.

Attachment of the dodder to the host plants was achieved four ways: (1) Germinated dodder seedlings were placed in 1.5ml micro vials filled with water, attached to the upper stem of a host plant, and placed in a growth chamber or greenhouse. (2) Germinated dodder seedlings were placed in the soil next to periwinkle plants. (3) Established dodder stems were clipped and placed in vials of water implanted in the soil next to a host plant. (4) At different times, young periwinkles were infected by placing them among dodder infested periwinkle plants.

Spore suspensions were prepared by rinsing the spores with distilled water directly from 7 to 12 day old cultures grown on TA at room temperature. Mycelium was removed by filtration through cheesecloth, and desired concentrations were obtained by dilution with distilled water.

Germination of the fungal spores at different concentrations was determined by spreading 0.1ml of spore suspensions containing either 0.1, 1.0, or 10 million spores per ml on water agar plates and holding at room temperature for 24 hours. Germination percentages were determined by observing 200 spores at 10x magnification with a compound microscope and counting the germinated spores.

Spore germination at different temperatures was tested by spreading 0.1ml spore suspension containing 1 million spores per ml on water agar plates, wrapping the plates in foil, and holding at 24, 28, or 30 C for 24 hours.

C. campestris was used for most tests. It was grown in the greenhouse, trimmed to the point of attachment on the host, then moved to growth chambers about seven days before each test to provide more uniform plants.

Inoculations were made with an aerosol sprayer and applied until runoff occurred. Spore concentrations for tests ranged from 3.5 to 7 million spores per ml. A 0.5% concentration of a surfactant (Soydex) was added to the spore suspension for one test. Control plants were sprayed with distilled water only or with Soydex only. At least three replications were utilized for each test. Immediately following inoculation, plants were placed in a dew chamber. Dew period temperatures for tests were 24 or 28 C; the temperature chosen for most tests was 28 C, based on good spore germination at this temperature. Length of dew periods ranged from 12 to 14 hours. After the dew period, plants were placed in growth chambers at 24, 28 or 32 C.

Quantification of disease for the initial tests was achieved by strip-

ping the entire dodder plant from the host plant, usually 7-8 days after inoculation, and measuring dodder stems from each host plant. Necrotic tissue (Any tissue completely shrunken and discolored and all tissues terminal to a necrotic lesion were designated as necrotic tissues.) of dodder stems was then measured. Total necrotic length was then divided by total stem length and multiplied by 100 for percent diseased tissue.

Quantification of disease for the later tests was achieved similarly, except inoculated tissues *only* was measured and all new growth of the dodder during incubation was excluded. This was done by marking certain stems of the dodder with ink before inoculation and retrieving those stems *only* for the measurements. These data were also taken 7-8 days after inoculation.

Quantification of disease for the host range tests was achieved by rating as follows: (-) = no infection or reaction, (+) = positive reaction.

Confirmation of pathogenicity of the fungus was checked by surface-washing infected tissue in a 10% clorox solution for 1 minute and placing the tissue on TA agar. All water checks were excluded from tabulated data.

RESULTS

Spores germinated best at 1 million spores per ml (40%) as compared to concentrations of 10 million (11%) or 0.1 million (37%) over a 24 hour period. The optimum temperature for spore germination over a 24 hour period was 24 to 28 C (33 and 34% respectively) compared to 17% at 30 C.

Disease symptoms began to appear on parts of the dodder stems 24 to 36 hours after inoculation. Stems first sagged, then developed small flecks, and finally collapsed, leaving only remnants of tips or midsection areas (Fig. 1). The most severe symptoms were observed during the fourth or fifth day after inoculation. After this period, severity did not increase under the test conditions.



Figure 1. Disease development on *Cuscuta campestris* four days after inoculation with *Colletotrichum gloeosporioides* f. sp. *cuscuta*.

The amount of disease (using figures which included all adventitious growth) appeared to be the greatest at 28 C, following the inoculation including soydex. With this surfactant, the average percent kill was 21.7%, compared to 12.4% at 24 C and 15% at 28 C.

In the last three temperature tests (using figures which did not include adventitious growth after inoculation), the amount of disease was greatest at 32 C. At 32 C, an average of 69% of the inoculated tissue was killed. In contrast, 40% to 47% was killed at both 28 C and 24 C. These data are summarized in Table 1.

Table 1. Kill of dodder tissue at three temperatures.

Centigrade	Regrowth Included	Percent
24	YES	13.8
	NO	44.0
28	YES	13.9
	NO	43.0
32	--	--
	NO	69.0

The most disease was observed on the California collection of *C. campestris*, with almost complete control obtained. Disease on the other three dodders appeared to be about equal with a moderate control level observed for all.

For the host range test of species other than dodder, all plants seemed unaffected by the fungus except for sweet potato (*Ipomoea batatas* [L.] Lam.). Spots or lesions appeared on the leaves a few days after inoculation, and the fungus was reisolated from infected tissue. All other species were resistant in this test (Table 2).

Table 2. Reaction of plants other than *Cuscuta* spp. to *Cgc*.

Family/species	Common name	Reaction
Apocynaceae		
<i>Vinca rosea</i>	Tall periwinkle	(-)
Chenopodiaceae		
<i>Chenopodium album</i> L.	Lambquarters	(-)
Convolvulaceae		
<i>Ipomoea coccinea</i> L.	Red morningglory	(-)
<i>Ipomoea lacunosa</i> L.	Pitted morningglory	(-)
<i>Ipomoea hederacea</i>	Entireleaf morningglory	(-)
(var. <i>integriscula</i> Gray)		
<i>Ipomoea batatas</i> (L.) Lam.	Sweet potato	(+)
Fagaceae		
<i>Quercus pelustris</i> muenchh.	Pin oak	(-)
Poaceae		
<i>Setaria viridis</i> L.	Green foxtail	(-)
Lauraceae		
<i>Cassytha</i> sp.	cassytha	(-)
Fabaceae		
<i>Aeschynomene virginica</i>	Northern jointvetch	(-)
<i>Glycine max</i> (L.) Herr.	Soybean (var. Lee74)	(-)
<i>Lespedeza stipalacea</i> Maxim.	Korean lespedeza	(-)
<i>Medicago sativa</i> L.	Alfalfa	(-)
Solanaceae		
<i>Lycopersicon esculentum</i> Mill.	Tomato (var. big boy)	(-)
<i>Nicotiana glutinosa</i>	"Tobacco"	(-)
<i>Nicotiana tabacum</i> L.	Tobacco (var. Kentucky 16)	(-)

Rating by observation only (+)=reaction, (-)=no reaction

DISCUSSION

These experiments demonstrated that the Chinese strain of *Cgc* would infect species of dodder indigenous to the United States. Though degree of control varied and complete control was rare, the fungus never failed to cause disease on all of the dodder plants in these test conditions.

It appears that this fungus strain may do better at warmer temperatures. Maximum disease development occurred at 30-32 C. Infection occurred at a dew period and temperature (24 to 28 C for 12 to 14 hr) that would be typical of much of the southern U.S. The fungus

grew well in culture and sporulated abundantly. The host range test needs additional study due to the reaction of sweet potato. Since *Cgc* is an exotic organism, it should be intensively tested for any indication of risk to economic crops where it might be used.

The primary limitation of the fungus was its inability to control the adventitious growth of the dodder plant. If we take into consideration the inoculated tissue only, the percent of control was much greater than if the entire dodder plant (including adventitious growth) was considered in calculating the control percentages. Since the nature of this weed demands that new growth be considered, the lower percentages are probably more realistic. The use of a surfactant improved control but did not destroy adventitious stems. Dodder haustorial coils, from which all new stems erupt, were rarely infected, but when they were, the entire dodder plant died. Environmental or other conditions that would optimize haustorial infections were not found in these tests.

The biology of the dodder plant makes it a difficult weed to control because the host-parasite relationship is complicated, and the dodder seems to follow closely its host's biology. Dodder may be more easily controlled on some hosts than on others. For instance, the *Alternaria* species used in the U.S.S.R. controlled dodder quite well on alfalfa (90%), but was much less effective on sugar beets (Ashton and Santana, 1976). It may be possible to tailor fungal strains to be host-specific on certain dodder-crop associations and suggest areas of investigation that should be studied, if this control measure is to be feasible on a commercial scale.

The potential for this fungus as a mycoherbicide for dodder in this country, as a result of these tests, appears to be low. Additional work, however, is justified because these tests proved that the fungus could be an aggressive pathogen. Research is needed to determine optimum pre- and post-inoculation conditions, better application procedures, and the possibility for the use of surfactants or other enhancement compounds. It should be possible to select more virulent strains of the fungus. In China, loss of virulence in culture was overcome by repeated strain selection (Gao *et al.*, 1985). Selection of strains more virulent to U.S. species of dodder would seem necessary to enhance the commercial feasibility of this fungus in this country.

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BACTERIOLOGICAL QUALITY OF PRIVATE WATER WELLS IN CLARK COUNTY, ARKANSAS

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ABSTRACT

Most private water wells in Clark County appeared to be contaminated by bacteria, apparently entering the wells from surface water seepage. Eighteen to 24% of the wells investigated were positive for fecal contamination. Deeper wells were less often contaminated. More than one-half of the wells sampled exceeded recommended limits of inorganic chemicals for safe potable water. High concentrations of iron and manganese were most common, exceeding recommended limits in more than 40% of the wells.

INTRODUCTION

This study was initiated because of interest expressed by the three student authors regarding the quality of water available from private wells. Wells throughout Clark County were analyzed for three bacterial parameters and fourteen chemical/physical parameters.

METHODS AND MATERIALS

Fifty wells were sampled during 1987-88. Bacterial analyses were by membrane filtration (Millipore HA 045) according to standard methods (APHA, 1985). Both typical and atypical colonies were counted for total coliform. The other two parameters measured were fecal coliform and fecal streptococcus. Culture media were m-Endo broth (Millipore), m-FC broth and m-Enterococcus agar (Difco). Quality control was maintained according to EPA guidelines (Bordner and Winter, 1978). Chemical analyses were according to standard methods (APHA, 1985). Only the chemical data from wells exceeding recommended concentrations are reported in this paper.

RESULTS AND DISCUSSION

Sampling was throughout the county and an attempt was made to sample wells in each of the approximately seventeen geological formations appearing at the surface within the boundaries of the county (USGS, 1976). The study was biased in regard to finding the property owner at home so that permission could be obtained before sampling the well.

Coliform bacteria were isolated from 78% of the wells tested. Since both typical and atypical colonies were enumerated, this parameter was considered to indicate surface water seepage into the well without consideration of fecal contamination.

Eighteen percent of the wells tested positive for fecal coliforms and 24% for fecal streptococci. These parameters are indicative of fecal contamination, and the data suggest 18-24% of the wells have seepage from septic tanks, barnyards, or other sources of animal wastes.

An inverse correlation between well depth and degree of bacterial contamination seemed to exist (Table 1). Deeper wells were less often contaminated by surface seepage.

A continuation of the study is planned to attempt to correlate variation in water chemistry with geologic strata, but data reported here relate only to those wells with inorganic chemical concentrations exceeding Environmental Protection Agency (EPA, 1976) and/or American Society for Testing and Materials (ASTM, 1979) recommendations (Table 2).

Table 1. Correlation of well depth with bacterial contamination.

Bacterial Parameter	Wells Tested Positive (%)		
	Depth (m)		
	less than 10	10-30	more than 30
Total coliforms	54	32	12
Fecal coliforms	16	8	0
Fecal streptococcus	24	8	0

Table 2. Wells exceeding inorganic chemical limitations

Chemical Parameter	Limit (mg/l)	Wells above limit (%)
Ammonium nitrogen	0.5*	10.2
Chloride	250.0*	2.0
Iron	0.3**	49.0
Manganese	0.05**	44.9
Nitrate nitrogen	10.0**	8.2
Sulfate	250.0*	6.1

*ASTM (1979).

**EPA (1976).

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More than 40% of the wells sampled exceeded limitations of iron and manganese, 10% exceeded the limit for ammonia, 8% for nitrate nitrogen, and 6% for sulfate. No correlation between well depth and chemical contaminants was observed, and data are inadequate at this time to relate to particular geologic formations through which the wells pass.

Data from the study indicated that most private water wells within Clark County are contaminated with bacteria from surface water. Deeper wells (100 ft or more) are more often free of contamination. Details of construction were not known for most of the wells but deeper wells are usually cased to a greater depth which would prevent surface water seepage into the well. Any amount of contamination may constitute a health hazard, and if a source of animal wastes in the vicinity of the well results in fecal contamination, the health hazard is compounded. Anyone considering the construction of a well might be properly advised to invest in a deep well and adequate casement to prevent surface water seepage. Home owners using an existing shallow well should consider installation of a chlorination system.

Inorganic chemicals considered in this study, particularly iron and manganese, may originate from soluble components of the geologic strata through which the well is drilled. Others such as ammonia and nitrate may be of surface origin from fertilizers or from bacterial degradation of contaminating organic compounds.

From a public health viewpoint, this study suggests greater care needs to be exercised in using private wells for potable water, and more effort should be directed toward the expansion of water districts so that treated water can be delivered to every household.

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ALLELOPATHIC OBSERVATIONS IN RICE (*ORYZA SATIVA* L.) TO DUCKSALAD (*HETERANTHERA LIMOSA*)

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ABSTRACT

More than 50 weed species infest drill-seeded rice in the U.S. and one of the most prevalent aquatic weeds is ducksalad (*Heteranthera limosa*). During the summer of 1988, a field experiment was conducted to identify rice accessions from the USDA/ARS rice germplasm collection for allelopathic effects to ducksalad. In this field experiment, 5,000 accessions were evaluated for allelopathic activity. Five to seven seeds of each rice accession were planted in hills about 75cm apart in two replications. Allelopathic activity was recorded as 1) radius of the area affected by allelochemical from the base of the rice plant and 2) percentage of weed control within the affected area. Ducksalad was rated at the panicle initiation stage of rice development. Of the 5,000 accessions that were evaluated, approximately 191 were identified as having evident allelopathic activity. The accessions that demonstrated allelopathic activity originated in 26 countries (Afghanistan, Argentina, Australia, Brazil, Columbia, Dominican Republic, France, India, Iran, Iraq, Israel, Italy, Japan, Malaysia, Mexico, Pakistan, Peru, Philippines, Portugal, Republic of Korea, People Republic of China, Soviet Union, Taiwan, Thailand, Turkey, and United States).

INTRODUCTION

Rice is one of the leading food crops of the world. In 1985, the value of rice production in the U.S. was over one billion dollars (Anonymous, 1986). However, annual losses due to weeds in rice have been estimated at about 17% of the potential production or one million metric tons valued at 205 million dollars (Chandler, 1981). More than 50 weed species infest direct-seeded rice in the U.S. (Barrett and Seaman, 1980; Smith *et al.*, 1977). Ducksalad (*Heteranthera limosa* [Sw.] Willd.), is one of the most frequently reported aquatic weeds in rice (Chandler, 1981; Smith *et al.*, 1977). Effective weed control programs for rice include preventive, cultural, mechanical, chemical, and biological practices (Smith *et al.*, 1977; Smith and Moody, 1979). The most recent and perhaps least exploited control practice is the biological method. In recent years, one biological strategy of weed control, allelopathy, has received increased attention. In 1977 it was estimated that the development of new technology from allelopathics "would benefit U.S. agriculture by two percent of its total production, or about two billion dollars annually" (Anonymous, 1977).

Allelopathy, the direct or indirect harmful effect of one plant on another through the production of chemical compounds that escape into the environment occurs widely in natural plant communities (Rice, 1974; Whittaker and Feeny, 1971). Allelopathy is postulated to be one of the major mechanisms by which weeds affect crop growth (Bell and Koeppel, 1972; Gressel and Holm, 1964; Rice, 1974). In addition to the existence of allelopathy in weeds, several workers have reported that crops such as rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), tobacco (*Nicotiana tabacum* L.), sunflower (*Helianthus annuus* L.), and oats (*Avena sativa* L.) release toxic substances into the environment either through root exudation or from decaying plant material (McCalla and Haskins, 1964; Patrick and Koch, 1958; Patrick *et al.*, 1963).

Putnam and Duke (1974) postulated that "wild ancestors" of existing crops may have possessed high allelopathic activity and this character was reduced or lost as they were hybridized and selected for useful characteristics. Fay and Duke (1977) evaluated 3,000 accessions of *Avena* spp. germplasm for production of scopoletin (6-methoxy-7-hydroxy coumarin), a chemical identified as the allelopathic agent in a wide range of wild plants, and found that four accessions exuded up to three times as much as 'Garry', a standard oat cultivar.

The objective of this study was to evaluate germplasm accessions from the rice portion of the USDA/ARS Small Grains Collection for allelopathic activity on ducksalad.

MATERIALS AND METHODS

A field experiment was conducted in 1988 to identify rice accessions with allelopathic properties to ducksalad as part of the USDA/ARS rice germplasm evaluation project. Approximately 5,000 accessions were seeded in hills in a 75 X 75 cm grid. Between five and seven seeds of each accession were planted in hills, with two replications from April 28 to April 30, 1988. The seedlings emerged between April 10 and April 12, 1988. The test was irrigated on May 13 and June 1, 1988 to insure uniform seedling emergence. A permanent flood was applied on June 15, 1988. The test was conducted at the Rice Research and Extension Center, Stuttgart, Arkansas, on a Crowley silt loam (fine montmorillonitic, thermic Typic Albaqualf) naturally infested with ducksalad.

Allelopathic activity to ducksalad was recorded from July 11 through July 20, 1988, or at the panicle initiation stage, for most of the accessions. Two methods were used to record allelopathic activity: 1) the radial area (cm) from the base of the rice plant that had reduced or no weed growth due to allelochemicals, and 2) the percentage of weed control within the affected area.

Plant height (cm), days to maturity, plant type, panicle type, hull cover or pubescence, hull color, lemma color, awning, lodging, and grain type were recorded for each accession. Plant height was measured in cm from ground level to the center of the mature panicle. Maturity was determined by calculating the number of days from the date of seedling emergence to the date that 50% of the panicles had emerged. Plant type, panicle type and lodging were recorded in the field. Grain type, hull cover, hull color, lemma color, and awning were recorded in the laboratory after threshing. The accessions were characterized as having extra long (>7.50 mm), long (6.61-7.50 mm), medium (5.51-6.60 mm) or short grain (<5.50 mm).

A total of 82 kg N/ha as urea was applied to plots based on a three-way split application. Thirty-one kg N/ha as urea were applied in June 14, 1988 when the seedlings were at the fourth true leaf stage of development. The remaining 41 kg N/ha were applied in equal increments on July 7, 1988 and 12 days later on July 26, 1988. The test was irrigated on May 13 and June 1, 1988 to insure uniform seedling emergence. A permanent flood was applied on June 15, 1988.

RESULTS AND DISCUSSION

Field observations from the replicated test identified 191 accessions, or about four percent of the 5,000 accessions with apparent allelopathic

Allelopathic Observations in Rice (*Oryza sativa* L.) to Ducksalad (*Heteranthera limosa*)

activity to ducksalad. The accessions that demonstrated allelopathic activity originated in 26 countries (Afghanistan, Argentina, Australia, Brazil, Columbia, Dominican Republic, France, India, Iran, Iraq, Israel, Italy, Japan, Malaysia, Mexico, Pakistan, Peoples Republic of China, Peru, Philippines, Portugal, Republic of Korea, Soviet Union, Taiwan, Thailand, Turkey and United States). The range in plant height among the 191 accessions that demonstrated apparent allelopathic activity to ducksalad was from 60 to 190 cm and the days to maturity, from seedling emergence to 50% heading, ranged from 70 to 141 days. The accessions included long, medium and short grain types.

Table 1. Origin, weed response, and plant characteristics of 10 germplasm accessions that demonstrated allelopathic activity to ducksalad (*Heteranthera limosa*).

Germplasm Identification	Country of Origin	Radial Mean Activity (mm)	Percent Weed Control	Plant Height (cm)	Grain ¹ Type	Days to ² Maturity	Seed ³ Coat Color
India AC 1423	India	17.5 a*	85.0 a	128 c	M	115 b	Red
NSSL 10/28 STP 8	U.S.	16.5 a	85.0 a	136 bc	L	90 cd	Lt.Br
Tono Brea 439	Dom. Rep.	16.5 a	85.0 a	160 a	L	137 a	Lt.Br
Phil MGVR	Philippines	15.0 a	90.0 a	160 a	M	141 a	Lt.Br
Red Khosha Cerma	Afghanistan	15.0 a	85.0 a	143 b	L	89 cd	Lt.Br
Donduni Kunluz	Afghanistan	15.0 a	85.0 a	135 bc	L	104 bcd	Lt.Br
Tsai Yuan Chon	Taiwan	15.0 a	90.0 a	137 bc	S	109 bc	Lt.Br
Mon Z Wuan	China	15.0 a	82.5 a	127 c	M	87 d	Lt.Br
IR 644 1 63 11	Philippines	15.0 a	85.0 a	71 e	M	91 cd	Lt.Br
NATO/9209 Sel//AROS/3/NROS	U.S.	15.0 a	85.0 a	96 d	M	90 cd	Lt.Br
Control	U.S. Cultivars	0.0 b	0.0 b	---	-	---	----

1/ Grain type: S = short, M = medium and L = long.

2/ Days to maturity: From seedling emergence to 50% of the panicles emerged

3/ Seed coat color: Lt. Br. = Light Brown

* Means within columns with the same letter are not significantly different at the 0.05 level.

There were 10 accessions (Table 1) that demonstrated a radius of activity greater than 15 cm and weed control within the area of activity that was greater than 80%. Other plant characteristics such as days to maturity, plant height, grain type, and seed coat color for the 10 accessions are also listed in Table 1.

The IBPGR-IRRI Rice Advisory Committees report on "Descriptors for Rice *Oryza sativa* L." (Anonymous, 1980) defines different plant characteristics in rice. The following plant descriptions for the 10 accessions are defined in the IBPGR-IRRI report. Tono Brea 439 and NATO/9209 Sel//AROS/3/NROS had an intermediate plant type; whereas the other eight accessions possess an open plant type. All of the accessions had an intermediate panicle type. NATO/9209 Sel//AROS/3/NROS has a glabrous (smooth) hull cover; whereas the other nine accessions had short hairs throughout the hull. Donduni Kunluz had a purple hull; India AC 1423 had a black hull; Phil MGVR had a mottled, speckled, or piebald hull; and the other seven accessions had a straw (yellow) hull. All of the 10 accessions had a straw colored lemma. Tono Brea 439, Phil MGVR, Tsai Yuan Chon, and NATO/9209 Sel//AROS/3/NROS did not have awns; NSSL 10/78 STP 8, Donduni Kunluz, Mon Z Wuan, and IR 644 1 63 11 had short awns and the seed were partly awned; whereas, India AC 1423 and Red Khosha Cerma had long awns and the seed were fully awned. IR 644 1 63 11 and NATO/9209 Sel//AROS/3/NROS had moderately strong culm strength; Mon Z Wuan had a culm strength between moderate and intermediate; Tono Brea 439 had a culm strength between weak and very weak; and the remaining six accessions had a very weak culm.

CONCLUSIONS

Approximately four percent of the 5,000 rice accessions that were evaluated for allelopathy to ducksalad demonstrated some allelopathic activity. A four percent frequency rate suggests that about 600 accessions in the rice collection may suppress ducksalad. The 191 accessions

that demonstrated allelopathic activity also exhibited genetic diversity for plant characteristics such as plant height, maturity, grain type, plant type, hull cover, hull color, and culm strength. Tests to isolate and identify the allelochemicals that are responsible for the allelopathic activity are presently being conducted.

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BLACKLAND PRAIRIES OF SOUTHWESTERN ARKANSAS

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ABSTRACT

The Blackland Prairie community type has been described in Texas; related communities exist in Alabama and Mississippi. The Arkansas variant of the community has not been described in detail. Since the Arkansas Natural Heritage Commission began a systematic inventory of the community in 1985, more than 36 remnants have been identified that retain substantial natural character. However, all show some degree of disturbance. Based on aerial photo interpretation, aerial inspection, and ground study, an initial description of the community is presented, including original distribution, soil, vegetation and relationship to similar communities of Texas, Mississippi, and Alabama.

INTRODUCTION

The Blackland Prairie Community in Texas has been described as a belt of prairie varying in width from 70km in the north to 12km in the south, extending in a northeast to southwest direction through the eastern part of that state (Collins *et al.*, 1975; Dyksterhuis, 1946; Hill, 1901; Kuchler, 1964). The term blackland refers to the deep mantle of black soil high in organic matter which occurs over a substrate of Cretaceous chalk or marl. It is generally dominated by *Andropogon gerardii*, *A. scoparius*, *Sorghastrum nutans*, and *Tripsacum dactyloides*. Virtually all of the Texas blackland prairie has been converted to cropland and pasture.

Cretaceous deposits similar to those of Texas occur along much of the northern edge of the Gulf Coastal Plain from Arkansas to Georgia. The best-known area is in Mississippi and Alabama, where "blacklands" or a "Black Belt" have been delineated (Kuchler, 1964; Shantz and Zon, 1924). These blacklands are characterized and mapped by the presence of alkaline soils; however, the vegetation of the Black Belt has been a topic of controversy. Shantz and Zon (1924) showed the area on their map as "tallgrass prairie". Rostlund (1957) disputed this, contending that "a natural prairie belt" in the Mississippi/Alabama blackland region was a "myth". Jones and Patton (1966), in a response to Rostlund, presented evidence that within the Black Belt, grassland was the characteristic vegetation on calcareous clay soil.

The vegetation of the Arkansas blacklands has never been comprehensively described. The vegetation of the Coastal Plain of southwestern Arkansas as a whole is characteristically Loblolly Pine - Hardwood Forest on the uplands and Bottomland Hardwood Forest in the floodplains of rivers. However, southwestern Arkansas was settled very early and has for over 150 years been subjected to extensive and intensive land uses that have so modified the landscape that it is difficult to find areas exhibiting a high degree of naturalness. There have also been few ecological studies within the area. This makes understanding the original character of the community difficult.

Because of the dearth of ecological information on southwestern Arkansas, the Arkansas Natural Heritage Commission determined it would increase inventory efforts within the region. The blackland prairie community was an early priority.

The purposes of this paper are to describe remnant grasslands of the Arkansas blackland region and to examine the relationship of this community to similar communities in Arkansas and elsewhere.

THE STUDY AREA

The Arkansas blackland region, that is, the region containing calcareous clay soils, lies primarily within the portion of the West Gulf Coastal Plain underlain by Cretaceous deposits (Foti, 1974). The blacklands are not one contiguous area, but instead consist of several discrete areas that are best shown on the General Soil Map of Arkansas (USDA, 1982). As shown in Fig. 1, blacklands occur within seven

counties of southwestern Arkansas on both the more usual Cretaceous substrate and also on a narrow strip of land underlain by the Midway Group of Tertiary age (Haley *et al.*, 1976).

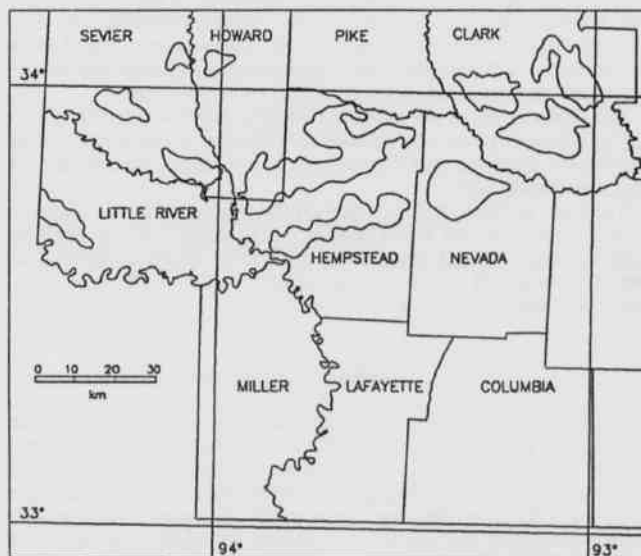


Figure 1. Blackland soil areas (USDA Soil Conservation Service, 1982).

DESCRIPTIONS OF REGIONAL VEGETATION

Most of the literature on the vegetation of the region is in the form of vegetation maps. The vegetation has been mapped by Kuchler (1964), who showed the area as Oak-Hickory-Pine forest in the uplands with Floodplain Forest in the streambottoms and a small area of upland Oak-Hickory Forest.

Many of the useful early scientific and historical descriptions were done by geologists. The earliest was provided by G.W. Featherstonhaugh (1835, 1844), who traveled the Old Military Road from Missouri to the Red River in 1834.

Featherstonhaugh (1844) described the physiognomy of the prairies near present-day Blevins in Hempstead County:

"...a chain of prairies running westward and parallel with Red River for a great distance, until the whole country becomes one vast prairie, devoid of trees, except those which grow immediately upon the watercourses. Some

of these prairies were mere bald spots of half an acre and more, whilst others contained several hundred acres, in every instance surrounded with a belt of timber and plants peculiar to the country...[the soil was]...black as charred wood and had a much more inky color than the rich vegetable mold usually found in low grounds...this portion of the country which had the quasi prairie character, was bottomed upon immense beds of rotten limestone, probably derived from the...remains of the mollusca I have named, since entire shells in a soft state are found embedded in the soft limestone."

Later, Owen (1860) and Dane (1929) provided increasingly detailed descriptions of the regional geology, while also touching on such relevant matters as relationship of vegetation and land-use to geology. Maps of value were produced by Langtree (1866), who compiled information from the land-survey plat sheets which included some prairies; Sargent (1884), who showed major forest cover types of Arkansas as well as the location of major prairie areas; and Branner and Hill (1888), whose geological map also showed several prairies. Sargent's map is the best known and shows two prairies along the eastern edge of the study area, and two more in Miller County just outside the blackland region.

Harper (1914) was taken to what was apparently a blackland prairie new Arkadelphia and said, "In crossing it rapidly I noticed essentially the same kind of soil and topography and treeless horizons and some of the same weeds and crops that characterize the geologically similar black belt or prairie region of Alabama and Mississippi."

The existence of the blackland prairie community was noted by Foti (1974) who recognized the Cretaceous region as a distinct section of the Coastal Plain Natural Division partly because of the presence of that community type.

The most detailed existing study of the vegetation of the region is an unpublished report in the files of the Arkansas Natural Heritage Commission, "The Arkansas Blackland Region" (Roberts, 1979). That report summarized the geology, geography, soils, origin, vegetation, flora, and the three sites known then.

METHODS

The historical and scientific literature sources, along with the known existing sites, were used to create an initial description of the blackland prairie community and other vegetation of the region. Using this initial description, aerial photography was examined to locate what were apparently the least disturbed areas within the region. These photos were examined in the county offices of the Soil Conservation Service (SCS) and Agricultural Stabilization and Conservation Service (ASCS), and in the offices of the Arkansas Highway and Transportation Department in Little Rock.

The criteria for evaluating naturalness of grassland areas on the photographs included absence of cattle trails, fences, or other obvious effects of grazing, a relatively uneven texture that would preclude the presence of improved pasture, and natural boundaries if within forest. Also, older aerial photographs, some as early as 1937, were consulted to evaluate past condition. The outlined areas were considered potential natural areas (PNA's), and made up the list of areas to be investigated in the field. The study area was examined from a light aircraft.

PNA's were inspected on the ground between 1985 and 1988. If an area was found to be highly disturbed, this fact was noted and the area was given no further attention. If the area appeared little disturbed, its dominant vegetation was qualitatively described, then entered into the Natural Heritage Commission database, and was used in further studies.

As field investigation provided increasingly-detailed knowledge of the distribution and character of blackland prairies in the study area, the portions that contained prairies, either high-quality or degraded, were identified and delineated. The presettlement character of these areas was examined by consulting microfilmed field notes of the Public Land Survey (PLS) of the General Land Office (GLO).

Ten of the less-disturbed prairies were selected for repeated visitation during 1986. Each was visited in April, June, and August; species lists compiled, and uncommon plants documented. In August the aerial cover of dominant species within six to 12 quadrates, 0.25m X 1.0m was estimated on each of these prairies, with data from all prairies pooled. This method was chosen to rapidly obtain data on community composition, although the sample was not adequate to compare individual prairies.

RESULTS AND DISCUSSION

POTENTIAL NATURAL AREAS

Within the study area, 295 potential natural areas (PNA's) were identified for further examination. Of these, 118 were determined to be blackland prairie remnants. An additional 49 were rocky glades or other non-blackland and openings in forest. Approximately 90% of the PNA's have been examined; the remainder are posted and permission to enter has not yet been granted. Of the blackland prairie PNA's, ground and aerial inspection showed that at least 36 retained substantial natural values; the others have been significantly altered.

DISTRIBUTION AND GEOLOGY

The distribution of blackland prairie remnants located by the inventory is shown in Fig. 2. The observed distribution of blackland prairie remnants does not coincide with that on any previous vegetation map. Most of the blackland prairie relicts lie within the blackland areas delineated on the SCS map (Fig. 1). However, a few lie outside the SCS blackland limits and some of the blackland areas shown on the SCS map contain no prairie relicts. Furthermore, relicts are not distributed uniformly over the SCS blackland areas, but rather in specific portions.

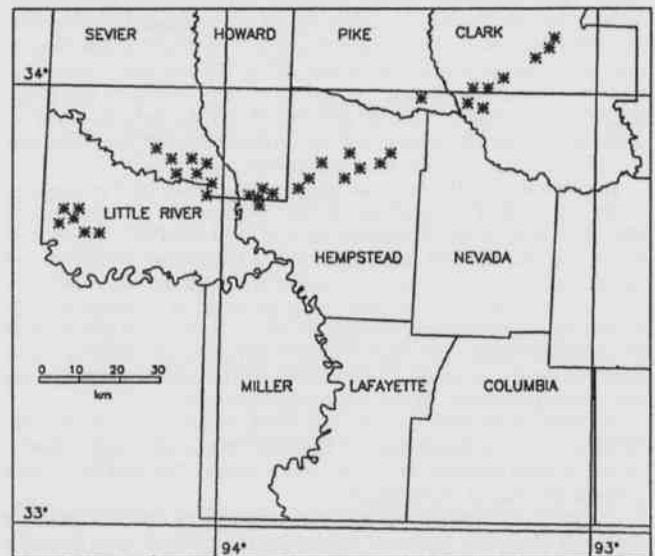


Figure 2. Locations of blackland prairie relicts.

The distribution of blackland prairies is most clearly understood in relation to geological substrates and topographic features. Numerous prairies have been located on these geologic formations: Saratoga Chalk, Marlbrook Marl, Annona Chalk and the Ozan Formation. Several have been found on Brownstown Marl. Few have been found on other formations. Distribution of the most important formations is shown in Fig. 3. Prairies have not been found on Arkadelphia Marl, a substrate

which forms deep black soils that seem to be the "classical" blackland soils. Nor have relicts been found on the Tertiary deposits where prairies were described at the time of settlement.

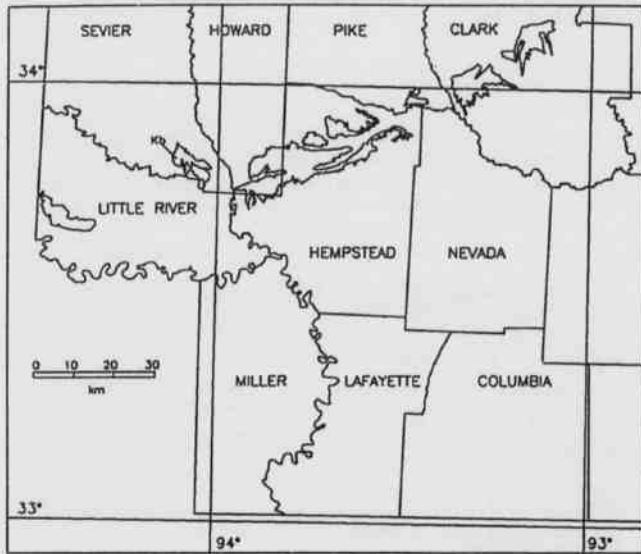


Figure 3. Distribution of geologic formations on which blackland prairie remnants occur. Outlined areas include Saratoga Chalk, Marlbrook Marl, Annona Chalk, and Ozan Formation, with a small area of Brownstone Marl (Kb) on which several prairies occur.

Prairies do not occur over the full exposure of the appropriate formations. They occur on the steep faces of ridges known as cuestas that are characteristic features of the study area. Cuestas are asymmetrical ridges with a steep slope and a shallow slope. On these steep slopes the underlying chalk or marl outcrops are mantled with only a thin layer of soil (Fig. 4). In the GLO land survey notes, it appears that the prairies originally occurred primarily on the steep slopes, but extended for a distance into the gentle slopes at the foot of a ridge. However, they were usually replaced by forest within a short distance of the foot of the slope. In all the existing prairie relicts, the base of the slope and the adjacent valley have been plowed.

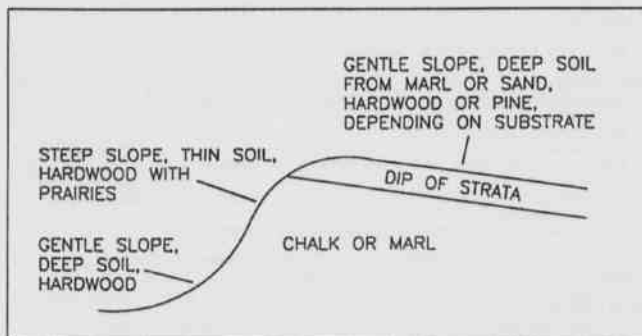


Figure 4. Relationship of forest and prairie to slope, substrate, and soil depth on cuestas.

Based on these geologic and topographic site factors a map of the original distribution of blackland prairies is presented in Fig. 5. Forests covered much of even these limited areas. However, those portions of

the Brownstone Marl and the Ozan Formation that apparently supported no prairies have been eliminated from this map. The two prairies on the eastern edge of the study area that frequently have been shown on other maps are not delineated in Fig. 5 because no relicts have been located and there are major differences in site between those areas and the prairies described here.

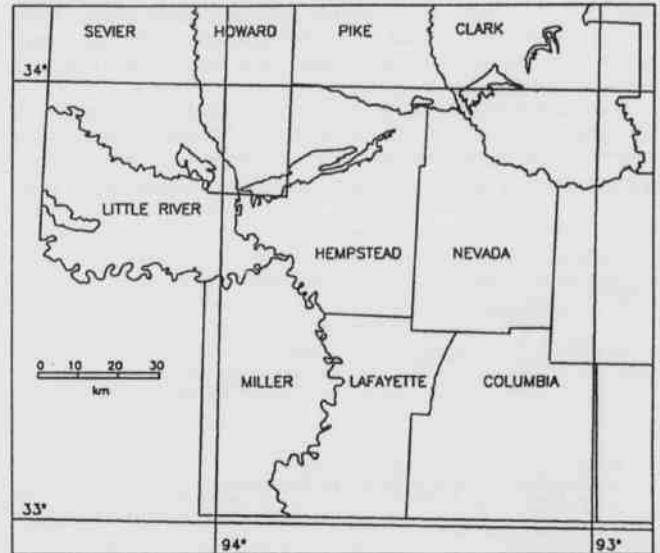


Figure 5. Original distribution of blackland prairies, based on geology, topography, and distribution of relicts.

PHYSIOGNOMY

As noted by Featherstonhaugh (1835) and verified by examinations of the GLO field notes and field observation of relicts, these prairies were typically small, ranging from less than an acre to a few hundred acres in size, separated by fringes of trees, shrubs and vines along watercourses.

In the GLO field notes, there were usually trees to mark the section corners, so the areas were not treeless. However, the distances to witness trees were typically rather long (often 15-20m), indicating the landscape was open. Prairie notations often took the form "2 prairies" or "4 small prairies". However, just north of present-day Columbus, several mile notes indicated "mostly prairie" and at two corners no trees occurred. This may therefore have been a prairie of several hundred acres.

SOIL

Blackland prairie remnants occur primarily on Sumter and Demopolis soil series. Some occur on Oktibbeha soils, but in these areas the soil appears to be one of the other two soils. Therefore, it is assumed that these are inclusions of the other soils within Oktibbeha. The descriptions of these two soils are summarized from USDA (1979).

Sumter clay soil occurs on slopes of three to 12 percent. It is classified as fine-silty, carbonatic, thermic Rendollic Eutrocrepts. It is moderately deep, well-drained, and gently to moderately sloping on hilltops and hillsides. Erosion has removed most of the topsoil. Typically, the surface layer is olive clay about 10 cm thick. The upper part of the subsoil is olive clay to a depth of about 45 cm, with pale olive mottled clay to 67 cm and light olive gray soft chalk to a depth of about a meter. Hard rippable chalk lies below. The soil is moderate in natural fertility and in organic matter. It is moderately alkaline throughout, and erosion hazard is severe.

GYMNOSPERMS

CUPRESSACEAE
Juniperus virginiana L. abundant invader

MONOCOTS

AMARYLLIDACEAE
Agave virginica L. uncommon
Hypoxis hirsuta L. Coville common

CYPERACEAE
Carex cherokeensis Schwein. Spring dominant

GRAMINEAE
Andropogon gerardii Vitman dominant moist areas
A. glomeratus (Walt.) B.S.P. uncommon
A. saccharoides Swartz common disturbed areas
A. scoparius Michx. dominant
A. virginicus L. uncommon disturbed areas
Aristida longispica Poir. common thin soil
Routeloua curtinendula (Michx.) Torr. in Marcy uncommon thin soil
Erianthus sp. uncommon
Panicum virgatum L. uncommon
Scleria sp. common
Sorghastrum nutans (L.) Nash common
Sporobolus asper (Michx.) Kunth uncommon
Tridens flavus (L.) Hitchc. common disturbed areas
Tripsacum dactyloides (L.) L. dominant deep moist soil

IRIDACEAE
Nemastylis geminiflora Nutt. common
Sisyrinchium sp. common

LILIACEAE
Nothoscordum bivalve (L.) Britt. early Spring dominant
Smilax bona-nox L. uncommon
Zigadenus nuttallii A. Gray

ORCHIDACEAE
Spiranthes lacera (Raf.) Raf.

CONDITION AND MANAGEMENT

Almost all relicts that have been located to date show evidence of grazing, either at present or on older photographs. Many are abandoned pastures. The only exceptions are probably some small prairies within forest. Nevertheless, the lack of weedy invaders on some of the formerly grazed prairies, and the presence of species that are rare statewide, provide evidence that grazing does not necessarily destroy the prairie. In many cases, however, grazing has been excessive and the prairie has probably been permanently damaged. Even on the prairies of higher quality, questions remain as to long-term impacts of past grazing.

Evidence of disturbance includes exotic species such as *Melilotus* sp. and woody species such as *Ilex decidua* and *Juniperus virginiana*. Of great concern is the presence of eroded spots on all the prairies. These spots may be smaller than a square meter to hundreds of square meters, even in the higher-quality prairies. The underlying chalk and marl, and hence the sites, are very erosion-prone and occur on sometimes-steep slopes. Overgrazing obviously can aggravate erosion problems. However, it is uncertain whether grazing has been the cause of the erosion problems of these sites, noted as early as Owen, 1860. The GLO notes do not contain specific references to erosion. There is no past history of conscientious management of this community, e.g., no prairies have been found that are being managed using fire or other standard prairie management techniques. Few native prairies have been found that are mowed for hay.

species that tolerate disturbances, as well as those that are found only on undisturbed sites. Notes in the list indicate aspect dominance, abundance, statewide rarity, observed response to disturbance, etc.

Species dominance of the blackland prairie community is presented in Table 2, and is based on estimated aerial cover within 113 plots on the 10 remnants of relatively high quality. As can be seen in this table, *Andropogon scoparius* is the overwhelming dominant on the prairies. It is more dominant on these prairies than on any other prairies in Arkansas. *Panicum virgatum*, a co-dominant on most of the other prairies in Arkansas, is uncommon on the blackland sites that remain. The status of these species indicate the extreme dryness of these sites.

COMPARISON WITH THE TEXAS AND MISSISSIPPI BLACKLANDS

Because of their geographical proximity and alkaline soils, the Arkansas and Texas blackland prairies share many species. Species such as *Astragalus crassicaerpus*, *Hypoxis hirsuta*, *Neptunia lutea*, *Dalea purpurea*, *Salvia azurea*, along with the dominant grasses, are typical of both areas. However, the prairies are quite different in overall character. The Texas prairies are (or were) expanses of prairie over deep fertile soil. The relatively high rainfall (75-115 cm/yr) and the high water-retention capability of the soil, give the prairie a "lowland grassland appearance even on upland, well-drained situations" (Collins *et al.*, 1975, p. 86). The Arkansas blackland receives as much precipitation as the Texas maximum, but the soils are thinner, well drained, and less fertile. These prairies are notably drier and smaller than other prairies in Arkansas and certainly do not have a lowland grassland appearance. It is probable that communities similar to those described here occur on comparable sites in Texas. Further study should be devoted to finding, describing, and relating these communities.

There may be more similarity between the blacklands of Arkansas and those of Mississippi and Alabama, even though the areas are geographically separated. The prairies there are small, located on thin soils over chalk on cuestas, and they occur on the same soil series as those in Arkansas. The descriptions of physiognomy and distribution of the Black Belt prairies cited before, are very similar to the patterns encountered in Arkansas. Since there are no floristic or ecological studies of those eastern blackland prairies, it is not possible at this time to determine how similar the plant communities are.

Table 2. Percent cover of major species on 10 prairie relicts, August, 1986. Data obtained from visual estimates of areal cover within 113 plots, 0.25m x 1.0m.

SPECIES	PERCENT COVER
<i>Andropogon scoparius</i>	50.2
<i>Sorghastrum nutans</i>	4.0
<i>Dalea</i> sp.	3.6
<i>Neptunia lutea</i>	1.9
<i>Desmanthus illoensis</i>	1.4
<i>Ratibida columnaris</i>	1.4
<i>Panicum virgatum</i>	1.2
<i>Hedyotis</i> sp.	1.1
<i>Rudbeckia hirta</i>	1.0
Miscellaneous species	11.7
Bare Ground	21.9

Miscellaneous species are those having a cover value less than 1 percent. Included are *Aristida* sp., *Juniperus virginiana*, *Rosa* sp., *Carex* sp., *Cacalia plantaginea*, and others.

A substantially different community, dominated by *Tripsacum dactyloides*, existed on the gentler, moister slopes at the bases of the ridges. No extensive example of this community has been found. A few small areas exist at the transition between the steep hill slopes and the gentler valley floors. In this transition zone and in mesic pockets higher on the slopes, *Sorghastrum nutans* and/or *Andropogon gerardii* also become dominant.

ACKNOWLEDGMENTS

William M. Shepherd and Jerry Roberts assisted with assembling the species lists. Max D. Hutchison, working under contract with the ANHC, assisted with photointerpretation.

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INTEGRATED CIRCUITS INTERCONNECT METALLIZATION FOR THE SUBMICRON AGE

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ABSTRACT

The interconnect metallization being used by the semiconductor industry has been aluminum or aluminum silicon. Aluminum silicon is being replaced by aluminum copper and aluminum copper silicon, due to its superior resistance to electromigration and hillock growth. This paper discusses the implementation of aluminum copper/silicon alloys in semiconductor processing, along with a review of the problems and advantages of the same.

INTRODUCTION

Aluminum has been used for a long time in the IC processing industry as an interconnect metal due to its cost and electrical properties. Tighter geometries and shrinking device sizes have led to increased current densities and multilevel capability requirement for interconnects. Keeping in view the above points, it is essential that factors such as: 1) electromigration, hillock growth (Herman *et al.*, 1972; Rey *et al.*, 1984) and 2) corrosion be considered for submicron geometries.

ELECTROMIGRATION, HILLOCK GROWTH

A number of studies (Weaver and Parkinson, 1971; Vandenberg and Hamm, 1981; Shih and Ficalora, 1981) has been done related to the dynamics of gold-aluminum intermetallic formation. This intermetallic formation subsequently leads to the phenomenon known as purple plague and Kirkendall voiding (Clifford *et al.*, 1974; Philofsky, 1970) resulting in weaker bonds causing electrical and mechanical failures. The mechanism of Al-Au interdiffusion is presented (Baglin, 1978).

Copper has been added to aluminum for a long time to reduce electromigration and the occurrence of failure due to stress induced voiding (Ames *et al.*, 1970). The void formation process is reduced due to the formation of the theta phase-Al₂Cu (Berenbaum and Rosenberg, 1971). Hence, the failure mechanism has been summarized as being mainly due to grain boundary electromigration which consists of nucleation and growth of voids. The table from d'Heurle and Ho (1978) shows how the addition of copper can dramatically increase the median time to failure. The addition of copper is known to inhibit grain boundary movement by a pinning mechanism (Herman *et al.*, 1972) where second phase particles in Al retard creep of the parent phase and therefore inhibit growth of annealing hillocks (Philofsky *et al.*, 1971).

IC PROCESSING AND CORROSION MECHANICS

The above advantages of the addition of copper are offset by certain disadvantages, the most important of which is corrosion. Al-Cu films especially have been found to be more susceptible to intergranular and pitting corrosion than pure Al (Totta, 1976). The prime source of corrosion was found to be chlorinated hydrocarbons which had decomposed in wafer cleaning baths. From corrosion studies on Al-Cu bonding pads, it was understood that the formation of Al₂Cu particles in Al-Cu was believed to be formed at the grain boundaries and this formation resulted in the film being depleted of Cu in the vicinity of the particle. This formed a shorted galvanic cell where the electrode potentials are reported to be -0.85 volts for Al and -0.73 volts Al₂Cu. The Al₂Cu acted as a cathode in contact with the aluminum which acted as an anode. In the presence of a suitable electrolyte, corrosion can occur. The corrosion of aluminum proceeds around the Al₂Cu particle until the aluminum is deposited and the Al₂Cu particle is electrically isolated (pit). The corrosion rate is dependent on the size and homogeneity of distribution of the particle. The corrosion product is believed to

be Al(OH)₃. In the presence of moisture, this forms a thin fluid which spreads over the metal surface around the pit. As the fluid dries up it forms a hard film which causes poor adhesion of the gold to the Al-Cu contact pad. It is noted that the distribution of copper is of critical importance and, thus, the homogeneity of Al₂Cu is a factor to be considered in the film processing (Thomas and Berg, 1985).

Corrosion also occurs during the etching of Al-Cu/AL-Cu-Si films. This is due to the fact that Cu forms CuCl with chlorine which in itself is relatively non-volatile below 175°C. Thus, Cu residues remain after these alloy films are dry etched. This makes Al-Cu more difficult to etch in chlorine plasmas. As the Cu concentration goes up, etching becomes a bigger problem (Wolf and Tauber, 1986) along with the problems associated with nonhomogeneity which leads to the formation of an electrochemical potential (Pramonik and Saxena, 1983).

Corrosion modeling has been done wherein corrosion activity is determined from the resistance rise of the corroded Al-Alloy region. It was shown that corrosion activity decreases as a function of time (Fan and McPherson, 1988). To counter this problem, the substrate temperature was increased to promote CuCl desorption and ionic bombardment of the surface is increased so that significant sputtering of residue occurred. Corrosion due to the galvanic cell effect takes over in the presence of moisture, which reacts with the chlorine residues forming HCl, which in turn plays the role of an electrolyte. Wolf and Tauber (1986) showed weight gain versus exposure time. It was clearly seen that with no Cu there was a negligible weight gain, where weight gain indicates the extent of corrosion. With Cu concentration greater than 1%, there was a significant weight gain change indicating increased corrosion activity. Using CF₄ right after the Cl₂ plasma etch is believed to replace the chemisorbed chlorine with fluorine, thus passivating the aluminum by the formation of nonhygroscopic AlF₃. Using CHF₃ on the other hand, is thought to deposit a polymer film over the Al, thereby sealing the surface (Wolf and Tauber, 1986). The impact of a post etch *in situ* with CHF₃ plasma passivation has been discussed (Fan and McPherson, 1988). It also is noted that thermal oxidation at 300°-350°C and 1 atm oxygen pressure for greater than 30-45 minutes was found to be effective in improving the corrosion resistance of the etched sample (Lee and Eldridge, 1981). Ritchie and Andrews (1981) state that the CF₄/O₂ plasma which may be used for passivation could cause corrosion. It was observed that two possible phenomena could be responsible. One was that Al is corroded spontaneously due to cathodic corrosion in the presence of electrolytic fluoride contamination (Comizolli, 1980) and secondly, it was reported that modification of the native aluminum oxide and change in its electrochemical behavior is possible due to the introduction of fluoride ions (Valand and Nilsson, 1977).

Hence, it was suggested that the plasma exposure time be increased so that chemisorption of oxygen will take place on the pads which would in turn block the reactive sites, thus retarding corrosion (Flamm, 1979).

The above discussion on corrosion has been deemed important since it has a direct relationship to bonding and reliability. It is critical that great care be taken during processing of integrated circuits. Delays in transferring lots from one process to another, especially after etching, should be avoided.

Precipitation influence on electromigration in Al-Cu thin films has been studied (Walker and Goldsmith, 1973) and it was concluded that the total homogeneous distribution of copper atoms in the films controlled the effective lifetime of the film. d'Heurle and Ho (1978) observed that failures of Al-Cu or Al-Cu-Si occurred in areas of the conductor that are depleted of Cu and in areas in the vicinity of large Al₂Cu precipitates. Formation of second phase particles have been studied in Al-Cu films (Mader and Herd, 1972), and it was observed that the film surfaces provided preferential nucleation sites for the equilibrium precipitate of theta-Al₂Cu.

GOLD BONDING TO Al/Al-ALLOY

A number of papers have discussed bonding methods for improving bonding integrity of Au to Al (Hund and Plunkett, 1986). Ball-bond integrity tests have been discussed (Harman, 1983). A bond failure mechanism for Al-Si was studied with the conclusion that silicon nodules formed resulted in points of high stress during bonding. A pad structure with Ti:W below Al-1%Si acted as a buffer to stress, which helped reduce cratering created by silicon nodules in the pad structure (Ching and Schroen, 1988). Removal of the deposited PSG layer from the pad structure decreased the susceptibility of the pad to cracking induced by the silicon nodules (Koch *et al.*, 1986). Also available are papers on effects of molding materials on Au-Al bonding (Khan *et al.*, 1988; Richie and Andrews, 1981).

It is the authors' understanding, after discussions and review of literature, that if fluorocarbons are used in the processing steps, it is desirable to expose with oxygen long enough for the oxygen to react with the Al-Cu contact pad and form a passivating layer, thus, preventing fluoride ions from corroding the metal.

METHODOLOGY FOR STUDIES OF BOND INTEGRITY

Bonding window studies of thermosonic wire bonding have been made with reference to Al; Al-Cu; Al-Si; and Al-Cu-Si systems. This method has been suggested as a powerful tool in conjunction with wire pull and ball shear testing for bonding studies (Berg and Mitchell, 1985). The criterion used was by visually inspecting sticking vs. nonsticking bonds. It was ascertained by using this criterion that a large fraction of the very weak bonds were identified. The thickness of Al; Al-1%Si; Al-1.5%Cu; Al-1%Si-1.5%Cu in this study was 1.2 micrometers. Six ultrasonic powers and three bonding times were evaluated. The ultrasonic powers and times were chosen to give many nonsticking bonds at low power-time combinations and many sticking bonds at the highest settings. The bonding windows are shown in Figure 5 of Berg and Mitchell (1985). It is clear that the bonding windows are sensitive to the change in metallization. Also presented in Figure 12 of Berg and Mitchell (1985), are bonding parameters data which showed percent nonsticking bonds vs. first bond force for Al-1.5%Cu metal. The best bond force is found to be 35g.

Other methods of improving gold ball bond reliability and testing are presented by Hund and Plunkett (1986).

CONCLUSION

The advantages involved in the use of Al/Cu/Si alloys offset its disadvantages. Copper concentration and also silicon concentration should be closely controlled with copper concentration kept below 4%. High silicon concentration can cause precipitation of silicon nodules contributing towards bond integrity degradation.

In conclusion, as long as the composition is well balanced, which depends on the need, Al-Cu/Si will be the interconnect metallization of the future submicron era.

ACKNOWLEDGMENTS

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The heart of the potentiostat is the two operational amplifiers and transistor network as shown in the output stage of Fig. 1. Amplifier 1 is a FET input amplifier which buffers the reference electrode voltage by minimizing any reference electrode current. Amplifier 2 is used to sum the constant voltage set by the programming voltage section and the voltage sensed by the reference electrode. As shown, the electrochemical cell completes the feedback path and any voltage changes at the reference electrode are coupled to the inverting input of amplifier 2 such that its output varies the electrochemical cell current via the two transistor (A and B) push-pull current booster. Thus, the controlled current output maintains a constant potential between the working electrode and the reference electrode. Transistors C and D were added to limit the cell current. If the user needs more current, a Darlington arrangement could be incorporated. A high voltage amplifier (amplifier 2) was used to increase the cell potential range.

In order to select a reference voltage, a stable source is required. Amplifiers 5 and 6 in the programming voltage section use a constant current configuration and a Zener diode's voltage stability to provide a stable input voltage for amplifier 5 and subsequently amplifier 2. A 10 turn potentiometer is used to vary the programming voltage. Resistors R1 and R2 can be adjusted to change the reference voltage range.

The second part of the circuit is the current monitor. Operational amplifier 3 and the two transistors attached to the output (pin 6), and RL (10 Ω , 1 watt) form a transresistance amplifier, better known as a current-to-voltage converter. The two transistors provide extra output current for the operational amplifier which is limited to a maximum of 12 ma. The input (pin 2) of operational amplifier 3 is forced to remain at ground potential by virtue of negative feedback. Any current flowing into the circuit is balanced by current leaving the input through RL; therefore, the voltage of the output of the transresistance amplifier is proportional to the input current which is the total current through the electrochemical cell. The resultant output voltage for amplifier 3 follows the relationship: $V_{out} = (I_{cell}) * RL$. The output of the transresistance amplifier is inverted and scaled by the inverting amplifier 4.

A double pole-double throw switch was added in the current monitor section. The double pole-double throw switch selects one of two circuit modes; current calibration or potentiostat operation. In the current calibration mode, a known current passes through the 300 ohm precision resistor to the current monitor circuit. A 5 kilo ohm 10 turn potentiometer is adjusted to give the correct voltage output to the current display.

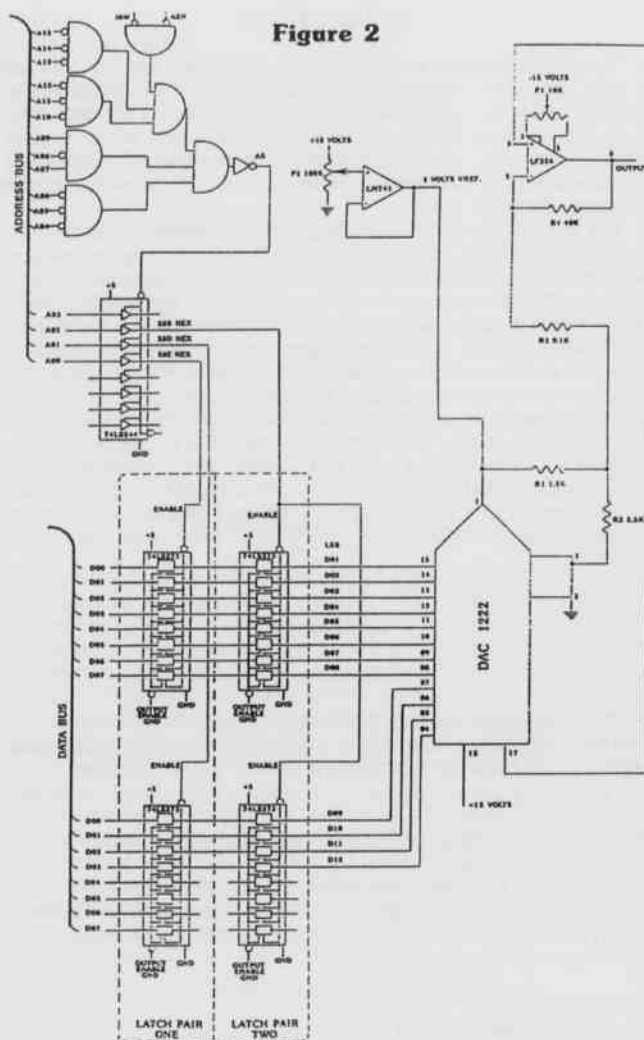
Two digital panel meters are required to monitor the reference voltage and the electrochemical cell current. The reference voltage was monitored at the output of the buffer amplifier 1 and the total cell current was monitored at the output of amplifier 4.

COMPUTER CONTROL FOR THE POTENTIOSTAT

A computer controlled digital-to-analog converter was added to the potentiostat to provide greater flexibility. A 12-bit National Semiconductor binary multiplying digital to analog converter DAC1222 (DAC) was chosen to provide the conversions. The 12-bit DAC allowed a resolution of 0.00244 volts per step over the chosen +5 to -5 voltage range. An interface was constructed between the computer and the DAC, as shown in Fig. 2, which consisted of an address decode and various data latches to support the DAC.

The interface between the computer and the DAC was designed so that it could be used with other IBM compatible computers. An IBM XT-compatible was used in this laboratory. The DAC interface required ± 15 volts at approximately 20 ma and +5 volts at approximately 130 ma. The ± 15 volts were provided by the potentiostat power supply section and the +5 volts were provided by the computer.

Output voltage data were taken directly off the data bus and loaded into two octal data latches (74LS373), one at a time. After both latches are loaded, a second pair of latches are pulsed and the voltage data are transferred to the DAC for conversion. Care was taken to ensure that the latches were in close proximity to the data bus. Small distances (less than one foot) will ensure clean, valid output voltage



data to the latches. The first pair of latches was used to change the 8-bit data bus of the computer to 12-bits required by the DAC. A second set of latches was used in this design since the DAC did not have an enable line for conversion initiation. Thus, the voltage data (all 12-bits) could be transferred to the DAC simultaneously permitting the DAC to convert the correct voltage data instead of two pieces of data. Two allow the 12-bit DAC bipolar voltage swings, a LF356 JFET input OP-AMP was used (Fig. 2). Equations for the resistors are (National Semiconductor Corp., 1982):

$$\begin{aligned} AV &= \text{Peak Voltage out} / \text{Voltage reference} \\ R4 &= (2 AV - 1) R \\ R1 &= R2 (AV - 1) / AV \\ R3 &= R1 :: R2, \end{aligned}$$

where $R = 10,000$ ohms, $R2 = 2500$ ohms, and the voltage reference = 2 volts.

Potentiometers were used for the resistance values to allow final adjustment of the DAC to eliminate any offsets caused by varying component tolerances. Resistor R2 was used to zero tolerance differences found in the linear op-amp while resistor R4 was used to adjust the \pm peak voltages in the design. Resistor R3 was found to be the most critical in allowed tolerances.

A basic program to control the potentiostat is included in Appendix 1.

A Versatile Potentiostat With Optional Computer Control

APPLICATIONS

This design provides a versatile instrument which may be used for a wide range of electrochemical applications. It is the intent of the authors to use the potentiostat as a teaching tool in a graduate level course in electrochemistry. In this role the instrument will be used to plate copper from solution, demonstrate electrolysis of solvents and ion migration, and generally familiarize students with the various aspects of electrochemical systems.

The current application of concern to the authors is the anodic oxidation of PCB's. It has been shown that PCB's may be oxidized at a ruthenium dioxide anode (Laule *et al.*, 1986). This initial success with 4,4' PCB has prompted work to optimize the reaction conditions. The reaction is carried out on a 5 ppm solution of PCB in a 50/50 acetic acid/water solution. An oxidizing agent such as potassium persulfate is added and the solution subjected to a potential of 2.00 volts versus a silver/silver chloride reference electrode for 48 hours.

The present work is an attempt to optimize the yield of the reaction at room temperature. The variables of concern are: time of reaction, applied potential, oxidant used, and exposure to UV radiation. Initial results indicate that the exposure of the reaction cell to UV light greatly enhances the yield of the reaction. Not enough data have been received to make any predictions as to the effect of other conditions on the reaction.

APPENDIX 1

```

'
' Computer Control for the Potentiostat 10-29-88
'THIS PROGRAM WRITTEN FOR THE QUICKBASIC COM-
'PILER. IT CAN BE MODIFIED TO FIT YOUR NEEDS.

```

- ```

' 1) Voltage entered (+5 to -5 volts) and converted to positive
' voltages only.
' 2) Voltage is converted to its decimal equivalent voltage 0.0024414
' volts per step
' 3) Voltage is converted to binary form " 1111111111 "
' 4) The high four and low eight are converted back to decimal
' 5) Voltage data loaded into the latches and transferred to DAC

```

```
start:
```

```

input " enter voltage ",volt
volt = volt + 5.00 :changes the voltage to positive only
volt = cint (volt/.0024414)
print "decimal convert volt ",volt

```

```

'
' converts voltage to binary
'
'

```

```

x = 2048:bin$ = " "

```

```
convert:
```

```

volt1 = (volt - x)
if volt1 >= 0 then bin $ = bin$ + "1"
if volt1 < 0 then bin $ = bin$ + "0"
if volt1 >= 0 then volt = volt1
x = x / 2
if x < 1 goto continue
goto convert

```

```

'-----
continue:

```

```

'-----
'
' after conversion voltage is in form " 1111111111 "
' low$ will take the right 8 bits for loading into latches
' high$ will take the left four for loading into latches
'-----

```

```

low$ = right$(bin$,8)
high$ = left$(bin$,4)

```

```

'-----
' convert low binary to decimal.
' conversion fo the lower 8 bits back to decimal for output to
' the latches
'-----

```

```

ll = 0 : bits = 1
for x = 1 to 8
shift$ = right$(low$,x): shift$ = left$(shift$,1)
if shift$ = "1" then ll = ll + bits
bits = bits * 2
next x
print "lowload ";ll;

```

```

'-----
' convert high binary to decimal
' conversion of the high 4 bits into decimal for loading into the
' latches
'-----

```

```

l2 = 0 : bits = 1
for x = 1 to 4
shift$ = right$(high$,x)
shift$ = left$(shift$,1)
if shift$ = "1" then l2 = l2 + bits
bits = bits * 2
next x
print "high load ";l2;

```

```

'-----
' the loading of the latches
' 654 dec = 28E hex load latch one
' 653 dec = 28D hex load latch two
' 651 dec = 28B hex strobe data to dat
'-----

```

```

print "loading the d / a ", q
out 654,11 ;'load one (1)
out 653,12 ;'load two (2)
print "output enabled " ,q
out 651,0 ;'output enable
goto start

```

```
end
```

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# DISTRIBUTION AND STATUS OF SHREWS IN ARKANSAS

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## ABSTRACT

Between January, 1988 and February, 1989 a total of 1300 pitfall traps were placed in 150 sites covering 43 counties in Arkansas. Over 290 small mammals and numerous amphibians, lizards, tortoises, and invertebrates were captured. Shrews accounted for 167 of the small mammals, and included *Blarina carolinensis* (116), *Cryptotis parva* (48), and *Sorex longirostris* (3). *B. carolinensis* is abundant in all habitats in the southeastern two-thirds of the state, *C. parva* is common statewide in grassy or brushy areas, and *S. longirostris* is considered to be uncommon, but is found in a variety of habitats. *B. hylophaga*, although not targeted, is found in the northwestern one-third of the state where it is considered to be common. *Notiosorex crawfordi* is only found in the extreme western part of the state and is considered to be rare.

## INTRODUCTION

Sealander (1979) reported four species of shrews (Insectivora: Soricidae) to inhabit Arkansas - southern short-tailed shrew (*Blarina carolinensis*), least shrew (*Cryptotis parva*), southeastern shrew (*Sorex longirostris*), and desert shrew (*Notiosorex crawfordi*). George *et al.*, (1981, 1982) concluded that the southern short-tailed shrew from the northwestern portion of the state was actually Elliot's short-tailed shrew (*B. hylophaga*). Thus, the state's soricid fauna currently consists of five species; of which, four have distributional ranges which terminate within the boundaries of the state.

The distributional ranges, population status, and biology of the soricids in Arkansas are not well delineated. This study was undertaken to determine the current geographical ranges and status of the soricids within Arkansas.

## METHODS AND MATERIALS

It has been well established that pitfall traps are superior to live and snap traps for sampling shrews (Brown, 1967; Pucek, 1969; Wolfe and Esher, 1981). Because they can be checked at irregular intervals, pitfall traps may allow coverage over a wider geographical area. Therefore, pitfall traps were used exclusively in this study. Pitfalls consisted of a plastic container measuring 19.0 cm in depth and 14.5 cm in diameter; three holes were positioned approximately 10 cm from the bottom of each trap to allow partial drainage. Approximately 200 cc of 4% formalin were placed in each pitfall to prevent escape and partially preserve specimens.

Pitfalls were placed in 150 trap sites, located in 43 counties of Arkansas (Fig. 1), between January, 1988 and February, 1989. These sites represented various habitat types including fence rows, old fields, clearcuts, pine forests, and mixed hardwood and pine forests. Five to 15 pitfalls were placed at each site. Pitfalls were examined every 10-20 days and left in place from 3 weeks to 2-3 months.

## RESULTS AND DISCUSSION

Throughout this study, 1300 pitfalls were set, resulting in over 40,000 trap-nights. In excess of 290 small mammals (Table 1), together with numerous amphibians, lizards, tortoises, and invertebrates were captured. A total of 167 shrews were identified, including the southern short-tailed shrew (*Blarina carolinensis*) - 116, least shrew (*Cryptotis parva*) - 48, and southeastern shrew (*Sorex longirostris*) - 3. No known specimens of Elliot's short-tailed shrew (*B. hylophaga*) or the desert shrew (*Notiosorex crawfordi*) were captured. Accounts for all Arkansas species follow:

Table 1. Small mammals captured in pitfalls between January, 1988 and February, 1989 in Arkansas.

| Species Captured                  | Number Captured |
|-----------------------------------|-----------------|
| Order Insectivora                 |                 |
| <i>Blarina carolinensis</i>       | 116             |
| <i>Cryptotis parva</i>            | 48              |
| <i>Sorex longirostris</i>         | 3               |
| Order Lagomorpha                  |                 |
| <i>Sylvilagus floridanus</i>      | 2 <sup>a</sup>  |
| Order Rodentia                    |                 |
| <i>Microtus ochrogaster</i>       | 43              |
| <i>M. pinetorum</i>               | 31              |
| <i>Peromyscus leucopus</i>        | 26              |
| <i>P. maniculatus</i>             | 17              |
| <i>Reithrodontomys fulvescens</i> | 6               |

a - Two baby cottontails were caught in adjoining pitfalls

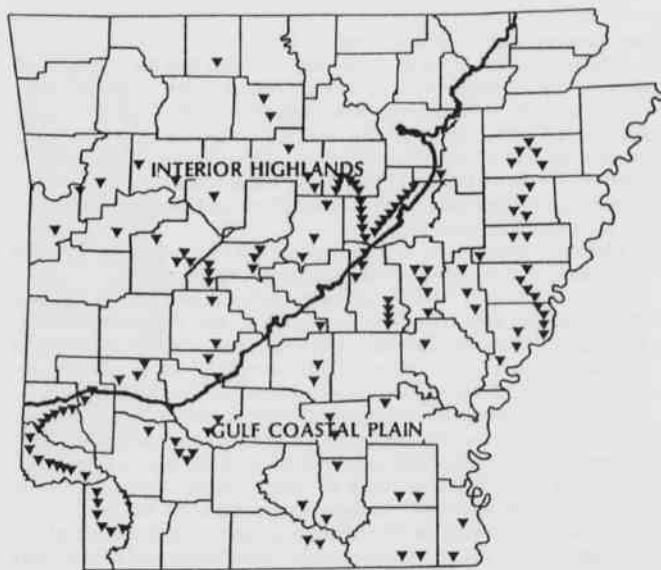


Figure 1. Trapping sites (triangles) where pitfalls were placed between January, 1988 and February, 1989.

**Elliot's short-tailed shrew - *Blarina hylophaga*:**

Previous to 1981, all short-tailed shrews in Arkansas were considered to be *B. carolinensis*. George *et al.* (1981, 1982) examined the taxonomic relationships within the genus *Blarina* and the status of *B. hylophaga* and determined that the short-tailed shrews in the northwestern portion of Arkansas should be included with *B. hylophaga*. Figure 2 illustrates the proposed ranges of *B. hylophaga* and *B. carolinensis* in Arkansas. However, the exact distributions and taxonomic relationship of shrews in the northwestern portion of the state are largely unknown. To allow for this, we included a rather broad contact zone between the two species. Studies on the systematics of short-tailed shrews in Arkansas are needed. The subspecies in Arkansas is *B. h. hylophaga*.

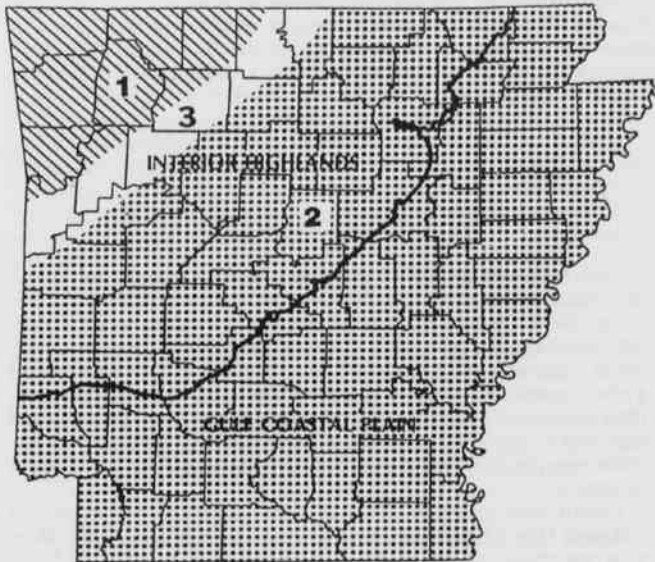


Figure 2. Proposed geographical distribution for *Blarina hylophaga* (1), *B. carolinensis* (2), and zone of contact (3).

Few pitfalls were placed in this species' supposed range and no known specimens of Elliot's short-tailed shrew were recorded in this study. Sealander (1979), however, reported that short-tailed shrews have been taken from most of the counties in northwestern Arkansas and he considered them to be abundant.

**Southern short-tailed shrew - *B. carolinensis***

A total of 116 southern short-tailed shrews were identified. This species was captured in every county and habitat trapped; they were most common in moist hardwoods or brushy areas. It was by far the most common species of shrew encountered, accounting for 70% of shrew specimens taken. As many as five *B. carolinensis* were found in a single pitfall and two or three were common. In several old fields, this species was captured together with the least shrew (*Cryptotis parva*), and in a honeysuckle (*Lonicera japonica*) thicket it was captured together with a southeastern shrew (*Sorex longirostris*).

Hall (1981) indicated there were two subspecies, *B. c. carolinensis* and *B. c. minima* in Arkansas. *B. c. carolinensis* primarily occurs in the interior highlands and extreme southwestern portion of the state, and *B. c. minima* occurs in the remainder of the Gulf Coastal Plain. Sealander (1979) proposed a broad zone of intergradation between the two subspecies. Preliminary studies (M. Kennedy, Memphis State University and D. Moore, Emporia State University, *per. comm.*) indicate that systematic relationships of subspecies in Arkansas is unclear and further study is needed.

Sealander (1979) reported the southern short-tailed shrew to be common and the most abundant shrew species in Arkansas. Results from this study confirm his observations.

**Least shrew - *Cryptotis parva***

The least shrew was the second most commonly encountered shrew, accounting for 29% of the shrew specimens examined. Whitaker (1974) reported that the least shrew may be communal, locally abundant, and inhabits grassy, weedy, and brushy fields. In this study, least shrews were found in old fields, along fence rows, and in early growth clear-cuts; none were taken from woodland areas. In addition, multiple catches were common, lending further evidence to the communal nature of this species.

This species is common statewide and has been recorded from most Arkansas counties (Fig. 3). New county records were added for Van Buren, Poinsett, St. Francis, Lee, Phillips, Monroe, Chicot, Faulkner, and Perry. The subspecies in Arkansas is *C. p. parva*.



Figure 3. Counties from which *Cryptotis parva* has been recorded. Triangles represent previously published records, and circles represent records from this study.

**Southeastern shrew - *Sorex longirostris***

Where sympatric, it has generally been reported that the southeastern shrew is less common than the southern short-tailed shrew and least shrew (Lowery, 1974; Brown, 1978; Sealander, 1979). However, many authors feel that secretive habits and inadequate trapping may result in apparent scarcity (French, 1975; Rose, 1980; Wolfe and Esher, 1981). Further, Wolfe and Esher (1981) found the southeastern shrew to be as common as the southern short-tailed shrew and more common than the least shrew in Mississippi.

Previous to this study, southeastern shrews had only been recorded from Benton, Washington, Polk, and Stone counties (Sealander, 1960, 1977, 1981; Graham, 1976). This study resulted in only three additional specimens; one each from Van Buren, Perry, and Pike counties (Fig. 4). Graham (1976) felt that the range of this species in Arkansas should only include the interior highlands. Sealander (1979) felt that this species occurred in all but the southwestern portion of the state, and Hall (1981) also included all but the extreme southwestern portion in his distributional map. Since the only specimens reported from Arkansas have been captured in the interior highlands, together with our lack of specimens, in spite of heavy trapping, from the delta region, Graham's conclusion would seem warranted. However, since this shrew has been shown to be locally common on the Mississippi and Tennessee side of the Mississippi River (Wolfe and Esher, 1981; M. Kennedy, Memphis State University, *per. comm.*), we feel that it will eventually be captured from the Arkansas delta region. The subspecies in Arkansas is *S. l. longirostris*.



Figure 4. Counties from which *Sorex longirostris* has been recorded. Triangles represent previously published records, and circles represent records from this study.

A number of studies have shown that the southeastern shrew is not particularly habitat specific (Caldwell and Bryan, 1982; Hamilton and Whitaker, 1979; Rose, 1980; Wolfe and Esher, 1981). In Arkansas, this species has been taken from a brushy dam site (Graham, 1976), mesic hardwoods along creeks (Sealander, 1977; present study), and in a honeysuckle thicket at the edge of a pine woods (this study). We conclude that this shrew can be found in a variety of habitat types over a large portion of Arkansas. Further, we consider the southeastern shrew to be relatively uncommon in Arkansas.

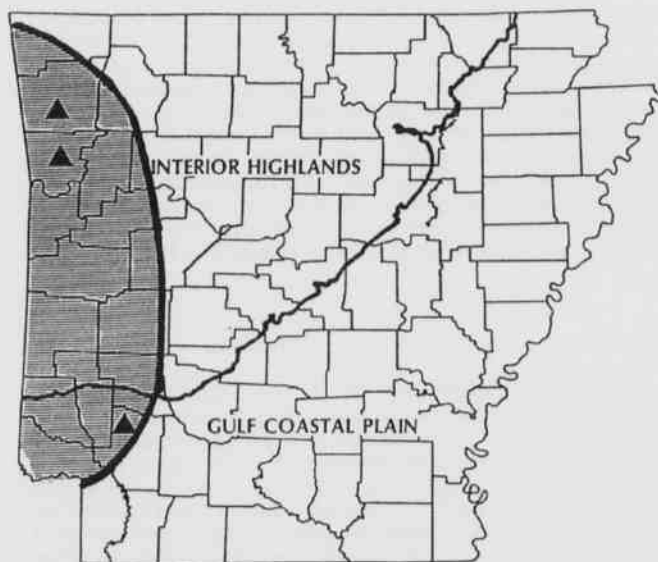


Figure 5. Distribution of *Notiosorex crawfordi*. Triangles represent previously published records.

#### Desert shrew - *Notiosorex crawfordi*

No desert shrews were captured in this study; however, because of their rocky habitats they were not specifically targeted. There have only been three recorded specimens of the desert shrew in Arkansas (Fig. 5). Preston and Sealander (1969) and Sealander (1952) reported one specimen each from Crawford and Washington counties. Recently, Steward *et al.* (1988) recovered two *Notiosorex* skulls from barn owl (*Tyto alba*) pellets in Hempstead County. The most northwesterly limits of this species' range include extreme western Arkansas. The desert shrew must be considered rare in Arkansas, and a need for further study of this species is indicated. The subspecies found in Arkansas is *N. c. crawfordi*.

#### ACKNOWLEDGMENTS

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**<sup>7</sup>Li NMR OF NORMAL HUMAN ERYTHROCYTES**R.P. GULLAPALLI,<sup>1</sup> R.M. HAWK,<sup>1</sup> and R.A. KOMOROSKI<sup>2</sup><sup>1</sup>Department of Electronics and Instrumentation  
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Little Rock, AR**ABSTRACT**

Lithium has been known to be an effective medication for people with bipolar disorder. The mechanisms of action of lithium in the brain is not very well understood. NMR spectroscopy and imaging are effective both in determining lithium levels in tissue and brain. We have monitored lithium levels in red blood cells. We have been able to separate intra- and extracellular compartments of lithium using shift reagents, thereby obtaining T<sub>1</sub>'s of both the compartments. Lithium uptake as a function of hematocrit was monitored weekly over a 3 week period. The time constant of 50 mM lithium uptake at 25 °C and 85% hematocrit was found to be 16.5 hrs. The time constant of 1.8 mM lithium uptake at 37 °C and 45% hematocrit was found to be 11.6 hrs. Experiments on the visibility of the quadrupolar nuclei indicate that it is only 74-90% visible and the visibility decreased with decreasing concentrations.

**INTRODUCTION**

Lithium salts have been used with considerable success in treating both depressive and manic recurrences of bipolar illnesses. Despite much research, little is known concerning the mechanism of action (Stern and Lydiard, 1987). We and others have detected *in vivo* <sup>7</sup>Li NMR signals from the brains of both rats and humans on lithium therapy (Komoroski *et al.*, 1989; Renshaw and Wicklund, 1988). It is important to know the intra- to extracellular ratio in the brain and, hence, the origin of the *in vivo* <sup>7</sup>Li NMR signals, because the presumed site of action is intracellular in the brain. A simple model where intra- to extracellular ratios of lithium have been determined and lithium transport studied is the red blood cell (Pettegrew *et al.*, 1987; Espanol, *et al.*, 1987; Hughes *et al.*, 1988; Mota de Freitas *et al.*, 1988). Apparently conflicting results concerning the <sup>7</sup>Li NMR visibility of intracellular lithium have been reported (Pettegrew *et al.*, 1987; Hughes *et al.*, 1988). We report studies of lithium transport across erythrocyte membranes, spin-lattice relaxation times, and NMR visibility at low concentrations of lithium.

**MATERIALS AND METHODS**

Lithium-7 spectra were acquired at 116.8 MHz on a General Electric GN-300 FT NMR spectrometer using 10 or 20 mm O.D. sample tubes. Spin-lattice relaxation times (T<sub>1</sub>) were measured using the inversion-recovery technique. Dysprosium tripolyphosphate was the extracellular shift reagent. A typical spectrum showing intra and extra peak separation is shown in Figure 1 after incubating red blood cells with lithium for 24 hours. One pint of fresh venous blood was drawn into citrate dextrose anticoagulant (CPDA-1) and typically was used in 1-2 days. Blood obtained from the UAMS Blood Bank was examined up to one month later. Erythrocytes were spun down and washed in a buffer containing 150 mM NaCl according to published methods (Espanol *et al.*, 1987).

**RESULTS AND DISCUSSION**

We obtained a <sup>7</sup>Li T<sub>1</sub> of 5.7 s for intracellular lithium at 25 °C, a value comparable to that previously reported (Espanol *et al.*, 1987). For packed erythrocytes at 25 °C, the extracellular T<sub>1</sub> was 6.5 s, substantially shorter than the 16.5 s reported previously at 13% hematocrit (Espanol *et al.*, 1987). At 37 °C the intra- and extracellular T<sub>1</sub> values are increased to 6.5 and 8.2 s, respectively. This latter value is reduced from the expected value of about 22 s, which we found for pure NMR buffer (see Table 1). The two-component behavior of the T<sub>1</sub> curves will not be a suitable method for distinguishing these compartments in the human brain *in vivo* because the intra- and extracellular T<sub>1</sub>'s are not greatly different.

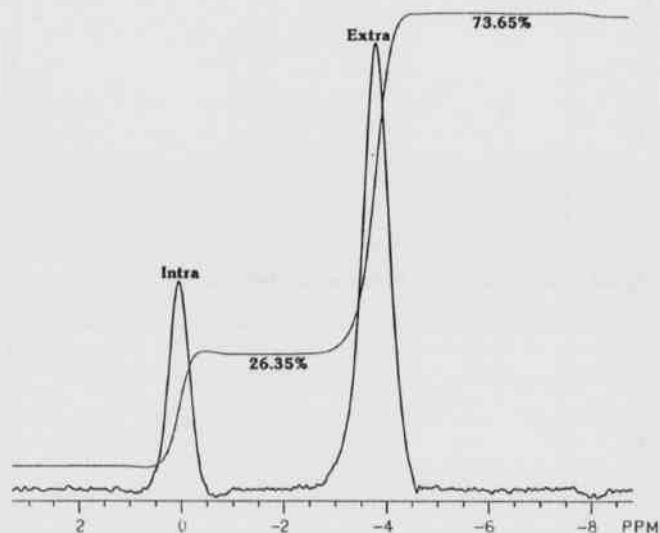


Figure 1. Intra- and Extra-Cellular Components Separated by Dysprosium Tripolyphosphate

Table 1. Spin Lattice (T<sub>1</sub>) Relaxation Times of Various Solutions.

**@25°C**

|                        |            |
|------------------------|------------|
| 1M LiCl                | 13.0 secs. |
| 3.5 mM LiCl            | 13.0 secs. |
| Extracellular Li (RBC) | 6.5 secs.  |
| Intracellular Li (RBC) | 5.7 secs.  |

**@37°C**

|                        |            |
|------------------------|------------|
| 1M LiCl                | 23.0 secs. |
| 50 mM LiCl             | 20.3 secs. |
| NMR Buffer             | 22.0 secs. |
| Extracellular Li (RBC) | 8.2 secs.  |
| Intracellular Li (RBC) | 6.5 secs.  |

$^7\text{Li}$  NMR of Normal Human Erythrocytes

As expected, the percentage of intracellular lithium decreases with decreasing hematocrit. The percentage of intracellular lithium increased substantially (100 - 200%) with aging from one to three weeks (see Figure 2). We have measured the kinetics of lithium transport into the cells for two situations. At 50 mM lithium (25 °C) and about 85% hematocrit (packed cells), we obtained a time constant of 16.5 hours, close to the 14.7 hours found by Pettegrew *et al.*, (1987). We first detected lithium about one-half hour into the experiment (see Figure 3). The kinetic data for 1.8 mM lithium at a level of 45% hematocrit and 37 °C are shown in Figure 4. These conditions closely approximate those expected clinically. The time constant was 11.6 hours. In this case, intracellular lithium was first observed 1.5 hours into the experiment.

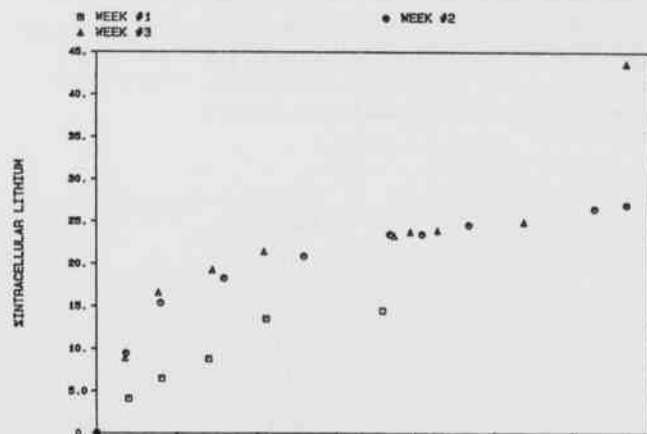


Figure 2. Li Uptake as a Function of Hematocrit

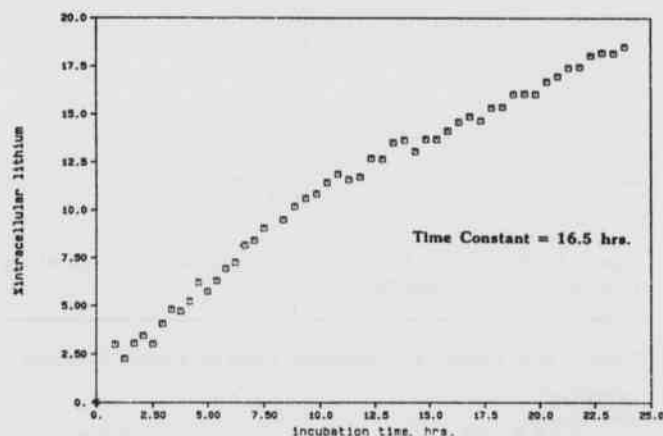


Figure 3. Kinetics of Li (50 mM) Transport in Packed Red Blood Cells at 25 °C

Spin-3/2 nuclei can exhibit reduced "NMR visibility" in motionally restricted environments (Bull, 1972). Such is common for  $^{23}\text{Na}$  but, in this regard, little is known about  $^7\text{Li}$ . Pettegrew *et al.* (1987) reported 98% visibility for the total signal from packed erythrocytes with about 33% intracellular lithium at 50 mM LiCl, whereas Hughes *et al.* (1988) reported full extracellular but reduced intracellular visibility at 40 mM LiCl. This reduced visibility is attributed to the binding of lithium to cell membrane and other cellular components.

We have examined the visibility of  $^7\text{Li}$  at several concentrations for packed red cells. At 40 mM, we found a total visibility of 88-90%, whereas at 1 to 10 mM, visibility was reduced to 74 to 84% (see Table 2). Our experiments do not permit separate determination of intra- and extracellular visibility. These results suggest that  $^7\text{Li}$  signals detection *in vivo* suffer from reduced visibility of one or both components.

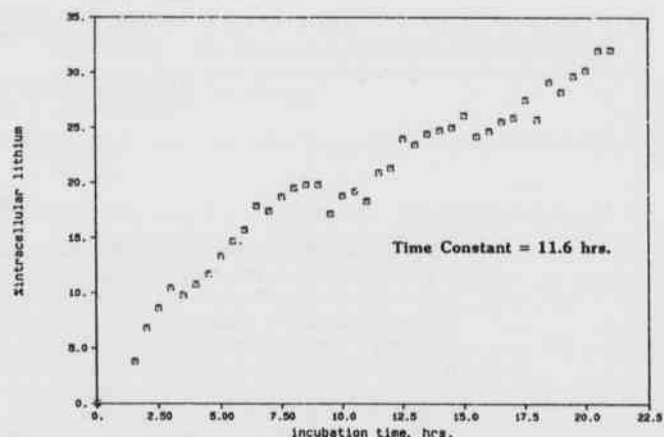


Figure 4. Kinetics of Li (1.8 mM) Transport in Red Blood Cells (45% Hematocrit) at 37 °C

Table 2. NMR 'Visibility' of Lithium in Erythrocytes

| Conc. of LiCl | % Total Visibility | % Intracellular Visibility * |
|---------------|--------------------|------------------------------|
| 40 mM         | 87.5               | 74.0                         |
| 10 mM         | 80.0               | 70.0                         |
| 5 mM          | 78.0               | 66.0                         |
| 1 mM          | 74.0               | 63.0                         |

\* assuming all the invisibility is due to intracellular compartment.

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# GENETIC AND PLANT GROWTH REGULATOR MANIPULATION OF RICE (*ORYZA SATIVA* L.) MESOCOTYL AND COLEOPTILE LENGTHS

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## ABSTRACT

Significant differences in mesocotyl lengths of semidwarf and non-semidwarf rice (*Oryza sativa* L.) cultivars were observed. However, the relationship between plant height and mesocotyl length was found to be due to linkage rather than pleiotropism. Seed treatments of gibberellic acid (GA<sub>3</sub>) significantly increased mesocotyl and coleoptile lengths in the laboratory study. The GA<sub>3</sub> seed treatments significantly increased stand density compared to the untreated control in the field study. However, no significant differences were observed for plant height at maturity or grain yields among the GA<sub>3</sub> treatments or the untreated control.

## INTRODUCTION

Semidwarf rice (*Oryza sativa* L.) cultivars are short in stature (80 to 120 cm in height at maturity), have short and more upright leaves, greater nitrogen (N) responsiveness without lodging, and higher grain yields relative to taller traditional rice cultivars (Turner *et al.*, 1982). Semidwarf rice cultivars are becoming more popular with rice producers in the southern states of Arkansas, Louisiana, Texas, and Mississippi. Semidwarf rice cultivars were seeded on 790,365 hectares in these states in 1988 (Matlick, 1988). Stansel (1984) stated that there may be problems with seedling emergence of semidwarf rice cultivars and changes in management practices may be required to maximize economic productivity of the semidwarfs.

Rice seedling emergence is a function of mesocotyl and coleoptile elongation. The mesocotyl in the rice seedling is the internode between the coleoptile node and the point of union of the root and the culm. The coleoptile is the cylinder-like protective covering that encloses the young plumule (DeData, 1981). Turner *et al.*, (1982) reported that poor stand establishment of semidwarf cultivars is largely attributable to short mesocotyl length, and further stated that the semidwarfs have shorter mesocotyls than traditional, non-semidwarf rice cultivars.

Mesocotyl and coleoptile lengths are genetically controlled but mesocotyl and coleoptile elongation are also influenced by seeding depth and environmental factors (Turner *et al.*, 1982). Kordan (1974) concluded that coleoptile elongation was enhanced by low oxygen (O<sub>2</sub>) levels while Chang and Bardenas (1965) reported that light inhibits mesocotyl elongation. Takahashi (1978) found that removing carbon dioxide (CO<sub>2</sub>) from the developing seedling decreased both coleoptile and mesocotyl elongation.

Turner *et al.* (1982) examined mesocotyl and coleoptile elongation of six rice cultivars at five seeding depths. Variability among cultivars existed for the mesocotyl and coleoptile elongation especially at different seeding depths. In general, the sum of the mesocotyl and coleoptile length equalled the seeding depth. However, the semidwarf cultivars 'Bellemont' and 'M101' did not emerge at the 10 cm seeding depth. Reduced emergence of these and other semidwarf rice cultivars at seeding depths exceeding 2.5 cm had led some researchers to theorize that there was a possible pleiotropic relationship between mesocotyl and coleoptile elongation and plant height.

Sunderman (1964) reported positive correlations between plant height and coleoptile lengths of winter wheat in three of five tests and coleoptile length was positively correlated with seedling emergence in one of two tests. Takahashi (1946) also found a positive correlation between coleoptile and culm length in barley but found was no relationship between coleoptile length and seedling emergence.

Stowe and Yamaki (1957) postulated that since maize dwarfs are caused by single gene defects, the dwarfness may be interpreted as a block in a biosynthetic pathway that ultimately leads to the production of gibberellin-like compound. They suggested that by adding gibberellic acid (GA<sub>3</sub>), these blocks may be overcome and the requirement for the growth substance satisfied. However, Allan *et al.* (1962) found that dwarf and semidwarf wheat cultivars were not induced to grow to normal height by injections of GA<sub>3</sub>.

Bird and Ergle (1961) found significant increases in seedling emergence and seedling height of cotton when cotton seed was treated with potassium gibberellate (75% GA<sub>3</sub>). The positive influence of the GA<sub>3</sub> was thought to be associated with the increased rate of hypocotyl elongation.

When our study was initiated, the association of semidwarf rice cultivars and inadequate stand establishment were known. Furthermore, the proposed theory was that the mesocotyl and coleoptile were pleiotropic with plant height. The objectives of the study were to: (a) evaluate the potential mesocotyl and coleoptile elongation of semidwarf and standard cultivars, (b) determine the relationship between mesocotyl and coleoptile elongation and plant height in parental rice germplasm, (c) evaluate enhanced germplasm developed by hybridization and selection to determine the relationship of mesocotyl and coleoptile elongation to plant height, and (d) evaluate GA<sub>3</sub> as seed treatments on rice for mesocotyl and coleoptile elongation, stand establishment, plant height, and grain yield.

## MATERIALS AND METHODS

Laboratory and field experiments were conducted from 1982 through 1988 to determine the mesocotyl, coleoptile, and total (mesocotyl + coleoptile) lengths of rice cultivars to determine the association of these parameters to plant height. Studies were also initiated to examine the effects of GA<sub>3</sub> at rates of 10, 50, and 100 mg ai/kg seed on seedling morphology, stand establishment, plant height, and grain yield. One kilogram of seed was treated with solutions of GA<sub>3</sub>. The seed lots were then dried to inhibit germination.

The rice germplasm utilized in the mesocotyl/coleoptile and plant height study were the very-short-season cultivars 'M101', 'L201', and 'Labelle', and the experimental line RU7703008 (NWRX//C19881/PI331581). The semidwarf germplasm was represented by M101 (an early maturing California cultivar) and RU7703008 (a sister line of USDA-Texas Agricultural Experiment Station midseason cultivar, Belle-



mont). The comparatively taller statured germplasm was represented by Labelle, developed by USDA-Texas Agricultural Experiment Station and L201, developed by California Cooperative Rice Research Foundation.

The rice cultivars used in the GA<sub>3</sub> seed treatment study included 'Lemont' and 'Rexmont', two semidwarf cultivars developed by USDA-Texas Agricultural Experiment Station and 'Mercury', a semidwarf cultivar developed by the Louisiana Agricultural Experiment Station. The data from the studies were statistically analyzed using the General Linear Mean Model (GLM) procedures provided by the Statistical Analysis System (SAS Institute, 1982).

#### Laboratory Experiments

The laboratory test was designed to determine the potential length of the mesocotyl and coleoptile of the germplasm sources when grown in darkness. The slantboard technique described by Jones and Peterson (1976) was used. Moistened blotters (13 X 18 cm) were placed on acrylic plates of the same size and 20 seeds were placed in a horizontal line, germ-end down, 1 cm apart and 4 cm from the bottom of the plate. The seeds were held in position by placing a single thickness of germination paper over the seeds. The plates that were holding the seed were moistened with distilled water. Plates, blotters, and adhering seed were placed in slotted acrylic racks which held 12 acrylic plates. The entire assembly was placed in a glass tray containing 1,000 ml of distilled water. The assembly was wrapped in two black polyethylene bags to maintain high humidity and to assure a uniform dark environment. The assembly was placed in a temperature controlled germinator in total darkness at 100% RH for 10 days at 25°C, after which the mesocotyl and coleoptile of each seedling were measured. The sum of the mesocotyl and coleoptile measurements comprised the total length. Each acrylic plate contained 20 uniform, mature seeds (five seeds/germplasm entry) which represented one replication. Also, the previously described technique was used in the GA<sub>3</sub> seed treatment study. However, a separate glass tray was used for each concentration of the GA<sub>3</sub>.

#### Germplasm Enhancement - Field Study

The four germplasm sources (M101, L201, Labelle and RU7703008) were hybridized in all possible combinations to determine if semidwarf recombinant plants which produce long mesocotyls and coleoptiles could be recovered. Parent and F<sub>1</sub> seed were hand planted in 4.7 m rows with seed being placed 15 cm apart within a row and 20 cm between rows on Crowley silt loam (Typic Albaqualf) at the Rice Research and Extension Center at Stuttgart, AR. Seeds were hand harvested from F<sub>1</sub> plants and individual F<sub>2</sub> seed were hand planted in the same design that was used for the parent and F<sub>1</sub> seed. Seeds from 340 randomly selected F<sub>2</sub> plants from each cross combination were hand harvested, threshed, and stored at 10°C and about 50% RH. These seeds were sown in two replications of 1 m rows using 19 cm row spacing. Heading date, percent lodging and average plant height (five plants/row) were measured and uniform, mature seeds from individual plants of the F<sub>2</sub> rows were evaluated as described in the materials and methods for mesocotyl and coleoptile length. Twenty-three semidwarf selections that produced long mesocotyls and coleoptiles were evaluated at two locations (four replications/location) in 1986 and 1987 for plant height, and mesocotyl/coleoptile length.

#### GA<sub>3</sub> Seed Treatment - Field Study

In 1988, field studies to examine the effects of GA<sub>3</sub> seed treatments on stand establishment, plant height, and grain yield were initiated at the Southeast Branch Experiment Station, Rohwer, AR. The semidwarf, Lemont, was seeded at 2.5 and 7.5 cm depths at a rate of 112 kg/ha in Desha silt loam (Vertic Haplaudolls). Plots were nine drill rows wide (15 cm spacing) and 4.6 m in length.

A split-plot experimental design was used with seeding depth as main plot and GA<sub>3</sub> seed treatments as subplots. Each treatment combination was replicated four times. Stand counts were taken in two 0.09 m<sup>2</sup> areas prior to flooding. At maturity, plant height was measured from the soil level to the top of the extended panicles at two locations in each plot. The plots were combine harvested and grain yields are expressed as 12% moisture.

## RESULTS AND DISCUSSION

### Laboratory Experiment

#### I. Mesocotyl/Coleoptile Evaluation of Parental Germplasm

The mesocotyl and total lengths of the two semidwarf genotypes, M101 and RU7703008, differed significantly from those of the taller genotypes, L201 and Labelle (Table 1). These data are similar to results reported by Turner *et al.* (1982), who found that semidwarf cultivars have shorter mesocotyl and total lengths than taller rice cultivars. Furthermore, the coleoptile length of L201 was significantly longer than the coleoptiles of RU7703008 and Labelle, but not that of M101.

Table 1. Mesocotyl, coleoptile and total (mesocotyl + coleoptile) lengths of four rice cultivars (laboratory experiment).

| Cultivar | Lengths in mm |            |       |
|----------|---------------|------------|-------|
|          | Mesocotyl     | Coleoptile | Total |
| M101     | 2.1           | 26.7       | 28.9  |
| 7703008  | 1.6           | 13.4       | 15.0  |
| L201     | 16.9          | 31.6       | 48.5  |
| Labelle  | 15.9          | 20.7       | 36.7  |
| LSD 0.05 | 2.9           | 7.6        | 6.2   |

The germplasm in this study exhibited genetic differences in mesocotyl and coleoptile length which could be advantageous in the subsequent development of semidwarf cultivars. Furthermore, the slantboard technique previously described would be useful in determining mesocotyl/coleoptile lengths in large segregating populations because the technique would permit the saving and transplanting of individual seedlings that possess the desired seedling vigor traits.

#### II. GA<sub>3</sub> Seed Treatments

The GA<sub>3</sub> seed treatments significantly increased mesocotyl, coleoptile and total lengths of the three cultivars compared to the untreated control (Table 2). The mesocotyl lengths of Rexmont at the GA<sub>3</sub> 10 mg/kg rate were significantly less than mesocotyl lengths at the GA<sub>3</sub> 50 and 100 mg/kg rate. No significant differences were observed among the GA<sub>3</sub> rates for mesocotyl lengths of Lemont or Mercury. Additionally, no significant differences were observed among the GA<sub>3</sub> rates for coleoptile or total lengths of any cultivar.

Table 2. Influence of GA<sub>3</sub> seed treatments on rice mesocotyl, coleoptile and total (mesocotyl + coleoptile) length of three rice cultivars (laboratory experiment).

| Seed Treatment<br>mg/ha | Lengths in mm |      |      |             |      |      |             |      |      |
|-------------------------|---------------|------|------|-------------|------|------|-------------|------|------|
|                         | - LEMONT -    |      |      | - REXMONT - |      |      | - MERCURY - |      |      |
|                         | Meso          | Cole | Tot  | Meso        | Cole | Tot  | Meso        | Cole | Tot  |
| Untreated Control       | 1.7           | 9.4  | 11.1 | 1.9         | 10.9 | 12.8 | 1.7         | 15.3 | 17.0 |
| GA <sub>3</sub> 10      | 16.3          | 26.7 | 43.0 | 6.9         | 23.8 | 30.7 | 6.9         | 33.4 | 40.3 |
| GA <sub>3</sub> 50      | 12.4          | 24.8 | 37.2 | 11.6        | 27.1 | 38.7 | 8.0         | 32.6 | 40.6 |
| GA <sub>3</sub> 100     | 15.8          | 22.6 | 38.4 | 9.5         | 19.8 | 29.3 | 8.3         | 33.0 | 41.3 |
| LSD 0.05                | 3.6           | 5.9  | 7.7  | 2.5         | 7.8  | 8.7  | 2.6         | 10.6 | 12.7 |

#### Germplasm Enhancement

This part of the study was designed only to identify semidwarf germplasm with mesocotyls and coleoptiles that were equal to the mesocotyl/coleoptile length of normal height germplasm. The test was not designed to determine the inheritance of mesocotyl or coleoptile elongation. Approximately 600 semidwarf plants (< 80 cm in height) from each of the six cross combinations were tested for mesocotyl/coleoptile elongation (Table 3). The greatest frequency of the desired plant type was identified in the RU7703008 X L201 (eight plants) and M101 X L201 (six plants) population (Table 3).

**Genetic and Plant Growth Regulator Manipulation of Rice (*Oryza sativa* L.) Mesocotyl and Coleoptile Lengths****Table 3.** Recombinant semidwarf germplasm that produces normal length mesocotyls and coleoptiles from six hybrid populations (germplasm enhancement experiment).

| Hybrid              | Recombinant Plants | Total Population |
|---------------------|--------------------|------------------|
| RU7703008 X L201    | 8                  | 609              |
| M101 X L201         | 6                  | 612              |
| RU7703008 X M101    | 3                  | 610              |
| L201 X Labelle      | 0                  | 597              |
| M101 X Labelle      | 3                  | 602              |
| RU7703008 X Labelle | 3                  | 601              |

The new germplasm and their parents were evaluated for plant height versus mesocotyl/coleoptile elongation in the field in 1986 and 1987. Two of the semidwarf lines, designated (RU7703008 X L201)-512 and (RU7703008 X L201)-513, from the crosses produced significantly longer mesocotyls than the semidwarf parent, RU7703008 (Table 4). There was no significant difference in plant height between the new germplasm and RU7703008, a sister selection of Bellemont. However, these three genotypes were significantly shorter than L201, the normal height parent. There was no significant difference in coleoptile length of L201 or the new germplasm; however these three genotype sources have significantly longer coleoptiles than RU7703008 (Table 4). Therefore, these data verify that plant height and mesocotyl/coleoptile elongation in rice are not due to pleiotropism but rather these two plant characteristics are controlled by separate but closely linked loci.

**Table 4.** Mean separation of plant height, mesocotyl, coleoptile and total (mesocotyl + coleoptile) length of parental and new semidwarf germplasm that has normal length mesocotyls and coleoptiles (germplasm enhancement experiment).

| Genotype             | Plant Ht<br>cm | Mesocotyl | Coleoptile | Total  |
|----------------------|----------------|-----------|------------|--------|
| L201                 | 90.5 a         | 26.8 a    | 29.8 a     | 56.6 a |
| (7703008 X L201)-512 | 78.5 b         | 16.7 b    | 35.1 a     | 51.8 a |
| (7703008 X L201)-513 | 84.5 b         | 14.2 b    | 29.4 a     | 43.6 b |
| 7703008              | 83.5 b         | 6.2 c     | 19.3 b     | 25.5 c |

P = 0.05

**GA<sub>3</sub> Seed Treatment - Field Study**

Analysis of variance indicated there was a significantly greater stand density at the 2.5 cm seeding depth compared to the 7.5 cm seeding depth (Table 5). However, there was no difference between the seeding depths for plant height at maturity or grain yield.

**Table 5.** Influence of seeding depth on stand density, plant height, and grain yield of Lemont rice averaged over GA<sub>3</sub> seed treatments.

| Seeding Depth<br>cm | Stand Density <sub>2</sub><br>plants/m <sup>2</sup> | Plant Height<br>cm | Grain Yield<br>kg/ha |
|---------------------|-----------------------------------------------------|--------------------|----------------------|
| 2.5                 | 221                                                 | 92.9               | 8110                 |
| 7.5                 | 137                                                 | 91.3               | 7955                 |
| LSD = 0.05          | 32                                                  | NS                 | NS                   |

The GA<sub>3</sub> seed treatments significantly increased stand density compared to the untreated control (Table 6). However, there was no difference among the GA<sub>3</sub> seed treatments for stand density. Furthermore, there was no difference for plant height at maturity or grain yield when the GA<sub>3</sub> seed treatments were compared to the untreated control. Although there were significant increases in mesocotyl, coleoptile, and total lengths from the GA<sub>3</sub> seed treatments as determined in the laboratory study, these seedling elongation effects were not observed at maturity.

**Table 6.** Influence of GA<sub>3</sub> seed treatments on stand density, plant height and grain yield of Lemont rice averaged over seeding depths.

| Seed Treatment<br>mg/kg | Stand Density <sub>2</sub><br>plants/m <sup>2</sup> | Plant Height<br>cm | Grain Yield<br>kg/ha |
|-------------------------|-----------------------------------------------------|--------------------|----------------------|
| Untreated Control       | 127                                                 | 92.3               | 7705                 |
| GA <sub>3</sub> 10      | 191                                                 | 92.8               | 8200                 |
| GA <sub>3</sub> 50      | 191                                                 | 91.3               | 7950                 |
| GA <sub>3</sub> 100     | 201                                                 | 92.1               | 8265                 |
| LSD 0.05                | 53                                                  | NS                 | NS                   |

**CONCLUSIONS**

These results indicate that there are differences in mesocotyl/coleoptile lengths of semidwarf and taller rice cultivars, but no pleiotropic relationship between plant height and mesocotyl elongation was found. Furthermore, two sources of germplasm [(RU7703008 X L201)-512, (RU7703008 X L201)-513] have been identified which have semidwarf plant height and mesocotyl/coleoptile potential that is similar to taller cultivars which can be utilized in rice breeding programs.

Also, the use of the GA<sub>3</sub> seed treatments can enhance mesocotyl, coleoptile, and total elongation in present semidwarf rice cultivars. Field studies with Lemont demonstrated that stand establishment can be increased with the use of GA<sub>3</sub> seed treatments.

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# AGE ASSESSMENT OF WHITE BASS FROM OTOLITHS, DORSAL SPINES, AND SCALES

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## ABSTRACT

Otoliths, dorsal spines, and scales of 85 white bass collected in 1987 from a pre-spawning group were used for age assessment. Agreement between spine and otolith ages were 78.3%, between scale and otolith ages was 68.2%. Unlike spine ages, percent agreement of scale ages with otolith ages decreased from small to large fish.

Length-frequency analysis in conjunction with ages assessed by the three calcified structures showed that dorsal spines and scales underestimated white bass ages compared to the otoliths. Clarity of the otolith annuli, even of the older fish, makes them a reliable source for white bass age assessment.

## INTRODUCTION

Age data are useful in fish growth and longevity studies and in making management decisions. In the past 50 years, fish scales have been the main source for age determination of freshwater fishes, but this method might not reveal the true age of slow-growing or older fishes (Carlander, 1987; Erickson, 1983). Indistinctness or compactness of annuli at the outer edges of scales makes them unreadable. Resorption of scales to provide calcium to fish during periods of deficiency and ovarian development adds to the difficulty of scale age assessment (Garrod and Newell, 1958; Simkiss, 1974). Age estimates using otoliths and bones, which have a higher priority for calcium utilization and have easily recognizable annuli, have been found more reliable than the scales, even in older fish (Simkiss, 1974; Beamish, 1979; Erickson, 1983; Sikstrom, 1983; Casselman, 1983). In contrast to the scales, otoliths continue to grow as the fish gets older (Beamish and McFarlane, 1987).

To our knowledge, scales are the only calcified structures to have been used in the age assessment of white bass (*Morone chrysops*) (Jenkins and Elkin, 1957; Forney and Taylor, 1963; Priegel, 1971; Yellayi and Kilambi, 1975), but as early as 1941, Frey and Vike (1941) reported the failure of annulus formation on white bass scales. The efficacy of various calcified structures in aging white bass was not determined. Therefore, the purpose of our study was to evaluate the feasibility of reliable age determination of white bass using calcified structures other than scales.

## MATERIALS AND METHODS

A total of 85 white bass was collected in March 1987 from the 11,400 ha Beaver Reservoir, Arkansas, by electroshocking and gillnetting. Total length (mm), weight (g), and sex were recorded for each fish. Scale samples obtained from an area near the tip of the appressed left pectoral fin just below the lateral line were cleaned, mounted between two glass slides and photographed by microfiche reader-printer. The annuli were counted from the photographs.

The second dorsal spine removed from the base and the excised otoliths (sagittae) were stored in alcohol and a 1:1 mixture of alcohol and glycerine, respectively. They were sectioned after epoxy embedding using a low speed circular saw. Two spine sections were discarded due to bad sectioning. The spine and otolith sections mounted on glass slides in Permount were examined for annuli under a Ken-a-Vision microprojector and photographed under a phase contract microscope. An annulus was assumed at the outer margins of the three calcified structures used in this study, even when it was not recognizable, as the white bass were collected from a pre-spawning population. Sexes were pooled for age analysis. Initially, the age determinations were made independently by the authors. The calcified structures were reexamined by both of us in cases of disagreement, to assign final ages.

## RESULTS AND DISCUSSION

The otolith annuli, even of the older white bass, were distinct and easy to enumerate (Fig. 1). Hence, otolith ages were used as the basis for comparison with spine and scale ages (Table 1). The otoliths and spines yielded six age groups, while five age groups were discernible from the scales.

Agreement between the spine and otolith ages was 78.3%. Excluding the age group VI because of small sample size, spine ages did not exhibit any trend in percent agreement with increased otolith ages (Table 1). For the age group II, the age overestimation by spines of some fish was by one year and for age groups III through VI, the spine ages were less than the otolith ages by one year for some of the white bass. The spine ages for the rest of the white bass were in agreement with the otolith ages. The underestimation of age by some dorsal spines was probably because of difficulty in locating the first annulus due to the vascularized core obliterating it (Prince *et al.*, 1986).

Agreement between scale and otolith ages was 68.2%. In comparison with otolith ages, percent agreement of scale ages decreased and age underestimation increased with increased otolith ages (Table 1). Some scale ages were one year less than the otolith ages for age groups III, IV, and V. The ages of the two fish in age group VI were underestimated by one and two years by the scale method. Some scale ages were more than otolith ages for groups II, III and IV with no trend. The discrepancies in scale age estimates occurred for all the otolith ages and were probably due to failure of formation, indistinctness and presence of supernumerary scale annuli. Frey and Vike (1941) reported that the annuli failed to form on the scales of the Kagonosa and Waubesa white bass populations when the fish did not grow because of food scarcity. We found the annuli on some scales hard to discern and enumerate, unlike the annuli on the otoliths.

The numbers of spine and scale ages were regressed on otolith ages by the least square method (Steel and Torrie, 1980). Details of the statistics are given in Table 2. Although correlation coefficients ( $r$ ) were significant, the regression coefficients ( $b$ ) were significantly less than the unit slope ( $b = 1$ ) and the Y-intercepts ( $a$ ) were significantly different from zero. Therefore, we concluded that the spines and scales underestimated white bass ages in comparison with otolith ages. The variances about regression line ( $S^2_{r.e.}$ ) indicated greater variability for scale ages than the spine ages compared to the otolith ages (Table 2). The otolith, spine, and scale annuli of the 404 mm white bass showed underestimation of age by the spine and scale methods (Fig. 1). The maximum scale age of four years recorded for the Beaver Reservoir white bass by Yellayi and Kilambi (1975) was probably an underestimate assuming the otolith ages represent the actual age.

The length distribution of the 85 white bass of this study was disjoint with 44 fish in the 215-270 mm, 35 fish in the 304-376 mm, and six fish in the 400-439 mm size groups, respectively (Table 3). Analysis of the length-frequency distribution by the probability paper method



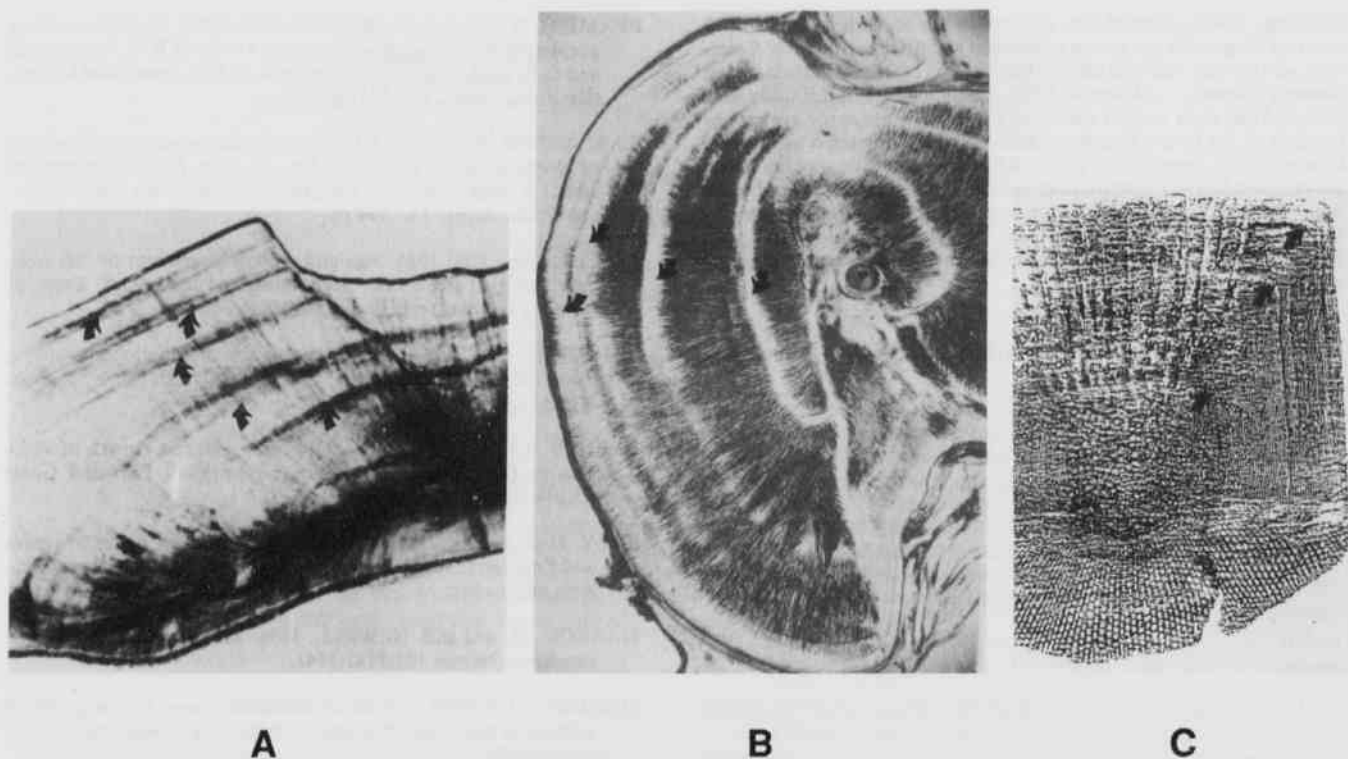


Figure 1. Otolith and dorsal spine sections, and body scale of 404 mm white bass.

- A. Otolith (40x)
- B. Dorsal Spine (41x)
- C. Body Scale (10x)

Table 1. Agreement and disagreement of spine and scale ages with otolith ages of white bass.

| Otolith age      | Number of fish | Percent   |               |              |
|------------------|----------------|-----------|---------------|--------------|
|                  |                | Agreement | Underestimate | Overestimate |
| <u>Spine age</u> |                |           |               |              |
| II               | 28             | 89.3      |               | 10.7         |
| III              | 22             | 72.7      | 27.3          |              |
| IV               | 26             | 73.1      | 26.9          |              |
| V                | 5              | 80.0      | 20.0          |              |
| VI               | 2              | 50.0      | 50.0          |              |
| <u>Scale age</u> |                |           |               |              |
| II               | 30             | 96.7      |               | 3.3          |
| III              | 22             | 54.6      | 31.8          | 13.6         |
| IV               | 26             | 61.5      | 34.6          | 3.9          |
| V                | 5              | 20.0      | 80.0          |              |
| VI               | 2              |           | 100.0         |              |

Table 2. Sample size (n), Correlation coefficient (r), regression coefficient (b), Y-intercept (a), standard errors of b and a (S.E. b and S.E. a), and variance about regression ( $S^2_{yx}$ ) of least square regression analysis with t-tests for  $b=1$  and  $a=0$ .

| Statistics | Regression of              |                            |
|------------|----------------------------|----------------------------|
|            | Spine ages on otolith ages | Scale ages on otolith ages |
| n          | 83                         | 85                         |
| r          | 0.92***                    | 0.85***                    |
| b          | 0.86**                     | 0.75**                     |
| S.E. b     | 0.0445                     | 0.0514                     |
| a          | 0.21*                      | 0.59***                    |
| S.E. a     | 0.1484                     | 0.1701                     |
| $S^2_{yx}$ | 0.1776                     | 0.2437                     |

Significance level,  $p^* \leq 0.05$ ;  $** \leq 0.005$ ;  $*** \leq 0.001$

## Age Assessment of White Bass From Otoliths, Dorsal Spines, and Scales

(Harding, 1949) indicated two age groups for the fish in the 215-270 mm size range with age group separation at 240 mm. In Beaver Reservoir, all two-year-old and older white bass of both sexes attain sexual maturity (Newton and Kilambi, 1969). The white bass of this study were collected just prior to their spawning migrations, hence the age groups I and II of the length-frequency analysis were designated as two and three year olds, respectively. The otolith, spine, and scale annulus counts also showed this size group comprised of two- and three-year-old white bass (Table 3). Two year olds constituted 68.2% of this size group by the otolith method and 73.8% and 79.6% by the spine and scale methods, respectively, thus the latter two methods underestimated the age of white bass.

Table 3. Age group frequencies in relation to length of white bass.

| Length group<br>(mm) | Number of fish in age groups |     |    |   |    |       |     |    |   |    |       |     |    |   |
|----------------------|------------------------------|-----|----|---|----|-------|-----|----|---|----|-------|-----|----|---|
|                      | Otolith                      |     |    |   |    | Spine |     |    |   |    | Scale |     |    |   |
|                      | II                           | III | IV | V | VI | II    | III | IV | V | VI | II    | III | IV | V |
| 215-224              | 3                            |     |    |   |    | 3     |     |    |   |    | 3     |     |    |   |
| 225-234              | 4                            |     |    |   |    | 3     |     |    |   |    | 4     |     |    |   |
| 235-244              | 10                           | 2   |    |   |    | 9     | 3   |    |   |    | 12    |     |    |   |
| 245-254              | 11                           | 6   |    |   |    | 12    | 3   |    |   |    | 12    | 5   |    |   |
| 255-264              | 1                            | 5   |    |   |    | 3     | 3   |    |   |    | 2     | 4   |    |   |
| 265-274              | 1                            | 1   |    |   |    | 1     | 2   |    |   |    | 2     |     |    |   |

The 340-376 mm group contained a single age group according to the probability technique. The annulus counts showed three and four year olds contributing 33.3% and 63.8%, respectively to the size group (Table 3). The relative composition of the four-year-old white bass by the otolith, spine, and scale ages was 74.3, 57.1, and 60.0%, respectively.

The spine and the scales underestimated white bass age, compared with the otolith age. The failure of the probability analysis in discerning more than a single age group was probably due to slow growth with length overlap of the constituent age groups.

The white bass in the 400-439 mm size was composed of five and six year olds by the otolith and spine aging; the scale analysis showed four and five year olds, thus underestimating the ages of the older fish (Table 3). The single age group designation by the probability technique was due to very small sample size.

Our study found variation in the otolith, spine, and scale assessed ages of white bass. The spines and scales underestimated the ages when compared with the otolith ages. It cannot be ascertained which of these three calcified structures depicted the true ages of white bass without validation through mark-recapture or by the study using known-age fish. However, otolith annuli were more distinct and easy to enumerate, even of the older fish, than the spine and scale annuli. We, therefore, assume that the otoliths provide a reliable source for aging white bass.

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# ARKANSAS' INCENDIARY WILDFIRE RECORD: 1983-1987

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## ABSTRACT

All wildfire reports from lands protected by the Arkansas Forestry Commission for the calendar years 1983 through 1987 were studied. The number of wildfires steadily increased from 2,185 in 1983 to 4,150 in 1987, burning a total of 27,146 hectares in 1987. Incendiarism on forested lands in 1987 comprised 77% of the total fires and 84% of the area burned. Incendiarism was responsible for 40% of all fires and 60% of the area burned in 1983, but increased to 54% of all fires and 69% of the area burned in 1987. In 1987, 80% of all incendiary fires on industry lands were started by local residents. Most incendiary fires occurred on Class 3 (52%) and Class-2 (27%) fire-danger class-days. More incendiary fires (64%) occurred during the spring fire season (January through June). The general public reported 66% the non-incendiary fires, but only 56% of the incendiary-caused fires. Implications of these findings for wildfire prevention programs are discussed.

## INTRODUCTION

There is strong archaeological evidence documenting man's use of fire (Brown and Davis, 1973). Innumerable uncontrolled encounters no doubt predate this evidence. While controlled combustion as a tool has molded modern civilization, uncontrolled fire tends to disrupt civilized patterns, destroy property and provoke primitive responses from humans. Reports of broad-scale conflagration, such as the Yellowstone National Park fires in the summer of 1988, kindle responses of fear, anger and wonder on the part of land managers, legislators and the general public. Few individuals remain without opinion in the face of such loss.

It is, perhaps, man's primal reactions to wildfire that have prompted its use as a weapon of fear and destabilization. The use of fire as an implement of terror is recorded in the Bible with Samson's raid using jackals carrying torches to incinerate Philistine crops (New English Bible, Judges 15:1-8). In 1943, the American and British Royal Air Forces used saturation fire bombing to create cataclysmic fire storms in Hamburg, Germany (Middlebrook, 1981). The successful intent was not only to destroy military targets, but to demoralize the German population. In a similar manner, incendiaries today may use wildfire as a weapon against landowners to redress real or perceived offenses. Incendiarism in response to changes in private land management practices is abundantly documented in Arkansas and elsewhere (Kluender *et al.*, 1988; Bradshaw and Huff, 1985; Bertrand *et al.*, 1970; Bertrand and Baird, 1975). Finally, some individuals set forest and open-land fires on their own and other's lands due to a misunderstanding of fire's ecological effects. Because many individuals who set fires are ignorant of fire behavior or over-estimate their ability to control fire, many fires escape control and culminate in wildfires. To fire-fighters, however, why fires were started makes little difference; smoke and heat have the same effect, regardless of ignition source. Fire-fighters and landowners are both apprehensive of wildfire due to its potential threat to life and property.

The number of wildfires and the frequency of incendiarism have been increasing in recent years (Kluender *et al.*, 1988; U.S. Forest Service, 1984). Despite active wildfire awareness campaigns, wildfire losses are increasing. Substantial losses continue to occur on both private and public ownerships. A complicating factor is that many rural southern communities tolerate and empathize with the acts of incendiaries (Bertrand *et al.*, 1970). When wildfire occurs, regardless of reason,

society ultimately loses through the direct and indirect costs passed back to the general public in insurance costs and increased taxes for protection.

In Arkansas the principal organization charged with wildfire suppression is the Arkansas Forestry Commission (AFC). The AFC is charged with wildfire detection and suppression as well as enforcement of the state's wildfire law (Act 85 of the 1935 Session of the Arkansas State Legislature, as amended). The AFC has collected information on individual wildfires since the 1930s.

This study was initiated to provide a better understanding of the extent and nature of incendiary wildfires in Arkansas. Specific objectives were to determine the patterns of incendiary occurrence in Arkansas and to identify emerging trends that may call for changes in forest use, fire use or fire management policy.

## METHODS

A data set, including the records of all reported wildfires on lands protected by the AFC in Arkansas for the period January 1983 through December 1987, was used (Arkansas Forestry Commission unpublished data). The individual fire records are developed from Individual Fire Reports (AFC Form 2410.1) which are filled out by suppression personnel immediately after a fire has been investigated. Information recorded for each fire included: year, county, origin (original fire or break-over), type of fire (forest, non-forest, or a mixture), day of the year, cause (e.g. incendiary, debris burning), who discovered the fire (aerial detection, tower or ground surveillance by the AFC, or the public), how the fire was extinguished (suppression, burn out, or rain out), acres burned and fire-danger class-day. Fire-danger class-day is an index of the total wildfire danger anticipated for an area and is rated on a scale of 1 to 5, with Class 1 indicating predictable fire behavior with little chance of spread and Class 5 extreme fire behavior with rapid spread. The data set included reports of 13,136 fires.

Analysis of the fire data was done using SPSS/PC+ (Norusis, 1988) and began with a multiple dimension frequency analysis. Because most variables were of a categorical nature, they were tested for significance using standard one-way and multi-way analysis of variance procedures against the principal dependent variables of interest (e.g. forest

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hectares burned). Additionally, testing of categorical variables against each other for significant association was done using Chi-square contingency tables. Statistical significance was accepted at the .05 level.

RESULTS AND DISCUSSION

Total wildfires increased from 1984 through 1987, with substantial rises in 1986 and 1987 (Table 1). Of greater importance, however, is that while the total burned area decreased from 1983 to 1985, it increased sharply during 1986 and 1987. Of the total area burned, the greatest proportion was consistently forested land (84%). Additionally, the average forested area burned per fire remained relatively constant (5 ha or 12 ac burned). Seventy-five percent of all fires were less than 4.2 ha (10 ac) in size, and 98% of all fires were 42 ha (100 ac) or less. The largest fire was 900 ha (2160 ac).

Table 1. Incendiary wildfires by property ownership and local residents in Arkansas, 1983-1987.

|                                                    |    | 1983   | 1984   | 1985  | 1986   | 1987   | MEAN   |
|----------------------------------------------------|----|--------|--------|-------|--------|--------|--------|
| All Lands                                          | N  | 2,185  | 1,650  | 1,981 | 3,170  | 4,150  | 2,627  |
|                                                    | Ha | 12,873 | 11,112 | 7,683 | 22,040 | 27,146 | 16,171 |
| Nonindustrial Private Lands                        | N  | 71%    | 70%    | 66%   | 69%    | 61%    | 66%    |
|                                                    | Ha | 73%    | 69%    | 64%   | 64%    | 58%    | 64%    |
| Industrial Lands                                   | N  | 26%    | 22%    | 29%   | 25%    | 32%    | 28%    |
|                                                    | Ha | 20%    | 21%    | 31%   | 30%    | 29%    | 27%    |
| Other Lands <sup>1</sup>                           | N  | 3%     | 8%     | 5%    | 6%     | 7%     | 6%     |
|                                                    | Ha | 7%     | 10%    | 5%    | 6%     | 13%    | 6%     |
| <b>Incendiary Fires</b>                            |    |        |        |       |        |        |        |
| All Lands                                          | N  | 870    | 638    | 848   | 1,558  | 2,233  | 1,229  |
|                                                    | Ha | 4,096  | 3,996  | 4,396 | 4,996  | 5,496  | 4,796  |
| Nonindustrial Private Lands <sup>2</sup>           | N  | 535    | 392    | 445   | 911    | 1,123  | 681    |
|                                                    | Ha | 5,789  | 4,422  | 2,357 | 8,049  | 9,703  | 6,064  |
| Industrial Lands <sup>2</sup>                      | N  | 280    | 168    | 350   | 531    | 947    | 455    |
|                                                    | Ha | 1,252  | 1,442  | 1,439 | 5,195  | 5,856  | 3,036  |
| Ha/Fire                                            |    | 10.8   | 11.3   | 5.3   | 8.8    | 8.6    | 8.9    |
|                                                    |    | 4.5    | 8.6    | 4.1   | 9.8    | 6.2    | 6.7    |
| <b>Incendiarity by Local Residents<sup>3</sup></b> |    |        |        |       |        |        |        |
| All Lands                                          | N  | 662    | 451    | 576   | 1,146  | 1,692  | 905    |
|                                                    | Ha | 72%    | 71%    | 68%   | 74%    | 76%    | 74%    |
| Nonindustrial Private Lands <sup>4</sup>           | N  | 386    | 277    | 294   | 667    | 815    | 488    |
|                                                    | Ha | 4,551  | 3,542  | 1,651 | 6,102  | 7,048  | 4,579  |
| Industrial Lands <sup>4</sup>                      | N  | 200    | 112    | 237   | 385    | 755    | 338    |
|                                                    | Ha | 71%    | 67%    | 68%   | 72%    | 80%    | 74%    |
| Ha/Fire                                            |    | 902    | 1,072  | 872   | 4,211  | 4,446  | 2,300  |
|                                                    |    | 72%    | 74%    | 61%   | 81%    | 76%    | 76%    |

<sup>1</sup> Mostly state ownerships, federal ownerships are not included in the data set.

<sup>2</sup> Proportions calculated using wildfires from the corresponding land ownership.

<sup>3</sup> A person who lives in the area but did not start the fire on their own land.

<sup>4</sup> Proportions calculated using incendiary fires from the corresponding land ownership.

These trends in wildfires were influenced in part by weather factors. Statewide, precipitation was usually below average monthly levels during the spring fire season (January through June), but variable and slightly above average during the fall fire season (July through December) (National Climatic Data Center, 1987). As an example, the fall of 1984

was exceptionally wet. The residual spring moisture in 1985 combined with a second abnormally wet fall accounted for the lower total area burned in 1985. The years 1986 and 1987 were opposites; 1986 had a dry spring and moist fall while the spring of 1987 was normal and the fall very dry, until December when precipitation increased dramatically.

SEASON, FIRE-DANGER CLASS-DAY AND DISCOVERED BY

Three percent of the incendiary fires occurred on Class-1 days, 27% on Class-2 days, 52% on Class-3 days, 13% on Class-4 days, and 5% on Class-5 days. These numbers were not significantly different from the proportions for all other wildfire causes, suggesting that incendiary fires do not select days with more severe fire-danger to set fires. Seasonally, 64% of all incendiary fires occurred during the spring fire season (January through June); 36% occurred during the fall fire season (July through December). Again, these proportions were not significantly different from those for all other causes. Sixty-three percent of the Class-1 and 53% of the Class-2 wildfires occurred days during the fall fire season. However, 64% of the Class-3, 77% of the Class-4, and 88% of the Class-5 day wildfires occurred during the spring season. In the spring, fuels are cured and winds are higher. Therefore, if a fire does occur, it will spread more rapidly, cause more damage, and cost more to suppress.

Significant differences were found among whom wildfires were detected by and their ignition source. The general public detected 56% of the incendiary fires and 66% of the other fires, while organized detection units such as the AFC and active patrols by industrial employees discovered 44% of the incendiary fires and 34% of the others. This information suggests the general public is more likely to report fires started by debris burning and other such causes, probably because these fires are more apt to occur on lands close to or used by them.

CAUSES OF AND RESPONSIBILITY FOR WILDFIRES

Incendiarism was consistently the principal cause of wildfires, averaging 47% of the fires and 64% of the area burned. The second most frequent cause was debris-burning fires that escaped control, averaging 27% of the fires and 18% of the area burned. Other identified causes were smokers (5.6%) equipment use (3.9%), children (2.5%), railroads (2.3%), lightning (2.2%), and campfires (1.4%). A miscellaneous category accounted for 8.7% of all wildfires. Wildfire statistics for the five-year period 1983-1987 in the southern U.S. showed incendiarism averaged 44% and debris burning 30% of the wildfires (U.S. Forest Service, 1988). In this respect, Arkansas differs little from the southwide average for 1983 to 1987.

However, our data show that the number of incendiary fires, the area burned, and their corresponding proportions are escalating (Table 1). The percentage of incendiary fires increased from 40% in 1983 to 54% in 1987. Similarly, the area burned by incendiary fires also increased from 60% in 1983 to 69% in 1987, with 1985 an exception due to the moist conditions.

Probably no trend in landowner/public relations concerns forest managers as much as the rising trend of incendiarism. Of the increase in wildfires from 1984 through 1987, 64% (1,595) were incendiary. Further, in the period 1986 to 1987, there was an increase of 980 total wildfires, 69% (675) of which were incendiary. Of these incendiary fires, 93% (628) were on nonindustrial private (212) and industry lands (416); 82% (518) of these fires were incendiarism by local residents (a person who lives in the area but did not start the fire on their own land).

Nonindustrial private landowners received the brunt of the incendiary fires in terms of number of fires and area burned (Table 1). However, as a proportion of wildfires on nonindustrial private lands, incendiary-caused fires were much less the similar percentages for industrial lands. Additionally, the average size of fires is higher on nonindustrial private lands than on industry lands.

Industrial landowners increasingly appear to be the target of incendiary fires, as the proportion of incendiary fires and area burned on industry lands increased with time. Moreover, proportionately more



fires on industry land are incendiary (average of 62% on industry vs. 39% on nonindustrial private), while incendiary and debris burning share the load on private land (debris burning averaged 14% on industry vs. 33% on nonindustrial private).

Local residents were responsible, on average, for 74% of all fires and 76% of the area burned by incendiary fires (Table 1). The proportions of local-resident caused incendiary fires and the area burned were similar on nonindustrial private and industrial lands, suggesting local residents showed little preference for land ownership when starting these fires.

#### IMPLICATIONS FOR PREVENTION

Wildfire investigation and enforcement is an assigned role of AFC District Foresters and Rangers who shoulder this responsibility as well as landowner assistance. Suppression costs are levied and collected from landowners who lose control of a fire. Additionally, active cases are developed on 15% of all fires each year.

Generally, enforcement activities, including investigation, active prosecution, conviction and settlements, are designed to reduce incendiary activities. When adequate evidence exists, criminal charges are filed on incendiary cases, with the burden of proof resting on the AFC. Upon conviction, damage awards may include suppression cost, property damages, court costs, fines and incarceration.

During the calendar year 1987 the courts convicted two incendiary cases (Arkansas Forest Commission, unpublished data). Each conviction resulted only in fines, court costs and suspended jail sentences.

Thus, little in the way of punitive deterrence awards was levied on these known incendiary cases. With 2,233 suspected cases of incendiary wildfire in Arkansas in 1987, this conviction rate underscores the difficulty of deterring incendiary by using law enforcement.

Donoghue *et al.* (1986) studied the potential for reducing incendiary in Arkansas based on models developed South-wide. The general relationship found was one of sharp decreases in incendiary after initial enforcement activities followed by rapidly decreasing results to additional efforts. Since settlements related to negligent debris-burners represent the majority of the total enforcement effort in Arkansas, incendiary will probably continue their activities unchecked.

Enforcement alone is not sufficient to keep the number of incendiary fires at manageably lower levels. Brown and Davis (1973) discuss regulating the use of public and private lands, and public education as equally important components of prevention activities. These activities are designed to break the chain of incendiary. They list the components of an incendiary fire as 1) a person, 2) with a motive for burning, and 3) incendiary devices. Incendiary devices can be as simple as a match, or a can of gasoline with an ignition device. Except in more sophisticated forms, incendiary devices cannot be denied the general population. Regulation of public use of high hazard areas removes persons from incendiary targets. Closing national forests and state parks in times of extreme fire danger are examples of this type of control. Educational activities are generally aimed at changing the reasons for starting fires and defusing incendiary activities through local social pressure.

Clearly, given the number of yearly incendiary fires in Arkansas, and the recent increases in such fires, more effective long-term solutions

to incendiary need to be developed. Based on the Donoghue *et al.* (1986) model, enforcement benefit-cost ratios fall rapidly after initial levels. But, since it is also obvious that enforcement cannot be abandoned, determining how much incendiary reduction to buy through enforcement is crucial.

Restricting use of forest land is an effective deterrent during intervals when high use corresponds with high fire danger (e.g. high fire-danger concurrent with fall hunting season). However, on nonindustrial private or industrial lands this may be construed as threatening to those practicing free access to traditional hunting areas.

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# HEMOGLOBIN SUBUNIT-SUBUNIT AFFINITY-DETERMINANT OF HEMOGLOBIN FORMATION

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## ABSTRACT

Hemoglobin A<sub>2</sub> is often elevated in  $\beta$ -thalassemia and decreased in  $\alpha$ -thalassemia. This might be due to hemoglobin subunit-subunit affinity variation. It has been inferred from the study of abnormal hemoglobins that the  $\alpha$  subunits have higher affinity for  $\beta$  subunits than for  $\delta$  subunits. However, only in one study has the affinity of  $\alpha$ ,  $\beta$ , and  $\delta$  subunits for each other been measured. In this work we have attempted to measure the hemoglobin subunit-subunit affinity with somewhat different approach, i.e., hybridization of hemoglobin A and A<sub>2</sub>. It is shown that hybridization and recombination of equal amounts of hemoglobins A and A<sub>2</sub> lead always to the formation of more hemoglobin A than A<sub>2</sub>. Incubation of pure  $\alpha$ ,  $\beta$ , and  $\delta$  subunits forms more hemoglobin A than A<sub>2</sub> as the availability of  $\alpha$  subunits declines. It is concluded that hemoglobin  $\alpha$  subunits have approximately four-fold higher affinity for  $\beta$  subunits than for the  $\delta$  subunits under these experimental conditions. This subunit-subunit affinity difference, which has been attributed to the variation in molecular electrostatic charges, explains the variation of hemoglobin A<sub>2</sub> levels in thalassemia syndromes.

## INTRODUCTION

Hemoglobin (Hb) A<sub>2</sub> level is often elevated in patients with  $\beta$ -thalassemia. Although its level is expressed as a percentage of the total Hb (HbA + A<sub>2</sub>) which is variable in these patients, thence a high degree of apparent variability of HbA<sub>2</sub> level, the absolute amount of HbA<sub>2</sub> is, however, increased (Weatherall, 1972; Bunn *et al.*, 1977). In  $\alpha$ -thalassemia, on the contrary, the level of HbA<sub>2</sub> is often decreased (McCormack, 1980). Higher electrostatic attraction between  $\alpha$  and  $\beta$  subunits than between  $\alpha$  and  $\delta$  subunits has been suggested as a cause of this phenomenon (Bunn and McDonald, 1983; Mrabet *et al.*, 1986; Bunn, 1987). However, in only one study affinity between  $\alpha$  and  $\beta$  and  $\alpha$  and  $\delta$  subunits was measured (Martinez and Menendez, 1982). The present work was undertaken to measure directly the Hb subunit-subunit affinity *in vitro*.

## MATERIALS AND METHODS

Paramercuribenzoic acid (p-CMB) was obtained from Sigma Chemical Company, St. Louis, MO. Diethylaminoethyl cellulose (DE-52) was obtained from Whatman, Kent, England.

Hemoglobin A was purified from fresh human red cells by CM-Sephadex - ion-exchange chromatography (Winterhalter and Huehns, 1964).  $\alpha$  and  $\beta$  subunits were prepared by incubation of HbA with p-CMB according to the method described by Bucci and Fronticelli (1965). HbA<sub>2</sub> was prepared by DEAE-Sephadex - ion-exchange chromatography (Huisman and Dozy, 1962).  $\delta$  subunits were prepared according to the following procedure. HbA<sub>2</sub> was gel filtered against diluted tris-HCl buffer. Two percent p-CMB solution in 2M glycine-KOH buffer, pH 8 and MgCl<sub>2</sub> were added so that the final concentrations of p-CMB and MgCl<sub>2</sub> were 0.013 M and 0.3 M respectively. The pH was adjusted to 5.6 with 1.5 M solution of glacial acetic acid. The solution was incubated under carbon monoxide (CO) at 4°C for about seven days. The Hb solution was gel filtered first against 5 mM NaCl solution and then against tris-phosphate-KOH buffer and applied to a column of DE-52 equilibrated with the same buffer. The column was washed with the same buffer containing (0.3 per cent v/v) of 2-mercaptoethanol under CO. After the  $\alpha$  subunits and the non-reacted HbA<sub>2</sub> were eluted, the native  $\delta$  subunits were eluted with 0.3 M sodium phosphate, pH 7.4. The purity of all Hb subunits was tested by cellulose acetate electrophoresis (Marengo-Rowe, 1965).

## HYBRIDIZATION OF HEMOGLOBIN A AND A<sub>2</sub>

Pure hemoglobin A and A<sub>2</sub> samples were dialyzed overnight against distilled water at 4°C. The concentrations of both hemoglobin solutions were adjusted to about three percent. 0.2 ml of hemoglobin A solution was mixed with 0.2 ml of hemoglobin A<sub>2</sub>. One-half was kept as control and the other half was dialyzed against sodium acetate buffer at pH 4.7 and 4°C. Recombination was carried out by dialysis of the sample for about 20 hours against tris-EDTA-boric acid buffer, pH 9 at 4°C. The percentage of various hemoglobins was determined by cellulose acetate electrophoresis and quantitation of the different bands.

## RECOMBINATION OF HEMOGLOBIN SUBUNITS

Various amounts of hemoglobin subunits ( $\alpha, \beta, \delta$ ) in concentration of 0.2 mM were mixed in such a way that the final proportions of different subunits of the total mixture was as is shown in the following: a)  $\alpha = \beta = \delta = 33.3$  percent; b)  $\alpha = 50$  percent,  $\beta = 25$  percent,  $\delta = 25$  percent; c)  $\alpha = 20$  percent,  $\beta = 40$  percent,  $\delta = 40$  percent; d)  $\alpha = 10$  percent,  $\beta = 45$  percent,  $\delta = 45$  percent. The mixture a, b, c, and d was dialyzed against tris-EDTA-boric acid buffer, pH 9, in order for the subunits to recombine (Huisman, 1977). At different time intervals, small samples were taken and were subjected to electrophoresis on cellulose acetate strip in order to separate HbA from A<sub>2</sub>. In order to quantitate the separated hemoglobins, the bands were cut and inserted in buffer to elute the hemoglobin. The eluted hemoglobins were quantitated by determination of absorption of 420 nm.

## RESULTS

When hemoglobin A and A<sub>2</sub> were hybridized and the final percentage of hemoglobin A and A<sub>2</sub> were measured, it was found that the proportion of hemoglobin A is higher in all 19 experiments performed in spite of wide variability of the results (Fig. 1).

Since the total of hemoglobin A and A<sub>2</sub> recovered was taken as 100 percent in every experiment, the percentages in each experiment are mirror images. It should be mentioned that there was no visible precipitate in any of the hemoglobin solutions after the hybridization.

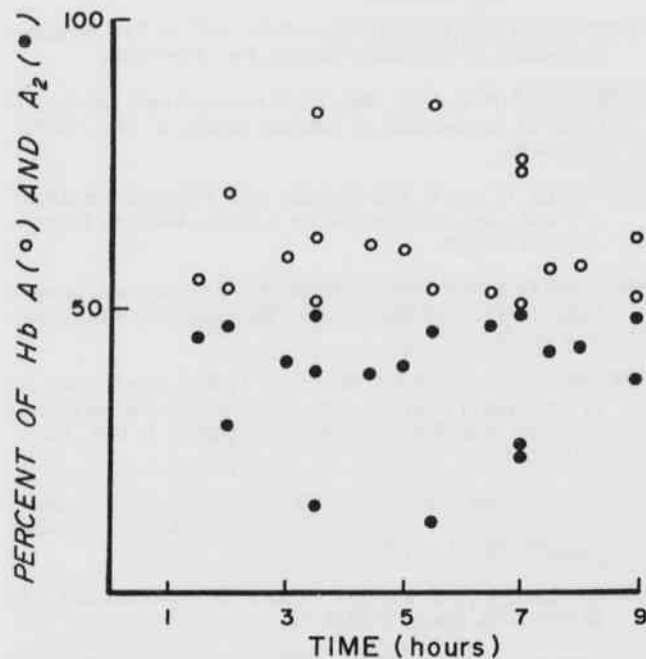


Figure 1. Separation and quantitation of hemoglobin A and A<sub>2</sub> by cellulose acetate electrophoresis after hybridization and recombination at various time intervals. The total quantity of hemoglobin A and A<sub>2</sub> is taken as 100 percent in every experiment.

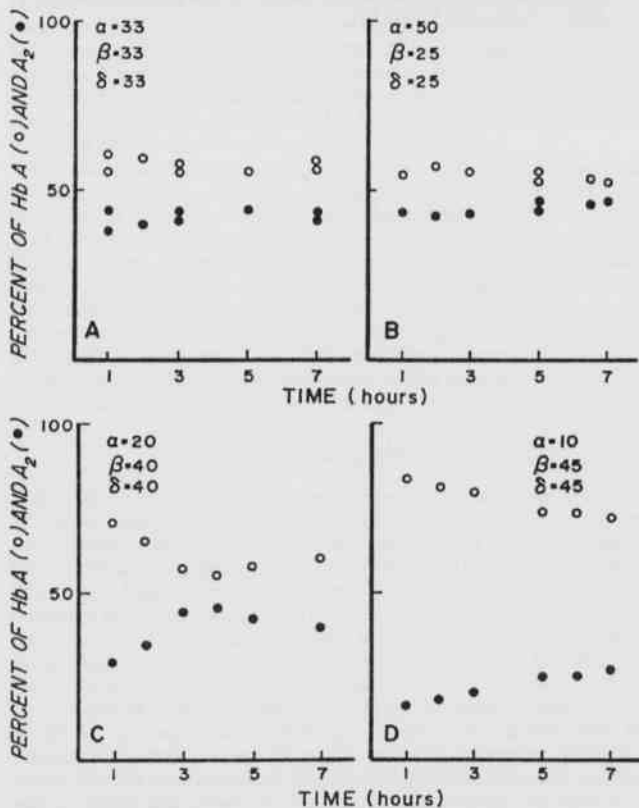


Figure 2. (A,B,C,D) Recombination of various hemoglobin subunits at different ratios of  $\alpha$ ,  $\beta$ , and  $\delta$  at various time intervals. Again, the total recovered hemoglobin A and A<sub>2</sub> were taken as 100 percent.

When isolated  $\alpha$ ,  $\beta$ , and  $\delta$  hemoglobin subunits were mixed and incubated in such a way that there were as many  $\alpha$  subunits as  $\beta$  and  $\delta$  subunits together, the proportion of HbA formed was somewhat higher than HbA<sub>2</sub> in all experiments (Fig. 2B). The reference point (100 percent) is derived by the addition of HbA and A<sub>2</sub> concentrations in each experiment. In this way the total sum of HbA and A<sub>2</sub> is always equal to 100 percent. This has the advantage that regardless of the concentration of subunits, the changes in the proportions of HbA and A<sub>2</sub> closely correspond to their synthesis. As the relative concentration of  $\alpha$  subunits to  $\beta$  and  $\delta$  subunits decreased, the proportion of HbA<sub>2</sub> decreased. In Figure 2A there is a slight deficiency of  $\alpha$  subunits. Where there were 33 percent of  $\alpha$  subunits, there was a tendency for slight decrease in HbA<sub>2</sub> formation. As the concentration of  $\alpha$  subunits decreased further, there was less and less HbA<sub>2</sub> formed (Fig. 2C,D). It should be noted that there was no appreciable loss or precipitation of subunits during the experiments. All solutions were visually clear and free from precipitates. The optical baseline also did not change significantly.

#### DISCUSSION

Hemoglobin A is a tetrameric molecule with two  $\alpha$  and two  $\beta$  polypeptide subunits which are usually synthesized in equal amounts in the red cell precursors. In  $\alpha$  and  $\beta$  thalassemias there is an excess of  $\beta$  or  $\alpha$  hemoglobin subunits respectively which precipitates and, through a complex mechanism, damages the cell membrane. One could expect that the absolute amount of hemoglobin A<sub>2</sub> should remain the same since there is a very small amount of  $\delta$  chains available. However, the amount of hemoglobin A<sub>2</sub> increases in most cases of  $\beta$  thalassemia and decreases in some cases of  $\alpha$  thalassemia (McCormack, 1980). Although this could be due to variation in synthetic rate of  $\delta$  chains, the experiments in this work suggest strongly that the  $\alpha$  subunits have a higher affinity for  $\beta$  subunits than for  $\delta$  subunits. This must play a significant role in the determination of HbA concentration *in vivo* similar to what has been reported by Shaeffer *et al.* in regard to HbA and S in patients with sickle cell trait (Shaeffer *et al.*, 1978). The magnitude of this affinity difference is not certain, but the results of experiments which are presented in Figure 2D where the concentration of  $\alpha$  subunits was the lowest suggest that the  $\alpha$  subunits have at least four-fold higher affinity for  $\beta$  than for  $\delta$  subunits. This is much lower than 7.5-fold difference shown by Martinez and Menendez (1982). It can be said that recombination properties of the isolated subunits might be different from those of the native Hb subunits. This might introduce errors in the results; however, it has been shown in the past that when Hb subunits are fresh and completely devoid of p-CMB, they behave in similar fashion as native subunits. Utmost care was exercised in these experiments to remove p-CMB completely from the subunits. Since the method of preparation of  $\delta$  subunits is different from that of  $\alpha$  and  $\beta$  subunits, it might bring an artifactual difference in the subunit affinity. This was probably not the case here because the  $\delta$  subunits are very stable and the presence of CO during the incubation confers to them more stability. One factor which has not been taken into consideration is the rate of monomerization of subunits which might affect rate of binding together. For example, the  $\beta$  subunits are in tetrameric form. In order that they combine with  $\alpha$  subunits, they have to monomerize. It is quite unlikely that *in vivo* the subunits are synthesized in the tetrameric form and then monomerize and combine with  $\alpha$  subunits. *In vivo*, the subunit synthesis and their combination to form hemoglobin are probably synchronized. Variation of affinity between various subunits has been attributed mainly to their charge difference, i.e., electrostatic attraction between relative positive and negative charges (Rahbar and Bunn, 1987). It has been inferred from the study of abnormal Hbs that the affinity of  $\alpha$  subunits is lower for  $\delta$  than for  $\beta$  subunits since  $\delta$  subunits have higher positive charge than the  $\beta$  subunits. In only one study the affinity between subunits has been measured. In the present study, we have demonstrated *in vitro* with a different approach that the higher affinity of  $\alpha$  subunits for  $\beta$  than  $\delta$  subunits might in fact be a major contributing factor in determination of the levels of Hbs A and A<sub>2</sub>. Whether the variation between Hb

subunit affinity is only due to variation in their electrostatic charges or whether other factors are involved is not known and needs further investigation.

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**ABSTRACT**

Tissue culture methods have been established to regenerate certain rice (*Oryza sativa* L.) cultivars, but regeneration of the rice cultivars widely grown in Arkansas has not been reported. This study has established an *in vitro* culture for the rice cultivars 'Nortai', 'Starbonnet', 'Mars', 'Tebonnet', 'Newbonnet', and 'Lemont'. Callus was induced in the dark at either 20 or 28 C from dehusked seeds cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 40 g L<sup>-1</sup> sucrose, 10 g L<sup>-1</sup> agar, 0.5, 1.0, or 2.0 mg L<sup>-1</sup> 2,4-dichlorophenoxyacetic acid (2,4-D) and adjusted to pH 5.7. After four weeks the calli were weighed, transferred onto MS medium containing no 2,4-D, and maintained in a 12-h photoperiod (65 uE m<sup>-2</sup> s<sup>-1</sup>) at 25 ± 2 C to induce plant regeneration. Callus production was best when cultured on a medium containing 1.0 mg L<sup>-1</sup> 2,4-D and incubated at 28 C. Plant regeneration was observed two to four weeks later. The percentage of calli regenerating plantlets varied with the cultivar and the callus induction treatment. Callus induction at 20 C on a medium with a 2,4-D level less than 2.0 mg L<sup>-1</sup> enhanced the regenerability of most cultivars. Regenerates were transplanted to soil and grow normally to maturity. This system can be helpful in improving rice cultivars with tissue culture techniques such as somaclonal variant selection and somatic hybridization.

**INTRODUCTION**

Improvement and development of superior crops to meet the needs of the world's growing population for food, fiber, fuel, medicine, and aesthetics requires the implementation of new as well as traditional techniques (Green, 1981). Rice (*Oryza sativa* L.) is the basic food of more than half of the world's population (Pathak, 1982). Traditional plant breeding has contributed greatly to the improvement of many rice varieties. Plant tissue culture, a technique of growing plant tissues *in vitro* in a nutrient medium under aseptic conditions, is an economical means for vegetative propagation that is becoming increasingly important as a tool in plant breeding.

Mutant selection, anther and pollen culture, and somatic hybridization are techniques that may be useful in rice improvement. The feasibility of these approaches is solely dependent upon the availability of a tissue culture system for the regeneration of a particular cultivar. Systems for *in vitro* regeneration of a number of rice cultivars have been established (Heyser *et al.*, 1983; Oard and Rutger, 1988; Raghava and Nabors, 1984; Siriwardana and Nabors, 1983); however, cultivars that are widely grown in Arkansas have not been included in these studies. Since genotypes differ in their ability to regenerate, our aim was to investigate suitable culture systems for the regeneration of several rice cultivars that are grown in Arkansas.

**MATERIALS AND METHODS**

Dehusked seeds of the cultivars 'Nortai', 'Starbonnet', 'Mars', 'Tebonnet', and 'Newbonnet' (cultivars developed by USDA/ARS and the Arkansas Agriculture Experiment Station), and 'Lemont' (a semi-dwarf cultivar developed by the USDA-ARS and the Texas Agriculture Experiment Station) were surface-sterilized in 95% ethanol for 30 sec, immersed in 2.6% hypochlorite for 20 min, and rinsed three times with sterile water. The mature embryos were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 0.4 mg L<sup>-1</sup> thiamine HCl, 100 mg L<sup>-1</sup> inositol, 40 g L<sup>-1</sup> sucrose, and 0.5, 1.0, or 2.0 mg L<sup>-1</sup> 2,4-dichlorophenoxyacetic acid (2,4-D). The medium was adjusted to pH 5.7 and solidified with 10 g L<sup>-1</sup> agar. The cultures were incubated in the dark at either 20 or 28 C for callus induction. After four weeks the calli were separated from the original explants and weighed with a digital microscale. To induce morphogenesis, the calli were transferred to MS medium without 2,4-D. The cultures were maintained at 25 ± 2 C in a 12-h photoperiod of cool-white fluorescent light (65 uE m<sup>-2</sup> s<sup>-1</sup>).

A three-factor factorial experimental design with 10 seeds per treatment was used to investigate temperature, hormone concentration, and cultivar response. The efficacy of the treatments was based on callus fresh weight and subsequent plant regeneration frequency. Regenerated plants were transplanted to potting soil, acclimated, and then transferred to a greenhouse.

**RESULTS AND DISCUSSION**

**CALLUS INDUCTION**

One week after initial incubation, callus growth was visible. Callus originated from the region subtending the primary shoot that arises initially as the seed begins to germinate. Germination soon ceased, but the callus continued to proliferate (Fig. 1). Callus growth was influenced by incubation temperature and 2,4-D concentration; those grown at 28 C yielded 0.090 g, significantly more than the 0.048 g of those cultured at 20 C at all 2,4-D concentrations. Fresh weights of the calli incubated

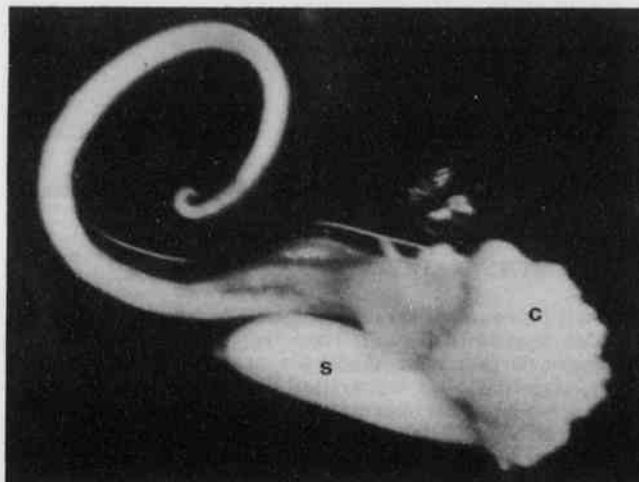


Figure 1. Rice callus (C) induction accompanied with seed (S) germination at initial stage.

## In Vitro Culture of Several Rice Cultivars

on the media with 0.5, 1.0, and 2.0 mg L<sup>-1</sup> of 2,4-D were 0.069, 0.086, and 0.052 g, respectively, at both temperatures. Calli induced by 0.5 and 1.0 mg L<sup>-1</sup> 2,4-D were not significantly different in weight, but those induced by 2.0 mg L<sup>-1</sup> 2,4-D were significantly smaller than calli induced by 1.0 mg L<sup>-1</sup>.

Callus growth differed significantly among the six cultivars (Table 1). Under the conditions of this experiment callus weight for Lemont was significantly less than that of all other cultivars. Furthermore, Nortai, and Starbonnet produced the most growth.

Table 1. The Response of Rice Cultivars to Callus Induction Treatments

| Cultivar   | Callus Weight (g) <sup>1</sup> |
|------------|--------------------------------|
| Nortai     | 0.091a <sup>2</sup>            |
| Starbonnet | 0.087a                         |
| Hars       | 0.076ab                        |
| Tebonnet   | 0.072ab                        |
| Newbonnet  | 0.057bc                        |
| Lemont     | 0.031c                         |

<sup>1</sup>Callus weight averaged over both induction temperatures (20 and 28 C) and 2,4-D concentrations (0.5, 1.0, and 2.0 mg L<sup>-1</sup>).

<sup>2</sup>Means followed by a common letter are not significantly different at 5% (LSD = 0.027).

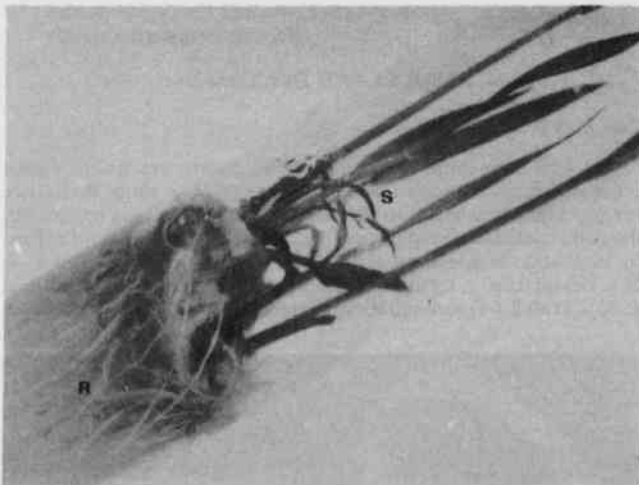


Figure 2. Plant regeneration from rice callus showing shoot (S) and root (R).

#### PLANT REGENERATION

Two to four weeks after callus-incubation on regeneration medium, plantlets regenerated (Fig. 2). These regenerated plantlets grew to maturity in the greenhouse. Shoot and root systems developed concurrently, suggesting plant regeneration via somatic embryogenesis, a phenomenon frequently observed in regeneration of monocotyledonous species (Nabors *et al.*, 1983). The percentage of calli that regenerated plantlets varied greatly depending on cultivar and callus induction treatments (Table 2). Generally, callus induction at the lower temperature of 20 C enhanced the regenerability of all cultivars except Starbonnet. Concentrations of 2,4-D in the callus induction medium lower than 2.0 mg L<sup>-1</sup> were more effective in producing regenerable calli.

Table 2. The Influence of 2,4-D Concentration, Callus Induction Temperature, and Cultivar on Regeneration Percentage.

| Cultivar   | % Plant Regeneration          |     |     |      |     |     |
|------------|-------------------------------|-----|-----|------|-----|-----|
|            | 20 C                          |     |     | 28 C |     |     |
|            | Concentration of 2,4-D (mg/L) |     |     |      |     |     |
|            | 0.5                           | 1.0 | 2.0 | 0.5  | 1.0 | 2.0 |
| Nortai     | 25                            | 0   | 0   | 0    | 0   | 0   |
| Starbonnet | 34                            | 25  | 0   | 67   | 25  | 0   |
| Hars       | 0                             | 25  | 25  | 0    | 0   | 0   |
| Tebonnet   | 40                            | 67  | 0   | 0    | 25  | 0   |
| Newbonnet  | 75                            | 50  | 34  | 0    | 0   | 25  |
| Lemont     | 20                            | 40  | 0   | 0    | 0   | 0   |

Cultivar response to callus induction did not correspond to plant regeneration. Nortai was superior in the amount of callus produced but showed the lowest plant regeneration frequency. A specific tissue culture system may be required not only for different species, but also for various cultivars of a single species. The necessity to define a unique set of conditions for the optimum culture of each rice cultivar was evident from the wide range in callus fresh weights produced from the six cultivars tested and from their differential responses in regeneration. Variability in callus weights, as well as in plant regeneration frequency among cultivars, could be attributed to genotypic variation.

#### CONCLUSIONS

This system demonstrates, for the first time, the suitability of these Arkansas and Texas rice cultivars for *in vitro* culturing. The regeneration of a given cultivar may be enhanced by particular modifications of the medium. This finding will allow the application of various tissue culture techniques to augment traditional breeding programs for rice improvement, such as the selection of favorable mutants at the cellular level.

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# SOIL MICROMORPHOLOGIC FEATURES OF HOLOCENE SURFACE WEATHERING AND A POSSIBLE LATE QUATERNARY BURIED SOIL, NORTHWEST ARKANSAS

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## ABSTRACT

Micromorphologic features of an alfisol developed in White River alluvium near Fayetteville, Arkansas are typical for this soil order. The A horizon has a relatively high organic matter content and an abundance of quartz sand grains with a silt and clay matrix. Voids are relatively common and some have been partly infilled. In contrast to the A horizon, the E horizon has less organic matter, larger voids, and some weak orientation of the clay matrix. The parent material for these horizons was deposited in the past 4,700 years and these pedologic horizons have formed since that time. In the underlying B horizon clay has accumulated in the form of grain coatings and caps and as void linings.

Translocation of clay into this horizon has relatively decreased the abundance of matrix silt and clay, and the amount of void space. The clay matrix that remains has extensively become oriented and some of the void space that remains is planar in shape. Both these features are partly responsible for the subangular blocky structure of this horizon. Deposition of this parent material began more than 8,000 to 10,000 years ago and was complete by 4,700 years ago. Many of the soil features have formed since 4,700 BP as the soil surface accreted upward.

The lower portion of the B horizon (2B) is developed in an older alluvial parent material, more than 10,000 years old. Some micromorphologic features suggest that the upper portion of this 2B horizon originally was an A/E horizon that has been modified after burial by subsequent weathering of the present ground soil. Some relict surface horizon features, such as relatively abundant voids, infilled vughs, and matrix, have persisted after burial. Other features characteristic of A horizons, such as organic matter, have been destroyed by oxidation. Many of the micromorphologic features in this 2B horizon have developed since burial, more than 10,000 years ago. Translocated clay features are abundant and partially mask the relict A/E horizon features. The lower part of the 2B horizon was a B horizon that continued to develop as a B horizon after burial. Translocated clay features are more abundant in this horizon than in the overlying relict A/E horizon.

## INTRODUCTION

Soil is formed by the interaction of climate, living organisms, parent material, and relief over a period of time (Harper *et al.*, 1969). The environment in which a soil is formed greatly affects the formation of that soil and its respective horizons and profile. The larger features are best seen and described in the field. Detail, significance, and interpretation of these features can be increased at a microscopic level of investigation called soil micromorphology. It is an invaluable tool for examining small-scale pedogenic processes and determining the sequence of those processes, using cross-cutting relationships and ghost structures.

The purpose of this study is first to megascopically and microscopically describe pedologic features of an alfisol developed in alluvial sediments. The second purpose is to interpret the genesis of these features. Finally, the maximum age of the features can be determined. From archaeological and sedimentologic information it is known that two stratigraphic layers are present and that the upper 75-90 cm has slowly accreted during the last 8,000 to 10,000 years (Guccione and Rieper, 1988). This aggradational environment may have affected soil formation by overthickening horizons, burying horizons, and/or overprinting new horizon features on older horizon features as the soil evolved.

## STUDY AREA

The study area is located along the White River near Fayetteville, Arkansas, in the south-central U.S. The White River Valley is located approximately four kilometers (2.5 miles) east of Fayetteville and includes a north-flowing river, a flood plain, and several terraces. Three branches of the White River head in the Boston Mountain Plateau to

the south and join to form a single channel just south of the study site (Fig. 1). At the study site, the White River makes a right-angle bend, changing from a north-flowing stream to an east-flowing stream (Fig. 1).

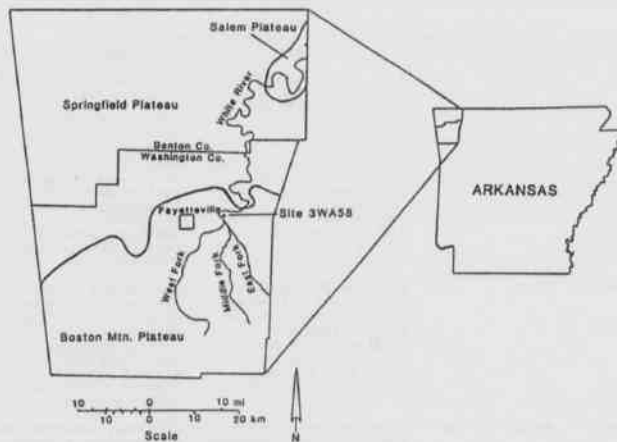


Figure 1. Location of study area.

Alfisols, ultisols, and mollisols are present in the White River Valley in the study area. Mollisols occur on the youngest and lowest part of the flood plain, alfisols occur on the older and higher part of the flood plain, and alfisols and ultisols occur on terraces. The Cleora (mollisol) and Razort (alfisol) soils are present on the flood plain at the study site. On the terrace, the moderately well-drained Savannah Soil, an

**Soil Micromorphologic Features of Holocene Surface Weathering and a Possible Late Quaternary Buried Soil**

ultisol, is developed in loam-textured overbank sediment and the underlying sandy clay loam-textured natural-levee sediment. Adjacent to the natural levee is a swale where the poorly drained Cherokee soil, an alfisol, is developed in silt loam and loam-textured distal overbank sediment. The paleochannel that was the source of the sediments was just west of the terrace margin.

The pedon used for this study was a Cherokee soil, which is classified as an alfisol (Harper *et al.*, 1969). Prominent subsurface zones of clay enrichment are formed by the accumulation of translocated clay from the horizons above. Translocation of the weathering products occurs during periods of moisture and precipitation of the weathering products occurs during alternating dry periods (Rust, 1983). The Cherokee soil is poorly drained, very slowly permeable, and develops on stream terraces (Table 1). It forms in alluvium and colluvium derived from acid sandstone, siltstone, and shale; and has a base saturation of 35-50 percent. Generally, the A horizon of the Cherokee soil is dark-gray (10YR 4/1) or dark grayish-brown (10YR 4/2) and the E horizon is light grayish-brown (10YR 6/2) or gray (10YR 5/1). Both have a silt loam texture and have a combined thickness of approximately 28 to 50 cm. The B horizon is mottled gray, brown, and yellowish-brown, plastic silty clay or clay and is 71 to 127 cm thick. Bedrock may occur at a depth of 152 to 365 cm (Harper *et al.*, 1969), but it was not penetrated in the 387 cm deep core used in this study.

**METHODS AND MATERIALS**

The pedon examined in this study was taken from a swale on a terrace at archeological site 3WA58 because soil descriptions, size analysis, radiocarbon dates, and archeological data were available (Lafferty *et al.*, 1988). A swale on the terrace was chosen to maximize the amount of sediment accumulation that occurred during soil formation. The site was cored using a truck-mounted Giddings coring device and the depth, thickness, and color of each horizon were noted (Table 1). Vertically-oriented samples of approximately 6 cm thickness were taken from each horizon at appropriate intervals. These samples were wrapped in cellophane and aluminum foil to ensure that the sample would remain moist and intact until the sample was impregnated with blue epoxy and a thin section was made. Eleven thin sections were analyzed using a petrographic microscope. Micromorphological features were point counted, using 100 points per slide.

Table 1. Cherokee Soil description.

| HORIZON | DEPTH (cm) | THICKNESS (cm) | DESCRIPTION                                                                                                                                                                         |                                                                                                                                                                                   |
|---------|------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|         |            |                | Field                                                                                                                                                                               | Micromorphology                                                                                                                                                                   |
| A       | 8-58       | 58             | Silt loam; dark yellowish brown (10YR 5/4); platy to medium moderate granular; few Mn coatings.                                                                                     | Granular, s-matrix of silt and clay; some vugh in-fillings; organic material; channels and vughs.                                                                                 |
| E       | 58-73      | 23             | Silt loam; dark yellowish brown (10YR 4/4) with common light gray (10YR 7/2) and a few yellowish red (5YR 5/8) mottles; weak coarse platy; Fe and Mn coatings.                      | Granular; s-matrix of silt and clay, uppermost presence of birefringence fabric (b-fabric); clay coatings; organic material; many large void spaces.                              |
| Bt1     | 73-99      | 26             | Loam; dark yellowish brown (10YR 4/4) with common coarse brownish yellow (10YR 6/8) reddish yellow (5YR 6/8), and light brownish gray (10YR 6/2) mottles; coarse subangular blocky. | Granular; s-matrix of silt and clay, some with b-fabric; large stained nodules; channel coatings; vugh in-fillings; deepest presence of organic matter; large voids, some planar. |
| Bt2     | 99-117     | 18             | Loam; yellowish brown (10YR 5/4) with abundant coarse reddish yellow (5YR 6/8) and grayish brown (10YR 5/2) mottles; medium subangular blocky.                                      | Granular; s-matrix, some with vesopic plasmic b-fabric; compound channel coatings, few vugh in-fillings; planar voids common.                                                     |
| Bt3     | 117-163    | 46             | Loam; yellowish brown (10YR 5/4) with coarse reddish yellow (5YR 6/8) and grayish brown (10YR 5/2) mottles; medium subangular blocky; Fe and Mn nodules.                            | Granular; s-matrix, some lattisepic b-fabric; compound vugh, grain, and chamber coatings, some Fe and Mn stains; grain and void coats; some vugh in-filling.                      |

|       |         |    |                                                                                                                      |                                                                                                                                       |
|-------|---------|----|----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| Bt4   | 163-283 | 68 | Dark yellowish brown (10YR 4/6) with some grayish brown (10YR 5/2) mottles; common Fe and some Mn nodules.           | Granular; s-matrix with b-fabric; abundant clay coats and some stress channel, vugh, and grain coats; few vughs and vugh in-fillings. |
| 2Bt1b | 283-288 | 77 | Dark yellowish brown (10YR 4/6) with indistinct light yellowish brown (10YR 6/4) mottles; common Fe and Mn coatings. | Granular; s-matrix with vesopic plasmic b-fabric; many compound void coats and some grain coats; few vughs and vugh in-fillings.      |
| 2Bt2b | 288-317 | 37 | Dark yellowish brown (10YR 4/4) with abundant coarse grayish brown (10YR 5/2) mottles; few Mn coatings.              | Granular; s-matrix with vesopic plasmic b-fabric; compound grain coats; vugh in-fillings.                                             |

**MICROMORPHOLOGY FEATURES**

The terminology applied to micromorphologic features in the literature is quite variable and the same terms are used in different ways by different researchers. To avoid confusion, the terms utilized in this research project are defined.

| TERM                     | DESCRIPTION                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| B-fabric                 | Birefringence fabric - fabric of the fine material in a soil thin section as evaluated by the pattern of birefringence of oriented clay grains (Fig. 8) (Douglas and Thompson, 1982).                                                                                                                                                                                                                                                                                                                    |
| Chambers                 | Spherical voids which are connected to channels and often contain faunal excrement (Kemp, 1985).                                                                                                                                                                                                                                                                                                                                                                                                         |
| Channels                 | Voids which may be linear but differ from planar voids in that they are generally cylindrical in three dimensions. Certain cross-sections are circular due to their tendency to change direction in relation to the thin section orientation. For this reason they are also frequently discontinuous in longitudinal extent (Fig. 2) (Kemp, 1985).                                                                                                                                                       |
| Clay Coatings            | Cutans, clay coats, argillans, etc. A modification of the texture, structure, or fabric at natural surfaces in soil materials due to concentration of particular soil constituents or in situ modification of the plasma. Cutans can be composed of any of the component substances of the soil material. Cutans are named according to the surfaces affected, composition and complexity of the cutanic material, and interpretation of the process of formation (Fig. 9) (Douglas and Thompson, 1982). |
| Coating                  | Layer of any substance covering a surface (Douglas and Thompson, 1982).                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Compound clay coatings   | Series of coatings of different composition around the same void or grain/aggregate surface (Douglas and Thompson, 1982).                                                                                                                                                                                                                                                                                                                                                                                |
| Grain coating            | Grain cutan - a cutan associated with the surface of a skeleton grain or other discrete unit (nodule, concretion, etc.) (Kemp, 1985).                                                                                                                                                                                                                                                                                                                                                                    |
| Grain capping            | Coating only on the upper surface of grains or aggregates (Kemp, 1985).                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Lattisepic plasma fabric | Plasma generally with a flecked orientation pattern that is, there are two short, discontinuous plasma separations usually oriented approximately at right angles to each other (Brewer, 1964).                                                                                                                                                                                                                                                                                                          |
| Mosepic fabric           | Abundant plasma separations with striated orientation that occur as isolated patches. These patches are unoriented with regard to one another (Brewer, 1964).                                                                                                                                                                                                                                                                                                                                            |



- Planar Voids Voids which are planar in three dimensions but appear linear and are continuous in thin section. They are of variable diameter and over long distances have common sharp changes in direction. They separate and occur within or across aggregates (Fig.8) (Kemp, 1985).
- Plasma That part of soil material which is capable of being or has been moved, reorganized, and/or concentrated by the processes of soil formation. It is a mobile, active part of the soil material. The plasma includes all the material, mineral or organic, of colloidal size and relatively soluble material which is not bound up in skeletal grains (Douglas and Thompson, 1982).
- S-matrix The material within the simplest (primary) ped or that composing apedal soil material in which pedological features occur. It consists of plasma that does not occur in pedological features. It does not include skeletal grains and voids.
- Vesicles Regular, smoothed voids which do not fit the criteria of any other types of void structures and may have equant, prolate, or oblate cross sections (Kemp, 1985).
- Vosepic Plasma that has a flecked orientation pattern, but plasma separations with striated orientation pattern occur parallel to adjoining natural surfaces. The striated orientation of the plasma separations is dominantly parallel to the walls of the voids, especially if they are planar (Brewer, 1964).
- Vugh infilling Soil material infilling a vugh (Figs. 6 and 10).

RESULTS AND DISCUSSION

MICROMORPHOLOGIC FEATURES

The A horizon is characterized by the accumulation of organic matter and the removal of unstable mineral material. In the Cherokee Soil the A horizon contains megascopic root fragments (Fig. 2), which are relatively abundant for an alfisol (6-8%) (Fig. 3). The mineral material is dominated by quartz mineral grains with a relatively large amount of silt and clay matrix (18%) (Fig. 4) and some iron staining. The matrix is light-colored, probably due to the partial removal of clay and Fe oxides. Many voids are present, including channels, vughs, and vesicles (Figs. 2 and 5). These form by burrowing of organisms and the formation of root pores. Some of the voids are subsequently infilled as bioturbation and pedoturbation continues, but the infilling preserves the void structure (Figs. 6 and 7). No translocated clay coatings are present (Fig. 8).

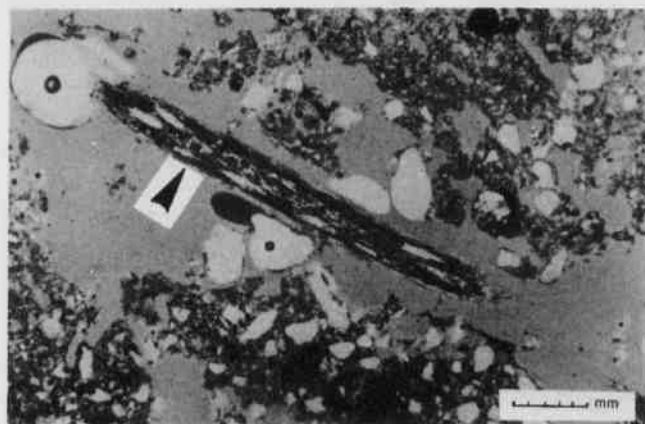


Figure 2. Plant root in channel, A horizon.

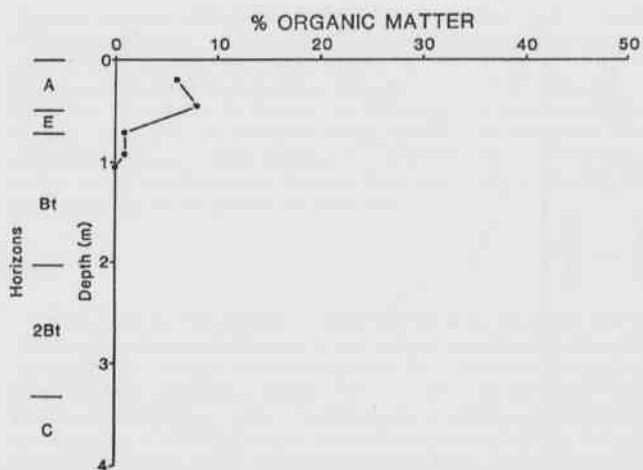


Figure 3. Graph of percent organic matter versus depth and soil horizon in the Cherokee pedon.

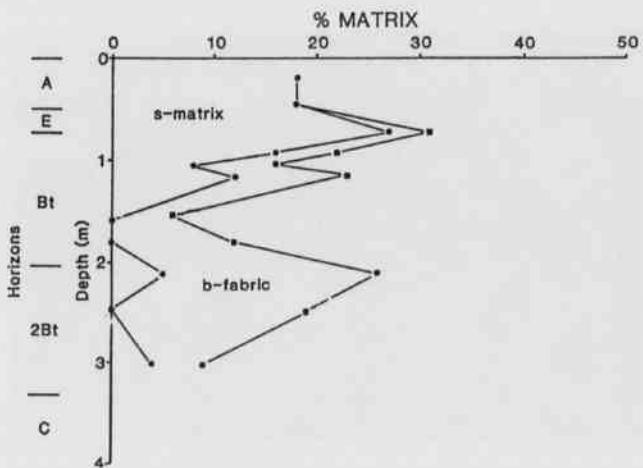


Figure 4. Graph of percents s-matrix and b-fabric versus depth and soil horizon in the Cherokee pedon.

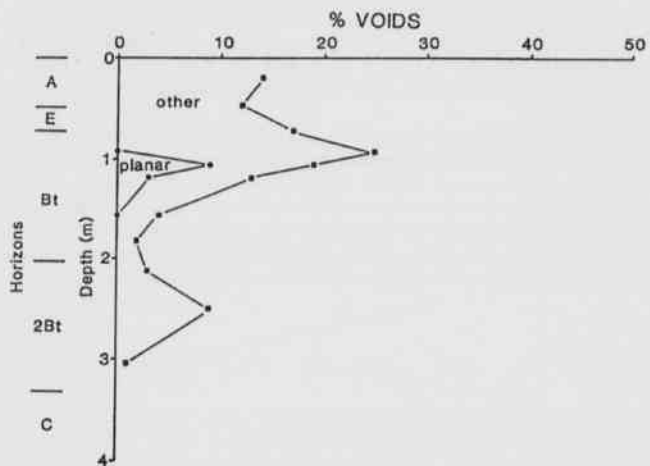


Figure 5. Graph of percents planar and other voids versus depth and soil horizon in the Cherokee pedon.

Soil Micromorphologic Features of Holocene Surface Weathering and a Possible Late Quaternary Buried Soil

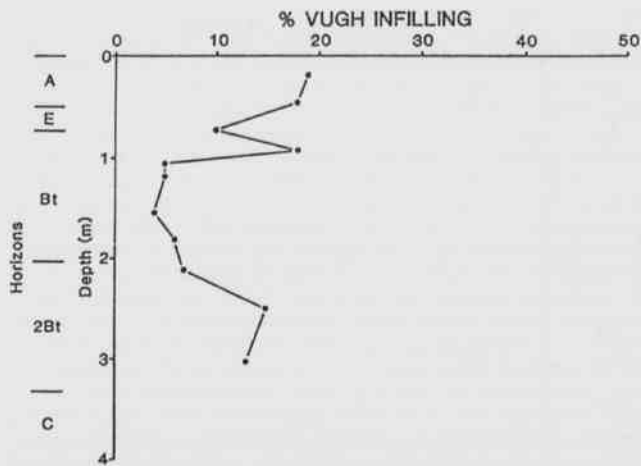


Figure 6. Graph of percent vugh infillings versus depth and soil horizon in the Cherokee pedon.

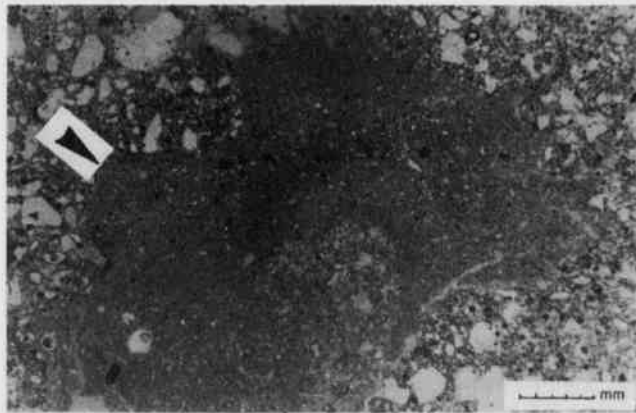


Figure 7. Vugh infilled with finer soil matrix, A horizon.

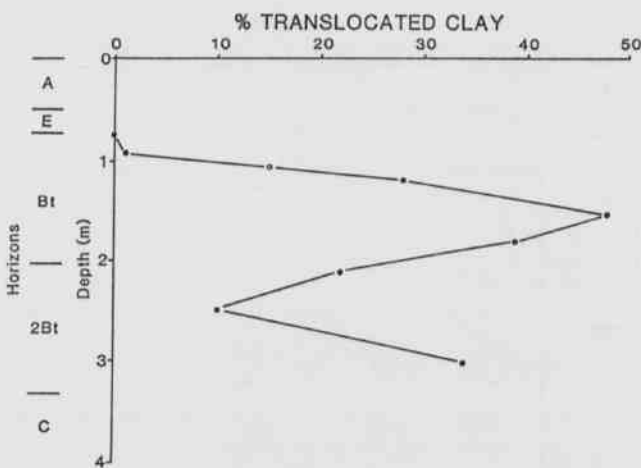


Figure 8. Graph of percent translocated clay features versus depth and soil horizon in the Cherokee pedon.

Like the A horizon, the E horizon is an eluvial horizon from which labile materials are removed, but unlike the A horizon it contains only a small amount (1%) of organic matter (Fig. 3) and the loss of clay and iron (Bullock and Thompson, 1985). The E horizon of this soil (Table 2) is also comprised of quartz grains within a very abundant silt and clay matrix (Fig. 4). Some of the clay in the s-matrix has become oriented forming birefringence fabric (b-fabric). Trace amounts of clay coatings, which become more abundant in the underlying B horizon, were noted (Fig. 8). Qualitatively, voids increase in volume and in size with depth, from the A to the uppermost part of the B horizon. Channels are the most abundant void structures preserved.

Table 2. Components of Cherokee Soil.

| HORIZON | DEPTH (cm) | GRAINS | MATRIX   |          |       | TRANSLOCATED CLAY | ORGANIC MATTER | VOIDS    |        |       | VUGH INFILLING |
|---------|------------|--------|----------|----------|-------|-------------------|----------------|----------|--------|-------|----------------|
|         |            |        | s-matrix | b-fabric | total |                   |                | channels | planar | total |                |
| A       | 14-23      | 43     | 18       | 8        | 18    | 0                 | 6              | 14       | 8      | 14    | 19             |
| A       | 45-51      | 45     | 18       | 8        | 10    | 0                 | 0              | 11       | 8      | 11    | 10             |
| E       | 67-75      | 41     | 27       | 4        | 21    | 0                 | 1              | 17       | 8      | 17    | 10             |
| Bt1     | 88-95      | 21     | 16       | 6        | 22    | 1                 | 1              | 27       | 8      | 27    | 18             |
| Bt2     | 103-107    | 43     | 8        | 8        | 14    | 15                | 0              | 13       | 9      | 21    | 5              |
| Bt3     | 118-124    | 21     | 13       | 11       | 22    | 28                | 0              | 18       | 3      | 13    | 5              |
| Bt3     | 134-138    | 38     | 8        | 6        | 6     | 48                | 0              | 4        | 8      | 4     | 4              |
| Bt4     | 174-183    | 48     | 8        | 13       | 13    | 39                | 0              | 2        | 8      | 3     | 4              |
| 2Bt2b   | 208-215    | 43     | 5        | 21       | 24    | 22                | 0              | 3        | 8      | 3     | 7              |
| 2Bt2b   | 244-252    | 45     | 8        | 18       | 18    | 13                | 0              | 9        | 8      | 9     | 15             |
| 2Bt2b   | 289-287    | 43     | 4        | 5        | 9     | 24                | 0              | 1        | 8      | 1     | 12             |

The B horizon is an accumulation of the weathered materials that have been removed from the overlying horizons. Processes that vertically transport clay dominate over processes that mix horizons or destroy clay (Bullock and Thompson, 1985). In this soil the B horizon has been subdivided into four horizons: Bt1, Bt2, Bt3, and Bt4. Only a minor amount of organic material (1%) is present in the uppermost Bt1 horizon and is comparable to that in the E horizon (Fig. 3). All of the horizons have quartz grains with a silt and clay matrix. This matrix is less abundant than that in the overlying E horizon and it decreases with depth (Fig. 4). However, the amount of matrix with b-fabric (Fig. 9) is greater than that in the E horizon, and it increases slightly with depth. Because the B horizon is the zone of clay accumulation, the amount of translocated clay features is relatively great (Fig. 8). It increases from a minimal 1% at the top of the horizon to 48% in the middle of the horizon and then decreases in the lower part of the horizon. These translocated clay features occur as grain and void coats and as grain caps. Compound clay coats (Fig. 10) are most abundant in the same horizons that contain the most translocated clay (Table 2). Channels and planar voids (Fig. 9) are present in the upper and middle part of the B horizon, but at depths where translocated clay increases, the abundance of voids and infilled vughs decreases (Figs. 5 and 6). Iron and manganese staining is also abundant in the central part of the B horizon.

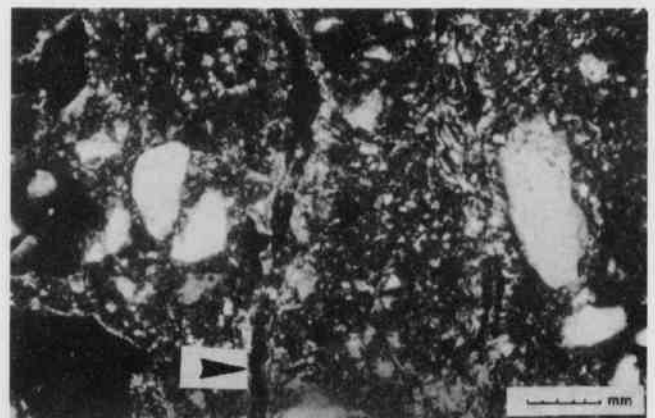


Figure 9. Planar void and b-fabric, Bt2 horizon.

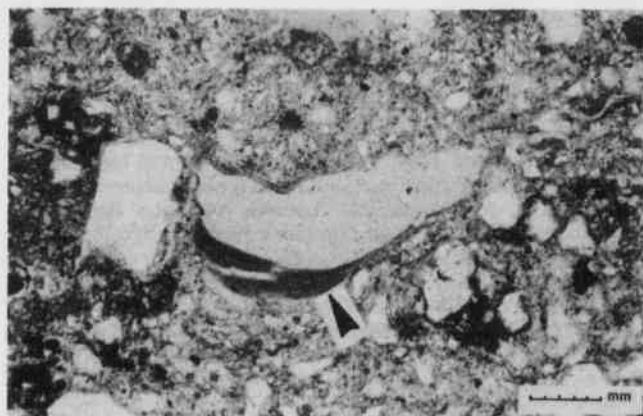


Figure 10. Compound clay coating in void, Bt1 horizon.

The lowest portion of the soil that was sampled is formed in a second parent material (2Bt1 and 2Bt2). This may be the upper part of a buried soil that has been welded to the overlying soil developing at the surface today. No megascopic organic material was present (Fig. 3). Many features in the 2Bt horizons are similar to the B horizons above. For example, translocated clay features are present in both the Bt and the 2Bt horizons, but the amount in the upper part of the 2Bt horizon is considerably less than that in the overlying Bt horizon (Fig. 8). The amount of total matrix, most of which has a b-fabric, is greatest in the upper portion of the 2B horizon (Fig. 4). This amount is comparable to that in the surface E horizon and is considerably more than that in the overlying Bt horizon. It decreases with depth in the 2B horizon to an amount comparable to that in the overlying B horizon. Voids and vugh infillings (Figs. 5, 6, and 11) increase in abundance in the 2B horizons compared to that in the Bt horizons, and are almost as abundant as in the surface A and E horizons.

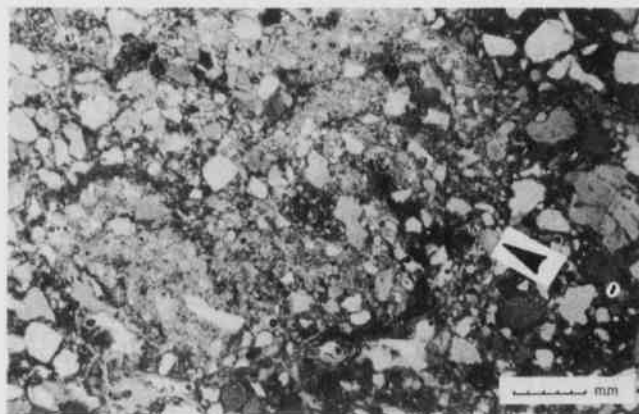


Figure 11. Vugh infilling, 2Bt1b horizon.

#### BURIED SOIL?

Soil micromorphologic evidence supports the hypothesis that the lower parent material was weathered prior to its burial by the upper alluvium. The abundance of matrix, voids, and vugh infillings in the upper part of the 2Bt horizon increases to values approaching that found in the A and E horizons. Conversely, the abundance of translocated clay features that are dominant in B horizons, are reduced in abundance in the upper part of the 2Bt horizon. This suggests that the material was originally an A/E horizon of a soil. Some of the micromorphologic

features characteristic of an epipedon (voids, infilled vughs, matrix) have been preserved, whereas other features (organic matter) have been destroyed. Subsequent burial and weathering of the present ground soil has superimposed some B-horizon features on the buried epipedon. In the lower part of the 2Bt horizon the abundance of translocated clay features increases and the abundance of features associated with epipedons decreases. This suggests that the horizon was a B horizon before burial and B-horizon features have continued to develop due to weathering of the present ground soil.

#### AGE

The age of the soil parent material examined in this study can be determined using stratified organic and archeologic material. Charcoal from a Middle Archaic cultural feature in the E horizon at a depth of 60 cm has been radiocarbon dated 4,700  $\pm$  100 years BP (Beta-19892) (Guccione and Rieper, 1988). Therefore, the sediment accumulated at a mean rate of 0.013 cm/year and soil micromorphologic features observed in the A and E horizons have developed in less than 4,700 years. Artifacts present at depths of 75-90 cm have been identified as Early Archaic projectile points which are thought to have been in use 8,000 to 10,000 years ago. Therefore, soil micromorphologic features in the lower E and upper B horizons have developed in less than 10,000 years. Because the sediment has slowly accreted during the Holocene, the B horizon features are probably younger than 10,000 years. If 30 cm of parent material had to accumulate before this parent material was buried deeply enough to develop B-horizon features, the translocated clay features would also have developed in less than 4,700 years.

#### SUMMARY

The micromorphologic features observed in this study are consistent with those expected in an alfisol (Figures 3, 4, and 6). The presence of organic material and relatively large voids and the absence of clay coatings in the upper three samples is characteristic of an ochric epipedon. The lack of organic material and abundance of translocated clay features in the middle five samples of the solum are characteristic of an argillic horizon. Both ochric epipedons and argillic horizons such as these are present in alfisols.

The evidence for a buried soil in this pedon is suggestive but is not conclusive. Soil micromorphologic features that are distinctive of surface horizons and have persisted after burial and are evidence for a buried soil. It is hypothesized that the upper 2B horizon was originally an epipedon that developed when alluviation temporarily ceased. This A/E horizon was later buried when alluvial deposition reoccurred. The present ground soil developed in this younger deposit and into the underlying buried soil. Clay was subsequently translocated into the buried A horizon by modern soil-forming processes. Although the A horizon was modified to a B horizon, not all of the original A horizon features, such as relatively abundant voids, infilled vughs, and s-matrix, were destroyed.

This hypothesized buried soil formed more than 8,000 years ago and was buried by younger overbank sediments, of which the upper 90 cm were deposited in the last 8,000 to 10,000 years and the upper 60 cm were deposited in the last 4,700 years (Guccione and Rieper, 1988). Slow aggradation during the Holocene has caused the modern soil horizons to become overthickened. Most, if not all, of the pedologic features in the upper 90 cm of the Cherokee soil have developed in less than 4,700 years.

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# CHARACTERIZATION OF RICE (*ORYZA SATIVA* L.) ROOTS VERSUS ROOT PULLING RESISTANCE AS SELECTION INDICES FOR DRAUGHT TOLERANCE

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## ABSTRACT

A technique described as Root Pulling Resistance (RPR) was used to evaluate genotypic differences in root growth and development of 50 rice germplasm accessions and cultivars. Several root characteristics in rice are associated with drought tolerance and avoidance capability of plants. The RPR measurements showed a significant positive correlation with maximum root length ( $r = 0.69$ ), root thickness ( $r = 0.75$ ), branching number ( $r = 0.75$ ), and root dry weight ( $r = 0.82$ ). Rice genotypes that had a high RPR value were identified as having longer, thicker, and denser root systems. The data indicated that high RPR measurements are strongly correlated with greater root penetration. Munji Sufaid Pak, IR 52 (IR5853-118-5) and Saunfia or Mabla Pak 329 had a significantly greater root length, root thickness, root number, root branching and dry weight as compared to IR 36. Also, there was no correlation between plant height and RPR. Furthermore, the data demonstrated that the RPR technique is ideal for selecting superior root systems and potential drought tolerant rice germplasm and cultivars.

## INTRODUCTION

Arkansas leads the nation in rice production, producing 41.0 percent of the rice in the United States. In 1987, over one million acres of rice was grown in Arkansas, with an average yield of 116 bu/acre (Arkansas Agricultural Statistical Service, 1987). The net returns per acre for rice is higher than for other major field crops such as soybean, wheat, sorghum, and cotton; however, the cost of rice production is also higher. Irrigation expenses account for approximately 15 percent of the total rice production cost. Rice utilizes a greater amount of irrigation water than any other crop in Arkansas, and that water is taken from the declining ground waters of the Quaternary Aquifer (East Arkansas Water Conservation Project, 1985).

Rice is economically and nutritionally an important crop in the world. Over half of the world's rice is grown under rainfed conditions; therefore, drought often has a major impact on yield (O'Toole and Soemartono, 1981). There has been only a limited amount of research conducted on evaluating cultivars for drought resistance although the importance of rice is appreciated worldwide (Hargrove and Cabanilla, 1979). Several researchers (O'Toole and Moya, 1978; Hasegawa, 1963; Parao *et al.*, 1976, 1977; IRRI, 1978; Ekanayake *et al.*, 1986) have reported that drought tolerance in rice is correlated with low transpiration rate and that rooting patterns are important in determining the degree of tolerance of rice to water stress. Researchers (O'Toole and Soemartono, 1981; Ekanayake *et al.*, 1986) have demonstrated the effectiveness of a technique which involves the measurement of root pulling resistance (RPR) among rice cultivars. This method evaluates the relative rooting efficiency. Root pulling resistance is the vertical force required to pull a plant from the soil and this technique has been widely used to assess the nature of root development in cereal crops (Nass and Zuber, 1971; Arihara and Crosbie, 1982).

The objectives of this study were to: a) determine the RPR and root characteristics of 50 germplasm accessions and cultivars, b) compare RPR at different growth stages of plant development, c) determine correlations between RPR and several root characters and d) select genotypes that have the highest RPR for field evaluation to determine their drought tolerance.

## MATERIALS AND METHODS

Fifty rice germplasm accessions and cultivars obtained from the USDA-ARS Rice Research and Extension Center at Stuttgart, AR were selected for this study. Two experiments were conducted at the University of Arkansas Pine Bluff Experimental Farm during the 1988 cropping season (June-October). The first experiment, seeded June 2, 1988,

received the normal flood irrigation and 56 kg N/ha as urea at pre-flood or when the rice seedlings had 4-5 true leaves. Twenty-nine additional kg N/ha as urea was applied when the internodes of Newbonnet elongated to about 10 mm, and another 29 kg N/ha were applied 10 days later. Experiment II, seeded June 7, 1988, was only irrigated prior to July 1 to assure seedling emergence, and was not irrigated after July 1, 1988; and the second experiment was fertilized only at pre-flood at the same rate as the irrigated experiment. The test was conducted on a Calloway silt loam. The 50 entries in each experiment were planted in a randomized complete block design with four replicates. The entries were seeded in 20 hills about 0.5 m apart in two rows. Plot size was 0.5 m X 4.0 m. The seedlings were thinned to one plant per hill at about the third true leaf stage.

The RPR was measured 30 and 60 days after 90 percent emergence. The seedlings had approximately 4-6 true leaves 30 days after emergence, and the plants were beginning the reproductive stage at 60 days after emergence. A clamp apparatus attached to a spring balance was used to pull the plants from the soil. This technique has been described in detail by O'Toole and Soemartono, 1981; and Ekanayake *et al.*, 1986.

A subsample of four plants in each replication was subsequently washed and taken to the laboratory where root characteristics were recorded. Maximum root length (cm) was measured as the longest root attached to the pulled plant. Only roots thicker than 1 mm were recorded as thick roots. A scale of 1 to 5 was used to rate root branching. One accession that produced almost no root branching (Pak-34) and another accession that had excessive root branching (Munji Sufaid Pak) were used to develop visual ratings of 1 and 5, respectively. These accessions were then used as standards to determine the root branching scores for the other accessions: 1 = almost no branching, 2 = root branching greater than 1 but less than the mean of 1 and 5, 3 = moderate branching or a mean visual branching between the root branching of Munji Sufaid Pak and Pak-34, 4 = root branching greater than three but less than five and 5 = excessive branching. Root dry weight in grams was recorded for each entry.

Analysis of variance and mean comparisons by LSD was conducted for the RPR measurements and root characteristics. Correlations among the traits were calculated using simple correlation analysis.

## RESULTS AND DISCUSSION

Plant height and days to 50% heading of five accessions with high, intermediate and low RPR are described in Table 1. Three of the accessions originated in Pakistan (Munji Sufaid Pak, Saunfia or Mabla Pak 329, and Basmati Sufaid Pak 187) and two accessions originated in the Philippines (IR 36 and IR 52 [IR5853-118-5]). The IBPGR-IRRI

Characterization of Rice (*Oryza sativa* L.) Roots Versus Root Pulling Resistance as Selection Indices

Rice Advisory Committee report on "Descriptors for Rice *Oryza sativa* L." (IBPGR-IRRI Committee, 1980) defines different plant characteristics in rice. The following descriptors (plant, type, panicle type, grain type, hull cover, hull color, sterile lemma color, seed coat color, awning, lodging and straw strength) are defined in the IBPGR-IRRI report. IR 36 had a spreading plant type; whereas, the other four accessions had an open plant type. All of the accessions had an intermediate panicle type, long grain, the hull cover had short hairs throughout, hull and sterile lemma color were straw yellow and the seed coat color was light brown. Staunfia or Mabla Pak 329 was partly awned and the awns are long; whereas, the other four accessions are partly awned but the awns are short. IR 36 was rated as having intermediate lodging resistance; whereas, Basmati Sufaid Pak 187 was rated as very weak and the other three accessions were rated as moderately strong.

Table 1. Plant height and days from emergence to 50% heading (maturity) for five accessions with high, intermediate and low root pulling resistance.

| Germplasm                | Ht(cm)    | Days to Heading |
|--------------------------|-----------|-----------------|
| Munji Sufaid Pak         | 123.05 a* | 142 b           |
| IR 52 (IR 5853-118-5)    | 82.50 b   | 147 a           |
| Saunfia or Mabla Pak 329 | 93.97 b   | 139 b           |
| Basmati Sufaid Pak 187   | 135.73 a  | 128 c           |
| IR 36                    | 57.05 c   | 124 c           |

\* Means within columns with the same letter are not significantly different at the 0.05 level.

Table 2. Mean comparisons among rice accessions for Root Pulling Resistance 30 and 60 days after emergence.

| Accessions               | 30 Day    | 60 Day    |
|--------------------------|-----------|-----------|
|                          | RPR Value | RPR Value |
|                          | KG        | KG        |
| Munji Sufaid Pak         | 22.3 a*   | 45.0 a    |
| IR 52 (IR 5853-118-5)    | 22.0 a    | 44.3 a    |
| Saunfia or Mabla Pak 329 | 11.6 b    | 39.6 b    |
| Basmati Sufaid Pak 187   | 9.6 c     | 32.2 c    |
| IR 36                    | 9.5 c     | 32.1 c    |

\* Means within columns with the same letter are not significantly different at the 0.05 level.

## ROOT PULLING RESISTANCE

The five accessions representing the high, intermediate and low RPR groups that were pulled at two dates are shown in Table 2. A greater force was required to pull the plants 60 days after emergence as compared to the force needed to pull the plants 30 days after emergence; however, the order of the RPR for the different germplasm accessions remained the same on both dates. For example, Munji Sufaid Pak required the greatest force to pull the plants at both 30 and 60 days after emergence; whereas, IR 36 required the least force to pull the plants at both 30 and 60 days (Table 2). Munji Sufaid Pak and IR 52 (IR 5853-118-5) were among the top 10 accessions that gave the highest RPR measurements at 30 days after emergence; whereas, Saunfia or Mabla Pak 329 was intermediate and Basmati Sufaid Pak 187 and IR 36 gave consistently low RPR measurements at 30 days after emergence. A similar trend was obtained for RPR measurements 60 days after emergence. In fact, the correlation coefficient between RPR at 30 days (RPR-1) versus RPR at 60 days (RPR-2) was 0.88 (Table 3). Because the plants were larger when the data were collected on the second sampling date (60 days after emergence) the measuring device was changed from a maximum load of 25-kg to a maximum load of 50-kg.

Table 3. Correlation coefficients between Root Pulling Resistance and plant characteristics.

| Plant Characteristics | r     |
|-----------------------|-------|
| Maximum root length   | 0.69* |
| Root thickness        | 0.75* |
| Root branching        | 0.75* |
| Root number           | 0.61* |
| Root dry weight       | 0.82* |
| RPR-1 vs. RPR-2       | 0.88* |

\* Significant at the 0.05 probability levels, N=50

## PULLED ROOT CHARACTERISTICS

The accessions having higher RPR measurements also had longer roots, thicker roots, greater root branching, greater root number and higher root weight as compared to those accessions having low RPR measurements (Table 4). For example, Munji Sufaid Pak which consistently gave high RPR measurements produced higher values for root characteristics than did Basmati Sufaid Pak 187 and IR 36 which gave low RPR measurements (Table 4). Table 2 shows that significant differences due to genotype were present for each RPR measurement regardless of the observation date. Therefore, these data suggest that root characteristics among the genotypes will differ regardless of sampling date but the relative order will remain the same from date to date.

Table 4. Mean comparison among five rice accessions for root characteristics.

| Accessions               | Root Length (cm) | Root Thickness (mm) | Branching (0-5) | Root Number | Dry Weight (grams) |
|--------------------------|------------------|---------------------|-----------------|-------------|--------------------|
| Munji Sufaid Pak         | 26.63 a          | 49.12 a             | 4.56 a          | 232.62 a    | 2.16 a             |
| IR 52 (IR 5853-118-5)    | 21.25 c          | 37.75 b             | 4.19 a          | 184.37 b    | 1.64 b             |
| Saunfia or Mabla Pak 329 | 22.43 b          | 28.00 c             | 2.75 b          | 106.87 c    | 1.08 c             |
| Basmati Sufaid Pak 187   | 15.30 d          | 16.12 e             | 2.19 c          | 60.75 d     | 0.44 d             |
| IR 36                    | 10.72 e          | 22.00 d             | 1.81 c          | 68.75 e     | 0.49 d             |

\* Means within columns with the same letter are not significantly different at the 0.05 level

## RELATION OF RPR TO ROOT CHARACTERISTICS

The RPR data were significantly correlated with the root characteristics. For example, the RPR measurements were significantly correlated with maximum root length ( $r=0.69$ ), root thickness ( $r=0.75$ ), root branching ( $r=0.75$ ), root number ( $r=0.61$ ) and root dry weight ( $r=0.82$ ) (Table 3). Some of the root characteristics were positively correlated with each other; however, additional data are needed before the effect of these mutual associations can be explained.

## CONCLUSIONS

The data in Tables 2 and 4, and the correlations presented in Table 3 demonstrate that RPR is an effective evaluation method for identifying plants with root systems having longer and thicker roots. The RPR measurements showed a significant positive correlation with maximum root length ( $r=0.69$ ), root thickness ( $r=0.75$ ), root branching ( $r=0.75$ ), root number ( $r=0.61$ ) and root dry weight ( $r=0.82$ ). The data indicated that high RPR measurements are strongly correlated with greater root penetration. Furthermore, there was no correlation between plant height and RPR. There was a significant correlation ( $r=0.88$ ) between RPR at 30 days after emergence as compared to RPR at 60 days after emergence.

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# COMPUTER-CONTROLLED PROGRAMMABLE PULSE GENERATOR FOR A JEOL JNM-FT-1A RADIO FREQUENCY AMPLIFIER SECTION OF A NUCLEAR MAGNETIC RESONANCE SPECTROMETER

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## ABSTRACT

A microprocessor controlled programmable pulse generator was specifically designed to interface with the JEOL JNM-FT-1A Radio Frequency (RF) section of a nuclear magnetic resonance (NMR) spectrometer. The programmable pulse generator was programmed and controlled by an IBM compatible computer. Static rams were utilized as the programmable pulse generator's on board memory which were used to store the pulse sequences. Pulse widths can be programmed from 0.2 microseconds to 14.3 minutes. The JEOL RF section is unique in that it allows the pulse generator to control the phase of the RF transmitted to the NMR probe and whether the sample will be decoupled. These two items set this programmable pulse generator apart from other pulse generators described in the literature and those systems which are commercially available. This programmable pulse generator was constructed to replace the old JEOL thumb wheel controlled pulse generator. Typical 180 degree pulse lengths for the JEOL NMR are approximately 20 microseconds.

## INTRODUCTION

A microprocessor controlled programmable pulse generator was specifically designed to interface with the JEOL JNM-FT-1A Radio Frequency (RF) section of a nuclear magnetic resonance (NMR) spectrometer. The programmable pulse generator was programmed and controlled by an IBM compatible computer. The programmable pulse generator was designed and constructed to replace the JEOL's thumb wheel controlled pulse generator. The programmable pulse generator had to meet the following requirements: (1) the length of any individual pulse could be varied from 0.2 microseconds to 14.3 minutes in increments of 0.2 microseconds. These times allow the programmable pulse generator to have high resolution and the long pulse widths allow the studying of relaxation times of various nuclei, (2) the pulse generator had to provide two phase shifting control lines that can be changed on every pulse which is an added feature on the JEOL RF section, (3) the pulse generator must provide the capability of running all the standard pulse sequences such as T1, Hahn Spin Echo, Carr-Purcell, and Meiboom-Gill (Becker, 1980), (4) an analog to digital converter (A/D) would have to be triggered by the pulse generator at varying times, (5) the pulse generator had to be computer controlled, (6) simple in design for a wide variety of user expertise, and (7) cost effective.

## PROCEDURE

Numerous devices were reviewed which included: (1) state-memory machine devices (Sidky *et al.*, 1988), (2) word programmer (Danese *et al.*, 1986), (3) an emitter coupled logic (ELC) (Thomann and Dalton, 1984), and (4) dedicated counter chains. This was a varied list, but none fulfilled the needs of our JEOL RF section. After the review was completed, a decision was made to design and construct the programmable pulse generator in-house. Our design required control lines to: (1) change the phase of the RF generator, (2) control the gated decoupling provided by the JEOL instrument, and (3) a trigger for the A/D converter. The above list of programmable pulse generators did not satisfy these requirements. Thus, a dedicated counter chain pulse generator was chosen as the easiest and the most cost effective method of achieving the desired 0.2 microseconds to 14.3 minutes specified in the design criteria; also, with the aid of static rams and a variety of control logic, all the other requirements were met.

The pulse generator took three separate paths before completion: (1) interface to the IBM compatible computer, (2) interface to the JEOL RF section, and (3) actual pulse generation. The IBM interface consisted of an address decode, data buffers, and data latches. Interface between the generator and the RF section utilized static rams and line drivers. The main component pulse generation combined a dedicated counter chain, static rams, a crystal oscillator, and control logic to achieve the desired pulse sequence.

Simplicity was the goal during the designing stage. Since numerous people would have access to this instrument, training time must be minimized.

## IBM INTERFACE

Pulse data were transferred from the computer to the pulse generator since the pulse generator was located in another enclosure a short distance from the computer and the superconducting magnet. An address decoder was constructed using ANDS, NOTS, TRI-STATE BUFFERS, and a FOUR to SIXTEEN BIT DECODER chip. Addresses A0 - A15, control lines I/O Write (IOW), and Address Enable (AEN) were used to decode hexadecimal addresses 380 - 38F. Sixteen control lines were used to latch onto the pulse data, clear the address counters of the static rams, increment the address counters, load the pulse data into the static rams, load pulse data into the counter chain, and to activate the pulse generator.

The first step in transferred data from the computer to the pulse generator was to decode the address bus of the computer (see Figure 1). Addresses hexadecimal 380 - 38F were chosen as valid user addresses for interfacing to the IBM computer (Eggebrecht, 1987). The IBM's computer architecture provides the user with two interfacing signal lines: I/O WRITE (IOW) and ADDRESS ENABLE (AEN). These two signals were used to validate the address and data on the bus. After the address was validated, it was decoded and then used as one of the control lines to control another activity listed above.

The user can write to any hexadecimal port address with the aid of software control. Only hexadecimal addresses 380 - 38F are decoded by the address decoder in Figure 1. The decoder functions only when both IOW and AEN are low, or simply, the decoder waits until the data and the address are valid on the bus before it decodes any address. The address then is decoded and the correct control line corresponding



to the address is switched low. When IOW goes from an active low to an active high, the control line goes high. This action also disables the decoder from functioning until the next address is written to the port.

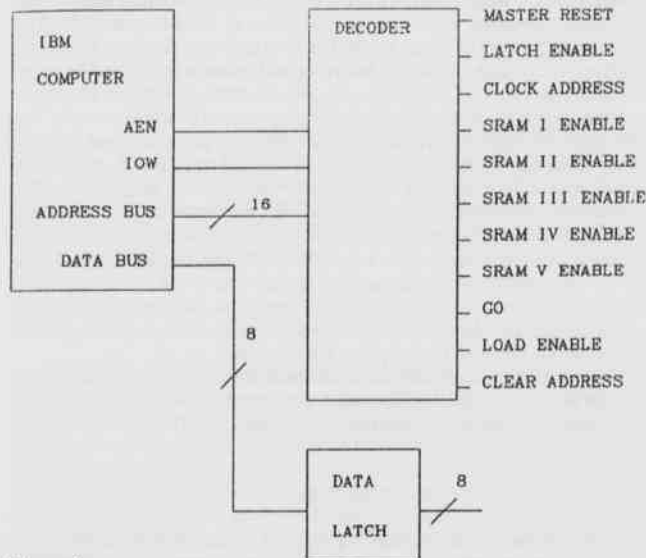


Figure 1

The address decoder also contains a data latch or a TTL 74LS373 octal "D" transport latch with tri-state output. The data latch on the address board kept the data bus signal lines from becoming too long. The data and address bus was kept as short as possible to minimize any noise and signal timing problems. The data contained in the latch can be used at anytime not just at the computer's clock rate. The data contained in the data latch can be transferred from the IBM interface to any of the pulse generator's static rams. The IBM interface now has decoded the address hexadecimal 380 with concomitant control line switching. The data are now ready to be transferred to the pulse programmer.

#### PULSE GENERATOR TO JEOL RF SECTION

The JEOL RF section required a pulse line, two phase lines, a gated or no gated decoupling line, and an analog to digital converter trigger. One static ram was dedicated to provide the output information needed. Line drivers and impedance matching circumvented any problems in transferring short pulses over long distances.

#### PULSE GENERATOR

The question was raised, "How do you generate 0.2 microseconds to 14.3 minutes of pulse time in a sequence that also contains phase shifting, A/D triggering, and gated decoupling information?" The answer was to use five static rams (2Kx8) as memory and to dedicate one of the rams as soft control logic for phase shifting, A/D triggering, decoupling, on/off, and start/stop information (see Figure 2). The other four static rams were combined with eight four bit down counters and a 10MHz crystal controlled oscillator. The crystal controlled oscillator is then divided by two to give the user a clock rate of 5MHz or 0.2 microseconds per clock cycle, which allows the counter chain 4.295 X 10E9 counts to time the pulses from 0.2 microseconds to 14.3 minutes. As an added feature the user can change the time per cycle with dip switches, selecting the increment of time needed for a specific experimental resolution.

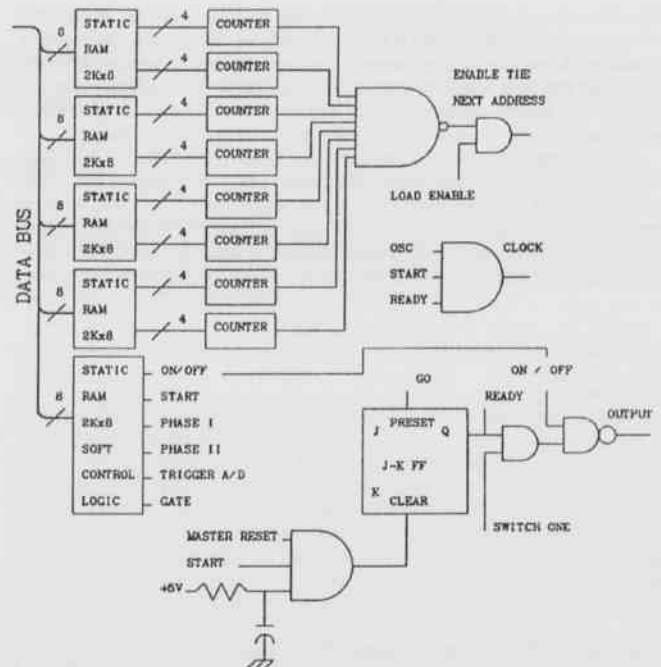


Figure 2

Two kilobytes were chosen so that the memory was deep enough not to limit the number of pulses that could be programmed by the pulse generator. The only problem with static rams is that they must be addressed to accept data. Three 74LS191 counters were used to generate the addresses for the static rams. Two control lines from the address decoder in the computer were dedicated to clear the address counters and to clock the address counters.

Four-bit presettable synchronous binary up/down counters, 74LS191, were chosen as the dedicated counters. These counters were chosen because they are programmable and they provide a signal line MAX/MIN (pin 12) that goes high when the counter has counted down to zero. The counter's ability to be presettable allows any value to be loaded. The counters were combined in a dedicated chain and the ripple through clock (pin 13) was used to complete the chain's clock requirements.

#### DESCRIPTION OF THE PULSE GENERATOR

Pulse or control data are loaded into the data latch in the computer after the address decoder strobes the address counter clear line thereby zeroing the address of the static rams. Data then are transferred from the computer into one of the corresponding static rams. The address decoder cycles the address clock control line which increments the address counters. The above process is repeated as needed.

The address decoder then toggles the load enable to allow transfer of the first bit of pulse data into the dedicated counter chain. At the end of loading the first bit of pulse data into the counter chain, the load enable control line also clocks the address counters and the next address is presented at the static rams to avoid signal timing problems. The next line pulsed is the "go" control line after which the pulse generator takes control. The go line sets the output on a JK flip-flop that remains high throughout the timing process. The high on the JK enables the master clock to start clocking the counter chain. The start control soft logic line also must be high for the master clock to clock the counter chain. The counter chain has been set in a count down mode.

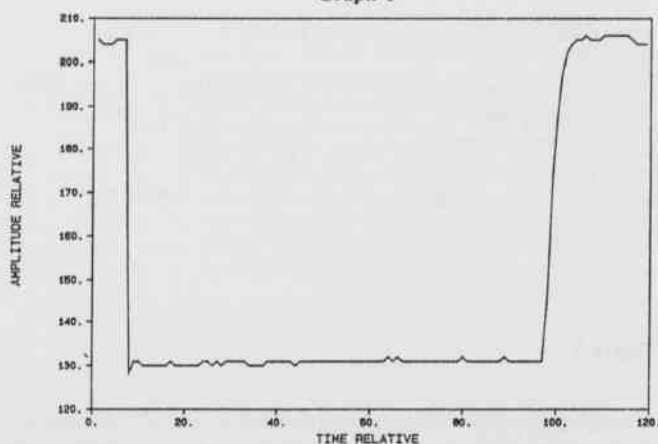
When the counters count down to zero, the MAX/MIN signal pin goes high. The pulse generator now loads the next sequence of pulse

**Computer-Controlled Programmable Pulse Generator for a JEOL JNM-FT-1A Radio Frequency Amplifier****RESULTS**

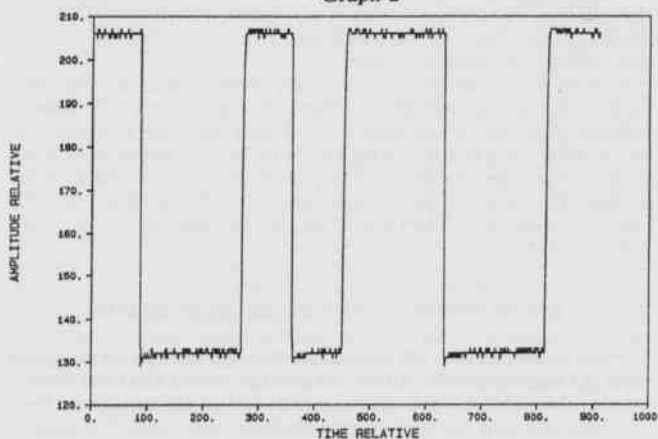
data into the counter chain and clocks the address counters at the end of this cycle. The MAX/MIN lines fall low and the counter chain starts counting down the next sequence. This repeats until the start control soft logic line goes low. The start control soft logic line was programmed by the user as the ending of the pulse sequence. The pulse generator will reset itself and wait until a new pulse sequence is entered.

Three things control whether the pulse is on or off. The JK flip-flop output must be high, the switch SW1 must open, or a high at the AND gate. If any of these are low, the pulse generator's output is forced high or the pulse is "off". The ON/OFF control logic line has final control on if the pulse is on or off. A high forces the pulse generator's output low thereby signaling that the pulse is on; a low has the opposite effect.

Graph 1



Graph 2



Several pulse sequences were entered and run in the pulse generator to verify that it would indeed satisfy the requirements listed above. The first pulse sequence was a simple one pulse of 10 microseconds. The results were plotted in graph 1 with the time and amplitude on the graph relative. The x-axis on the 10 microsecond pulse was plotted to show fall and rise times of the pulse. As can be seen, the edges are sharp and the slope very steep. The steep slope was a very desirable result. The next pulse sequence entered and run on the pulse generator was a triplet pulse sequence consisting of 20 on, 10 off, 10 on, 20 off, and 20 on (times were microseconds). The results were plotted in graph 2. The triplet sequence timings were accurate and the sequence demonstrated a multiple pulse sequence in the pulse generator. Several variations of the basic pulse sequences were entered into the pulse generator and run. All the sequences were verifying the requirements of the pulse generator. The pulse generator also was able to trigger the analog to digital converter chosen for the JEOL NMR Spectrometer. The triggering of the A/D will be verified in the software section.

Digitalization of the pulse sequences was accomplished with a LeCroy Digital Storage Oscilloscope then transferred via RS232C to a VAX computer and plotted on a Hewlett Packard plotter.

**CONCLUSION**

Soft and hard control logic were used in the timing of the pulse sequences. The counter chains and associated memory were used to control the amount of time until the next address was clocked. In other words, the static ram that contained the soft control logic actually controlled when the pulse was on or off and the counter chains provided the timing data for switching to the next address.

The programmable pulse generator can be modified for use with other NMR spectrometers by selecting the control lines that are unique.

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# REPRODUCTION OF *LINDERA MELISSIFOLIA* IN ARKANSAS

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## ABSTRACT

Field reproduction was investigated in several populations of the endangered dioecious species *Lindera melissifolia*. Fruit set was light and erratic, and even where fruiting was heavy no effective seed bank was found. Field transplantation of greenhouse-grown seedlings was successful for one growing season. Rhizome sprouting was widespread, and produced rapid recovery from fire. Without enhancement of natural reproduction, the species will probably decline.

## INTRODUCTION

Pondberry, *Lindera melissifolia*, is a federally and State endangered shrub known in Arkansas from only a few populations in three areas of the northeastern part of the state (Wright, 1989). Individual stands of this dioecious species grow as understory shrubs in bottomland hardwood forest, but only where temporary ponds occur in depressions between old dunes formed from glacial outwash (Saucier, 1978). Ponds are typically well isolated from each other, due both to natural topography and to agricultural modification of the landscape.

Pondberry stems are shortlived (Tucker, 1983) but replace themselves readily by rhizome sprouts. Survival of the species requires that existing stands maintain themselves and if possible spread to other ponds. Sexual reproduction may be ineffective, judging from the frequency and size of single-sex clones (Wright, 1989). Apparent preponderance of male clones suggests that survival may be related to difference in allocation of resources between males and females, as has been observed for other dioecious species (Agren, 1988; Doust and Doust, 1988). Populations are prone to disturbance from fire and from wood cutting, although their topographic position has tended to protect them from destruction through land clearing. This study focused on sexual reproduction and response to disturbance of several field populations in 1987-1989.

## METHODS

1) Fruit set was observed in 1988 and compared with observations the previous two seasons. 2) The 1988 seed bank was assessed in sections of the forest floor beneath plants that had fruited heavily in 1987. On July 22 six plots (totalling 0.47 m<sup>2</sup>) of forest floor were removed to depths of 8-10 cm, and placed intact in the greenhouse. Seedling and rhizome sprout emergence were observed over an eight month period. 3) 100 fruits were sown in the mineral A horizon of an upland forest in November 1987. Germination was assessed in 1988 and 1989. 4) 1987 fruit germinated in the lab at greater than 90% when seed coats were removed (Wright, 1989). These were grown in the greenhouse during winter and spring of 1988. From late April through early June a total of 64 seedlings was transplanted to a pond containing an existing population as water receded. Transplants were watered seven times between May 12 and September 29, and their survival monitored. 5) Three stands were monitored for recovery from fires that killed above-ground stems between November 1987 and May 1988.

## RESULTS

1) Fruit set varied from year to year. Fruit set in one stand was extremely heavy in 1987, with an estimated 100,000 fruits set (Wright, 1989). This amounted to 2000 fruits per m<sup>2</sup>. In 1988 the stand produced no more than 2000 fruits. Stands that had fruited well in 1986 had low fruit production the next two years. Stems that had fruited heavily in 1987 were prone to dieback for about half their length. Of

six populations observed over three seasons, three had only male plants and thus bore no fruit.

2) An effective natural seed bank was not demonstrated. Forest floor samples grown in the greenhouse from July 1988 to March 1989 produced no new seedlings, although the samples came from an area estimated to have produced about 1000 fruits the previous season. During that same time 94 new rhizome sprouts emerged from the samples. There were two seedlings of the current year present in the samples when they were dug, and no signs that any seed had already germinated and died.

3) Fruits sown in the upland forest in November 1987 produced no seedlings in 1988. In May and June 1989, five seedlings emerged (out of 100 fruits planted).

4) Seedling transplantation from the greenhouse to a native stand was successful except where plants were placed too high on the pond bank. Survival to September 29 was as follows:

|                          |            |                         |
|--------------------------|------------|-------------------------|
| Top of bank . . . . .    | 1/10, 10%  | (transplanted April 26) |
| Bank slope . . . . .     | 14/20, 70% | (transplanted April 26) |
| Edge of bottom . . . . . | 11/16, 69% | (transplanted June 2)   |
| Lower bottom . . . . .   | 16/18, 89% | (transplanted June 14)  |

Seedlings generally attained heights of around 10cm, with little new growth after transplantation except for seedlings in the lower bottom, several of which grew to double their pre-transplantation height. The lower bottom was a few cm below the lowest elevation of the existing pondberry stand in this pond.

5) Pondberry plants that burned during the dormant season (November 1987 fire) resprouted as soon as the pond receded late in May. One stand with 109 stems before the fire produced 134 from new growth. In another burned stand extending up a pond bank, vigorous regrowth by associated shrubby and herbaceous vegetation outpaced pondberry about two to one in rate of stem elongation.

## DISCUSSION

During the years 1986-88, pondberry in Arkansas did not fruit heavily. All-male stands (Wright, 1989) had no fruit, and of those stands with female plants only one stand fruited heavily in one year. Whether due to failure of pollen transfer, pollen inviability, cyclic fruiting or other causes, fruit production was clearly low. A parallel exists with *Neviusia alabamensis*, another rare plant with a range similar to that of *Lindera melissifolia*, which in Arkansas has not been seen to produce fruit (Long, 1986). Other clonal dioecious species are known to produce abundant fruit (Doust and Doust, 1988; Lloyd, 1984), suggesting that low fruit production may be a critical factor among life history events for pondberry.

Very low seedling production also characterizes pondberry (Wright, 1989). This is corroborated by failure of the seed bank to reveal any viable seed, even though some hundreds of fruits would have been shed on the seed bank plots. It is possible that the occasional new seedlings germinated where I disturbed the forest floor while harvesting the year

Reproduction of *Lindera melissifolia* in Arkansas

before, and therefore, some viable seed sown into disturbed soil may germinate. This is suggested by the 5% germination after two winters where fruit was sown in mineral soil. Possibly low seedling recruitment is sufficient in species with strong vegetative reproduction, yet it appears that in some populations females have died out completely, indicating total failure of seedling recruitment. It may also be that lower allocation of resources to reproduction had favored males, as has been found for species of *Rubus* (Agren, 1988), *Rhus* (Doust and Doust, 1988), and other plants (Lloyd, 1984).

The transplant study gives clear indication that stands can be enhanced or recovered by transplanting greenhouse-grown seedlings into moist pond bottoms and banks. Precipitation in 1988 was below average for the summer months in Arkansas, totalling 265mm unofficially in Conway from June through September, yet transplants survived the season with minimal watering.

As is true for many species (Wright and Bailey, 1982), recovery from fire was robust, both for pondberry and for its associates. Since there is little competition in the understory for most of the populations (Wright, 1989), fire should be no problem. However, where better drainage or breaks in the tree canopy promote vigorous understory growth, competitors may well outgrow pondberry after fire, and at other times as well.

In summary, *Lindera melissifolia*, as long as its ponds with their forest canopies remain intact, should do well where it is now established. However, if the canopy trees are removed and a fire follows, severe impacts to the stand will occur. The stands have little capacity to increase genetic variability within populations by sexual reproduction, and no real ability to colonize new locations. Present distribution of sexes indicates that one or another of these hazards has over the years reduced populations. There is every likelihood this will continue unless management by establishing new seedlings or ramets counteracts natural attrition.

ACKNOWLEDGMENTS

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# THE BATS OF THE OUACHITA MOUNTAINS

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## ABSTRACT

A survey was conducted from June, 1982 through January, 1989 to determine the occurrence of bat species in the Ouachita Mountain region of west-central Arkansas and southeastern Oklahoma, with emphasis on censusing lands managed by the USDA Forest Service, Ouachita National Forest. Seven genera and 13 species of bats in the families Vespertilionidae and Molossidae were captured. Species represented included: *Eptesicus fuscus*, *Lasiurus noctivagus*, *Lasiurus borealis*, *Lasiurus cinereus*, *Lasiurus seminolus*, *Myotis austroriparius*, *Myotis keenii*, *Myotis leibii*, *Myotis lucifugus*, *Myotis sodalis*, *Nycticeius humeralis*, *Pipistrellus subflavus*, and *Tadarida brasiliensis cynocephala*.

## INTRODUCTION

From June, 1982 through January, 1989 we conducted a survey of bat species occurring in the Ouachita Mountain region of west-central Arkansas and southeastern Oklahoma, with major emphasis on the Ouachita National Forest.

The Ouachita Mountains, located in west-central Arkansas and southeastern Oklahoma (Fig. 1), have been folded and vaulted as well as uplifted, exhibiting an east-west, ridge-and-valley landscape with elevations ranging from 80 to 860 meters above sea level. The soils, developed from sandstone, shale, novaculite and chert, are dry to droughty and range in texture from loam to clay (Pell, 1983). Second-growth (50-70 years old) mixed hardwoods (*Quercus/Carya*) occur on more mesic north slopes, with dryer south slopes vegetated by second-growth shortleaf pine (*Pinus echinata*) forest types. The region contains a significant number of rivers and streams, and several thousand small lakes and wildlife waterholes, all providing riparian foraging habitat. Almost all first and second order streams and some third and fourth order streams have intermittent flow during dryer summer and fall months. However, most third and fourth order streams are perennial (USDA Forest Service, 1983).



Figure 1. Location of Ouachita Mountains (backslashed area) and study area (crosshatched area).

A majority of the land within the Ouachita Mountains is owned by private timber companies and the USDA Forest Service, Ouachita National Forest. Hot Springs National Park also occurs within the region. Timber harvest activities have created a very diverse landscape,

both horizontally and vertically, ranging from early successional seral stages (regeneration areas) to areas of older growth spatially arranged over the landscape.

Due to the geology of the region, solutional caves are essentially nonexistent. Bear Den caves, located in Winding Stair Mountain, LeFlore County, southeastern Oklahoma, occur in an outcrop belt of a massive sandstone unit and were formed by a number of factors, the most important being gravitational sliding and slumpage of sandstone. These caves have more than 365 meters of passageway and represent the only known caves from the study area (Puckette, 1974-75).

Additional subterranean habitat exists in the area in the form of abandoned mining drifts. Ranging from shallow test holes to extensive linear and T and L-shaped tunnels, these excavations are utilized by a variety of vertebrate species (Heath *et al.*, 1986; Saugey *et al.*, 1988c). Data from one recently discovered drift in Sevier County, Arkansas are reported in this study.

Some of the earliest information regarding distribution of bats of the genus *Myotis* in the study area were reported by Glass and Ward (1959). The bulk of the information concerning bats of the Ouachita Mountains of southeastern Oklahoma is the result of recent studies by Caire (1986), Caire *et al.* (1986), Hardisty *et al.*, (1987), and Stevenson (1986). Similarly, early studies of cave dwelling species by Sealander and Young (1955), and Davis *et al.* (1955), and more recent investigations by Heath *et al.* (1983, 1986) and Saugey *et al.* (1983, 1988a, 1988b, 1988c), have provided much additional information on the bat fauna of the Arkansas portion of the region.

The primary purposes of this study were to provide distribution and natural history data concerning the "tree bats" of the Ouachita Mountains and supplement existing cave and mine observations of bats with surface records. Additional information concerning cave and mine use is also reported.

## MATERIALS AND METHODS

Collection was primarily by mist netting as described by Tuttle (1976). A total of 60 net nights (a net night is one mist net opened into the capture position for the evening's activities) was generated on 29 different dates, with one to five net nights per date. Nets of different lengths (5.5, 12.8, and 18.3m) were erected and opened into the capture position prior to dusk and checked at five-minute intervals. Actual netting periods varied from four to 10 hours depending upon time of year. Netting primarily occurred over shallow pools of third and fourth order streams and at the entrances of abandoned mining drifts. Each bat was removed from the net, and species, sex, age, reproductive condition, time of capture, and ambient temperature were recorded. In addition, one abandoned mining drift and four fracture caves known to be used as hibernacula were visited on at least one occasion during winter months to record their use. Voucher specimens were retained as necessary and are deposited in Museum of Zoology Collections at the University of Arkansas at Little Rock (UALRMZ) and Arkansas State University, Jonesboro (ASUMZ).

## The Bats of the Ouachita Mountains

Assignment of bats into age classes was determined by closure of phalangeal epiphyses. Bats were designated as juveniles on the basis of small overall size, as well as visual observation of incomplete ossification of epiphyses.

Reproductive condition of males was determined by the position of the epididymides, and size and location of testes. Scrotal males were characterized by complete descent of the epididymides into pigmented sheaths dorsolateral to the tail and by the presence of enlarged testes. Females were diagnosed as pregnant by palpation of fetuses and examination of an obviously enlarged abdomen. Lactation was determined on the basis of teat examination.

## RESULTS

## FAMILY VESPERTILIONIDAE

Big brown bat, *Eptesicus fuscus* (Palisot de Beauvois): A year round resident, the big brown bat appears to be well distributed throughout the Ouachita Mountains, especially in towns and areas adjacent to clusters of manmade structures including pump houses, picnic pavilions, and dwellings. Abandoned mining drifts were also utilized as temporary roosts or hibernacula (Heath *et al.*, 1986; Saugey *et al.*, 1988c).

A number of maternity colonies were located during this study. The most unique maternity colony was located in Perry County in the seams between concrete sections of the dam impounding the Fourche la Pave River and creating Lake Nimrod. A colony of 30 adult females and non-volant juveniles was first observed during June 1984. According to local residents, the site had been used for a number of years. Periodic observations since 1984 have revealed continued use as a maternity site, with bats present during spring, summer, and fall.

The largest maternity colony or group of maternity subcolonies was discovered during the summer of 1984 when a bat infestation involving this species and the Brazilian free-tailed bat was recorded from a housing project in Hot Springs, Garland County, Arkansas (Saugey *et al.*, 1988a,b). Over one thousand individuals were removed and destroyed, or simply excluded from structures by a professional exterminator and Hot Springs Animal Control.

On 5 May, 1987, a maternity colony of 50 females and two males were excluded from the attic of a rental cabin at Lake Ouachita State Park near Hot Springs. Bats were collected by mist netting as they exited the cabin to begin foraging activities. Although all females were palpated and determined to be pregnant, only 23 females were weighed and forearm lengths recorded; mean weight (N=23) was 23.25g (range: 15.25-26.0g) and mean left forearm length (N=23) 48.6mm (range: 45.6-50.1mm). The two males weighed 12.5g and 14.5g.

The smallest maternity colony, composed of eight pregnant females (each with two fetuses) and one adult male, was discovered roosting in a small separation between a chimney and exterior wall of a home in Hot Springs on 18 May 1988. Mean female weight was 26.1g (range: 23-28.75g), and mean forearm length was 48mm (range: 45.8-50.9mm). Fetuses included 10 males and six females, and the mean weight was 2.3g (range: 0.6-3g), and the mean forearm length was 12.8mm (range: 7.2-15.1mm).

Several additional small colonies were discovered, but due to the time of the year visited, their status as maternity colonies could not be ascertained. These included a colony discovered during the renovation of an old school building in Norman, Montgomery County; a small colony in the Womble Work Center, U.S. Forest Service, Mt. Ida, Montgomery County; 15 individuals in a horse barn outside Hot Springs; and a colony of 14 males and 25 females in a pavillion at Shady Lake Recreation Area, Polk County, Ouachita National Forest. The colony at Shady Lake shared its roost with a small colony of the Brazilian free-tailed bat, *Tadarida brasiliensis cynocephala*.

Only eight specimens were captured during mist netting activities in heavily forested areas, with six of these captured in one evening. Conversely, 66 *Eptesicus* were netted over a small pool within Hot Springs National Park (Saugey *et al.*, 1988a), an area surrounded by homes, buildings, and other manmade structures.

Although over 800 *Eptesicus* were observed or examined during this

study, most of these specimens were associated with the large infestation in the Hot Springs housing project. Heath *et al.* (1986) reported specimens from abandoned mining drifts in the Ouachita Mountains of Arkansas, and Caire (1986) reported 39 adults, subadults, and juveniles netted in riparian areas and at Bear Den Caves, but did not locate a maternity roost in southeastern Oklahoma.

Silver-haired bat, *Lasiorycteris noctivagans* (LeConte): The first silver-haired bat reported from the study area was by Heath *et al.* (1983), when a 15.0g female was mist netted over a pool in Polk County, Arkansas on 9 October 1982. Heath *et al.* (1986) reported the occurrence of a 9.25g, scrotal male found hibernating in an abandoned Cinnabar mine in Pike County, Arkansas.

Since these reports, 10 additional individuals (nine males and one female) have been collected from the study area in Arkansas. Interestingly, all male specimens of this bat, with the exception of the specimen reported from the mine shaft, have been collected in spring. Eight of the nine males were collected between 11 April and 3 May 1987 by mist netting two localities in Garland County (representing new county records for Arkansas), and five of these eight were collected on the evening of 11 April 1987. The ninth male was collected over Brushy Creek in Polk County on 6 May 1983. None of these individuals were scrotal. Mean weight of males (N=9) was 8.77g (range: 7.25-10.0g; SD=0.81) and mean left forearm length (N=8) was 41.0mm (range: 39.0-42.6mm; SD=0.98). The single female specimen was recorded from the porch of a Garland County residence in December, 1986. Caire (1986) collected a single adult male on 22 May 1985, the first known specimen from southeastern Oklahoma.

*Lasiorycteris* encountered during this study began to forage early in the evening. Times of capture were remarkably similar with 77% (N=7) occurring between 2041 and 2310 hours. Fifty-five percent (N=5) were captured between 2200 and 2240 hours.

This species may also forage in pairs or small groups (Barbour and Davis, 1969). On 11 April and 3 May, 1987, two males were netted simultaneously and within a few centimeters of one another as they flew over a pool.

Although considered to be migratory (Barbour and Davis, 1969), the occurrence of a hibernating male in January and the December occurrence of the female specimen indicate some individuals overwinter in the study area.

Red bat, *Lasiurus borealis* (Muller): The red bat was more frequently mist netted than any other species, with 386 individuals captured. Although all of our netting was conducted in riparian areas, this bat was observed to utilize almost every available foraging area from ridgetops to densely wooded timber stands, regeneration areas, powerline rights-of-way, highways, and old logging roads. This lack of preference by red bats for any particular foraging habitat was also observed by Lacki and Bookhout (1983) in the Wayne National Forest in Ohio. On several occasions during late summer and early fall, mist nets were set at the entrances of abandoned mining drifts resulting in the capture of males. Such behavior by lasiurine (tree) bats in the Ouachita Mountains may be common, especially during autumn swarming and mating activities. Cassidy *et al.* (1978) reported red bats in late summer and early autumn swarms with several species of cave-dwelling bats at the entrances of Ozark caves, and Saugey *et al.* (1978) reported the remains of 140 red bats in an Arkansas Ozark cave.

Male red bats were netted with greater frequency than females, often at a 3:1 ratio. LaVal and LaVal (1979) reported similar findings in Missouri and Saugey *et al.* (1988a) reported similar results from Hot Springs National Park, Arkansas.

Capture rates for this species were quite variable. On some occasions only one or two specimens would be captured, while on the next night, in the same general area and under seemingly similar conditions, many individuals would be captured.

On 13 August 1983, 130 red bats were mist netted over a shallow pool of Dutch Creek in Yell County. This pool was located in an open area adjacent to a low water bridge, with trees on one bank and an extensive gravel bar on the other. Temperatures recorded during netting activities ranged from 29 C at dusk to 22 C at 0130 hours of the

14th. A majority of captures occurred at a temperature of 24 C spanning a three hour period; a time probably coinciding with normal foraging. Ninety-three males and 37 females were captured in just over four hours. LaVal and LaVal (1979) observed such concentrations of red bats in Missouri during the month of July, and Baker and Ward (1967) reported similar aggregations in southeastern Arkansas during August. Such late summer/early fall concentrations of red bats may be equivalent to swarming behavior exhibited by species that frequent caves.

On numerous occasions during netting activities on 13-14 August, three or more bats would strike the net simultaneously and within a few centimeters of one another. Examination of these clusters typically revealed two or more males and one female, suggesting males may have been pursuing females for breeding purposes. Saugey *et al.* (1988a) documented similar behavior in the evening bat, *Nycticeius humeralis*, during fall netting activities in Hot Springs National Park.

Breeding activity was observed on two occasions, both of which were initiated in flight. In September, 1982, a copulating pair of red bats was observed on the parking lot of Garland County Community College in Hot Springs. The male was mounted dorsally and clenched the nape of the female's neck in his mouth, maintaining tension throughout the episode.

Information on the hibernation of red bats in the Ouachita mountains was restricted to winter (December through March) observations of specimens "smoked" from their hibernacula during prescribed burning activities on the Ouachita National Forest. Red bats were often observed on the ground, still in a state of near torpor, attempting to crawl or fly after becoming overwhelmed by smoke or perhaps stimulated into arousal by the elevated air temperatures resulting from burning leaf litter and other debris. Exact winter roost preference was difficult to ascertain since bats were observed beneath both shortleaf pine and various species of hardwood trees on both north and south aspects. However, a majority of the sightings were made on north aspects or in drainages where temperatures tend to remain cooler and more constant during the hibernation period. Selection of such sites may help conserve stored energy reserves by preventing hibernating bats from unnecessarily arousing when true ambient temperatures are below temperatures at which lasurine bats become active, as might occur on south aspects due to radiant heat. Interestingly, all red bats observed during winter months were males.

Several instances of mortality were observed during the study. The remains of numerous red bats were observed along roadways where bridges spanned streams with suitable flyways. This indicates automobiles may account for some limited mortality. Saugey *et al.* (1988a) observed attempted predation on a male red bat entangled in a mist net by the screech owl, *Otis asio*, during mist netting activities in Hot Springs National Park. One unique case of mortality was discovered when a dehydrated male red bat was found hanging from a strand of barbed wire enclosing a horse pasture near the community of Jessieville in Garland County. The barb had penetrated the uropatagium at such an angle as to preclude escape when the bat attempted to fly. Wisely (1978) reported similar observations for the hoary bat, *Lasiurus cinereus*.

Heidt *et al.* (1987) reported that between 1982 - 1986, bat rabies in Arkansas averaged 10% of animals submitted to the Arkansas Department of Health. During this period, 258 red bats were submitted for testing, and 44 (17.1%) were positive. The following counties in the Ouachita Mountains reported one or more positive red bat: Garland, Logan, Perry, Pulaski, Saline, Scott, Sebastian, Sevier, and Yell. Other species of bats which had specimens test positive from the Ouachita Mountains included: *Eptesicus fuscus* (Garland, Pulaski and Scott counties), *Nycticeius humeralis* (Pulaski County), *Lasiurus cinereus* (Logan and Pulaski counties), and *Tadarida brasiliensis cynocephala* (Garland County).

Red bats apparently communicate with one another while foraging. During mist netting activities, it was rather common for red bats to circle about and/or land on cages or pillow cases in which captured bats were constrained. This behavior has also been reported by Baker and Ward (1967) and Saugey *et al.* (1988a).

The red bat appears to be well distributed and very common

throughout the Ouachita Mountains of Arkansas; having been captured in considerable numbers from Garland, Logan, Montgomery, Perry, Polk, Scott and Yell counties. Caire (1986) considered this species to be one of the most commonly encountered bats of southeastern Oklahoma.

Hoary bat, *Lasiurus cinereus* (Palisot de Beauvois): Initially reported from the Ouachita Mountains of Arkansas by Gregg (1937), the distribution of the hoary bat was greatly expanded by Heath *et al.* (1983) when specimens were reported from Logan, Montgomery, and Polk counties. Saugey *et al.* (1988a) reported the captures of a large pregnant female on 1 June 1983, and a scrotal male (20.0g) and large female (26.0g) on 26 August 1983 in Hot Springs National Park, Garland County.

During this study, 30 hoary bats (12 females and 18 males) were captured. The captures occurred primarily during two distinct periods - May/June and August/September. Similar collection dates were reported by Baker and Ward (1967) for southeastern Arkansas, Gardner and McDaniel (1978) in northeastern Arkansas, and Caire *et al.* (1986) for the Ouachita Mountains of southeastern Oklahoma. Although specimens were captured outside these periods, and specimens of the hoary bat have been recorded throughout the year in Arkansas, these capture dates appear to indicate migration through the area; Zinn and Baker (1979) have discussed the migratory character of this bat.

Six of the 12 females captured were pregnant. On 6 May 1983, a pregnant female weighing 34g was captured; three weeks later on 28 May, four pregnant females with a mean weight of 33.8g (range: 32.0-35.5g) were mist netted while foraging over a stream; and on 5 June 1984, a pregnant female was captured that weighed 35.0g. Females captured during other times of the year included: 5 September 1982 (28.0g); 26 August 1983 (27.0g); and 19 April 1987 (29.0g). Caire *et al.* (1986) reported the capture of a pregnant female (containing a single well developed embryo) in late May and a lactating female in mid-June.

Two non-scrotal males were mist-netted during spring and early summer. One of these males, captured on 19 April, weighed 19.6g and the other, taken on 15 June, weighed 28.0g. The remaining 16 males, captured primarily in late August and early September, ranged in weight from 19.5g to 31.0g and included both mature adults and young of year. All males captured during this period were either partially or fully scrotal.

Although considered a tree bat (Barbour and Davis, 1969), a male hoary bat was netted at the entrance of an abandoned mine in Polk County on 3 September 1982 (Saugey *et al.*, 1988c). Occurrences of this species in caves of the Ozark region of northern Arkansas have been previously reported (Grove 1974, Saugey *et al.*, 1978).

Caire *et al.* (1986) refuted the description of this species as a late flyer by documenting capture times. They found times varied from 2055 to 0440, with 69% of specimens captured prior to 2400 hours and 38% prior to 2200 hrs. While exact time of capture was not recorded for all specimens in this study, our data (N = 13) support their contention in that 76% (N = 10) of our specimens were captured prior to 2400 hours and 38% (N = 5) of these were captured before 2200 hours. Time of capture ranged from 2040 hours to 0123 hours. Nets were monitored continuously while in the capture position, but were not left up all night.

This bat was recorded from Garland, Montgomery, Polk, Scott, and Yell counties.

Seminole bat, *Lasiurus seminolus* (Rhoads): First reported from Arkansas by Sealander and Hoiberg (1954), this bat was considered to occur in the Gulf Coastal Plain over most of the southern two tiers of counties (Baker and Ward, 1967; Sealander, 1979; Hall, 1981). Heath *et al.* (1983) reported the capture of an adult female (3 September 1982) at the entrance of an abandoned mining drift in Polk County, extending the range of this species 57km north of its previous marginal records and into the Ouachita Mountain area of the Interior Highlands. Since that time, four additional specimens were captured in Arkansas during the summer of 1983.

On 30 July 1983, a scrotal male was mist-netted over a pool of Jack Creek in Jack Creek Recreation Area, Logan County (T4N-R27W-SO2). This specimen extended the range of the species approximately 71km



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north of the 1982 location (ASUMZ # 10363). On 13 August 1983, two scrotal males (ASUMZ #'s 10393/10394) were netted among a large aggregation of red bats (previously discussed) over a pool of Dutch Creek in Yell County (T4N-R25W-S29). A scrotal male was captured, along with 45 red bats, over a pool of Blocker Creek in Garland County (T1N-R21W-S34) on 27 August 1983. This capture was made approximately 83km east/northeast of the initial mine entrance record and was still far north of specimens previously reported in the literature. These additional records have greatly expanded the known range of this species within the Ouachita Mountains of Arkansas (Fig. 2).

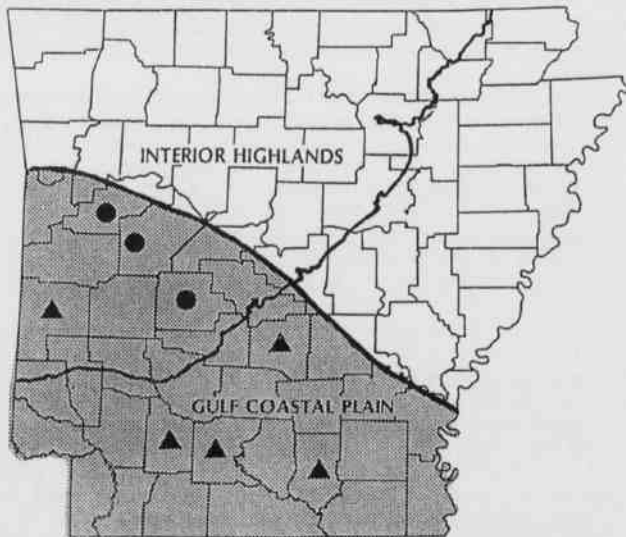


Figure 2. Arkansas distribution of the seminoe bat (*Lasius seminohus*). Triangles represent counties with previously published records; circles represent counties from which bats were taken in this study.

Southeastern bat, *Myotis austroriparius* (Rhoads): The first specimens of the southeastern bat collected in Arkansas came from the Ouachita Mountains when Davis *et al.* (1955) reported the occurrence of this bat from an abandoned mining drift in Garland County. Heath *et al.* (1986) reported the occurrence of a hibernating colony (numbering 150 individuals of both gray and red color phases) from an abandoned Cinnabar mine on a peninsula in Lake Greeson, Pike County. On 16 January 1989, a hibernating colony of 15 individuals of both sexes and color phases was found in an abandoned mine in northern Sevier County, Arkansas, in the Athens Plateau subdivision of the Ouachita Mountains. Suitability of this mine as a hibernaculum was apparently due to multiple entrances that provided continuous movement of air and an internal temperature several degrees cooler than that found in mines with single openings (14.4 - 16.6 C). The hibernaculum was shared with 30 *Pipistrellus subflavus* and five *Eptesicus fuscus*.

Caire (1986) did not capture this species in the Ouachita Mountains of southeastern Oklahoma, although this bat was recorded from the area by Glass and Ward (1959). Steward (1988) and Steward *et al.* (1986) reported additional specimens of this bat from Arkansas counties within and adjacent to the study area. The Southeastern bat is listed as a Category 2 candidate for possible listing as a threatened or endangered species in the U.S. Federal Register (1989).

Small-footed bat, *Myotis leibii* (Audubon and Bachman): Only one specimen of the small-footed bat was known from the Ouachita Mountain region (Hall, 1981) until Caire (1986) and Stevenson (1986) reported captures of six individuals from Bear Den Caves in LeFlore County of southeastern Oklahoma during the summer and fall. McDaniel *et al.* (1982) stated this species was the rarest and least known bat in the southern Ozarks, where caves are plentiful, and Caire (1986) stated this bat was probably restricted to cave areas and the paucity of caves in

southeastern Oklahoma make the few caves there critical to its survival and presence in the area.

Heath *et al.* (1986) did not encounter this species during investigations of abandoned mining drifts. This bat's habit of hibernating in drafty open mines and caves may render most abandoned mines in the Ouachita Mountain region unsuitable due to their relatively warm temperatures (15-16.5 C) and restricted air movement. Steward *et al.* (1986) did not report this species from the caveless region of southwestern Arkansas.

On 19 January 1989, five individuals were observed hibernating in Bear Den Caves. All roosted solitarily in relatively restricted areas of cave breakdown. Temperatures at roost sites varied from 9.5 to 15 C. Two individuals (a male and female) were examined: the male weighed 4.5g and had a left forearm measurement of 33.7 mm. The occurrence of these bats in summer, fall, and winter indicates this species is found in the study area throughout the year. The small-footed bat is a Category 2 federal candidate species.

Little brown bat, *Myotis lucifugus* (LeConte): Sealander and Young (1955) first reported the occurrence of this bat from specimens observed in an abandoned mine in Garland County. Heath *et al.* (1986) reported a single male specimen from a different mine in Garland County. During this study, only one little brown bat, a female, was captured in a mist net at 2120 hours on 30 July 1983 over a pool of Jack Creek, Logan County. One additional specimen, a male, was found hibernating with a small cluster of the endangered Indiana bat (*Myotis sodalis*) on 16 January 1989, in Bear Den Caves, LeFlore County, southeastern Oklahoma. Glass and Ward (1959) reported one specimen from southeastern Oklahoma, but extensive work by Caire (1986) and Stevenson (1986) failed to document additional specimens from the area.

Keen's bat, *Myotis keenii* (Merriam): Thirteen Keen's bats (11 males/2 females) were mist netted from locations in Garland, Montgomery, Polk and Yell counties. These sites are widely spaced and probably indicate the species is well distributed over the area even though rarely encountered.

Heath *et al.* (1986) reported this species from 12 different abandoned mining drifts in the Ouachita mountains. The largest hibernating aggregation consisted of 12 bats and the largest group encountered at other times of the year was a group of 57 pregnant females. Sealander and Young (1955) also reported this species from an abandoned mining drift.

An adult male, mist netted on 6 May 1983, weighed 7g. Additional adult and subadult males, mist netted in riparian areas and at the entrances of abandoned mines in September and October, ranged in weight from 5.5-7g, and all were scrotal. Two postlactating females were captured on 16 July 1983.

Occurrence of Keen's bat in the Ouachita Mountains of southeastern Oklahoma was originally reported by Glass and Ward (1959). Stevenson (1986) and Caire (1986) reported the capture of a total of 328 adults, subadults, and juveniles from riparian areas and cave entrances in southeastern Oklahoma.

Indiana bat, *Myotis sodalis* (Miller and Allen): The Indiana bat was not mist netted during this study, nor was it reported from abandoned mining drifts (Heath *et al.*, 1986). Although reported from a cave in Pushmataha County in southeastern Oklahoma by Glass and Ward (1959), neither Caire (1986) nor Stevenson (1986) reported this species from southeastern Oklahoma after extensive mist netting of riparian areas and examination of caves and buildings.

On 16 January 1989, a hibernating cluster of seven Indiana bats was discovered in Bear Den Caves, LeFlore County, Oklahoma. The cluster, composed of both males and females, and a single male *Myotis lucifugus*, was located six meters above the cave floor where the air temperature was 9.5 C.

Indiana bats may have used these caves as hibernacula in previous winters, but may have gone undetected because the two major studies of the bat fauna in southeastern Oklahoma were conducted in summer and fall (Caire, 1986; Stevenson, 1986). In addition, mist netting of riparian areas may not result in the capture of this bat which was shown



to forage extensively in forested hillsides and ridgetops in Missouri (LaVal *et al.*, 1977; LaVal and LaVal, 1980).

The discovery of this hibernating cluster may indicate the occurrence of this species in the Ouachita Mountains during other times of the year, although females are known to travel considerable distances from hibernacula to maternity sites (LaVal and LaVal, 1980). Recent investigations of habitat utilization by this species in Illinois (Gardner *et al.*, 1989) revealed use of living red oak, white oak, post oak, and hickory trees as roost trees, and use of a shagbark hickory snag as a maternity site; all of which occur in the Ouachita Mountains. The need to retain existing snags, together with the creation of snags with large diameters, have been addressed in the draft Land and Resource Management Plan for the Ouachita National Forest (USDA Forest Service, 1989).

Evening bat, *Nycticeius humeralis* (Rafinesque): A total of 158 evening bats was examined from the study area. Twelve were mist netted in riparian areas, and the remainder occurred in two maternity colonies located in the attics of older homes in Hot Springs.

Specimens mist netted in forested areas included 10 males and two females collected from widely spaced areas in Garland, Logan, Montgomery, Perry, Polk, and Scott counties. Two of these males, collected in May, weighed 8.5g and 10g, and a subadult male collected in July weighed 9g. Males collected in September weighed 12g, 13g, and 14g and a subadult male weighed 9.75g. One of the two females captured was pregnant and weighed 13.0g.

The largest of the two maternity colonies was examined on 15 July 1982, and contained an estimated 150 individuals. A total of 109 individuals (39 adult females, 37 juvenile females, 33 juvenile males) was captured, examined, and released. Several adult females were still lactating and all juveniles were volant.

The smaller maternity colony, estimated to contain 100 individuals, shared their maternity site with a maternity colony of *Tadarida brasiliensis cynocephala* (Saugey *et al.*, 1983). Initial examination of the site on 11 June 1983, revealed a number of pregnant females. Although many bats were present, only four could be captured due to the inaccessible nature of the roost. Weights for these pregnant females were 10.5g, 11.5g, 11.75g, and 12.25g. A female that gave birth while we were in the roost weighed 8.25g immediately after parturition. Two newborns, one male and one female, both weighed 2.25g and had left forearm lengths of 17mm. When re-examined on 1 July, both volant and non-volant juveniles were present. Seven non-volant males had left forearm lengths (LFA) ranging from 28-32mm, and weighed from 4.5-5.5g. Non-volant females (N=3) had LFA ranging from 27.3-33mm, and weighed 4.25-6.5g. Volant juvenile males (N=3) had LFA measurements ranging from 31-33.5mm, and weighed 5-6.5g. Volant juvenile females had LFA measurements ranging from 31.9-35.2mm, and weighed 5.5-6.75g.

This colony was observed for the final time on 13 July when most juveniles were observed to be volant and very difficult to capture. Seven juveniles were captured, however, five of which were volant. One non-volant juvenile male had a LFA measurement of 34.6mm; one non-volant female had a LFA measurement of 36.8mm; three volant juvenile males had LFA measurements ranging from 31.5-34.8mm; two volant females had LFA measurements of 34.9mm, and 36.8mm. Three post-lactating adult females were captured and weighed 8g, 10g, and 10.5g. Some females were presumed still to be lactating as evidenced by the presence of non-volant young. The simultaneous occurrence of volant and non-volant young during the period 1 July through 13 July, and presumably beyond for several more days, indicated parturition within the colony was protracted.

Eastern pipistrelle *Pipistrellus subflavus* (F. Cuvier): The eastern pipistrelle may be the second most common bat species in the forested areas of the Ouachita Mountain region. Caire (1986) found this bat to be fairly abundant in eastern Oklahoma, and Stevenson (1986) reported significant numbers of this species captured using mist nets and Tuttle traps (Tuttle, 1974) at Bear Den Caves. Heath *et al.* (1986) reported this bat from every abandoned mining drift at all times of the year with one mine harboring 600-800 hibernating individuals annually.

During this study, 39 eastern pipistrelles (26 males/13 females) were

mist netted over pools in riparian areas and at abandoned mining drift entrances. The largest number captured in any one evening was six, and on numerous occasions none were captured even though other species were numerous.

Adult males weighed as little as 4g in early April (upon emerging from hibernation) and 6g in early fall. Adult females weighed 5.25g in early May, and a pregnant female captured 15 June 1984 weighed 7.75g.

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Brazilian free-tailed bat, *Tadarida brasiliensis cynocephala* (L. Geoff. St-Hilaire): The Brazilian free-tailed bat has been found in two widely spaced localities within the study area. Saugey *et al.* (1988b) reported on the distribution and status of this species in Arkansas. This study included a major infestation of an apartment complex by this species and *Eptesicus fuscus* in Hot Springs, and the occurrence of a small colony located in the main pavillion area of Shady Lake Recreation Area, Ouachita National Forest, Polk County. Saugey *et al.* (1988a) also reported mist netting seven specimens in Hot Springs National Park.

Hardisty *et al.* (1987) captured 39 specimens at an apparent maternity site in the Forest Heritage Center building in Beavers Bend State Park, McCurtain County, Oklahoma, and Steward *et al.* (1986) reported specimens from several Arkansas counties south of the study area.

This species appears to be closely associated with man, readily utilizing suitable manmade structures. Interestingly, this species was not mist netted in riparian areas nor was it found to use abandoned mining drifts (Heath *et al.*, 1986).

#### DISCUSSION

Data reported in this and other studies of the area demonstrate that the Ouachita Mountain region has a rich and diverse bat fauna represented by 13 different species. Undoubtedly, the diversity of habitats found within the Ouachita Mountains (including vertical and horizontal distribution of different seral stage conditions, mixtures of pine, hardwood, and pine-hardwood mixed forest types, plentiful water and associated riparian areas, caves, and abandoned mines) contributes to an abundance of suitable foraging, hibernating and roosting sites.

The bat fauna represent an integral component of Ouachita Mountain ecology. Most species are currently represented by fairly wide spread and adequate populations; however, the Indiana bat is endangered, and the southeastern and small-footed bats are Category 2 federal candidates for possible listing as threatened or endangered species. Bats, particularly those inhabiting caves and mines, represent an extremely vulnerable faunal element (Saugey *et al.*, 1988c). Protection of caves, abandoned mining drifts, and associated epigeal habitats, and their designations as "key" wildlife habitat components have been recommended by Caire (1986) and Saugey *et al.* (1988c). The importance of these habitat types to bats and their disproportionate use have been discussed by Gates *et al.* (1984) and Maser *et al.* (1979).

Presently, 13 of the estimated 58 species of mammals believed to occur within the Ouachita Mountain region are bats, representing a significant component (22%) of the mammalian fauna. Clearly, marginal habitats of importance to bats such as caves and abandoned mines, and diverse and spatially distributed forest types and age classes, appear to have contributed to the rich bat fauna of the region. Protection, maintenance, and enhancement of these habitat components must be considered in all phases of planning and implementation of management activities in the Ouachita Mountain area.

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# CONCEPT ASSOCIATION

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## ABSTRACT

The complement to decomposition in scientific research is composition. In human language computing, composition is achieved by way of semantic association and the generation of strings of entities. That generation of strings takes place progressively: e.g., strings of symbols (words), strings of strings (sentences), strings of strings of strings (paragraphs), etc. The mathematical (topological, graph-theoretic) analysis of Roget's Thesaurus (1962) has opened a door onto a broad vista of potential achievements in such areas as artificial intelligence and expert systems, through the analysis of concept association, or concept composition.

For the purposes of natural language (e.g., English) concept association, words are salient. But the importance of words as bearers of concepts has come to be recognized, within recent linguistic paradigms, as something of a "spin-off" from efforts to describe the structure resulting from combining symbols and word strings into sentences. As Chomsky (1965) described sentence structures, syntactic part-of-speech designations and rules for acceptable syntax were primary; semantics comprised interpretations mapped onto the syntax. But Chomsky popularized the tree-graph representation of sentences, intending it as a description. Nonetheless, as he showed (still arguing that it was a description), once a descriptive tree is available, it is possible not only to ascend the tree (e.g., from the word, table, to the part-of-speech, noun, to the composite category, noun-phrase [in combination with, for example, an article], to the composite category, sentence [in combination with a verb phrase]), but it is also possible to start at the top (sentence) and generate the rest of the tree by a series of rewrite rules (e.g.,  $S \rightarrow NP + VP$ ). One can hypothesize that once the idea of generating sentences gains power, one begins to consider what in fact seems to happen, or be uppermost in our minds, when engaged in such generating. Most of us do not think first of such strings as Determiner-Noun-Verb-Determiner-Noun but rather of some concept(s), or information to be conveyed in the form of words or collections of words. Not surprisingly, some of Chomsky's students became known for their work in generative semantics; more recently still, linguists schooled within the transformational paradigm are speaking of lexical-relation grammars. Thus, within a paradigm growing out of the earlier structuralist paradigm, with emphases in both cases upon the syntax of strings of strings (sentences), there is now a strong emphasis upon the lexicon, upon the words, and their meaning, strung together into sentences.

With the paragraph (strings of strings of strings), we enter the realm of discourse analysis — of trying to explain why we perceive a paragraph, or a chapter, or a book as unified, or 'holding together' as a single piece of text. For the English language, one of the more rigorous analytical systems was developed by Halliday and Hasan (1979), who explored and listed the kinds of "ties" that bind texts together. Although some of the general categories are at least meta-syntactic (e.g., the category of reference, especially pronominal reference), in fact the emphasis is upon the lexicon, upon words which are actually present, as is the case in most of the categories employed, or are assumed to be present, as in elided segments that repeat some aspect of earlier content. Clearly, a pronoun such as "he" will refer to some specified single male elsewhere in the discourse; hence, the two instances will refer to the same individual and, in the act of doing so, bind the text together. Under the category of "substitution," words such as "thing" are made concrete by other words (as with "This is the thing...") and, of course, there are many types of ties comprising words that are closely related semantically, i.e., they can be used in very similar contexts.

Within the field of artificial intelligence, and very notably artificial intelligence as embodied in expert systems, there is a great need for good natural language interfaces, sometimes only as "front-ends" but

sometimes comprising the backbone of a given system. For the development of sophisticated tutoring systems or computer-based consultants or any interactive system for which the preferred mode of interaction is a natural language such as English, significant progress can only derive from much more conceptually agile (and therefore more wide-ranging as to domain) systems than are available today. The research discussed here focuses upon large general-purpose lexicons, notably thesauri and more particularly, Roget's International Thesaurus, Third Edition (1962), which have the great advantage of being culturally-validated — that is they have been used by, in the case of Roget's, English-language speakers for many decades and are thus descriptive of the language as it is used, in part because they are also prescriptive. This research is directed toward building a foundation for intelligent systems that can range as widely as the semantic space for an entire language permits them to. Further, it has long been evident that approaches to natural language knowledge representation are so laborious as to daunt entire generations of graduate students likely to be involved in building those representations — hence, given the approach advocated by many of building conceptual structures "from scratch," the possibility of intelligent and expert systems able to work within any but very restricted domains is remote. It seems desirable, therefore, to redouble our efforts to use, at least as a basis for a domain-transcendent natural language knowledge representation, the very large lexicons already available which people find adequate for many semantic discriminations.

After examination of a number of lexicons, our research focused in upon Roget's, which has both an explicit and implicit structure, both deriving from concept association. The explicit structure is hierarchical (N.B., that the Roget's used here is not set up like a dictionary) with seven or eight levels, depending upon how they are specified. At the top are eight very general categories and at the bottom of the hierarchy are the individual words, grouped together with other, closely semantically associated, words in a semicolon group (i.e., the boundaries are formed by semicolons). An example of such a group would be: inspiration, inhalation, indraft or indraught, inflow, inrush, sufflation, insufflation, afflation, afflatus. The implicit structure depends upon the multilocality of a number of words in the Thesaurus — a given word may appear in a number of different places in the Thesaurus, thus providing the means for rulefully traversing the Thesaurus cross-hierarchically. Bryan (1973) has produced an elegant mathematical model of the Thesaurus which, among other properties, defines aspects of the implicit structure, including chains, stars, and neighborhoods.

Two questions immediately come to mind concerning a resource like Roget's: 1. Is it really a reliable guide to English semantic space as defined by cultural usage? and 2. Can it provide the types of relationships needed in computer-based natural language implementation?

In answer to the first question, empirical research suggests that the Thesaurus can be accurately regarded as the skeleton for English-speaking society's collective associative memory. Briefly, the ways in which this conclusion has been tested are: A. the determination of when the initial characters in an English word are functioning as a prefix;



our working hypothesis was that when a possible prefixed word form and its possible stem occur close together within the Thesaurus, they probably can be considered as a stem and a prefixed form; (This assumption was borne out in a large majority of cases, while at the same time the Thesaurus correctly showed, e.g., that the words "vent" and "prevent" cannot be grouped together as stem and prefixed form. Thus, as a measure of 'semantic distance' between English words, the Thesaurus is useful in a satisfying way in this test [Warfel, 1971-2; Sedelow, 1969; Sedelow, 1985a; Sedelow, 1985b, 1988]); B. early experiments with content-analytic programs using the Thesaurus showed that it provides semantic clustering conformal with experience and expectations as to usage patterns (Sedelow and Sedelow, 1969); C. starting from a low level (indicated by semi-colon boundaries, hence called semicolon groups) with the syntactic subset comprising verbs, navigation of 'chains' based on the topological model (Bryan, 1973; Patrick, 1985; Sedelow and Sedelow, 1986) produced distinctions among homographs — a very important achievement, given the pervasiveness of ambiguity in multi-domain natural language knowledge-bases, user queries, and system responses, and thus problems involving disambiguation; D. a distribution of the so-called Chinese simplicia, as categorized by Karlgren (1923), against categories in Roget's showed semantic gaps conformal with observations made more 'anecdotally' by scholars comparing aspects of Chinese and English (e.g., book and private conversation of Bloom [1981]); E. research exploring the interaction between the Thesaurus and abstracts of articles in the 1985 SCAMC (Symposium on Computer Applications in Medical Care) Proceedings which produces a conceptual overview of the abstracts based on intersections between textual context and thesaurus concepts — for which the results are quite satisfactory (Brady, 1981); F. a distribution of the UNIX Spelling Dictionary against terms occurring in the Thesaurus shows a very high correlation with the grouping of entries in the Thesaurus as to semi-colon group, paragraph, category, etc. A distribution of the Oxford Advanced Learner's Dictionary against the Thesaurus also has produced a very high correlation; G. inasmuch as the sentence "Time flies like an arrow" is a classic in discussions of ambiguity, it is worth noting that the Thesaurus, again interacting with the text of the sentence (Brady, 1989), produces the reading that seems often to come to mind first, i.e., the speed with which time goes by.

These studies of the Thesaurus — studies that range over many different kinds of problems/applications and many types of text — suggest that it is appropriate to conclude that the initial working assumption as to the potential usefulness of a culturally validated resource such as Roget's has been substantiated with specific reference to Roget's. We do not claim that the Thesaurus is 'perfect' — we can ourselves generate examples of desirable modifications and additions that are well grounded theoretically — but it has performed very well in a number of tests over rather wide-ranging discourse domains.

The answer to the second question — Can the Thesaurus provide the types of relationships needed in computer-based natural language implementation? — must be brief. It is our hope that the associative structure of the Thesaurus will lend itself to the kinds of approaches suggested by genetic computing (Walbridge, 1989) and natural computation (Richards, 1988) thus obviating the need for some of the structures currently being used for natural language interface programs. Insofar as additional relationships are desirable, some of them can be derived from the Thesaurus as it stands. For example, the hierarchical structure will specify IS-A (taxonomic) relationships and can help deal with the issue of "inheritance" (as in frames). Insofar as stereotypes (e.g., user expectations in a given context), in their more general sense, are culturally-prescribed and induced, the networks of associated terms in the Thesaurus ought to provide good indices to stereotypes; at least as they function to elicit knowledge of the user, one or two nodes in the net should serve to "haul in" sets of related terms which might either exist in the user's current knowledge base, and thus help characterize the user, or which could be tutorially added, and thus help inform the user. These are domains within which we now are proposing to do research, along with a number of others which time and space prevent describing. As is evident, though, concept association is the key to our approach as it is, we believe, the key to natural language communication and understanding.

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# DENTAL PATHOLOGY IN SELECTED CARNIVORES FROM ARKANSAS

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## ABSTRACT

The occurrence and kinds of dental pathology in wild carnivore populations in Arkansas were investigated through examination of 1295 skulls of bobcat (*Felis rufus*), river otter (*Lutra canadensis*), gray fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), and raccoon (*Procyon lotor*). Pulpitis or abscesses in broken or otherwise exposed teeth were noted in all species, but bobcats had the fewest exposures while otter and gray fox had the most. Osteomyelitis occurred in all species except the bobcat. Caries were noted in the coyote, raccoon, and gray fox. Otter and coyote had alveolar thinning, and coyote had enamel dysplasia and maxillary sinusitis.

## INTRODUCTION

The effect of dental pathology on wildlife populations has received little attention, possibly because of an impression that due to evolved food habits and life styles wild animals are relatively free of disorders of dental tissues (Robinson, 1979). Because few studies have been conducted to evaluate the occurrence or the effects of dental pathology on wild populations, little is known concerning its prevalence or role in population regulation.

The condition of teeth directly affects nutritional status, thereby affecting behavior, reproduction, and longevity. Occurrence of dental pathology is logically a function of age due to increased exposure to mechanical, chemical, and bacterial attacks from the external environment. The fracture of a tooth may expose the pulpal tissue producing pulpitis, and the spread of infection along pulpal tissue may produce a periapical abscess. Erosion of subadjacent bone produces a fistulous tract and local osteomyelitis. Extension of this tract may produce maxillary sinusitis (Robinson, 1979). Interestingly, Stirling's (1969) study of Weddell's seals (*Leptonychotes weddelli*) indicated a correlation between tooth wear and adult mortality.

The present investigation was conducted to provide data on the kinds and prevalence of pathology in dentitions of bobcat (*Felis rufus*), river otter (*Lutra canadensis*), coyote (*Canis latrans*), raccoon (*Procyon lotor*), and gray fox (*Urocyon cinereoargenteus*) from Arkansas as represented in the Collection of Recent Mammals, Arkansas State University Museum of Zoology (ASUMZ). Knowledge of the frequency of pathological dentitions may aid in the study of population health and longevity.

## MATERIALS AND METHODS

A total of 1295 skulls (202 bobcat, 93 river otter, 150 coyote, 645 raccoon, and 205 gray fox) was examined for caries, fractures, pulp cavity exposures, abscesses, fistulae, excessive attrition (tooth wear), and other indicators of dental pathology. Most of these specimens were collected during the regular Arkansas trapping seasons (approximately December and January) of 1978 through 1982. Randomness of the sample was assumed because no specimens were obtained or rejected specifically because they showed some form of pathology. The only biases should be those incurred through the method of harvest, mostly shooting and trapping.

Frequencies of pathological conditions expressed as percentages of the sample are misleading. Juvenile dentitions have been exposed to risk for only a short time and consequently seldom show abnormality

(see Schultz, 1935; Hershkovitz, 1970). Therefore, frequency reflects age structure rather than the prevalence of dental disease in the adult population. For this reason, the importance of dental disease was analyzed with consideration for age structures. Results of this investigation are considered to be minimum representations because less progressed infections could not be noticed in prepared skulls. Also, dentitions with sharp-broken teeth were not included because such breaks may have occurred while the animal tried to free itself from the trap.

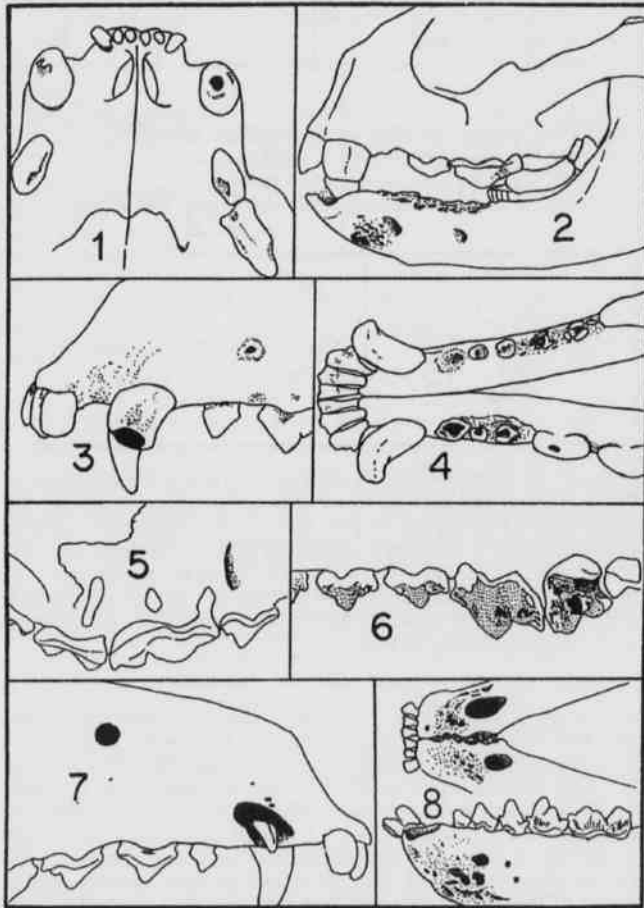
Table 1. Distribution of exposed cheek teeth in selected Arkansas carnivores; u = upper, l = lower teeth.

| Species                         | C    | P1 | P2 | P3 | P4 | M1 | M2 | Total |
|---------------------------------|------|----|----|----|----|----|----|-------|
| <i>Felis rufus</i>              | u 6  | -- | -- | 1  | 0  | 0  | -- | 7     |
| N = 202                         | l 7  | -- | -- | 2  | 0  | 2  | -- | 11    |
|                                 | 13   |    |    | 3  | 0  | 2  |    | 18    |
| <i>Lutra canadensis</i>         | u 24 | 14 | 20 | 22 | 10 | 2  | -- | 92    |
| N = 93                          | l 20 | -- | 18 | 19 | 7  | 4  | 0  | 68    |
|                                 | 44   | 14 | 38 | 41 | 17 | 6  | 0  | 160   |
| <i>Canis latrans</i>            | u 3  | 0  | 2  | 0  | 0  | 0  | 0  | 5     |
| N = 150                         | l 4  | 2  | 2  | 1  | 0  | 0  | 0  | 9     |
|                                 | 7    | 2  | 4  | 1  | 0  | 0  | 0  | 14    |
| <i>Procyon lotor</i>            | u 24 | 9  | 10 | 10 | 10 | 8  | 6  | 77    |
| N = 645                         | l 15 | 6  | 9  | 5  | 7  | 8  | 7  | 57    |
|                                 | 39   | 15 | 19 | 15 | 17 | 16 | 13 | 134   |
| <i>Urocyon cinereoargenteus</i> | u 24 | 23 | 42 | 51 | 22 | 6  | 0  | 168   |
| N = 205                         | l 12 | 37 | 44 | 45 | 34 | 17 | 3  | 192   |
|                                 | 36   | 60 | 86 | 96 | 56 | 23 | 3  | 360   |

## RESULTS AND DISCUSSION

**Bobcat** — Only eight of 202 bobcat dentitions were diseased. Seven of these were caused by mechanical breakage, most often of a canine, although premolars and a molar were also involved (Table 1). Bobcats have the fewest teeth (28) of North American carnivores and therefore are likely most affected by tooth loss. Disease in any major tooth could cause appreciable difficulty to the individual by terminating the function. Species with two or more premolars or molars may still retain some premolar or molar function if one of these teeth is lost, but this is not

true for bobcats. The relatively low incidence of disease in bobcat dentitions was similarly noted by Hall (1940), who suggested the reduced number of small teeth and the decreased chances of injury due to the presence of few teeth (law of probability) as explanations. In our sample, more progressed infections of the pulp cavity led to abscesses in the left upper canine of ASUMZ 7719 (Fig. 1), and the right lower molar of ASUMZ 7633, and pulpitis only was evident in four specimens.



Figures 1-8. Dentition of Arkansas carnivores expressing pathology. 1 - Exposed left C<sup>1</sup> of bobcat (ASUMZ 7719); 2 - extreme dental fragmentation and osteomyelitis of river otter (ASUMZ 7237); 3 - broken left C<sup>1</sup> of a coyote with periapical fistula (ASUMZ 7454); 4 - traumatic loss of premolars in a coyote (ASUMZ 7331); 5 - alveolar thinning through right maxillary wall at carnassial region of a coyote (ASUMZ 6934); 6 - right maxilla of coyote with enamel dysplasia and caries (ASUMZ 6912); 7 - abnormal eruption of the right C<sup>1</sup> with a periapical fistula in a coyote (ASUMZ 2786); 8 - large fistulae at C<sup>1</sup> of raccoon, ventral and lateral views (ASUMZ 8198).

Wear is an obvious function of age, but two specimens had unusual forms of wear. The outer surface of the right upper canine of ASUMZ 8684 was chipped off, probably as a result of a sharp downward bite against a hard object, but the pulp cavity was not exposed. The upper and lower canines of ASUMZ 7633, a 13-year-old specimen as determined by cementum analysis, developed too closely opposed. Friction over time had seriously eroded the opposing surfaces exposing the pulp cavities and resulting in pulpitis and possibly periapical abscesses of all canines.

*River otter* — Otter are known to crack mussel shells with their teeth (Morejohn, 1969) and thereby risk breakage, especially of the premolars, of their 36 teeth. Several otter dentitions contained broken or diseased teeth (Table 1). Estimated ages of otters with exposed pulp cavities indicated that all age groups, including young-of-the-year, experienced exposures. In accordance with age-specific risk, younger otters had fewer exposures than older individuals. Canines were almost always involved, but the first two premolars were less often severely affected than the third premolars (note: the otter does not possess the lower first premolar).

The short jaws and strong masseter muscle provide the otter with a powerful bite. Sharp bites to break hard objects often chip teeth and sometimes result in exposures, most notably of the canines and high cusps of the fourth upper and third lower premolars, which first contact hard objects. Young permanent dentitions are more susceptible to these mechanically-induced exposures because juvenile pulp cavities are larger (Kuehn and Berg, 1983) and therefore more easily reached through chipping. Major chipping, in which 2-4 mm diameter chips were lost from the entire anterior surface of canines, was observed in four specimens.

Extreme dental pathology was exhibited by ASUMZ 7237 (Fig. 2); a 4+ year-old female (age determination by cementum analysis may be biased downward when based on diseased teeth) that possessed no healthy teeth. Only the right third lower incisor was present and most of the upper incisors showed pulpitis. All canines were broken and abscessed, and osteomyelitis had progressed on the right upper and both lower canines. The fistulous tracts of the lower canine region exposed the canine roots. The right fourth upper premolar and left third and fourth upper premolars were the only intact ones, but even these had pulpitis and abscesses. Both upper molars were broken and abscessed. The fragmented right first lower premolar was the only lower premolar present, and osteomyelitis was quite apparent. Both first lower molars were severely fragmented and abscessed with prominent fistulae present on the buccal aspect of the right mandible. Only the last lower molars were intact.

Alveolar thinning was found in three specimens (3.5% of sample) and was relatively minor — the posteroexternal root of the first upper molars showed only slightly through the floor of the orbit. Beaver *et al.* (1981) noted this condition in 23.3% of their sample of otter from Maryland. Because this condition is believed to be caused by pressures of mastication, it is possible that harder prey available to estuarine otters may be responsible for the observed disparity in occurrence.

*Coyote* — Of the carnivores examined, the coyote and gray fox possess the greatest number of teeth (42), providing the most opportunities for disease. Trauma-induced pathology is probably less likely to occur in the coyote than in the fox because the coyote's teeth are larger. Accordingly, exposed pulp cavities in coyotes were few (Table 1). Pulp cavities of incisors from ASUMZ 2786, 6466, and 7454 were exposed and infection had produced abscesses in 2786 and 7454. Further, ASUMZ 2786 and 7454 had abnormal occlusal wear by the third upper incisors on the lower canines, accounting for lower canine exposures on 2786. ASUMZ 7454 also suffered traumatic loss of the left upper canine in early life, causing a large exposure and a severe abscess with osteomyelitis (Fig. 3). Only peg-like roots of most of the premolars remained in an additional specimen, ASUMZ 7331 (Fig. 4), which had developed abscesses and osteomyelitis. Infection was not apparent, but ASUMZ 6934 (Fig. 5) had alveolar thinning around the roots of the upper fourth premolars and the first and second upper molars. Alveolar thinning is normally associated with these teeth in other mammals (Smith *et al.*, 1977; Beaver *et al.*, 1981).

Colyer (1936) found carnivores to contain few caries and Hall (1940) found caries to be present only in bears, attributing the observation to a high-sugar diet. However, Schitoskey (1971) found caries to be common in feral nutria, *Myocastor coypus* (a rodent). Caries were found in two coyotes, ASUMZ 6466 and 6912. Caries on 6466 occurred on both left upper and both right lower molars, and on the left and right fourth upper premolar, and on the right upper first molar. Attrition had exposed the dentine and probably allowed high-sugar foods (e.g., persimmons) to cause caries. Similarly, caries in the carnassial region



## Dental Pathology in Selected Carnivores From Arkansas

of specimen 6912 were attributed to exposed dentine, but without an attrition etiology.

Specimen 6912 (Fig. 6) demonstrated enamel dysplasia. The incisors had the most normal enamel covering of the dentition, decreasing in quality from the first to the third incisors. The gingival one-third to one-half of the canines possessed enamel, and some enamel was present in scattered locations toward the apex. The canines were the only teeth with root dwarfism, which made them loose and easy to remove. Paracones and paraconids of the first premolars were of exposed dentine, and the fourth lower premolar showed abnormal eruption. Scattered chalky enamel was discernable on the premolars. Normal enamel was present only on the ridge of the molars with the paracones, metacones, protocones, and talons (and their lower homologs) composed of dentine with deposits of chalky enamel scattered over them. The condition was very similar in appearance to the genetic condition termed amelogenesis imperfecta (Colby *et al.*, 1971); however, siblings were not available for verification of that etiology. Similar enamel disorders have been reported for primates (Colyer, 1936; Jones and Cave, 1960; Molnar and Ward, 1975), but, to our knowledge, not in carnivores.

Another form of dental disease was apparent in ASUMZ 2786. The right upper canine did not erupt from the alveolus but grew through the inner wall of the maxilla, into the nasal conchae, and posteriorly to the plane of the anterior area of the third upper premolar. At this point a 5 mm fistula appeared through the maxilla at the root tip of the abnormal canine (Fig. 7). Maxillary sinusitis was undoubtedly a complication.

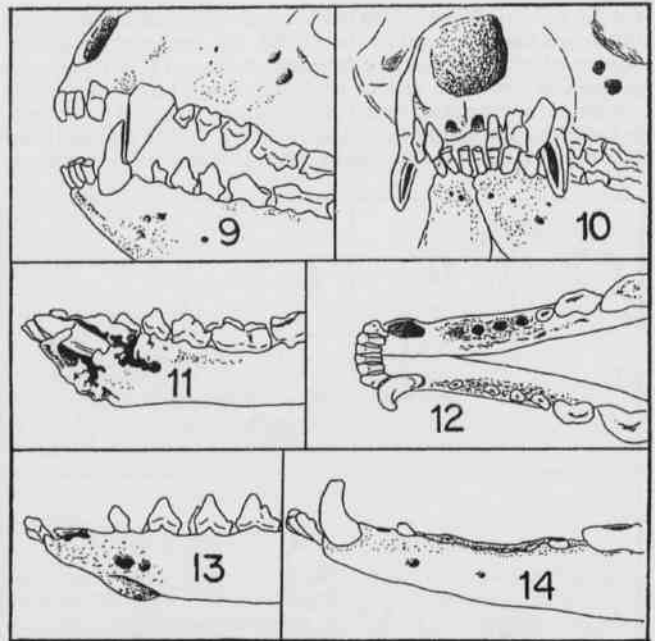
**Raccoon** — Raccoons possess 40 teeth, the smaller premolars of which were the most often broken in diseased specimens. Canines were the most commonly exposed teeth (Table 1). The presence of exposures in individuals seemed relatively uncommon (3.9% of sample) considering the large sample of 645 specimens. However, approximately 72% of the sample were young-of-the-year, leaving 181 specimens of our sample as assumed adults, of which 13.8% had pulpal exposures.

Chipping or more traumatic breakage of canines, premolars, and occasionally molars was evident in 13 specimens. All of these traumas had resulted in abscesses of varying sizes. Small maxillary fistulae were observed at the canine root tip of ASUMZ 8177, 8198, and 8539. Progressed osteomyelitis occurred in 4535 and 8198. Numerous fistulae, up to 7 mm maximum diameter, appeared in the area of the lower canines of 8198 (Fig. 8). Specimen 4535, a juvenile, had extreme bone resorption around the right lower canine while the infection around the left lower canine was so severe that bone destruction had practically separated the post-canine mandible from the pre-canine and symphyseal portion (Fig. 11). Hence, the left mandible was flexible and could not be functional for mastication.

Moderate degrees of osteomyelitis were encountered in seven additional specimens. In all cases these were explained through attrition. Raccoons are omnivorous and their diet gradually erodes the low cusps of their teeth. Erosion eventually results in dentine and finally pulp cavity exposure, providing the precondition for pathological events. In time, pulpitis leads to bone destruction. The inclusion of fruits in the diet helps explain the observation of caries in the more proximal premolars of eight old (5+ years) specimens, in which attrition was a factor.

Misposition of the left lower canine of ASUMZ 9136 was effected by the failure of the corresponding lacteal to be disposed. The permanent canine was forced to erupt posterior to its normal position, causing some malocclusion. This was a young specimen, and further complications had not yet appeared.

Prevalence of an unusual pathological condition affecting raccoon canines is especially noteworthy. Upper and lower canines do not normally come in direct apposition, but many specimens in the sample showed shear on the posterior face of the lower and on the anterior face of the upper canines. Assuming genetic implications, the effect of this phenomenon could be significant to a population if shear resulted in pulp cavity exposure leading to more severe pathological conditions. Affected individuals were expected to show greater wear with increasing age, and 202 specimens for which age estimates were available were



Figures 9-14. Dentitions of Arkansas carnivores expressing pathology. 9, 10 - Lateral and anterior views of raccoon canines demonstrating shear sufficient to expose the pulp cavity of the canines; 11 - extreme bone resorption from infection around the left C<sub>1</sub> of a raccoon (ASUMZ 4535); 12 - traumatic fragmentation of lower premolars with osteomyelitis in a gray fox (ASUMZ 6708); 13 - periapical fistulae at left C<sub>1</sub> of a gray fox (ASUMZ 8239); 14 - complete loss of premolars and scalloped dentary of gray fox.

used to test that assumption. Specimens from this sample were ranked on a scale of 0-3 according to the amount of wear, where 0 = no wear, 1 = noticeable faceting (approximately 0.5 mm diameter), 2 = appreciable faceting (approximately 1.0 mm diameter), and 3 = wear sufficient to expose the pulp cavity (Fig. 9, 10). Frequency within this system (from 0-3) was 138, 44, 15, and 5, respectively. Specimens of age one or older were considered to be capable of showing wear. Of the 138 of rank 0, 68 were old enough to show faceting. Hence, 48.5% of the adult sample were apparently predisposed to the condition and 3.8% (specimens of rank 3) were exposed to infection. Thus, the shearing phenomenon probably does not cause dental disease in enough cases to be a significant factor in raccoon population biology due to loss of life or reduction in fitness. Four additional individuals of unknown age had shear-exposed canines.

Generally, degree of wear was a function of age although some younger specimens had high rank and some older individuals had low rank. The mean age of rank 1 specimens was 1.7 years (range 0-8), compared to 2.9 years (range 1-7) for rank 2 and 4.8 years (range 3-6) for rank 3.

**Gray fox** — Gray foxes are probably more susceptible to mechanically-induced dental disease because of their number of teeth (42) and the relative delicacy of their premolars in comparison to the other carnivores examined. Forty-eight specimens (23.4%) had exposures, but 45.9% of the adults were affected. Gray foxes in Arkansas seldom reach five years of age, and their dentitions indicate that attrition may restrict longevity to approximately that age. Many of the exposures, especially when affecting the carnassials and molars, were explained by attrition. However, exposure by traumatic fragmentation or complete loss of teeth was also noted in younger specimens (and no doubt was also a factor in older dentitions which were explained by attrition).



Two individuals (ASUMZ 6708, 8239) were affected by osteomyelitis. Bone destruction in 6708 (Fig. 12) was noted along the jaws where the first three upper and lower premolars had been totally broken. Two larger fistula (3-5 mm diameter) were present at the root tip of the lower left canine of 8239 (Fig. 13).

Examination of Table 1 reveals that the second and third premolars are the most commonly affected teeth. Apparently, some aspect of gray fox behavior affects dental condition. Fifteen of the dentitions exhibited a near complete loss of premolars 1-3 in all jaws. Of these, 10 specimens had eroded the gingival tissues exposing the bone and had also scalloped into the dentary (Fig. 14). Sex was known for eight of these specimens, seven females and one male. Because of the skewed sex ratio, it may be that some aspect of female fox behavior, perhaps denning habits, predisposes females to dental trauma. Presumably, such tooth loss in either sex is caused by 'sawing' with the premolars against roots, limbs, bones, or other hard objects. We conjecture that den construction by females results in this phenomenon or perhaps physiological demands for calcium during gestation and lactation weakens the teeth and renders them more fragile in females.

One specimen (0.7% of sample) was affected by caries of the first upper molars. This was an older specimen in which attrition had exposed the dentine and pulp cavity. Abscesses of the upper molars were a complication.

#### CONCLUSIONS

The occurrence and importance of dental pathology to wild carnivores is related to age and the number and size of teeth. Bobcats possess the fewest (but generally largest) teeth, and the occurrence of dental disorders is low. Bobcats from the sample lived to be 15 years old. Coyotes have the most teeth of the carnivores examined but their teeth are generally large and dental disease was infrequent. Raccoons and gray foxes have several relatively delicate premolars and incidence of disease was highest for these. Raccoons may live to be eight years old and foxes five years. In both cases, dental attrition indicates these ages approach the functional maximum. Therefore, it appears that dental pathology may be influential as a limiting factor on longevity of individuals in some carnivore populations. However, this study also shows that animals can survive with a variety of dental problems. If dental pathology reduces an individual's success in predation, fitness may be affected if energetic requirements for reproductive success cannot be acquired or handled efficiently. At present, the effect of dental disease on fitness is not known.

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# CONCEPT DECOMPOSITION

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## ABSTRACT

Historically, perhaps the most general paradigm for scientists has been decomposition — as with, e.g., 'elements' in chemistry, and the basic structures/processes of theoretical physics. Knowledge representation research is encouraging a somewhat comparable activity in computer science, by way of the study of knowledge representation structures and knowledge representation systems. Symbol sets, rules of usage (including divergent inferencing engines), and conceptual primitives are among the entities involved in this process of decomposition.

Years of association with research in the history of science have eventuated in a conspectual overview of the character of science at large and in a perceived sense of science's Grand Design — including concept decomposition as well as other distinctive properties (Sedelow & Sedelow, 1978; 1979). Despite traditional associations for the term design, that Grand Design is not an individual invention, but, rather a 'social invention' (Ogburn and Thomas, 1922). It is not the work of any one individual, nor even in its entirety the fully conscious plan of a set of such, but, rather, the only partially intended, accreted result of a process Koestler (1959) spoke of tropically as in some respects even sonambulistic. To a computer scientist, in that perspective science itself may be 'ultimately' decomposable into the behavior of a Turing Machine (Sedelow, 1980), or a Bavel Power Tier Automation (Bavel, 1988). Thus, although conventionally there is an ascription of conscious intent, much of the detail of science, both in the large and in fine, does not require a positing of consciousness (Gregory, 1987) and of deliberate intent to account for patterns we find/invent in the output of the scientific enterprise; some indication of that approach is to be found in the published papers already cited on the formalization of historiography and the analysis of science as discourse, especially with reference to the highest level in the scientificity hierarchy.

Aside from the major scientific achievements in China in earlier centuries (Needham, 1954) as well as the notable achievements of early Islamic science, and taking science as at least predominantly an Occidental enterprise — and in the longer perspective of world history perhaps the Occident's primary accomplishment — it does seem apparent that the most general scientific paradigm (in Kuhn's, 1970, earlier view of the role of paradigms) has been decomposition. That emphasis on decomposition in the Grand Design of science is evident both by way of the examination of the history of the individual sciences and also by way of the study of the contrastive possibility of an emphasis on systemic understanding.

Efforts to enhance the intellectual and technical power of computer science as an academic discipline (Sedelow, 1989) encounter numerous adamant obstacles, many of them consequences of the pay-off matrix in computer applications — which is so loaded as to reward short-term, purely technique, trivia at the expense of scientific depth. Even in a computer science specialty so necessarily concerned with 'ideas' as expert systems, and artificial intelligence, that scientifically disadvantages condition has obtained. Nonetheless there are those like John McCarthy who, while indubitably making shorter-term 'practical' contributions (e.g., the language LISP), have persisted in an effort to achieve such generality of results (e.g., on the predicate calculus, as presented in his Turing Lecture, 1987) as contribute to that cumulateness crucial to the growth of science.

In the domain of human language computing, and more specifically expert systems, there is in the Little Rock division of the University of Arkansas system an on-going effort — which has been aided by grants from The National Science Foundation, the Office of Naval Research, the U.S. Air Force, and The Exxon Educational Research Foundation — to contribute to that depth of result which distinguishes science from technology, as well as to accord with that grand design in science which fosters decomposition. More specifically, and as also developed by S. Sedelow (in press), some of that effort is directed to the study of whole 'natural' languages as knowledge representation structures employable on the computer.

In the aftermath of the consensual recognition of the non-productivity of the General Problem Solver (GPS) of Simon, Newell, and Company (on the Computer Science faculty at Carnegie-Mellon University), there developed an enthusiasm for specific problem solvers highly engineered to accommodate massive informational pump-priming, so that a minimum of learning (contra Rosenblatt and Bloch's 'Perceptron') by the computer-based system was required. Unfortunately for the development of computer science, and for the development of science generally, a domain-specificity/generic-reasoning contrast was not problematicized, with the result that now more than a generation of computer science students have been professionally enculturated with the notion that in building cognitively robotic (Sedelow, 1988) or artificially intelligent systems one has to opt for domain narrowness in order to avoid a dead-end in an impoverished and barren abstractness. But now we can begin to see our way clear to attaining the advantages of that breadth of scope sought by the builders of the General Problem Solver at the same time that — through modelling the semantic structure of a decomposed whole human language — we can engage in the specific human language transforms which are at the core of expert systems and numerous cognitive robotic applications (Marr, 1982).

At the moment the expert systems of AI might more accurately be described as artificial idiots savants. While to build an artificial idiot savant is a considerable accomplishment — after all, an idiot savant specialized to playing chess is demonstrating considerable skill, no matter what may be his/her limitations otherwise — nonetheless it does not demonstrate that capacity for general-purpose symbol manipulation that we speak of as a manifestation of at least verbal and logico-mathematical intelligence.

In the process of building expert systems numerous types of decomposition are employed, irrespective of whether the systems in question are domain-narrow or domain-transcendent (Sedelow and Sedelow, 1988). Among the types of entities which knowledge representation specialists examine are symbol sets (such as an alphabet or a number system). There also is decomposition into rules of usage governing what constitutes an acceptable string of items made up from a symbol set, as well as decomposition into well-formed formulas, inferencing engines, etc. It is now also possible to decompose the meaning space — in the sense of a mathematical space — created by a human language at any given stage of its evolution. If, as in type-token mathematics, a distinction is made between types and tokens, the types as gathered together for a language and then sorted alphabetically comprise a dictionary's main entries.

The word types used in the definitional components of that dictionary could be regarded as a set of primitives (Sedelow and Sedelow, in press). Now if, one way or another — whether in trees, in semi-rings, or with some other discrete mathematical structure (webs, for example), perhaps not yet invented — an effort is made at least partially to order that set of primitives, the result is a semantic space structure for the language as a whole which also provides a basis for comparing one language with another (Sedelow, 1988), even if those comparisons have to be made with the aid of a supra-binary (multi-valued) logic, such as rough sets (Grzymala-Busse and Sedelow, 1988).

Many years of research stand behind the formalized symbolic processes (necessarily only glancingly) referred to here, and in Sally Yeates Sedelow's paper in this Proceedings. Perhaps the best single introduc-

tion to further knowledge of this work would be by Sedelow and Sedelow (1986); Sedelow and Sedelow (1987); and Sedelow and Sedelow (in press).

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# PREPARATION OF A SERIES OF PYRIDYL PHENYLUREAS OF POTENTIAL AGRICULTURAL INTEREST

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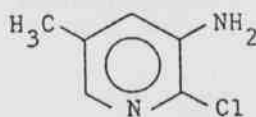
## ABSTRACT

Substituted phenylurea derivatives of 3-amino-2-chloro-5-methylpyridine and 5-amino-2-chloro-3-methylpyridine were prepared by treating the amines with appropriately substituted phenyl isocyanates. Structure-confirming spectral data are also presented.

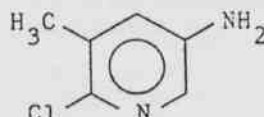
## INTRODUCTION

For several years we have synthesized various types of compounds of agricultural interest. Many of these compounds were screened for herbicidal and fungicidal activity by commercial agricultural research laboratories. A few compounds had desirable effects on specific weeds or plant fungi, but none were considered broad enough in spectrum to receive additional attention.

Since several of our halobenzamido derivatives of 3-amino-2-chloro-5-methylpyridine (I) (Setliff and Rankin, 1988) and 5-amino-2-chloro-3-methylpyridine (II) (Setliff and Palmer, 1987) exhibited moderate fungicidal activity toward wheat leaf rust, we have now prepared for screening other derivatives from these amines, namely phenylureas Ia and IIa.

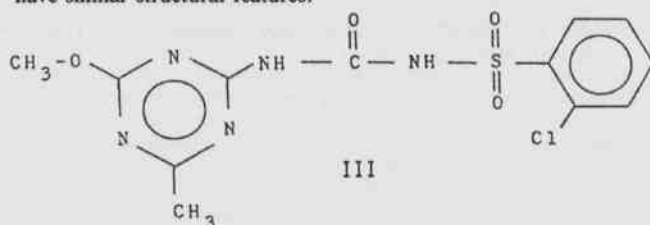


I



II

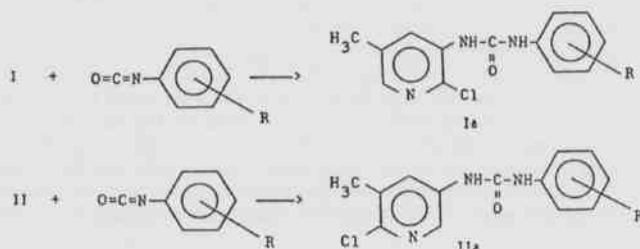
It is noteworthy that chlorosulfuron, (III) one of E.I. duPont's leading herbicides, contains a urea function, in addition to a nitrogen heterocyclic portion, halogens, and a methyl side unit. Our compounds have similar structural features.



III

## MATERIALS AND METHODS

The phenylurea derivatives Ia and IIa were prepared by the reaction of amines I and II with the appropriately substituted phenyl isocyanates. Dry chloroform served as the solvent for the reaction.



The substituted phenyl isocyanates were purchased from Aldrich Chemical Company and were technical grade. Amines I and II were prepared in our laboratory as previously described (Setliff, 1970). All phenylureas were recrystallized from aqueous ethanol. Melting points were taken on a Mel-Temp apparatus and are uncorrected. Infrared spectra were taken on a Perkin - Elmer 1430 Spectrophotometer equipped with a Model 7300 Data Station with samples prepared as KBr disks. Proton nuclear magnetic resonance spectra were determined in dimethylsulfoxide -  $d_6$  on a Varian EM 360 or a Varian XL 300 instrument with tetramethylsilane as internal standard. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee or Desert Analytics, Tucson, Arizona.

The preparations of the phenylureas were carried out as follows. Amine I or II (0.5g) was dissolved in dry chloroform (5 ml) in a 25 ml round - bottom flask. The appropriately substituted phenyl isocyanate (1.0 ml) was then added by syringe, and the resulting mixture was stirred under gentle reflux for 15 min. The precipitated solid (Ia or IIa), which rapidly formed, was filtered, washed with cold chloroform, and then recrystallized from aqueous ethanol.

Table 1. Experimental and Infrared Spectral Data for Phenylurea Derivatives Ia from 3-amino-2-chloro-5-methylpyridine (I)

| R                         | Yld (%) | M.P. (°C) | IR, $\nu$ , $\text{cm}^{-1}$ |      | Elemental Anal. Cal'd % (Found %) |            |              |
|---------------------------|---------|-----------|------------------------------|------|-----------------------------------|------------|--------------|
|                           |         |           | N-H                          | C=O  | C                                 | H          | N            |
| H                         | 50      | 217       | 3287                         | 1636 | 59.54(59.70)                      | 4.58(4.68) | 16.03(16.01) |
| <i>o</i> -Cl              | 47      | 231       | 3290                         | 1640 | 52.52(52.65)                      | 3.70(3.72) | 14.14(14.10) |
| <i>m</i> -Cl              | 74      | 221       | 3285                         | 1637 | 52.52(52.76)                      | 3.70(3.73) | 14.14(14.16) |
| <i>p</i> -Cl              | 60      | 246       | 3295                         | 1640 | 52.52(52.78)                      | 3.70(3.65) | 14.14(14.13) |
| <i>p</i> -F               | 58      | 234       | 3284                         | 1635 | 55.71(55.71)                      | 3.70(3.85) | 15.00(14.87) |
| <i>o</i> -CF <sub>3</sub> | 37      | 208       | 3298                         | 1645 | 50.91(50.99)                      | 3.33(3.26) | 12.73(12.63) |
| <i>m</i> -CF <sub>3</sub> | 52      | 227       | 3346                         | 1713 | 50.91(50.94)                      | 3.33(3.43) | 12.73(12.70) |
| <i>p</i> -CF <sub>3</sub> | 41      | 230       | 3349                         | 1659 | 50.91(51.18)                      | 3.33(3.43) | 12.73(12.62) |
| 2,4 - diF                 | 61      | 238       | 3288                         | 1640 | 52.35(52.48)                      | 3.37(3.36) | 14.09(14.15) |

## RESULTS AND DISCUSSION

Nine phenylureas were prepared from both amine I and amine II (Tables 1,2). Yields, although adequate, were generally better with the less sterically hindered amine II. Carbon, hydrogen, and nitrogen elemental analyses fell within acceptable limits of the calculated values. The infrared spectra exhibited the expected N-H and C=O absorption bands. In most cases the phenylureas Ia from amine I (Table 1) show lower frequencies for these absorptions than the phenylureas IIa from amine II (Table 2). This suggests a greater degree of hydrogen bonding in the Ia molecules.



Table 2. Experimental and Infrared Spectral Data for Phenylurea Derivatives IIa from 5-amino-2-chloro-3-methylpyridine (II)

| R                         | Yld (%) | M.P. (°C) | IR, $\nu$ , $\text{cm}^{-1}$ |      | Elemental Anal. Cal'd % (Found %) |            |              |
|---------------------------|---------|-----------|------------------------------|------|-----------------------------------|------------|--------------|
|                           |         |           | N-H                          | C=O  | C                                 | H          | N            |
| H                         | 72      | 220       | 3397                         | 1708 | 59.54(59.20)                      | 4.58(4.58) | 16.03(15.92) |
| <i>o</i> -Cl              | 73      | 213       | 3373                         | 1709 | 52.52(52.43)                      | 3.70(3.74) | 14.14(13.92) |
| <i>m</i> -Cl              | 64      | 226       | 3385                         | 1710 | 52.52(52.43)                      | 3.70(3.74) | 14.14(14.25) |
| <i>p</i> -Cl              | 66      | 263       | 3394                         | 1711 | 52.52(52.51)                      | 3.70(3.76) | 14.14(14.10) |
| <i>p</i> -F               | 83      | 243       | 3393                         | 1711 | 55.71(55.52)                      | 3.70(3.84) | 15.00(14.85) |
| <i>o</i> -CF <sub>3</sub> | 49      | 235       | 3292                         | 1643 | 50.91(50.73)                      | 3.33(3.24) | 12.73(12.65) |
| <i>m</i> -CF <sub>3</sub> | 74      | 211       | 3393                         | 1711 | 50.91(50.82)                      | 3.33(3.30) | 12.73(12.63) |
| <i>p</i> -CF <sub>3</sub> | 80      | 253       | 3390                         | 1712 | 50.91(50.80)                      | 3.33(3.40) | 12.73(12.73) |
| 2,4 -<br>diF              | 71      | 308       | 3403                         | 1709 | 52.35(52.21)                      | 3.37(3.37) | 14.09(14.16) |

The proton NMR spectra for both series of compounds showed a sharp three proton singlet at 2.3 ppm for the methyl protons, and a complex array of multiplet signals in the range of 7.0 to 9.5 ppm for the collective aromatic and amido protons.

#### ACKNOWLEDGMENT

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# THE MAMMALS OF SOUTHWESTERN ARKANSAS PART II. RODENTS

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## ABSTRACT

This study investigated the composition and habitat affinities of the mammalian fauna of southwestern Arkansas. The study area was comprised of the 21 counties located south and/or west of and including Pulaski County. The previously existing data set pertaining to the mammals of Arkansas was notably incomplete and this study area in particular, was poorly known mammalogically. Specimens were collected by standard trapping and salvage methods throughout the study area. The mammals considered during this study were limited to those species meeting a set of criteria designed to eliminate species that had been introduced or artificially maintained. This study has accumulated records of 25 species of rodents; over 1500 specimens have been recorded; and a total of 95 new county records have been documented.

## INTRODUCTION

A reliable understanding of an area's ecosystem can not be accomplished unless it is known of what that ecosystem is comprised biotically and abiotically. This realization is accentuated by the increase in the number of local, state, and federal agencies that have been studying aspects of the environment beyond those involving only recreational or economic considerations. These agencies agree that the more complete the knowledge of a studied area, the more efficiently the area can be managed and utilized. Consequently, many states are attempting to compile a complete data set of their natural resources. In Arkansas, the need for information on resources is intense. The present study, then, attempts to increase the data set available on the mammalian resources of southwestern Arkansas (an area previously only poorly known mammalogically).

There are relatively few studies on the distribution of mammals in southwestern Arkansas. For the state as a whole, the information available ranges from nonexistent to nearly complete depending on the species and area of concern. Black (1936) made the earliest study of the distribution of Arkansas mammals. His study was limited to northwestern Arkansas and contained limited habitat information.

Dellinger and Black (1940) attempted the first statewide inventory of Arkansas mammals. However, this study included little information on the mammals of southwestern Arkansas and almost no information on the habitats of mammals. The data were often restricted to one or two specimens from as many locations.

One of the first systematic distributional studies for southern Arkansas was conducted by Baker and Ward (1967). They reported on the distribution of nine species of bats in the southeastern portion of the state (Bradley, Cleveland, and Drew counties).

Sealander (1956, 1979) made the first comprehensive studies attempting to establish accurate distributions for the Arkansas mammalian fauna. His distributions were based on previous literature records, his personal collections, the collections of other researchers, and reports of sight observations. Although quite valuable, Sealander's book lacked data from many areas, particularly from southwestern Arkansas.

The purposes of the present study were to establish, as completely as possible, the current distributions of the mammalian species found in southwestern Arkansas and to present data on habitat affinities of these mammals.

## STUDY AREA

The study area comprises 21 counties located south and/or west of, and including, Pulaski County. Within this area of the state, habitats vary widely from the rolling hills and rocky outcroppings of the Ouachita Mountains in the northern portion, to forested hills and cultivated tracts of land, to the sandy flood plains found throughout the southern portion of the area.

Habitat characteristics examined included: predominant vegetation, substrate composition, topography, successional stage, and developmental stage. These characteristics were not considered on an individual basis, but instead were considered to be attributes of overall habitat at any given location. These data could be valuable for evaluating the possibility of encountering a desired species of mammal based on habitat parameters.

The dominant vegetation of the study area included oak-hickory climax forests, loblolly pine forests, cedar glades, brush and grass fields, and agricultural cropland. Substrates varied from deep sands and clays of the flood plains, to rocky cliffs and outcroppings of the mountains, and to tracts of rich loams with scattered swampy areas.

The term "developmental stage" refers to the extent to which a site has been changed from its normal ecological condition by the activities (intentional or inadvertent) of man. These activities include commercial, private, or governmental developments, such as city growth, road construction, farming, and development of recreational areas.

For the purposes of this study, mammals were included if they met the following criteria:

1. The species is not a recently escaped exotic.
2. The species has not been recently introduced or (reintroduced) to the area by other than those avenues naturally open to native species.
3. The species' presence in the area is not a result of current or very recent artificial management or control procedures (i.e., the stocking of game animals into the area from some other area).
4. The record(s) is(are) not likely to be considered spurious.

Finally, only those sight records from acceptably knowledgeable persons and for those species for which misidentification is not likely were used. In most instances, sight records were corroborated by the capture of the same species from a nearby area.

## METHODS

Numerous field collections were conducted throughout the period of this study by various individuals including Arkansas Game and Fish personnel, university personnel, public school teachers and their classes, graduate students, and other knowledgeable laypeople.

A variety of collection methods were necessitated by the different habits and life-styles of the mammals encountered in the study area. Sight identification of and collection of some road-kill specimens, particularly of larger species, resulted in many of the records accumulated for larger mammals and for those difficult to obtain by traditional collection methods. Sight identification of living specimens was made on only a few occasions, when accurate identification was assured.

Small terrestrial mammals were most often collected with commercially available kill and live traps. These traps were placed strategically

and were baited with a variety of mixtures, including commercial peanut butter, fat and grease, meat, vegetables, fruits, and other edible items.

Considerable information was provided by analysis of a collection of owl pellets removed from a roost in Hempstead County (Steward *et al.*, 1988).

Specimens were prepared as standard museum skin and/or skeletal preparations. The specimens were deposited in the Arkansas State University Collection of Recent Mammals.

The habitat composition of the study area was determined by a variety of methods. In most cases, a brief description of the habitat in which the specimen was collected was included with the standard collection data. In those cases where habitat information was not available, the most probable habitat for the collection site was determined. This determination involved consulting published reports for the specific area, personal knowledge of the site, and review of detailed maps and/or aerial photographs of the area when available. Ultimately, habitat information was used to determine the range of "usual" habitats for a given species. Additional sources of distributional and habitat data included the few published literature records, records of the Arkansas Department of Health, and the Collection of Recent Mammals at Arkansas State University.

## RESULTS AND DISCUSSION

More than 1500 rodent specimens were collected during this study. Voucher specimens now exist for 20 species of rodents found in southwestern Arkansas. Nomenclature and phylogenetic relationships utilized in this study are as reported by Jones *et al.*, 1986.

### SPECIES' ACCOUNTS

#### Order Rodentia Family Sciuridae

*Tamias striatus* (Linnaeus), the Eastern Chipmunk. This species is common in deciduous forests, ranging from open to solid stands, with rocky outcrops and deposits. Cover areas, such as rock and log piles and brush, are used as shelter and observation posts (Sealander, 1979; Snyder, 1982). Sealander (1979) reported this species from five counties of the study area (Table 1). Three specimens have been collected from forested areas in Pike County. This species is limited in its distribution within the study area by its requirements of open forested areas providing brush and rock piles.

*Marmota monax* (Linnaeus), the Woodchuck. Woodchucks are usually found in open areas such as fields, along forest edges, and in overgrown fence rows. They were rarely found in heavily wooded areas. Sealander (1979) reported this species from Grant, Hempstead, Hot Springs, Montgomery and Pike counties of the study area. No additional records resulted from this study.

*Sciurus carolinensis* Gmelin, the Gray Squirrel. This squirrel inhabits the denser deciduous forests, those composed chiefly of oak-hickory stands, although they may also be found in oak and conifer forests (Sealander, 1979). There are no previously documented records of this species from the study area. Never-the-less, this is a very commonly seen animal in the area, both alive and as victims of the automobile. We have recorded this species from eight counties of the study area, and nine specimens were collected (Table 1).

*Sciurus niger* Linnaeus, the Fox Squirrel. The fox squirrel is found in more open hardwood forests and is more abundant in upland forests than in bottomlands (Sealander, 1979). The fox squirrel does not compete well with its relative, *S. carolinensis* and usually will be displaced by the gray squirrel if their ranges overlap (Lowery, 1974). This species is less common than the gray squirrel. We have recorded this species from three counties of the study area and have only two specimens (Table 1).

*Glaucomys volans* (Linnaeus), the Southern Flying Squirrel. The preferred habitat of the flying squirrel is dense forest areas, preferably oak-hickory associations, that are near water (Sealander, 1979). Dolan and Carter (1977) reported that the habitat of this squirrel is best

described as a deciduous forest. This small squirrel is a rarity to most people of the area and they are usually unaware of its actual abundance. Heidt (1977) reported that this species commonly used nesting boxes in a study in Saline County. We have 11 specimens from five counties of the study area (Table 1). All of these specimens were taken from human dwellings.

Table 1. Sciuridae, Geomyidae and Cricetidae (Part I) from Southwestern Arkansas.

| County\Species | 1  | 2 | 3 | 4  | 5   | 6  | 7    | 8 | 9   | 10 |
|----------------|----|---|---|----|-----|----|------|---|-----|----|
| Calhoun        | -  | 1 | 1 | -  | -   | -  | -    | - | -   | -  |
| Clark          | -  | - | - | -  | -   | -  | -    | - | -   | -  |
| Columbia       | -  | 2 | - | 7  | 22* | 17 | 127* | * | 64  | -  |
| Dallas         | -  | 1 | 1 | -  | *   | -  | -    | - | -   | -  |
| Garland        | *  | X | - | 1  | *   | *  | *    | - | -   | -  |
| Grant          | -  | - | - | -  | -   | -  | -    | - | *   | -  |
| Hempstead      | *  | - | X | -  | *   | 19 | 13*  | 3 | 120 | -  |
| Hot Springs    | -  | - | - | -  | *   | -  | -    | - | 1   | -  |
| Howard         | *  | - | - | -  | -   | 1  | 2    | - | 3   | 1  |
| Lafayette      | -  | - | - | -  | 2   | 2  | 17   | - | 15  | 5  |
| Little River   | -  | - | - | -  | -   | -  | *    | - | -   | 1  |
| Miller         | -  | 2 | - | 1  | 1*  | 2* | 69*  | - | 56  | 1  |
| Montgomery     | -  | - | - | -  | -   | -  | 86   | - | 2   | 5  |
| Nevada         | -  | 2 | - | -  | 5*  | -  | 14   | - | 7   | 2  |
| Ouachita       | -  | - | - | 1  | 6*  | *  | 37*  | - | 8   | 4  |
| Pike           | 3* | - | - | 1  | -   | 3* | 12*  | - | 5   | 4  |
| Polk           | *  | 1 | - | -  | -   | *  | *    | - | -   | -  |
| Pulaski        | -  | - | - | -  | *   | 5* | 7*   | - | 2*  | 1  |
| Saline         | -  | - | - | *  | *   | -  | -    | - | *   | -  |
| Sevier         | -  | - | - | -  | -   | -  | -    | - | -   | -  |
| Union          | -  | x | - | -  | 4*  | -  | 3*   | - | -   | -  |
| Total          | 3  | 9 | 2 | 11 | 40  | 49 | 387  | 3 | 283 | 25 |

#### LEGEND

1. *Tamias striatus* 2. *Sciurus carolinensis* 3. *Sciurus niger* 4. *Glaucomys volans*  
 5. *Geomys bursarius* 6. *Orzomyomys palustris* 7. *Reithrodontomys fulvescens*  
 8. *Reithrodontomys humulis* 9. *Sigmodon hispidus* 10. *Neotoma floridana*

\* indicates a previous literature record for the species in that county.  
 X indicates a sight record of the species in that county.

#### Family Geomyidae

*Geomys bursarius* (Shaw), the Plains Pocket Gopher. This species of gopher is found only in areas having soils capable of supporting its fossorial life style. Gophers require soils low in clay (<30%) and higher in sand (>40%). High water tables, those within 1.5m of the surface, also exclude this animal. These conditions have greatly restricted the spread of this species (Lowery, 1974; Sealander, 1979). Gophers have been collected from localities previously cleared of timber, such as lawns, fields and farms. In the study area, its collection was limited to areas with sandy loams as the basic soil type. Sealander (1979) reported this species from 11 counties of the study area. We have 40 additional specimens and one documented additional county (Table 1).

#### Family Castoridae

*Castor canadensis* Kuhl, the Beaver. Jenkins and Busher (1979) observed that, besides the need for a stable body of water, the main determining factor of a site's suitability for habitation was the availability of food. Beavers feed on a variety of woody and herbaceous plant species. Aspens and willows were particularly favored (Jenkins and Busher, 1979). We have been told by numerous residents of the area that this species is relatively common. Beavers are said to be a nuisance in some of the lowland farm areas, but they are considered little or no problem by most people. We saw only one beaver while traveling in the study area, that being on Lake Hamilton near Hot Springs in Garland County. On another occasion we found a beaver dam while mist netting on the Saline River in Saline County.

## The Mammals of Southwestern Arkansas Part II. Rodents

## Family Cricetidae

*Oryzomys palustris* (Harlan), the Marsh Rice Rat. This rodent is most notably a wetland creature and will seldom if ever be found in a dry area. It normally inhabits thick vegetation found along ditches, lakes and ponds, marshes, and fields that are predominantly wet. Submarginal areas such as thickly overgrown fields and pine and bottomland forests are sometimes used (Lowery, 1974; Sealander, 1979; and Wolfe, 1982). We have collected this species in a variety of habitats throughout the study area. It is commonly found along the edges of cover areas, such as rights-of-way and forest-field ecotones and has been collected occasionally in or near man-made structures and trash dumps. This species has been reported from Pulaski (Dellinger and Black, 1940), Garland, Miller, Ouachita, Pike and Polk counties (Sealander, 1979). We have 49 specimens of this species from seven counties of the study area, including four new counties (Table 1).

*Reithrodontomys fulvescens* J.A. Allen, the Fulvous Harvest Mouse. There is some disagreement on the preferred habitat of this species. Lowery (1974) reported that overgrown fields and fence rows, thickets on the edge of woodlands, and occasionally dense grass fields may harbor this species. Sealander (1979) also listed these habitats, but added that they should border aquatic areas. He further stated that desert-like habitats are used by the species further south. Spencer and Cameron (1982) observed that grass-shrub field associations are the primary component of this mouse's habitat. The fulvous harvest mouse can be found in abundance throughout the study area. It utilizes habitats ranging from wet to relatively dry, and has been collected in large numbers from the rocky slopes of many Ouachita pine forests. Grass and brush fields, thickets (particularly honeysuckle), and trash dumps are also common habitats of this species. McDaniel *et al.* (1978) reported this species from Pulaski, Ouachita, and Little River counties; and Sealander (1979) reported it from Columbia, Garland, Hempstead, Miller, Pike, Polk, and Union counties as well. We have 383 specimens from 11 counties, four of which constitute new county records (Table 1).

*Reithrodontomys humulis* (Audubon and Bachman), the Eastern Harvest Mouse. This rodent is usually found in fields that have been taken out of use, briar thickets, overgrown ditches, and honeysuckle thickets (Lowery, 1974). Fields with a cover of tall thick grasses such as sedges or Johnson grass are commonly used (Sealander, 1979). Tumison *et al.* (1988) reported the collection of 32 specimens from the campus of Southern Arkansas University. These specimens were removed from a short-grass field and highway right-of-way. During the present study, three additional specimens were recovered during the examination of barn owl (*Tyto alba*) pellets collected from a roost in Hempstead County. The projected foraging area of the owls was composed of brush and low grass fields and cultivated tracts of land, as well as a small town. A railroad right-of-way was located in the foraging area. This species is not as common as *R. fulvescens*.

*Peromyscus attwateri* J.A. Allen, the Texas Mouse. Rocky outcrops and cliffs are the preferred habitats of this mouse in some regions; however, in the Ouachita Mountains, the Texas mouse is associated with pine-hardwood forests, steep slopes of forested areas, and rocky cedar glades and ravines (Sealander, 1979). Brown (1964) reported this species from juniper-grass glades in the Missouri Ozarks, and specifically on steep bluffs along stream courses. The Texas mouse is not common except at a few locations of optimal habitat. Optimal habitat includes the rocky, forested slopes of the central Ouachitas and similar areas, where Texas mice may be found in great abundance. Sealander (1979) reported this species from Garland, Howard, Polk and Pulaski counties. A total of 92 specimens was collected from Montgomery and Pulaski counties during this study (Table 2).

*Peromyscus gossypinus* (Le Conte), the Cotton Mouse. This is a mouse of the bottomland hardwood areas and stream banks with rock and brush piles, thickets, logs, and other sources of cover (Sealander, 1979). Wolfe and Linzey (1977) added mesic and hydric hammocks and swamps to the list of habitats in which this species is found. The cotton mouse is also reported to be found in various pine associations (Ivey, 1949; Shadownen, 1963). The cotton mouse is one of the most commonly encountered rodents of the study area and can be found in almost all habitats. It has been collected from the lowlands as well as

the highlands, and typically resides along forest edges, rights-of-way, in fields, and trash dumps and is a common pest in outlying buildings and homes. The cotton mouse was first reported in the area by Dellinger and Black (1940) from Pulaski County. Sealander (1979) reported the cotton mouse from Garland, Howard, Miller, Montgomery, Polk, and Union counties. We have accumulated 179 specimens of this species from these and seven additional counties (Table 2).

Table 2. Cricetidae (Part II) and Muridae from Southwestern Arkansas.

| County\Species | 1  | 2   | 3 | 4  | 5   | 6   | 7 | 8  | 9   | 10 |
|----------------|----|-----|---|----|-----|-----|---|----|-----|----|
| Calhoun        | -  | -   | - | -  | -   | -   | - | -  | -   | -  |
| Clark          | -  | -   | - | -  | -   | -   | - | -  | -   | *  |
| Columbia       | -  | 34  | - | -  | 30  | -   | - | 18 | 79  | -  |
| Dallas         | -  | -   | - | -  | -   | -   | - | -  | -   | *  |
| Garland        | *  | 3*  | - | 1* | -   | -   | - | -  | -   | -  |
| Grant          | -  | -   | - | -  | -   | *   | - | -  | -   | *  |
| Hempstead      | -  | 3   | - | -  | 3   | 75  | - | 20 | 1   | *  |
| Hot Springs    | -  | -   | - | -  | -   | -   | - | -  | -   | *  |
| Howard         | *  | 3*  | * | -  | 1*  | 1   | - | -  | 11  | -  |
| Lafayette      | -  | 12  | - | 2  | 3   | 1   | - | -  | 3   | -  |
| Little River   | -  | 4   | - | -  | 2   | -   | - | -  | -   | *  |
| Miller         | -  | 26* | - | 3  | 5   | 21  | 6 | 4  | 36  | *  |
| Montgomery     | 86 | 10* | 1 | 1  | 6   | 48  | - | -  | -   | *  |
| Nevada         | -  | 2   | - | -  | 2   | 1   | 1 | -  | 1   | *  |
| Ouachita       | -  | 35  | - | 2  | 29  | 8   | - | 1  | 11  | *  |
| Pike           | -  | 37  | - | 2  | 11  | *   | - | 2  | 4   | *  |
| Polk           | *  | 3*  | - | *  | -   | 3*  | - | -  | -   | *  |
| Pulaski        | 6* | 1*  | - | -  | -   | 1*  | - | -  | 1   | *  |
| Saline         | -  | -   | - | -  | -   | *   | - | -  | 2   | -  |
| Sevier         | -  | -   | - | -  | -   | -   | - | -  | -   | *  |
| Union          | -  | 6*  | - | *  | 18  | *   | - | 1  | 3   | *  |
| Total          | 92 | 179 | 1 | 11 | 110 | 159 | 7 | 46 | 152 | -  |

## LEGEND

1. *Peromyscus attwateri* 2. *Peromyscus gossypinus* 3. *Peromyscus leucopus*  
 4. *Peromyscus maniculatus* 5. *Ochrotomys nuttalli* 6. *Microtus pinetorum*  
 7. *Rattus norvegicus* 8. *Rattus rattus* 9. *Mus musculus* 10. *Myocaster coypus*

\* indicates a previous literature record for the species in that county.  
 X indicates a sight record of the species in that county.

*Peromyscus leucopus* (Rafinesque), the White-footed Mouse. This mouse is most commonly found in deep woods or in the brush along the forest borders (Lowery, 1974). This mouse is reported to be scarce in grass fields in some northern populations (Lackey *et al.*, 1985). Cover areas of brush and rock piles, thickets and fallen logs in the somewhat drier forested uplands are thought to be the normal habitat for this species in Arkansas (Sealander, 1979). Although Sealander (1979) thought that the distribution of the white-footed mouse was statewide, we have only one specimen from Montgomery County. Montgomery (1984) reported collecting this mouse in Howard County. The white-footed mouse appears to be rare in areas to the south of the Ouachita Mountains where it is apparently displaced by the cotton mouse.

*Peromyscus maniculatus* (Wagner), the Deer Mouse. This mouse is an inhabitant of open fields and is not found in forested areas. The deer mouse is commonly found in fields with cover grasses, or along fence rows, road and railroad rights-of-way, and croplands (Sealander, 1979). He also reported this species from Garland, Polk, and Union counties. During this study, 11 specimens were collected from six counties of the study area, five of which are new county records (Table 2). Although uncommon, this species has a wide distribution in the study area.

*Ochrotomys nuttalli* (Harlan), the Golden Mouse. Lowery (1974) reported that this species is usually found in pine-hardwood forests. Sealander (1979) stated that moist bottomland forests and stream banks with adequate cover are the preferred habitat of the golden mouse. Thickets of honeysuckle, cane, briars, vines, and rock-strewn areas are preferred as cover (Linzey and Packard, 1977, and Sealander, 1979). Montgomery (1984) reported the only record of this species from the study area, from Howard County. However, this species is common within the study area and was collected at almost all locations offering at least marginal habitat. Thickets, usually of honeysuckle, field and



forest edges, rights-of-way, and dumps were common habitats for this species. One hundred five specimens of the golden mouse were collected from 11 counties of the study area (Table 2). Strangely, this species was not collected from the northeastern portion of the study area.

*Sigmodon hispidus* Say and Ord, the Hispid Cotton Rat. This species is often encountered in old fields, thickets, and grassy ditches. Open areas that provide adequate cover, such as idle fields, ditches, fence rows and rights-of-way with thick grass and brush are suitable for this rat. The cotton rat will inhabit pine forests but seldom hardwood forests, though it may be found in border thickets and is also said to avoid wet areas (Lowery, 1974; Sealander, 1979). Areas of grasses and forbs are common habitat preferences (Fleharty and Mares, 1973; Goertz, 1964; Goertz and Long, 1973; Kaufman and Fleharty, 1974). The cotton rat is very common throughout the study area and has been collected from forest and field edges; fence rows; trash dumps; briar, brush and log thickets; and from fields composed of low grass, high grass, and/or brush communities. Pierce and Kirkwood (1977) reported this species from Saline and Grant counties. A total of 283 cotton rat specimens was collected from 12 counties of the study area (Table 1).

*Neotoma floridana* (Ord), the Eastern Woodrat. This is a common resident of hardwood bottomlands. The wood rat avoids dry areas and is rare or nonexistent in upland areas of pine forest (Lowery, 1974). The preferred habitat of this species, in the Ouachita Mountains, is rock crevices, caves, and rock piles. Elsewhere, they are known to inhabit areas of dense brush cover, buildings, and hollow logs (Sealander, 1979). The wood rat is found at scattered locations about the study area. It is usually found in moist to wet areas, particularly those associated with river and stream systems. Areas offering adequate cover such as overgrown fields and forest are common habitats of the woodrat. A few specimens were collected from rocky outcroppings. Twenty-five specimens from nine counties have been collected (Table 1).

*Microtus ochrogaster* (Wagner), the Prairie Vole. This vole is said to inhabit only open grassland areas and will not be found in forests (Sealander, 1979). The range of this species is limited to the extreme northeast portion of the study area. Sealander (1979) reported this vole from Pulaski County and we have 16 additional specimens from this county.

*Microtus pinetorum* (Le Conte), the Woodland Vole. Sealander (1979) reported that the habitat requirements of this vole rest mainly in a need for thick mats of ground cover. Smolem (1981) also reported the need for thick ground cover and added that the soil should be well-drained. Lowery (1974) added that this vole is not known to inhabit pine forests. Otherwise it may be found in areas as diverse as overgrown fields, fence rows, and moist woodlands. The woodland vole is much more abundant than its relative, *M. ochrogaster*. The woodland vole has been collected from a variety of areas providing the necessary cover. Forested and brush-covered areas, rights-of-way, and grass and rock fields have been common habitats for this vole. Sealander (1979) reported this species from Pike, Polk, Pulaski, and Union counties of the study area, and Pierce and Kirkwood (1977) reported the woodland vole from Saline and Grant Counties as well. A total of 244 specimens was collected from nine counties, seven of which represent new county records (Table 2).

*Ondatra zibethicus* (Linnaeus), the Muskrat. This rodent is an aquatic mammal found along slow moving water sources such as marshes, lakes, ponds, rice fields, ditches, canals, reservoirs, and swamps (Lowery, 1974; Sealander, 1979). This species can remain undetected in an area for some time, provided it does not become destructive. The muskrat has not been reported from the area, and we failed to document its presence. However, based on accounts related to us from residents of the area, it is obvious that this species is a relatively common resident of the many lowland waterways and ponds, lakes, and reservoirs throughout the study area.

#### Family Muridae

This family is unique in that all the members were introduced to the North American continent unintentionally. Murids have become so well established that they are now considered a natural part of the ecosystem and, therefore, are included in this study.

*Rattus norvegicus* (Berkenhout), the Norway Rat. This species of rat is less closely commensal with man than its relative *R. rattus*. It is found in great abundance wherever man has established residences. Populations can grow to large numbers when the necessary conditions of food and space are not controlled. Any building may offer shelter to this rat as well as fields and meadows, if cover and food are available (Lower, 1974; Sealander, 1979).

The Norway rat is not overly common in the study area and has been encountered more often in the wild than in developed areas. A total of seven specimens was collected from two counties of the study area (Table 2).

*Rattus rattus* (Linnaeus), the Black Rat. This rat is closely associated with man and is more aggressive than is *R. norvegicus*. The black rat will usually displace its relative when they come in contact, and is more often found dwelling in man-made structures than in the wild (Lowery, 1974; Sealander, 1979). The black rat is more common in southwestern Arkansas than is its relative, *R. norvegicus*. However, as common as it is, it has not yet been collected from a habitat that is not in some way associated with man's presence. This species has been collected from six counties of the study area, and a total of 46 specimens has been taken (Table 2).

*Mus musculus* Linnaeus, the House Mouse. This species is the only example of its genus currently found in the United States (Jones *et al.*, 1986). As with the other members of the family, the house mouse is usually closely related with man and his structures. In structures where control methods are limited or impossible, such as barns, sheds, and large stores, populations may be large (Lowery, 1974). This mouse also inhabits fields and pastures in the wild (Lowery, 1974). The house mouse is a common rodent in the study area. It has been found at numerous locations and in various habitats. In the wild it has been found in fields, forests, along rights-of-way and waterways, and in almost all trash dumps. Though undoubtedly common to all of the study area, only 154 specimens were collected from 11 counties (Table 2).

#### Family Erethizontidae

*Erethizon dorsatum* (Linnaeus), the Porcupine. The only specimen was recorded from Sevier County by Clark (1985). Reynolds (1957) reported that porcupines are usually confined to vegetated riparian habitats, although Woods (1973) reported that the habitat preference of this species varies according to what is available in the area.

#### Family Myocastoridae

*Myocastor coypus* (Molina), the Nutria. The nutria is similar to the muskrat in habits and needs and can be found in much the same habitat. Areas of slow moving or standing water with large amounts of aquatic and semiaquatic vegetation are preferred (Sealander, 1979). Bailey and Heidt (1978) and Sealander (1979) reported the only documented records of this species from Arkansas (Table 2). Based upon these records and conversations with residents of the study area, we believe that the nutria is a common species to the lowland waterways of the Gulf Coastal Plain.

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# THE MAMMALS OF SOUTHWESTERN ARKANSAS PART III. CARNIVORES.

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## ABSTRACT

This study investigates the composition of the carnivore fauna of southwestern Arkansas and presents data on habitat affinities. The study area is comprised of the 21 counties located south and/or west of and including Pulaski County. The previously existing data set pertaining to the mammals of Arkansas was notably incomplete and the study area, in particular, was poorly known mammalogically. Specimens were collected by standard trapping and salvage methods throughout the study area. Species considered during this study were limited to those meeting a set of criteria designed to eliminate species that had been introduced or artificially maintained. This study has accumulated records of 11 species of carnivores present in the study area and proposes the presence of one other; over 2100 specimens have been recorded; and a total of 81 new county records has been documented.

## INTRODUCTION

A reliable understanding of an area's ecosystem can not be accomplished unless it is known of what that ecosystem is comprised biotically and abiotically. This realization is accentuated by the increase in the number of local, state, and federal agencies that have been studying aspects of the environment beyond those involving only recreational or economic considerations. These agencies agree that the more complete the knowledge of a studied area, the more efficiently the area can be managed and utilized. Consequently, many states are attempting to compile a complete data set of their natural resources. In Arkansas, the need for information on resources is intense. The present study, then, attempts to increase the data set available on the mammalian resources of southwestern Arkansas (an area previously only poorly known mammalogically).

There are relatively few studies on the distribution of mammals in southwestern Arkansas. For the state as a whole, the information available ranges from nonexistent to nearly complete depending on the species and area of concern. Black (1936) made the earliest study of the distribution of Arkansas mammals. His study was limited to northwestern Arkansas and contained limited habitat information.

Dellinger and Black (1940) attempted the first statewide inventory of Arkansas mammals. However, this study included little information on the mammals of southwestern Arkansas and almost no information on the habitats of mammals. The data were often restricted to one or two specimens from as many locations.

One of the first systematic distributional studies for southern Arkansas was conducted by Baker and Ward (1967). They reported on the distribution of nine species of bats in the southeastern portion of the state (Bradley, Cleveland, and Drew counties).

Sealander (1956, 1979) made the first comprehensive studies attempting to establish accurate distributions for the Arkansas mammalian fauna. His distributions were based on previous literature records, his personal collections, the collections of other researchers, and reports of sight observations. Although quite valuable, Sealander's book lacked data from many areas, particularly from southwestern Arkansas.

The purposes of the present study were to establish, as completely as possible, the current distributions of the mammalian species found in southwestern Arkansas and to present data on habitat affinities of these mammals.

## STUDY AREA

The study area comprises 21 counties located south and/or west of, and including, Pulaski County. Within this area of the state, habitats vary widely from the rolling hills and rocky outcroppings of the Ouachita Mountains in the northern portion, to forested hills and cultivated tracts of land, to the sandy flood plains found throughout the southern portion of the area.

Habitat characteristics examined included: predominant vegetation, substrate composition, topography, successional stage, and developmental stage. These characteristics were not considered on an individual basis, but instead were considered to be attributes of overall habitat at any given location. These data could be valuable for evaluating the possibility of encountering a desired species of mammal based on habitat parameters.

The dominant vegetation of the study area included oak-hickory climax forests, loblolly pine forests, cedar glades, brush and grass fields, and agricultural cropland. Substrates varied from deep sands and clays of the flood plains, to rocky cliffs and outcroppings of the mountains, and to tracts of rich loams with scattered swampy areas.

The term "developmental stage" refers to the extent to which a site has been changed from its normal ecological condition by the activities (intentional or inadvertent) of man. These activities include commercial, private, or governmental developments, such as city growth, road construction, farming, and development of recreational areas.

For the purposes of this study, mammals were included if they met the following criteria:

1. The species is not a recently escaped exotic.
2. The species has not been recently introduced or (reintroduced) to the area by other than those avenues naturally open to native species.
3. The species' presence in the area is not a result of current or very recent artificial management or control procedures (i.e., the stocking of game animals into the area from some other area).
4. The record(s) is(are) not likely to be considered spurious.

Finally, only those sight records from acceptably knowledgeable persons and for those species for which misidentification is not likely were used. In most instances, sight records were corroborated by the capture of the same species from a nearby area.

## METHODS

Numerous field collections were conducted throughout the period of this study by various individuals including Arkansas Game and Fish personnel, university personnel, public school teachers and their classes, graduate students, and other knowledgeable laypeople.

A variety of collection methods were necessitated by the different habits and life-styles of the mammals encountered in the study area. Sight identification of and collection of some road-kill specimens, particularly of larger species, resulted in many of the records accumulated for larger mammals and for those difficult to obtain by traditional collection methods. Sight identification of living specimens was made on only a few occasions, when accurate identification was assured.

Specimens were prepared as standard museum skin and/or skeletal



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preparations. The specimens were deposited in the Arkansas State University Collection of Recent Mammals.

The habitat composition of the study area was determined by a variety of methods. In most cases, a brief description of the habitat in which the specimen was collected was included with the standard collection data. In those cases where habitat information was not available, the most probable habitat for the collection site was determined. This determination involved consulting published reports for the specific area, personal knowledge of the site, and review of detailed maps and/or aerial photographs of the area when available. Ultimately, habitat information was used to determine the range of "usual" habitats for a given species. Additional sources of distributional and habitat data included the few published literature records, records of the Arkansas Department of Health, and the Collection of Recent Mammals at Arkansas State University.

## RESULTS AND DISCUSSION

This study has resulted in the collection of more than 2100 specimens and the accumulation of numerous sight records from various points in the study area. Voucher specimens now exist for nine species of carnivores found in southwestern Arkansas. Nomenclature and phylogenetic relationship utilized in this study are as reported by Jones *et al.*, 1986.

Species' Accounts  
Order Carnivora  
Family Canidae

*Canis latrans* Say, the Coyote. The coyote resides in areas of open brushlands and idle fields with forest borders while avoiding dense forests (Lowery, 1974; Sealander, 1979). This canid is abundant throughout the study area. Coyotes are able to adapt to almost any habitat type, from farms and fields, to the residential areas of some of the larger cities. This species can undoubtedly be found in all counties of the study area, although, we have only 68 records from seven counties (Table 1) and a sight record from Miller Co., all of which are the first published records for this species in the area.

Table 1. Carnivores from Southwestern Arkansas

| County\Species | 1  | 2 | 3   | 4    | 5  | 6  | 7 | 8   | 9   |
|----------------|----|---|-----|------|----|----|---|-----|-----|
| Calhoun        | 1  | - | 6   | -    | -  | -  | - | *   | -   |
| Clark          | 1  | - | 27  | 20*  | -  | -  | X | 11* | 26  |
| Columbia       | -  | - | 11  | 5    | -  | -  | X | 1*  | -   |
| Dallas         | -  | - | 3   | 8    | -  | *  | - | 8*  | 17  |
| Garland        | 17 | - | 122 | 50   | 19 | *  | X | 5*  | 29  |
| Grant          | -  | - | 2   | 4    | -  | *  | - | *   | 6   |
| Hempstead      | -  | 1 | 76  | 216  | 2  | *  | X | 5*  | 5   |
| Hot Springs    | -  | - | 39  | 11   | 1  | -  | X | 7*  | 19  |
| Howard         | -  | - | 20  | 22   | -  | -  | X | 1*  | 20* |
| Lafayette      | -  | - | 3   | 1    | -  | -  | X | *   | -   |
| Little River   | -  | - | 10  | 289  | X  | -  | - | -   | 4   |
| Miller         | X  | - | 2   | -    | -  | -  | 1 | *   | -   |
| Montgomery     | 45 | - | 207 | 56   | -  | *  | X | 4*  | 82  |
| Nevada         | -  | - | 16  | 3    | -  | -  | X | *   | 1   |
| Ouachita       | 1  | - | -   | 1    | -  | -  | 1 | *   | 1   |
| Pike           | 3  | 1 | 70  | 308  | 11 | *  | X | 2*  | 35  |
| Polk           | 1  | - | 29  | 27   | -  | *  | X | *   | 24  |
| Pulaski        | -  | - | 33  | 18   | -  | 2* | - | 2*  | 6   |
| Saline         | -  | - | 6   | X    | -  | -  | X | *   | -   |
| Sevier         | -  | - | -   | 14   | -  | *  | X | *   | 8   |
| Union          | -  | - | 4   | -    | -  | *  | - | *   | 8   |
| Total          | 68 | 2 | 648 | 1055 | 33 | 2  | 2 | 46  | 291 |

## LEGEND

1. *Canis latrans* 2. *Vulpes vulpes* 3. *Urocyon cinereoargenteus*  
4. *Procyon lotor* 5. *Mustela vison* 6. *Spilogale putorius*  
7. *Mephitis mephitis* 8. *Lutra canadensis* 9. *Felis rufus*

\* indicates a previous literature record for the species in that county.  
X indicates a sight record of the species in that county.

*Vulpes vulpes* (Linnaeus), the Red Fox. The preferred habitat of this fox is open oak-pine uplands with fields and pastures. Areas of dense cover are avoided by the red fox (Lowery, 1974; Sealander, 1979). The distribution of the red fox is poorly known for the study area. Sealander (1979) proposed a statewide distribution of this fox while stipulating that it would be scarce in southern Arkansas.

Residents of the area claimed to have seen this fox on several occasions, but these claims were not substantiated. Single specimens were collected from both Pike and Hempstead counties.

*Urocyon cinereoargenteus* (Schreber), the Gray Fox. Like its relative, *V. vulpes*, this fox can usually be found to inhabit oak-pine uplands with open fields (Lowery, 1974). Hall (1981) reported that the gray fox was closely associated with deciduous forest. Sealander (1979) reported that the gray fox prefers rocky, forested areas with dense undergrowth, but will also utilize hardwood forests, bottomland forests, and farmlands with fields and cover areas. A total of 684 specimens has been collected from 18 counties of the study area (Table 1).

## Family Procyonidae

*Bassariscus astutus* (Lichtenstein), the Ringtail. This animal prefers to live in areas of rocky slopes and bluffs with brushy cover, normally no more than half a kilometer from water. It occasionally inhabits wooded areas with hollow trees as den sites (Sealander, 1979). Sealander (1979) reported this species from Clark, Dallas, Miller, Montgomery, and Pike counties of the study area. No additional records resulted from the present study.

*Procyon lotor* (Linnaeus), the Raccoon. The noted and often reported ability of the raccoon to adapt to almost any habitat type creates difficulty in ascertaining its preferred habitat. Sealander (1979) reported that the raccoon prefers bottomland hardwood forests and, though seldom far from water, they may be found in many habitats, such as swamps, upland forests, residential areas, and farmlands. Dellinger and Black (1940) reported the only published record of this species from the study area from Clark County. A total of 1055 raccoons was collected from 19 counties of the study area (Table 1). This species is very common in the study area and has been encountered in habitats ranging from swamps to residential backyards, and is plentiful in the uplands and the lowlands.

## Family Mustelidae

*Mustela frenata* Lichtenstein, the Long-tailed Weasel. Sealander (1979) stated that "...this species is most abundant in areas with high pocket gopher populations." He also reported that areas of brush cover, fence rows, upland forests, forest borders, and bottomland forests along water courses may be used as habitat by this weasel. The long-tailed weasel is undoubtedly rare and extremely secretive in the study area. Its favorite food source, the gopher, is present in large numbers.

*Mustela vison* Schreber, the Mink. The one key habitat requirement for this animal is its need for a waterway, such as a stream, pond, lake, or swamp (Lowery, 1974). Sealander (1979) added that water sources with suitable cover such as brush, windfalls, or other debris are preferred by this species of weasel. As was true for its relative, *M. frenata*, records of the mink have not been published for the study area. Unlike the weasel though, the mink is rather common. Thirty-three specimens were collected from four counties of the study area (Table 1), in addition to a sight record in Little River county.

*Spilogale putorius* (Linnaeus), the Eastern Spotted Skunk. Lowery (1974) reported that this skunk will make its abode in sheltered locations such as hollow logs, abandoned buildings, lumber piles, tree roots, and burrows. It prefers rocky areas with crevices where fields, pastures, fence rows, forests and forest edges are used. Wet or dense timber areas are usually avoided (Sealander, 1979). Sealander (1979) reported this skunk from 10 counties of the study area (Table 1). We have additional records of only two specimens from Pulaski County.

*Mephitis mephitis* (Schreber), the Striped Skunk. Lowery (1974) reported that this skunk chooses much the same habitats as *S. putorius*:



abandoned burrows, stumps, rubbish piles, and old buildings. Sealander (1979) reported that the spotted skunk inhabits open areas, fence rows, and wood lots that are not far from water and it prefers rock cavities for den sites. This species is a common sight as roadkills along the roadways of the study area. We have specimens of this skunk from two counties and sight records from 13 additional counties of the study area (Table 1).

*Lutra canadensis* (Schreber), the River Otter. This animal is limited in habitat by its aquatic life style. Otters require open water courses and prefer those that are bordered by timber. The diet of the otter is made up of mostly reptiles, amphibians, fish, and aquatic invertebrates (Lowery, 1974; Sealander, 1979). Polechla (1987) reported that the otter is common throughout the study area. Sealander (1979) reported specimens from Union County and sight records from 14 additional counties. Tumilson *et al.* (1981) reported specimens from all counties of the study area, with the exception of Little River County. We have 46 specimens from 10 counties of the study area (Table 1).

#### Family Felidae

*Felis concolor* True, the Mountain Lion. This cat is a creature of the deep forest and may be found in dense upland and bottomland hardwood forests and swamps (Lowery, 1974). Areas in which it can avoid man are most favored by the mountain lion and include swamps, dense forests and rough uplands (Sealander, 1979). Currier (1983) defined the habitat of the mountain lion as having become limited to those areas that offer a large enough home range, available prey, and seclusion. Sealander (1979) reported that the last mountain lion killed in the study area occurred in Montgomery County in 1949. If this species still occurs in the study area, it does so in very small numbers and in those areas isolated from the presence of man.

*Felis rufus* (Schreber), the Bobcat. Rocky outcrops and canyons are the preferred habitat of this species, although dense forested areas, swamps, and semi-open areas are used also (Lowery, 1974; Sealander, 1979). This species of cat is rather common in the study area. We have 291 specimens from 16 counties (Table 1). It is most common in the forested areas, usually along streams and other water sources.

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# IMAGE PROCESSING INSTRUMENTATION FOR *GIARDIA LAMBLIA* DETECTION

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## ABSTRACT

Currently, the identification and enumeration of *Giardia lamblia* cysts are based upon microscopic methods requiring individuals proficient in this area. It is a tedious process which consumes time that could be constructively used elsewhere. This project attempts to alleviate that burden by employing a computer to automatically process Indirect Fluorescent Antibody (IFA) prepared slides using digital image processing techniques. A computer controlled frame grabber, in conjunction with a CCD TV camera mounted on the epi-fluorescence microscope phototube, captures the light intensities of the objects in view under the microscope objective. The captured image is stored as pixels, with each pixel having a numerical value that can be altered using linear contrast enhancement and bit-slicing to emphasize the cysts and eliminate the majority of unwanted objects from the image. The altered image is then analyzed by a vector trace routine for typical area and perimeters characteristic to *Giardia lamblia* cysts. Objects in the image matching these characteristics are most likely cysts and are added to a running tally of the number of cysts present on the slide.

## INTRODUCTION

Discovered by Leeuwenhoek in his own stools over 300 years ago and regarded as nonpathogenic, the organism *Giardia lamblia* has only within the last three decades been identified for its detrimental effects on public health. Giardiasis has been coined "traveler's disease", affecting travelers, campers, hunters, fishermen — anyone drinking from untreated or inadequately treated water supplies (Lin, 1985). Affliction can also occur through the fecal-oral route due to poor hygiene practices (Polis *et al.*, 1986). Individuals react to giardiasis differently. Some may be asymptomatic, others may have acute or chronic symptoms (AWWA, 1985). It remains as a leading waterborne intestinal disease in the United States (Lin, 1985).

*Giardia lamblia* is a single-cell flagellated protozoan having a two-stage life cycle; a reproductive stage called a trophozoite, and a dormant stage called a cyst. After an individual ingests a cyst, stomach acid triggers the hatching of two trophozoites (AWWA, 1985). The trophozoites multiply in the duodenum, jejunum and upper ileum by binary fission and, in a severe infection, attach themselves with a sucking disk to nearly every epithelial cell (Linn, 1985). The inability to absorb fats and other nutrients disrupts the normal intestinal activities, causing abdominal distention, diarrhea, flatulence, belching, nausea, vomiting, anorexia, fatigue, and cramps (AWWA, 1985). Periodically the trophozoites detach and travel through the bowel, forming cysts which are shed through the stools of the individual.

Recently, amendments to the Safe Drinking Water Act, public law 93-523, state that the EPA will promulgate criteria for (1) filtration of surface water supplies, and (2) disinfection for all water supplies in the U.S. The "surface water treatment rule" was proposed 3 November 1987, revised 6 May 1988, and finalized in the fall of 1988. It states that treatment must achieve at least 99.9% removal and/or inactivation of *Giardia* cysts and 99.99% removal and/or inactivation of enteric viruses. Water producers will be in violation if they do not conform by fall 1992. In order to comply, water treatment plants need a quick and efficient way to check their treated water.

Detection of *Giardia lamblia* cysts, be it for clinical diagnosis or water treatment purposes, exists in some commonly used techniques revolving around microscopic examination. They include bright-field microscopy such as staining, and indirect fluorescent antibody (IFA) methods. Bright-field microscopy is a tedious and time consuming procedure requiring individuals proficient in these areas. The IFA procedure results in an identification process specific to *Giardia* cysts that marks them with fluorescein isothiocyanate (FITC) dye via an antibody to *Giardia* cysts. The dye, which has its peak fluorescence near 535nm (green), simplifies the identification of the cysts (Nikon, 1985).

The objective of this project is to use the IFA method in conjunction with digital image processing techniques to create a cost effective instrumentation package that can automatically identify and count *Giardia lamblia* cysts and possibly help water producers meet the new water treatment regulations.

## MATERIALS AND METHODS

Presently, our system consists of a Nikon Labophot Microscope in an epifluorescence configuration with a mercury light source and camera mount. A Computar series 3800 CCD (charged coupled device) black and white camera is placed on the camera mount to capture the magnified image. The relative light intensities of the image are converted to voltage signals and sent to a frame grabber which is housed in an AT&T 6300 computer. Here, the voltage signals are further converted to binary values between 0 to 63, with each value dictating a specific shade of gray; 0 represents the black end limit; 63 represents the white end limit. When these values are sent to a video monitor, they control the intensities of individual picture elements (pixels) to recreate a black and white "picture" of the image in view under the microscope.

## IMAGE ENHANCEMENT

The frame grabber provides the added benefit of allowing video images to be altered for enhancing selected features. Image processing software has been written to allow only a selected band of intensities to be displayed, satisfactorily emphasizing or deemphasizing targeted objects. Linear contrast enhancement and bit-slicing are techniques that employ such tactics and are fundamental in the algorithms written for use in our project.

Briefly, linear contrast enhancement modifies a given band of intensities by calculating and using the proximity ratio of each intensity within the band relative to the entire dynamic range of intensity levels. This produces a prominent visual contrast in the video image. Bit-slicing takes a given band and converts only intensities in that band to full white saturation. Anything above or below this band is converted to full black saturation.

In all the cyst recognition methods developed, each requires the objects in the video to be in full white saturation (i.e., the intensity of every pixel in the object to be the binary equivalent of 63). This is the job of the bit-slicing technique. However, the proper range of intensities needs to be determined first so the cysts are emphasized while at the same time eliminating most, if not all, of the noncyst objects from

the data. Recognizing that the eye is not highly sensitive to gray scale variations, this was accomplished by investigating the raw video image of the cyst and acquiring the binary values of the pixels near its perimeter. A program was written especially for accessing the values of any pixel by moving a cursor controlled video cross-hair over the desired pixel. For the *Giardia lamblia* cyst in Fig. 1 which was taken at x1000 magnification, the near optimal band for bit-slicing is from six to 15. Fig. 2 is the result of bit-slicing within this band.



Figure 1. Image of fluorescing *G. lamblia* cyst at x1000 magnification.

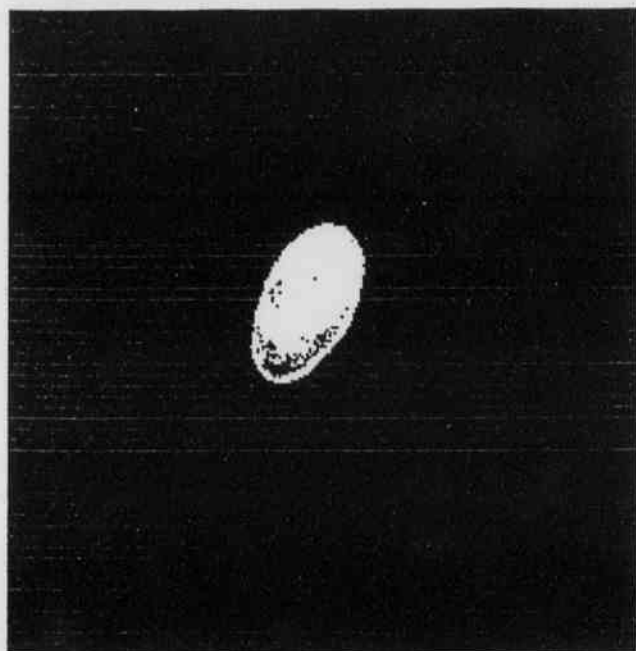


Figure 2. *G. lamblia* image modified by bit-slicing within an intensity band of 6 to 15.

The description of three methods that have been tried in the software development of an adequate *Giardia lamblia* cyst recognition algorithm follows. Area and perimeter calculations and cyst accumulation counts are automatically displayed on the computer monitor. The model cyst mentioned in these methods will eventually be created by taking the average areas and perimeters of a large sample of actual cysts. Because this project is still in the preliminary stages, the parameters of the model cyst were estimated from a small sample of cysts.

Method 1 assumes that the image contains no foreign objects after using the bit-slicing capabilities of the frame grabber. The ideal video image should reflect either a relatively black screen when no cysts are present or clusters of white pixels representing the effective areas of cysts (Fig. 2). By counting the total number of white pixels from the video image, dividing that by the number of pixels that make up the model cyst and allowing for slight variations in cyst size, the number of cysts in the image can be estimated. From experimental data, the model cyst at x1000 magnification has an effective area of approximately 2040 pixels. Although more data need to be taken, a difference of 40% from the model cyst pixel count is allowed. The area count for this cyst is 1725 pixels.

Method 2 introduces the comparison of perimeter pixel counts to achieve better cyst verification. This method was also designed around an ideal image. To save processing time, the computer program automatically locates the object on video and isolates it by placing a box of quarter-intensity pixels around it. Centering the box around the cyst is accomplished by finding the first and last rows the cyst occupies and calculating the row halfway between these two outer rows. This center row is scanned to find the first and last pixels, whereby the pixel midway between the two outer pixels is calculated and the box is centered around this pixel. The interior of the box is scanned first by columns, then by rows, to identify the first and last pixels it encounters to mark with half-intensity pixels indicating the perimeter of the cyst. Finally, all pixels within the box are inspected (Fig. 3). The number of half-

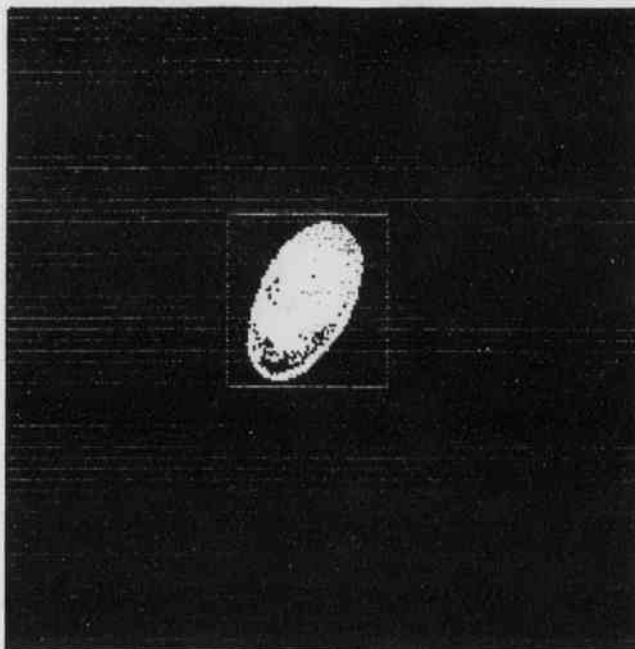


Figure 3. *G. lamblia* image processed using Method 2.

intensity pixels is the perimeter count and the combined number of half-intensity and full-intensity pixels is the area count. By dividing these numbers by area and perimeter counts of the model cyst — 2040 pixels and 146 pixels, respectively — it is concluded whether or not the object is a cyst. Again, variation in cyst size was considered. A 40% difference from the area and a 20% difference from the perimeter of

the model cyst was allowed. For the cyst in Fig. 3 the area count is 1725 pixels and the perimeter count is 144 pixels.

Method 3 considers the more likely image where background objects will be present regardless of the extensive use of processing techniques to emphasize the cysts (Kitchin *et al.*, 1983).

Before this method is implemented, the image is first processed using an automated ends-in search contrast enhancement algorithm which was recently integrated into this method. It looks at the histogram of pixel intensities of the video image, analyzes it, and performs linear contrast enhancement in a range of intensities relative to a predetermined percentage of pixels to be saturated either black or white as calculated from the cumulative distribution function. The cumulative distribution function represents the percentage of pixels with intensities at or below a given intensity (Green, 1989). After some trial and error, it was determined that the lower intensity limit be 3.17% below 100% (i.e., the intensity where 96.83% of the pixels have intensities below this intensity), and the upper intensity limit be at 100%. This corresponds to an intensity band from five to 15 over which the linear contrast enhancement is performed. Once this is completed, the image is bit-sliced over the entire range of pixel intensities leaving the remaining objects as clusters of white pixels (Fig. 4).



Figure 4. Results of using linear contrast enhancement and bit-slicing in Method 3.

The next step is to create an edge matrix of half-intensity pixels in a second 64K memory area which represents possible perimeters of objects in the current image. Each pixel is organized to be the upper left pixel (hereafter called the primary pixel) of a group of four pixels. Organizing them as such requires the groups to overlap each other, assuring a continuous transition into the edge matrix. Each group of four pixels is compared to four specific pixel patterns representing cases where the primary pixel cannot be regarded as the exterior of an object. If no match is made, the primary pixel is indicated as an edge with a half-intensity pixel in the edge matrix. This continues until the last group of four pixels in the image is analyzed. The edge matrix is then swapped with the image on video (Fig. 5). Each pixel in the current video is inspected until it reaches a half-intensity pixel assumed to be the perimeter of an object from the image. From this pixel a specific vector search pattern which moves in a counter-clockwise direction is used to locate the next half-intensity pixel. Eight different vector directions are allowed in a search (Fig. 6). The decision on which vector direction will



Figure 5. Corresponding edge matrix to image in Figure 4.

begin the search depends on the last vector movement that found the current perimeter pixel (Figs. 6 & 7). Upon identifying the next half-intensity pixel, it is changed into a full-intensity pixel. This continues until the search returns to the originating perimeter pixel of the object. The next object is located and the vector search is repeated (Fig. 8).

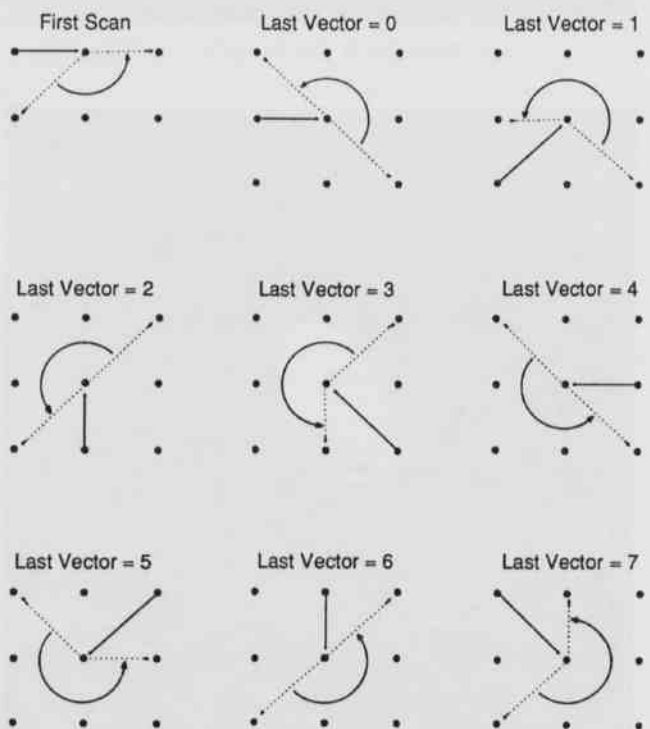


Figure 6. Vector search patterns used in determining the outer boundaries of objects in the edge matrix.





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# DISTRIBUTIONAL SURVEY OF THE EASTERN COLLARED LIZARD, *CROTAPHYTUS COLLARIS COLLARIS* (SQUAMATA: IGUANIDAE), WITHIN THE ARKANSAS RIVER VALLEY OF ARKANSAS

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## ABSTRACT

A distributional survey of the eastern collared lizard, *Crotaphytus collaris collaris*, was conducted in the Arkansas River Valley of Arkansas during the summer of 1988. Thirty-four sites in 10 counties were searched. Only three localities yielded colonies of lizards; all were found south of the Arkansas River. This species has a discontinuous distribution in central Arkansas; habitat depletion and overzealous collection may have contributed to the extirpation of lizards at formerly-reported localities in the Arkansas River Valley of Arkansas.

## INTRODUCTION

A number of publications have provided a wealth of information on the present distributions of herpetofaunal species of the Interior Highland Region (an isolated mountainous uplands spread unevenly over the states of Arkansas, Kansas, Missouri, and Oklahoma); these include works by Anderson (1965), Collins (1982), Conant (1975), Dellinger and Black (1938), Dowling (1957), Johnson (1987), Schuier *et al.* (1972), Schwardt (1938), Smith (1961), and Webb (1970). Only a few papers have considered, in any detail, the paleo-ecological and climatological impact of the post-Wisconsin glacial period on these present-day distributions (Auffenberg and Milstead, 1965; Cole, 1971; Dowling, 1956, 1958; Holman, 1974; Porter, 1972; Smith, 1957). The late glacial climatic episode (12,000 to 9,000 yr. B.P.), also called the pre-Climatic Optimum (pine) Phase (Smith, 1957), was followed by a period of warming and aridity resulting in desert and prairie extensions into the central United States. This latter Xerothermic Phase occurred between 6,000 and 3,000 yr. B.P. (Cole, 1971; Smith, 1957) and established the Prairie Peninsular Corridor for herpetofaunal dispersal (Auffenberg and Milstead, 1965). It was during this period that xeric-adapted species of plants and animals (e.g., prickly pear cactus and the eastern collared lizard) moved from the Great Plains eastward to become residents in xeric habitats in Arkansas.

Although recent work on the life history and ecology of the eastern collared lizard, *Crotaphytus collaris collaris*, in northern Arkansas (McAllister, 1980a, 1980b, 1985; McAllister and Trauth, 1982, 1985; McAllister *et al.*, 1985; Trauth, 1974, 1978, 1979) qualifies this species as one of the most thoroughly-studied reptiles inhabiting the state, no details have been published on any aspect of its biology elsewhere in Arkansas. Conant (1975) illustrated the range of *C. c. collaris* to be throughout the Interior Highland Region; however, few locality records exist for the lizard along the Arkansas River or in the Ouachita Mountains. In the White River drainage system of the Ozark Plateau of northern Arkansas, collared lizards are commonly found on arid, rocky, sandstone/limestone "cedar glades" where they spend much of the daytime foraging for insects or basking on rock perches overlooking their territories. Because these lizards are large, colorful, active by day, and conspicuous in their habitats, they are quite vulnerable to natural predators (mainly birds and snakes) as well as to human extirpative activities. One of the most destructive practices by man is the prevention of periodic fires on glades; consequently, encroachment by the oak-hickory forest association occurs and reduces suitable environmental conditions for the existence of collared lizards.

No recent distributional information has been published on *C. c. collaris* in the Arkansas River Valley of Arkansas. Collections from Conway County (in the vicinity of Petit Jean State Park), Logan County (near Mt. Magazine), and Pulaski County (on Pinnacle Mountain) are on record at the Carnegie Museum dating back to 1944 and 1945. Only two records for collared lizards are available from the Ouachita

Mountains of western Arkansas (Polk and Montgomery counties; see Dellinger and Black, 1938; Dowling, 1957). In this report, I provide information on the geographic distribution of *C. c. collaris* within the Arkansas River Valley from Little Rock to Fort Smith. This type of biological inventory furnishes a database for future studies designed to monitor changes in unusual environments (Smith *et al.*, 1984) and is beneficial to sound conservation management.

## MATERIALS AND METHODS

Thirty-four sites in 10 counties along the Arkansas River were included in this study (Fig. 1). Fieldwork was conducted from late May to early August, 1988. Both natural as well as man-made habitats were visited. Habitats were searched on sunny days during the optimal activity period (10:00 a.m. to 3:00 p.m.) of the lizards. Most habitats were photographed, and the general condition of the environment was recorded. Lizards collected as voucher specimens were deposited in the Arkansas State University Museum of Zoology (ASUMZ).

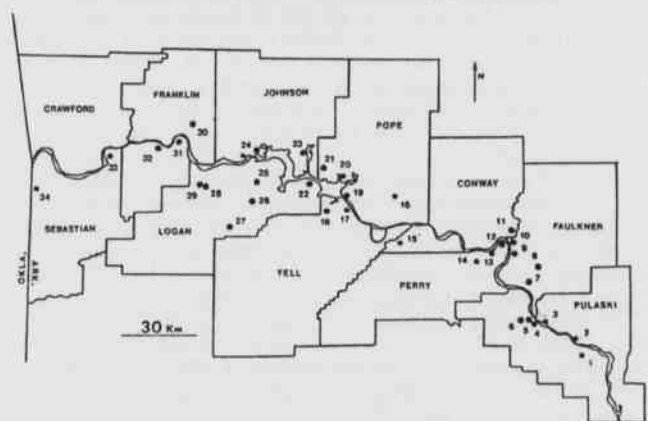


Figure 1. Map of counties along the Arkansas River from Little Rock to Fort Smith showing the location of habitats visited during this survey of the eastern collared lizard, *Crotaphytus collaris collaris*.

## RESULTS AND DISCUSSION

Only three sites (15, 22, and 25) contained populations of collared lizards, and all three were located south of the Arkansas River. One, (15) in the vicinity of Petit Jean State Park, was a natural habitat while the other two populations were associated with sandstone rock quarries. The following provides a brief description of each site.

1—Rock quarries and open rock outcropping (Granite Mountain), ca. 1.6 km N Woodyardville, Pulaski Co. (T1N, R12W, S34). Workers at this expansive rock quarry just south of Little Rock had never observed "mountain boomers" (collared lizards) at the quarry. A rocky glade (igneous rock) was examined, and there was no evidence of collared lizards.

2—Rock quarry (vicinity of Riverview Park), North Little Rock, Pulaski Co. (T2N, R12W, S28; 29). This abandoned quarry lies along the bank of the Arkansas River and has a rocky interior suitable for collared lizards. The entire inner perimeter of the quarry was examined, and no sign (scats or fecal pellets on top of rocks) of the lizards was observed.

3—Rock quarry (Round Hill), ca. 3.2 km SE Maumelle, Pulaski Co. (T2N, R13W, S3). This active rock quarry had no collared lizards according to heavy machine operators (including one person who had worked at the quarry for 17 yrs.).

4—Rock quarry (Fulk Mountains, Pinnacle Mountain State Park), Pulaski Co. (T2N, R14W, S1). This quarry site was suggested to me by one of the park naturalists (although he had never seen any collared lizards there). The site was an excellent potential habitat, and a few fence lizards (*Sceloporus undulatus*) were observed but no collared lizards. In general, the presence of basking fence lizards on the tops of conspicuous rocks in a rocky habitat usually indicates the absence of collared lizards or that they are present in low numbers.

5—Pinnacle Mountain (Pinnacle Mountain State Park), Pulaski Co. (T2N, R14W, S3). Collared lizards were collected from Pinnacle Mountain around 40 yrs. ago by collectors passing through the state. No recent records exist according to park officials. This site may have been subjected to overcollection prior to the formation of the state park. I climbed the east and west face trails to the summit and observed potential habitat for the lizards. Park officials discourage hiking beyond designated trails; therefore, the possibility of a population still existing somewhere on the mountain is plausible.

6—Maumelle Pinnacles (east pinnacle), Pulaski Co. (T2N, R14W, S5). The two pinnacles with crests running east-west lie just west of Pinnacle Mountain. I transversed the rocky summit of the easternmost pinnacle, a distance of ca. 250-300 m. The habitat is very steep and rugged, yet collared lizards could have existed there. Hiking activities on these pinnacles could have led to the demise of collared lizards in this terrain. The westernmost pinnacle was not investigated.

7—Reynolds Mountain, ca. 4.8 km W Mayflower, Faulkner Co. (T4N, R14W, S15). A distinct clearing in the hardwood forest on the south-facing slope of this mountain consisted of exposed ledges and rocky formations that could have supported a small population of collared lizards at one time was examined. A thick layer of grasses covered areas between rocky places, and a basking fence lizard was collected at this site.

8—Rock quarry, 6.1 km N Mayflower, Faulkner Co. (T4N, R13W, S5). This abandoned quarry site, located at the easternmost rim of a northward extension of Reynolds Mountain, was well suited for collared lizards. A small lake was present at the back of the quarry and was probably used as a swimming hole for local residents. Parts of the site were being used as a dump and a firing range.

9—Rock quarry, 1.2 km S Toad Suck Ferry Lock & Dam, Faulkner Co. (T5N, R14W, S18). This isolated and abandoned quarry was probably worked during the construction of TSF Lock & Dam. The site, an excavated V-shaped trench (100 m long), was partially filled with water. While parts of the quarry were capable of harboring collared lizards, the isolated nature (surrounded by woods) of this site would restrict migrations by collared lizards.

10—Cadron Ridge bluff (at the Arkansas River), Faulkner Co. (T5N, R15W, S1). This shear bluff overlooking the Arkansas River and active shale and sand pits situated just north of the bluff could not support a collared lizard population. Fence lizards were present at the site. Perkins (1928) reported collecting collared lizards on and near Cadron Ridge, west of Conway.

11—Shale pit, ca. 2.4 km SE Menifee, Conway Co. (T6N, R14W, S30). Although situated on a southwest-facing exposure of a small ridge, this arid pit was commonly used as a firing range. No collared lizards were observed.

12—Rock quarry (Jefferson Mountain at the Arkansas River), Perry Co. (T5N, R15W, S2). This active site can be easily seen from Site 10; however, I could not gain access to this quarry.

13—Rock quarry, ca. 0.5 km SE Stony Point, Perry Co. (T5N, R15W, S16). This extensive, two-tiered abandoned quarry has been used as a dump site for years; however, areas within the interior are well suited for collared lizards.

14—Rock quarry, 1.9 km E Houston, Perry Co. (T5N, R16W, S35). This isolated and abandoned quarry was constructed in a similar fashion as Site 9. The sloping banks form a trench that could support collared lizards.

15—Rock outcroppings (Petit Jean Mountain), near SW edge of Petit Jean State Park, Conway Co. (T5N, R18W, S6). I hiked about 200 m west of Highway 155 S through thick vegetation and underbrush to a shelfrock area commonly called "turtle rocks" because of the dome-shaped appearance of the outcroppings. I discovered four adult collared lizards in a very small restricted area. I photographed, measured, and toe-clipped three of the lizards; all were released back into the habitat. Juvenile collared lizards probably emigrate quickly from this site after hatching as it could not support a very large population. The shed skin of an eastern coachwhip (*Masticophis flagellum*), a predatory snake on collared lizards, was observed at the site. This remote population on Petit Jean Mountain will remain small because of the limited local habitat.

16—Carrion Crow Mountain, ca. 3.2 km W Atkins, Pope Co. (T7N, R19W, S13; 14). The southern edge of this mountain has an extensively-exposed rocky bluff. Unfortunately the hardwood forest has intruded to near the very edge of the bluff, effectively devouring suitable habitat for collared lizards. I investigated ca. 150 m of the bluff habitat. People living on the mountain stated that as children (in the 1920's) they saw collared lizards on the mountain.

17—Dardanelle Rock (Dardanelle Mountain), Dardanelle, Yell Co. (T7N, R20W, S30). This rock exposure (mostly a slab) contained very few loose rocks that would normally provide shelter for collared lizards. Fence lizards were common in the habitat.

18—Mount Nebo (Mount Nebo State Park), 9.6 km SW Dardanelle, Yell Co. (T7N, R12W, S18). Mount Nebo exhibits shear bluffs on all sides. The rocky exposures are similar to situations found at the east face of Petit Jean Mountain. I have yet to find collared lizards inhabiting shear bluff areas in Arkansas.

19—Rock quarries (Dardanelle Lock and Dam), near Russellville, Pope Co. (T7N, R20W, S18). Corp of Engineers' park personnel advised me that these quarries were mostly full of water. Access roads to the quarries were posted and gated. Workers at the park had never seen collared lizards in the area.

20—"40-acre rock", rock outcropping (Pleasant View Mountain), 5.6 km NW Russellville, Pope Co. (T8N, R21W, S24). This extensive, south-facing rocky exposure was one of the most promising natural habitats investigated. Local people told me that they had never seen collared lizards at the site. I hiked several times over most of the expansive habitat which featured cedar glades and numerous scattered small rocks near the forest edge without observing any collared lizards.

21—Rock quarry, 1.6 km N Georgetown, Pope Co. (T8N, R22W, S18). This small sandstone quarry contained several fence lizards, but there were no signs of collared lizards.

22—Rock quarries (River Mountain), ca. 4.0 km NW Delaware, Logan Co. (T8N, R22W, S27). Much of the forest habitat was mixture of hardwoods and pine; the soil was very sandy. Gas wells dotted the habitat. I visited three sandstone quarries and found collared lizards at two of them. Less than 10 lizards were observed; however, I did not explore all of the potential habitat for lizards. One voucher specimen (ASUMZ 11772) was collected at the site.

23—Bee Bluff (overlooking Dardanelle Reservoir), 2.6 km NE Knoxville, Johnson Co. (T9N, R22W, S28). This shear bluff had no suitable habitat that would support a collared lizard population.

24—Tick Hill (at the Arkansas River), 1.3 km SW New Spadra, Johnson Co. (T9N, R24W, S27). The south-facing slope of this rock-strewn mountain which faces the Arkansas River exhibited small patches of open habitat. Fence lizards and five-lined skinks (*Eumeces fasciatus*) were abundant.



25—Rock quarries (Prairie View Mountain), ca. 2.4 km N Midway, Logan Co. (T8N, R24W, S34; 35). This complex of active and abandoned quarries contained a large population of collared lizards. At only one other location in Arkansas (Bull Shoals Dam quarry site, Flippin, Marion Co.) had I ever observed more collared lizards. These mostly sandstone quarries (surface rock) occupy around a 3 km tract of land running east-west on the top and south-facing slope of the mountain. The number of collared lizards in a 0.5 km circuit of quarry are was 28; in general, this underestimates the total population of an area by about 50%. Moreover, only 25% of one region of the quarry habitat was examined. This population represents the westernmost site for collared lizards in this study. The continued activity of quarry operations is not likely to harm this population. Two voucher specimens (ASUMZ 11615, 11616) were collected.

26—Rich Mountain (Ozark National Forest), Logan Co. (T7N, R24W, S28-30). This flat-topped mountain running east-west is a stepping stone terrace to Magazine Mountain which lies to the southwest. The mountain was important because collared lizards had been reported from it during the 1940's (Carnegie Museum Herpetological Collection Nos. 24584-85; 24593). As with many sites previously discussed, the natural habitats favoring collared lizards have, through time, been overtaken by successional vegetation. Few large rocks for basking activity and shelter were observed, and large barren tracts of land or cedar glades which typify collared lizard habitats were absent.

27—Magazine Mountain (Ozark National Forest), Logan Co. (T6N, R25W, S20-22). Several places on the top of Magazine Mountain were searched because of the previous records in the literature of collared lizards. Although no lizards were found, talus slides at the base of the summit's bluffy escarpment may harbor lizards. No evidence of these lizards could be ascertained on any cliffs or ledges, such as on Cameron Bluff on the north rim. Several six-lined racerunners (*Cnemidophorus sexlineatus*) were observed in the various habitats.

28—Short Mountain, 1.0 km N Paris, Logan Co. (T7N, R26W, S3-5). This isolated knob rises sharply above the surrounding plain; it has a rim of rocky cliffs and outcroppings. The area near the summit was searched, but this site may be too rugged to support collared lizards.

29—Horseshoe Mountain, ca. 6.0 km NW Paris, Logan Co. (T8N, R27W, S36). Horseshoe Mountain is another isolated knob similar to Short Mountain. High rocky cliffs face to the south. I did not investigate the site.

30—Rock quarry, ca. 4.0 km NE Ozark, Franklin Co. (T10N, R26W, S26). This active quarry showed no signs of collared lizards.

31—Arkansas River bluff, ca. 3.0 km E Webb City, Franklin Co. (T9N, R27W, S9). This isolated bluff overlooking Ozark Lake (Arkansas River) stretches around 2.0 km. Again, high, steep bluffs usually do not support collared lizard populations, and I did not attempt to investigate the habitat.

32—Rock quarries, ca. 2.5 km NE Cecil, Franklin Co. (T9N, R26W, S24). These shallow quarries scattered throughout the area are associated with gas fields. Surprisingly, no collared lizards were observed in this open habitat. One large eastern coachwhip was observed as it hunted among the rock piles.

33—Rock quarry, at the junction of Arkansas Highway 96 and Courthouse Slough (near Arkansas River), Sebastian Co. (T9N, R29W, S1). This abandoned quarry was filled with water. Little area outside the quarry proper was suitable for collared lizards.

34—Gravel pit (George Mountain), ca. 4.8 km S Fort Smith, Sebastian Co. (T7N, R32W, S17). This shale pit was not suitable for collared lizards.

This survey suggests that collared lizards are not as common or ubiquitously distributed in the array of suitable habitats within the Arkansas River Valley in Arkansas. The isolated enclaves of these lizards on the rocky slopes of Petit Jean Mountain may represent one of the few remaining relictual populations occupying an ancestral habitat. The large quarry populations in Logan County demonstrate the vigor of collared lizards in man-made habitats. Collared lizards are not presently a threatened species in Arkansas. Yet, habitat depletion poses a severe threat to their continued existence in the Arkansas River Valley. Because *C. c. collaris* invokes a curiosity and fascination in all who view it, measures to protect the lizard and its natural habitat may eventually

be warranted. To continue to identify new populations of this species, especially in remote habitats within the Interior Highland Region, will add to a database for future conservation considerations. Then, only through monitoring populations will researchers be able to critically evaluate the status of the lizard as a natural component of Arkansas' native herpetofauna.

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# FEMALE REPRODUCTIVE TRAITS OF THE SOUTHERN LEOPARD FROG, *RANA SPHENOCEPHALA* (ANURA: RANIDAE), FROM ARKANSAS

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## ABSTRACT

Reproductive characteristics of female southern leopard frogs (*Rana sphenoccephala*) were studied from specimens collected in northeastern Arkansas. Egg masses collected from breeding habitats were also examined. A communal ovipositional site was discovered in March 1988 and possessed over 75 egg masses within a 3 m square area. Clutch size from necropsied females averaged 2958.7 eggs, whereas number of eggs per egg mass averaged 2106.5 eggs. Egg diameter averaged 1.76 mm and was similar to populations reported previously in Texas. Positive correlations existed between mean ovum size and female body size and between clutch size and female body size. Negative correlations were found between mean egg diameter and mass per egg as each varied with number of eggs per egg mass. The reproductive traits of *R. sphenoccephala* are basically similar to other ranid species.

## INTRODUCTION

In many anuran species with complex life cycles, patterns of reproduction are explainable by a clarification of quantitative relationships that exist between female reproductive parameters (Salthe and Duellman, 1973; Salthe and Mecham, 1974). When viewed together along with reproductive mode and other life history traits, these patterns reveal predictable reproductive strategies (Duellman and Trueb, 1986). Salthe and Duellman (1973) showed that even among taxonomically-diverse anuran species, positive correlations are found between ovum size and female body size or snout-vent length (SVL), clutch size and female SVL, and ovum size and size of hatchlings. A negative correlation between clutch size and ovum size is generally the rule (for specific exceptions, see Collins, 1975). These correlations may also characterize intraspecific size-fecundity relationships (Duellman and Trueb, 1986; Berven, 1988).

The southern leopard frog (*Rana sphenoccephala*), a member of the *R. pipiens* complex of the family Ranidae, is distributed throughout the southeastern and southcentral United States. This common species generally breeds in the late winter or early spring, but the species may breed at all times of the year in the southern portions of its range (Conant, 1975; Mount, 1975). Two distinct breeding periods (early fall and late winter) were observed by Caldwell (1986) in South Carolina. McAlister (1962) reported on variation in ovum size in Texas populations, while Caldwell (1986) summarized information on selection of egg deposition sites and on communal oviposition in South Carolina. General accounts on eggs are mainly limited to egg mass size (Smith, 1961; Rugh, 1964; Mount, 1975; Martof *et al.*, 1980; Johnson, 1987). In the present study, I investigated the reproductive biology of *R. sphenoccephala* specifically to address relationships among several female reproductive parameters.

## MATERIALS AND METHODS

Nearly all of the female *R. sphenoccephala* examined in this study ( $n = 36$ ) were collected from late January to mid-March, 1987 in northeastern Arkansas. Several additional specimens were taken during the same period in 1985 and 1986. Specimens were killed in a dilute chlorotone solution, fixed in 10% formalin, and stored in 70% ethanol. Snout-vent lengths (SVL) of females were recorded from preserved animals using vernier calipers. On 12 March 1988, egg masses ( $n = 49$ ) were collected from three locations in Craighead County, Arkansas. These sites were separated by no more than 30 km, and each was situated adjacent to (and just outside) the eastern levee of the St. Francis River (see Breeding Habitat in RESULTS).

Clutch size of each gravid female as well as egg mass size were calculated by estimation using gravimetric techniques. Ovaries were removed from the abdominal cavity and placed into vials of 70% ethanol. At the start of mensuration, each ovary was blotted and allowed to briefly air dry. The entire ovary was then weighed on an analytical balance; this was followed by the weighing of a sub-sample of ova (approximately 150) from the same ovary. Clutch size was estimated by dividing the ovarian mass (to the nearest 0.01 g) by the sample mass and multiplying this quotient by the number of ova in the sub-sample. The value obtained in this manner overestimates the number of ova per ovary because of the additional mass within the ovary (trapped interstitial water and ovarian stroma). To determine a percent error, all ova of a single ovary from six specimens were counted. (Ovaries were air dried and gently pulverized to release ova.) The average percent error was  $21.9 \pm 3.6$  (range, 16.9 to 29.7). This percentage was deducted from the original overestimated number of ova and yielded a final clutch size for each female. The estimation of the number of eggs per egg mass was conducted in a similar fashion. Egg masses were removed from 10% formalin, blotted dry, and allowed to briefly air dry. Each egg mass was weighed (to the nearest 0.01 g); then, as above, a sub-sample of eggs was weighed, and the number counted. The total number of eggs was derived by dividing the mass of each egg mass by the mass of the sub-sample and multiplying by the sub-sample egg count. Unlike the estimation of clutch size, no percent error was determined mainly because evaporation of water was negligible during the rapid preparation and weighing of each sub-sample.

Egg diameters were determined by measuring 10 eggs for each of the above egg mass sub-samples using an ocular micrometer (see Berven, 1988). Following preservation, yolked ovarian follicles were highly irregular in shape and were, therefore, not measured in the present study. Specimens and egg masses are deposited in the Arkansas State University Museum of Zoology. Statistical data (means) are accompanied by  $\pm$  two standard errors.

## RESULTS

### Breeding Habitat

The breeding habitats of *R. sphenoccephala* observed in this study were located at the margins of cultivated fields. These tracts of land sloped toward the levee of the St. Francis River and created lentic pools of temporary water. Globular egg masses of approximately 10 cm in width and 5 cm in depth were found laid either attached to submergent and emergent vegetation or, at some sites where no vegetation existed, were laid unattached. Water depth generally varied from 20 cm to 50

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cm. All egg masses collected for study were taken from small, isolated egg clusters of from two to five egg masses. Eggs of these masses had begun embryonic development, but they had not progressed beyond gastrulation. On 12 March 1988, a communal egg deposition site was discovered (Fig. 1). This site was found within several km of other collection sites visited on the same day. The breeding habitat differed from other areas mainly by the abundance of vegetation (predominantly broom sedge, *Andropogon* sp.). Over 75 egg masses were counted in an open area (Fig. 1B) of approximately three square m. The eggs were hatching at this site and were left undisturbed; however, by early June, the site was completely dry as a result of severe drought conditions, and the population was decimated.



Figure 1. Breeding habitat of *Rana sphenoccephala* in northeastern Arkansas. A. Communal breeding site, 3.5 km W Childres, Craighead County. Levee of St. Francis River in background. B. Close up of foreground in A showing a large aggregate of egg masses in approximately 25 cm of water.

#### Reproductive Parameters

The average clutch size of 39 female *R. sphenoccephala* ranging from 51 to 89 mm SVL was  $2958.7 \pm 328.7$  (range, 1700-5537), whereas clutch mass averaged  $6.68 \pm 1.02$  g (2.37-15.37). The number of eggs per egg mass averaged  $2106.5 \pm 171.7$  (1289-3366). The mass per egg ( $22.82 \pm 3.61$  mg; 15.73-52.02) was over 20 times as great as the average ovum mass ( $2.00 \pm 0.28$  mg; 1.40-3.66). Egg diameter averaged  $1.76 \pm 0.04$  mm (1.40-2.52) and was similar to the values reported by McAlister (1962) in Texas. Average mass of 49 egg masses was  $65.31 \pm 4.54$  g (20.29-105.32).

#### Quantitative Relationships

The relationships between average ovum mass and female SVL and

clutch size and female SVL are shown in Fig. 2; each reveals a significant positive correlation. The coefficient of determination in Fig. 2A ( $r^2 = 0.55$ ) predicts that most of the variability in ovum mass is accounted for by female SVL; however, in Fig. 2B ( $r^2 = 0.50$ ), only 50% of the variation in clutch size is a result of female SVL.

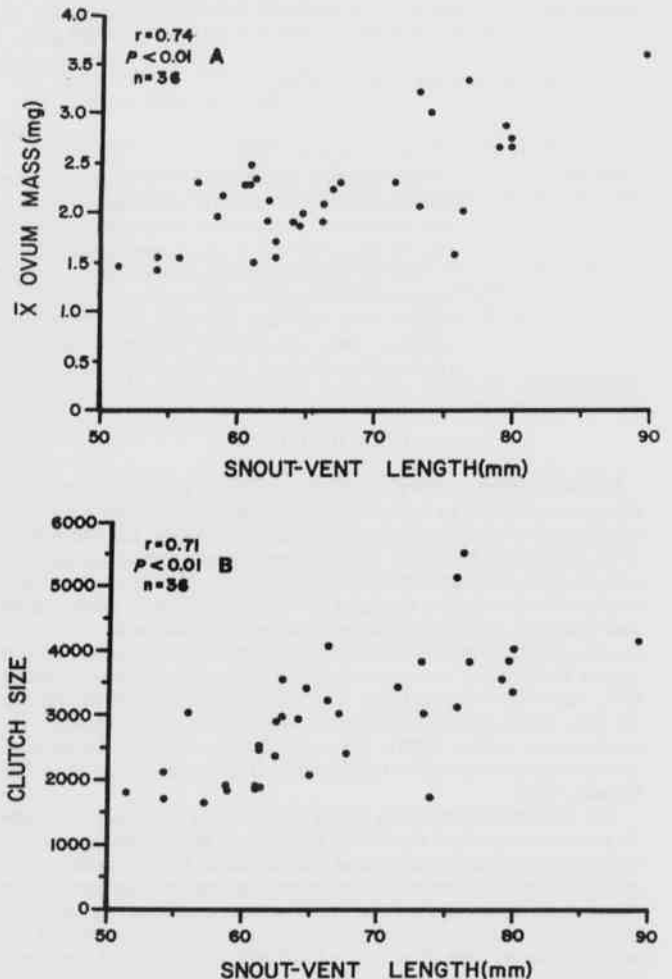


Figure 2. Relationships between average ovum mass and snout-vent length (A) and clutch size and snout-vent length (B) in *Rana sphenoccephala* from northeastern Arkansas.  $r$  = correlation coefficient.

In Fig. 3, average egg diameter (A) and mass per egg (B) are shown as a function of the number of eggs per egg mass; these relationships indicate inverse (negative) correlations with only the latter being significant. The coefficient of determination in each case (0.04 and 0.22, respectively) indicates that the variability in these  $y$ -axis parameters (i.e., egg diameter and egg mass) is not explained by their linear relationship to the number of eggs in each egg mass.

In Fig. 4, the relationship between the number of eggs per egg mass is plotted as a function of mass of each egg mass. Although the correlation is positive and significant, the coefficient of determination (0.44) predicts that only 44% of the variability in egg mass number is accounted for by increases in the mass of egg masses.

The clutch size-clutch mass relationship (not shown) reveals a significant positive correlation ( $r = 0.79$ ;  $n = 36$ ;  $P < 0.01$ ) and can be inferred from the relationships in Fig. 2. The coefficient of determination (0.62) predicts that 62% of the proportion of variability in clutch size is attributed to its relationship to clutch mass.



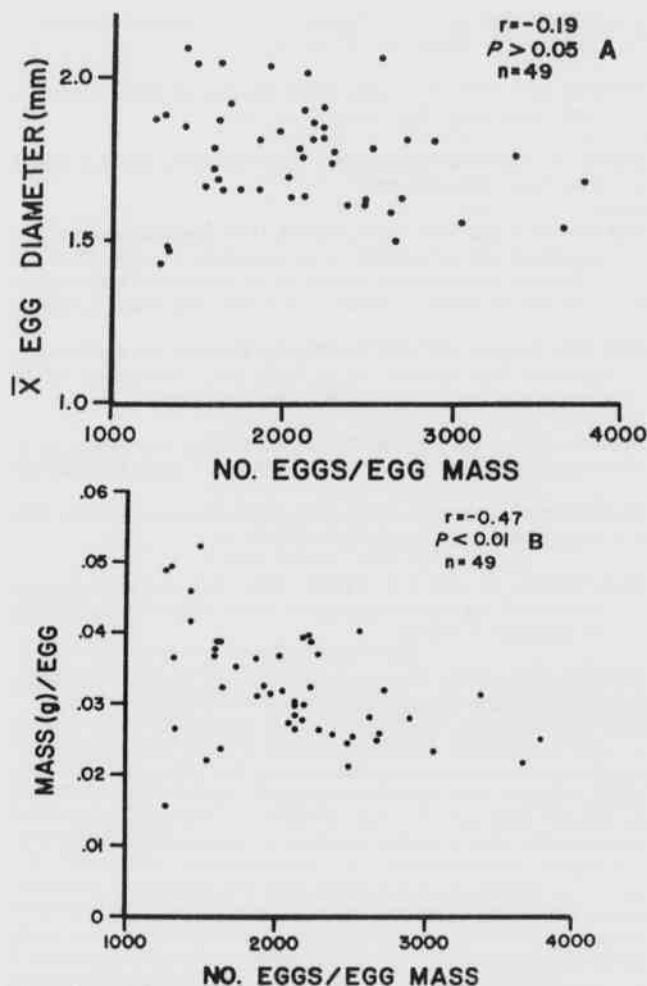


Figure 3. Relationships between average egg diameter and number of eggs per egg mass (A) and mass per egg and number of eggs per egg mass (B) in *Rana sphenoccephala* from northeastern Arkansas.  $r$  = correlation coefficient.

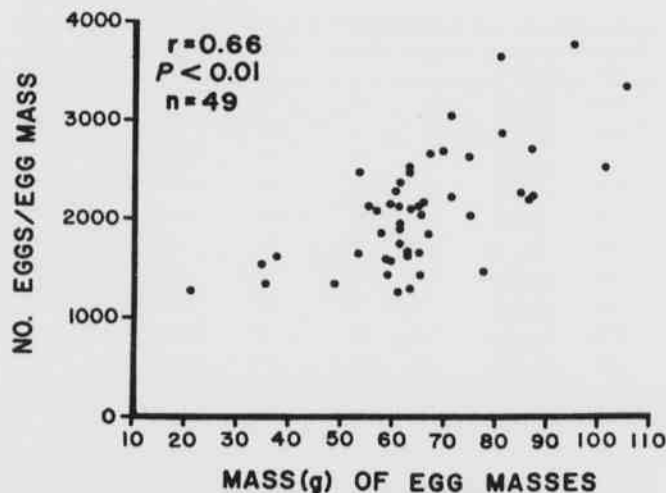


Figure 4. Relationship between the number of eggs per egg mass and the mass of each egg mass in *Rana sphenoccephala* from northeastern Arkansas.  $r$  = correlation coefficient.

## DISCUSSION

Caldwell (1986) hypothesized that *R. sphenoccephala* resorted to communal egg mass deposition to gain a thermal advantage for the development of eggs and embryos (a behavioral adaptation common in several northern latitude ranid species; see Seale, 1982; Waldman and Ryan, 1983) during cold weather breeding. She also stated that during warm weather, isolated egg masses are laid. The presence of appropriate materials to which *R. sphenoccephala* can attach egg masses will also influence the choice of egg mass deposition site. In my study, the location of the communal ovipositional site appeared to be related to the vegetational characteristics of the breeding habitat; i.e., the communal site was heavily vegetated, whereas the collection sites for isolated egg masses were sparsely vegetated. Male choice of calling sites within the actual breeding grounds could determine where females will eventually lay eggs (Wells, 1977). Although a male lek mating system has been proposed for *R. catesbeiana* (Emlen, 1977), no studies have shown that this is the case in *R. sphenoccephala*.

The reproductive traits of female *R. sphenoccephala* in northeastern Arkansas indicate that body size varied positively with ovum mass (and inferred ovum size) and clutch size (and inferred clutch mass). Collins (1975) found similar relationships in *R. sylvatica*, *Hyla versicolor*, and *Bufo americanus* in Michigan as did Berven (1988) for *R. sylvatica* in Maryland. My data support the general conclusions of Salthe and Duellman (1973); yet, contrary to Salthe and Duellman (1973), my data predict that clutch size in *R. sphenoccephala* is positively correlated to ovum size (also found by Collins, 1975). Larger females produce larger clutches with larger ova than do smaller females. Recently, Berven (1988) indicated that an inverse relationship existed between mean clutch size and egg size in all size classes of *R. sylvatica*. In other words, he found that younger females produced larger clutches with smaller eggs (vice versa in older females). In *R. sylvatica*, this would suggest that younger females oviposit their entire complement of eggs into a single mass, whereas older females oviposit more than once in a breeding period (as stated by Davis and Folkerts, 1986). I did not age the *R. sphenoccephala* in my study (although skeletochronological aging techniques are available for *R. pipiens*; see Leclair and Castanet, 1987) and, therefore, cannot make direct inferences about age classes as did Berven (1988). However, an examination of Fig. 3B shows an inverse relationship between mass per egg and number of eggs per egg mass which tends to support his findings. Presumably, as female *R. sphenoccephala* age and grow larger, they begin to partition their clutches into several egg masses which would exhibit large egg size. Small-to-intermediate sized females would oviposit their entire clutches of small eggs. If these assumptions are valid, one would expect to find egg masses exhibiting a minimal number of eggs but would also exhibit a continuum of size, mass, or volume from small to large. This condition is demonstrated in Fig. 4 as there is a four-fold increase in mass per egg mass (approximately 20 g to 80 g) with no accompanying increase in minimal egg number.

## CONCLUSIONS

Significant positive correlations exist between ovum size and female SVL and clutch size and female SVL in the southern leopard frog, *R. sphenoccephala*, from northeastern Arkansas. Negative correlations occur between mean egg diameter and size of egg mass and mass per egg and size of egg mass (the latter being significant). Smaller females appear to lay egg masses of a larger size and have smaller eggs, whereas larger females partition their clutches into several masses (each with relatively large eggs). A communal oviposition site observed in this study differed from other egg-laying sites by a preponderance of submergent vegetation. Reproductive traits in *R. sphenoccephala* are basically characteristic of other ranid species and most anurans.

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# REPRODUCTION AND LARVAL DEVELOPMENT IN THE MARBLED SALAMANDER, *AMBYSTOMA OPACUM* (CAUDATA: AMBYSTOMATIDAE), FROM ARKANSAS

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## ABSTRACT

Field investigations of the reproductive biology and larval growth and development in *Ambystoma opacum* from northeastern Arkansas began in early October, 1987 at the onset of nesting activities, and concluded in early May, 1988 when larval transformation occurred. The onset and timing of clutch deposition were documented. Clutch size averaged 107.1 eggs and was not significantly correlated with snout-vent length (SVL). The incubation period was estimated to be 90 days. Larvae grew at an average rate of 6.3 mm SVL per month. Metamorphosis, in early May, occurred at an average SVL of 30.5 mm. Larval development was recorded by noting the chronological appearance of digits on limb buds as well as noting the change in body color patterns. Premetamorphs characteristically retained a ventrolateral row of white spots throughout development. Postmetamorphs eventually lost these spots and developed a color pattern totally unlike adults.

## INTRODUCTION

Numerous studies have documented various aspects of the life history of the marbled salamander, *Ambystoma opacum*, throughout its range in eastern North America (Anderson, 1967). Nesting activities, reproductive potential, and larval growth are among the most common life history traits examined in this species (Petranka and Petranka, 1980). Although photographs of adults or nests with brooding females are depicted in the herpetological literature, authors usually seldom bother with illustrating premetamorphs or postmetamorphs temporally. Moreover, few studies have been conducted on this species west of the Mississippi River, and little information other than distributional records (Black and Dellinger, 1938; Dowling, 1957) is available on this species in Arkansas. This report presents information on the reproductive habits and larval growth of *A. opacum* in northeastern Arkansas.

Table 1. Reproductive parameters of 10 nesting female *Ambystoma opacum* from Craighead County, Arkansas. Ranges are in parentheses.

| ASUMZ No.      | SVL (mm)    | Total Length (mm) | Body Mass (g) | Clutch Size  | Clutch Mass (g) | Egg Diameter (mm; n = 10 per clutch) | Nest Volume (cm <sup>3</sup> ) |
|----------------|-------------|-------------------|---------------|--------------|-----------------|--------------------------------------|--------------------------------|
| 9107           | 68.3        | 120.6             | 9.6           | 131          | 6.9             | 5.4 ± 0.32<br>(5.0-6.8)              | 106.3                          |
| 9108           | 61.7        | 112.7             | 8.0           | 87           | 3.6             | 5.8 ± 0.44<br>(5.2-7.3)              | 90.6                           |
| 9109           | 66.2        | 124.5             | 11.3          | 100          | 3.5             | 5.6 ± 0.18<br>(5.2-6.1)              | 17.0                           |
| 9110           | 62.1        | 113.0             | 8.4           | 124          | 9.5             | 5.3 ± 0.10<br>(5.0-5.3)              | 57.7                           |
| 9111           | 59.5        | 106.7             | 7.7           | 93           | 7.0             | 5.4 ± 0.25<br>(5.0-6.2)              | 37.6                           |
| 9112           | 66.2        | 120.7             | 8.3           | 117          | 10.0            | 5.7 ± 0.55<br>(4.4-7.9)              | 104.1                          |
| 9119           | 61.2        | 110.5             | 6.7           | 97           | 4.1             | 5.6 ± 0.44<br>(4.6-7.2)              | 24.6                           |
| 9120           | 62.5        | 112.5             | 8.6           | 107          | 6.7             | 5.3 ± 0.20<br>(4.9-6.0)              | 12.2                           |
| 9121           | 65.5        | 114.3             | 7.4           | 108          | 6.9             | 5.5 ± 0.29<br>(4.9-6.4)              | 141.8                          |
| 9122           | 60.1        | 110.5             | 7.5           | 107          | 7.1             | 5.3 ± 0.20<br>(5.0-5.9)              | 14.5                           |
| Average Totals | 63.3 ± 1.09 | 114.6 ± 3.51      | 8.4 ± 0.82    | 107.1 ± 8.72 | 6.5 ± 1.40      | 5.4 ± 0.11                           | 60.6 ± 29.6                    |

## MATERIALS AND METHODS

Field investigations of *A. opacum* were conducted in northern Craighead County, Arkansas, from early October, 1987 through early

May, 1988. All adults, eggs, and larvae were collected in or near temporary ponds or depressions which were found in abandoned gravel pits on Crowley's Ridge (a low, narrow, eroded ridge that contrasts sharply with the surrounding alluvial floodplains within the Mississippi River Alluvial Valley). A series of postmetamorphs were also examined from other localities in either Greene or Jackson counties. Ten nests with attending females were studied at one gravel pit site to determine reproductive parameters (Table 1). Adult SVL as well as other adult and larval measurements were rounded to the nearest 0.1 units. Although males (n = 7) and nesting females (n = 10) along with their eggs were removed from the primary study site, many nesting sites were left undisturbed in order to determine time of hatching as well as subsequent larval growth. The ponds were periodically sampled for larvae between late January and early May. All specimens were sacrificed in a 20% chloretoe solution, fixed in 10% formalin, and preserved in 70% ethanol. Statistical data are followed by ± 2 SE. Specimens are deposited in the Arkansas State University Museum of Zoology (ASUMZ).

## RESULTS AND DISCUSSION

### Reproductive Traits

Female *A. opacum* were observed in gravel pits in early October and had constructed nests prior to the onset of rainy weather. Nests were generally spherical-to-oblong depressions within a red clay substrate with most found beneath trash (old mattresses, boards, rotting logs, etc.). By 15 November, all nests had been submerged by as much as 1.0 m of water. Adult nesting females were larger ( $\bar{x}$  = 63.3 ± 1.9 mm SVL; Table 1) than the adult males ( $\bar{x}$  = 56.0 ± 4.6 mm SVL; range, 51.2 - 64.8) collected within the immediate vicinity of nesting sites. In two instances, adult males were found together with brooding females, and the SVL of these pairs (male followed by female) and the female's clutch size (in parenthesis) were 64 and 68 (131) and 51 and 61 (97). No spermatophores were observed in or around nesting cavities.

Routine searching of optimal nesting situations revealed that only one egg clutch was laid between 8 and 10 October, and the female had departed from the clutch by 16 October (rainwater partially submerged her nest between 11 and 14 October). We also observed two other females that had abandoned their nests during a period of intermittent rain; nests were found under a mattress on 15 October. This site had been previously searched on 12 October, and no specimens were observed. Both females had left their nests by 27 October, and rainwater eventually inundated the entire site by 1 November.

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Female *A. opacum* lay one egg clutch per reproductive season (Noble and Brady, 1933). We found the average clutch size to be  $107.1 \pm 8.7$ , and clutch size was not significantly correlated with SVL ( $r = 0.2$ ;  $P > 0.05$ ).

## Larval Growth and Development

Growth of larval *A. opacum* from samples taken from a single nesting site (pond) were monitored. Larvae normally hatch between 15 and 19 mm in total length (Brandon, 1964); the incubation period in northeastern Arkansas was estimated to be 90 days. Larvae grew at an average rate of 0.2 mm per day or approximately 6.3 mm per month (Fig. 1). Metamorphosis occurred by early May with an average SVL of  $30.5 \text{ mm} \pm 1.4$  and an average total length of  $49.2 \pm 1.7 \text{ mm}$ .

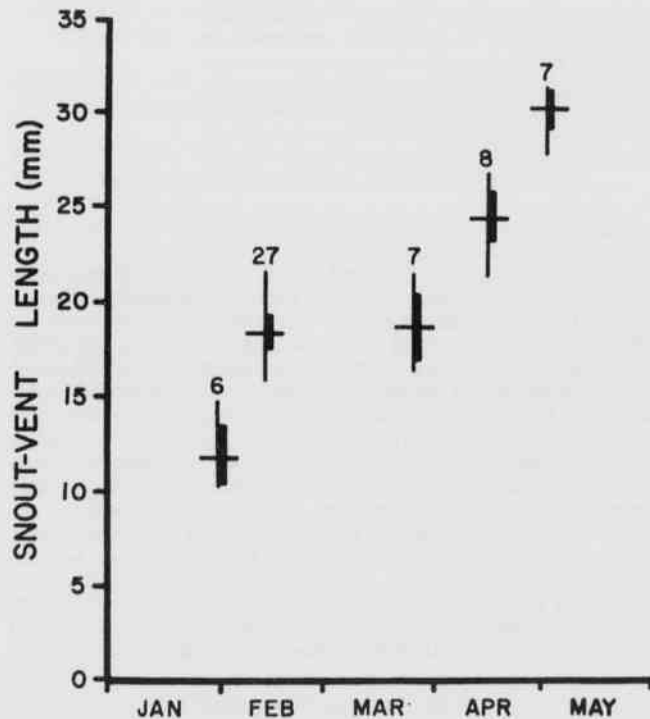


Figure 1. Larval growth in *Ambystoma opacum* from northeastern Arkansas. Numerals = sample sizes; horizontal lines = means; vertical lines = ranges; vertical bars =  $\pm$  two standard errors.

Larval development was analyzed by noting the chronological appearance of digits on the anterior and posterior limb buds. In late January, most larvae exhibited forelimbs possessing from two to four toes (four the normal complement), whereas the hindlimb varied from an absence of toes to the presence of four or five toe buds (five the normal complement). By mid-March, the forelimbs had four well-developed, elongated toes, but the hindlimbs had only four well-developed toes. All toes on both pairs of limbs were present by mid-April.

Variation in coloration of larvae and postmetamorphs is shown in Fig. 2. Young larvae smaller than 15 mm SVL (Fig. 2A) were uniformly dark dorsally. A series of distinct, circular light spots (associated with the lateral line system) lie on the ventrolateral surfaces between the limbs (one for each costal fold); ventral areas of the head, throat, and belly were mostly transparent. As development proceeds, the dark pigmentation becomes less intense on the entire body and especially on the tail fin of larvae approximately 20 mm SVL (Fig. 2B). A dorsal row of lateral line organs becomes obvious at this size as they are surrounded by circular areas mostly devoid of pigmentation. Ventro-

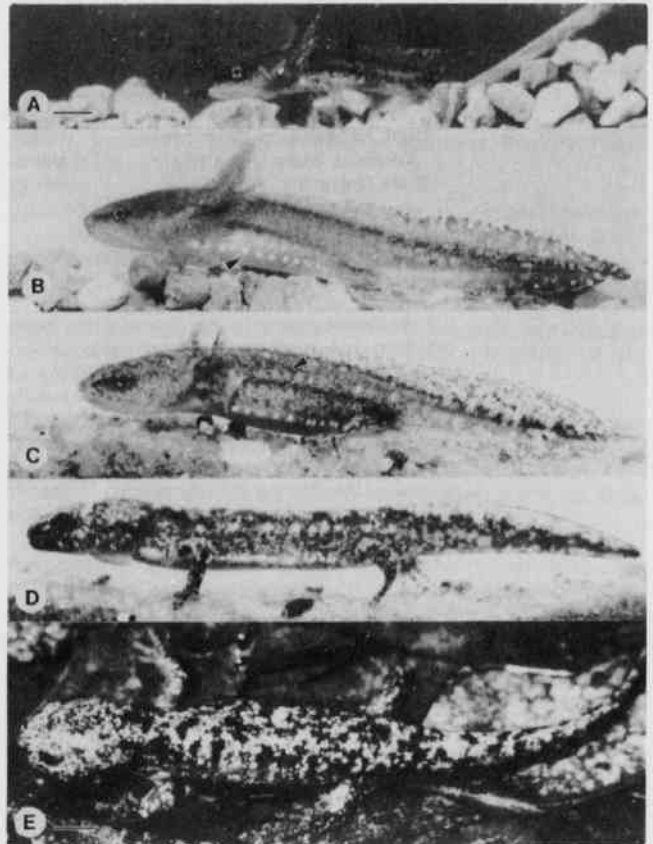


Figure 2. External appearance of larval (A, B, and C) and post-metamorphic (D and E) *Ambystoma opacum* from northeastern Arkansas. Pointer in B denotes ventrolateral series of white spots and in C, the dorsolateral spots associated with the lateral line organs. See text for further explanations. Line in A = 3 mm for A - D; line in E = 4 mm.

lateral white spots remain prominent. By mid-April, larvae have grown to around 25 mm SVL, patches of dark pigmentation are found on the head and face, and melanophores have aggregated on the body to create a mottled appearance (Fig. 2C). Larvae also begin to exhibit varying degrees of a light yellowish-green coloration. The dorsal spots of the lateral line system and the ventrolateral white spots are obvious at this stage. Pigmentation within the tail fin takes on a reticulated appearance. Immediately following transformation in early May, postmetamorphs (Fig. 2D) continue to possess the two linear series of light spotting on the body, and ground color varies from olive green to grayish black. At around 35 mm SVL in mid-May, postmetamorphs (Fig. 2E) have lost all signs of a keeled tail (as seen in Fig. 2B). Ground color of the entire body is now black, and areas of intense white flecking are scattered from snout to tip of tail on the dorsal aspect. This juvenile coloration bears little resemblance to the coloration of the adult marbled salamander (see Conant, 1975; Duellman and Trueb, 1986; Trauth and Richards, 1988). Postmetamorphs may retain the ventrolateral series of white spots for over a week after transformation (Anderson, 1967).

A comparison of the reproductive traits and larval development of *A. opacum* from northeastern Arkansas with previous accounts on the species throughout its range (see reviews in Anderson, 1967; Petranka and Petranka, 1980) revealed no major variations. For example, average clutch size and female SVL in Mississippi ( $\bar{x} = 107.3$  and  $62.5$ , respectively) were essentially the same as our findings (Walls and Altig, 1986). Size at metamorphosis in Alabama ( $32.5 \text{ mm SVL}$ ; Petranka and Petranka, 1980), Kentucky ( $33.8 \text{ mm SVL}$ ; Keen, 1975), and Maryland



(34.2 mm SVL; Worthington, 1968) was similar to our results. In addition, time of metamorphosis was March to April, May, and May, respectively, in these studies and did not differ from our results.

Larval growth rates in *A. opacum* are variable under natural conditions as low temperatures during winter months may inhibit growth (Hassinger *et al.*, 1970). We noted that larval growth at our primary study site slowed dramatically between mid-February and mid-March. Also, our observations on the development of the limbs and toes were similar to data presented by Hassinger *et al.* (1970) for New Jersey populations, although larvae from Arkansas had their full complement of toes slightly earlier.

Our presentation of variation in larval external morphology via photographs clearly illustrates the chronological changes in color pattern that take place during ontogenetic development in *A. opacum*. Viosca (1924) and Anderson (1967) described color patterns of pre- and postmetamorphic individuals, but they provided no photographs or illustrations of these stages. Bishop (1943) pictured both phases; however, his larval photograph presented no diagnostic features. Duellman and Trueb (1986) showed a premetamorph (p. 157) and an adult and postmetamorph (p. 185). Our series of photographs are the only published data to summarize external coloration and diagnostic features of larval and postmetamorphic specimens of *A. opacum*. The presence of the ventrolateral series of white spots has been used as an identification aid in this species (Brandon, 1964; Anderson, 1967; Altig and Ireland, 1984). We suggest that since other *Ambystoma* larvae (e.g., *A. annulatum*, *A. maculatum*, and *A. talpoideum*) may also exhibit a similar spotting pattern (at least in young larvae), some caution be exercised when making field identification of larvae in sympatric situations.

#### ACKNOWLEDGMENTS

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# GENERAL NOTES

## PHOTOREACTIVATION OF CHROMATID DELETIONS INDUCED BY UV-IRRADIATION OF G1 PHASE HAMSTER X *XENOPUS* HYBRID CELLS

Some amphibian cells are replete with photoreactivating enzyme and are capable of photoreactivating a relatively high level of UV-induced division delay damage, lethal damage and damage leading to chromosomal aberrations (Biological photoreactivation) (Regan *et al.*, 1968; Griggs and Bender, 1972; Griggs and Orr, 1979; Griggs and Payne, 1981). Biological photoreactivation has not been clearly demonstrated in placental mammalian cells (Cleaver, 1974). However, relatively low levels of photoreactivating enzyme activity (removal of pyrimidine dimers from DNA) have been detected in some mammalian cells (Sutherland *et al.*, 1974; Sutherland *et al.*, 1976). Recently, Kulp *et al.* (1985) and Bohlender *et al.* (1987) attempted to elucidate the differences in amphibian and mammalian photoreactivation (PR) mechanisms by studying interactions of the mechanisms in the photoreversal of UV-induced damage leading to cell death in hamster (V79) X *Xenopus* (A8) hybrid cell lines. It was anticipated that the *Xenopus* genomes in the hybrids would produce PR enzyme in sufficient concentration and of such a nature as to efficiently photoreactivate UV-induced lethal damage (pyrimidine dimers) in both hamster and *Xenopus* DNA, and the level of PR observed for the hybrid lines would closely approach the high level observed for the *Xenopus* parental lines. To the contrary, the levels of PR exhibited by the hybrids did not closely approach that of the *Xenopus* lines. To assist in the interpretation of this unexpected observation, Bohlender *et al.* (1987) compared the levels of PR of chromatid deletions induced by UV in selected hamster and *Xenopus* chromosomes of the hybrid. The results suggested that the hybrid lacked the ability to efficiently photoreactivate deletions in hamster chromosomes; implying that the relatively low level of PR of lethal damage manifested by the hybrid cells might be a consequence of their inability to efficiently PR UV-induced primary damage (pyrimidine dimers) in hamster DNA. However, the key data supporting this suggestion were indirect and limited, being results of an experiment to compare the percentages of deletions induced by UV in a marker hamster chromosome and a marker *Xenopus* chromosome (of equal length) that could be photoreactivated. Since not all segments of vertebrate DNA of equal length appear to be equally accessible to the induction of UV damage or associated repair (e.g., Smith, 1987), the question of the extent to which the results found with this pair of heterologous chromosomes are representative of the entire hybrid genome appropriately arises.

It was assumed that an enlightening (if not convincing answer to this question might be obtained if, following a given UV or UV + PR treatment of a set of hybrid cells, the sum total of the chromatid deletions occurring in the hamster chromosomes of the set could be compared with the sum total of chromatid deletions occurring in the *Xenopus* chromosomes of the set. We describe here an attempt to effect such comparisons.

Performance of the comparisons required a dependable technique for distinguishing hamster chromosomes from *Xenopus* chromosomes in the same hybrid metaphase spread. The technique developed was a modification of conventional Giemsa banding techniques. Briefly hybrid metaphase spreads on microscope slides were subjected to the following three treatments in the order listed: (1) Incubate for 90 minutes at 60° C in 2 X SSC (17.5g NaCl + 8.8g sodium citrate per liter distilled water), then air dry. (2) Incubate for 15 minutes at 4° C (ice bath) in trypsin solution (1:300 crude power in 0.9% NaCl in distilled water). (3) Stain for 10 minutes in 2% Giemsa (Gibco). This technique adequately distinguishes the hamster chromosomes from the *Xenopus* chromosomes in hybrid cells, as illustrated in Figure 1.

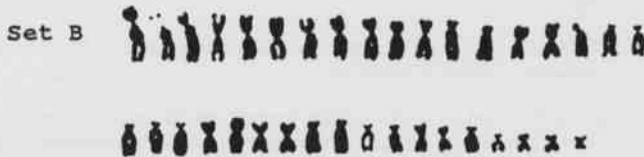


Figure 1. Differential Giemsa banding of the karyotype of a typical V79B3 (hamster) X A86 (*Xenopus*) hybrid cell. The banding technique was developed to distinguish V79B3 chromosomes (A, banded), from A86 chromosomes (B, not banded) in hybrid metaphase spreads.

Table 1. PR of chromatid deletions induced by UV in the chromosomes of G1 phase V79B3 X A86 hybrid cells.

| Experiment number* | UV fluence ( $J/m^2$ ) | PR fluence ( $J/m^2$ ) | Number cells scored | Mean number of deletions/trial ( $\pm$ standard errors) observed in |                  |
|--------------------|------------------------|------------------------|---------------------|---------------------------------------------------------------------|------------------|
|                    |                        |                        |                     | V79B3 chromosomes                                                   | A86 chromosomes  |
| 1                  | 0                      | 0                      | 1500                | 4                                                                   | 2                |
| 2                  | 0                      | 25,000                 | 1500                | 3                                                                   | 3                |
| 3                  | 5.0                    | 0                      | 1500                | 71.2 $\pm$ 9.1                                                      | 119.3 $\pm$ 12.3 |
| 4                  | 5.0                    | 25,000                 | 1500                | 67.2 $\pm$ 6.1                                                      | 28.6 $\pm$ 5.1   |
| 5                  | 10.0                   | 0                      | 1500                | 260.3 $\pm$ 11.1                                                    | 420.4 $\pm$ 16.1 |
| 6                  | 10.0                   | 25,000                 | 1500                | 240.4 $\pm$ 7.8                                                     | 70.1 $\pm$ 10.2  |
| 7                  | 15.0                   | 0                      | 1500                | 441.5 $\pm$ 17.3                                                    | 701.2 $\pm$ 24.6 |
| 8                  | 15.0                   | 25,000                 | 1500                | 381.2 $\pm$ 14.1                                                    | 93.4 $\pm$ 7.1   |

\*Each experiment consisted of three trials with 500 cells scored in each trial.

Conventional techniques for cell synchronization, irradiations and aberration analysis (Griggs and Orr, 1979; Griggs and Payne, 1981) were coupled with the banding technique to perform experiments for comparing frequencies of chromatid deletions occurring in the hamster and *Xenopus* chromosomes of sets of V79B3 X A86 cells, which were exposed to UV and/ UV + PR in G1 phase. Results of these experiments are shown in Table 1. The V79B3 X A86 line possessed a stable karyotype throughout the experimentation, with approximately 96% of the cells containing the entire complements of hamster and *Xenopus* chromosomes (22 and 36 respectively); however, as has been the case with previous hamster X *Xenopus* lines, the V79B3 X A86 line began to lose hamster chromosomes after about 100 cell passages (Kulp *et al.*, 1985). The PR scheme used in these experiments was essentially the same as the "optimum" PR scheme for the line studied by Bohlender *et al.* (1987) (i.e., Temperature-24° C; fluence rate-10w/m<sup>2</sup>; total fluence-25,000 J/m<sup>2</sup>). Comparison of the results of experiments 3, 5, and 7 indicates that a given fluence of UV induces a substantially higher number of deletions in the *Xenopus* chromosomes than in the hamster chromosomes of a set of hybrid cells; e.g. in experiment 3, hamster deletions/*Xenopus* deletions is approximately 71/119 = .60. However, when the sum of the lengths of the A86 chromosomes and the sum of the length of the V79B3 chromosomes, found in a set of hybrid metaphase spreads, were determined by ocular micrometer (data not shown), and compared; the ratio (sum of V79B3 lengths/sum of A86 lengths) was approximately .57. Thus, if it is assumed that segments of *Xenopus* and hamster G1 phase chromosomes of equal length contain equal lengths of vertebrate DNA, then *Xenopus* DNA and hamster DNA would appear to be about equally sensitive to chromatid deletion induction by UV. Comparison of the results of experiments 3, 5, and 7 with the results of experiments 4, 6, and 8 respectively, indicate that the V79B3 X A86 cells photoreactivated a much higher level of UV-induced damage leading to deletions in the A86 chromosomes than in the V79B3 chromosomes. For example, results of experiments 5 and 6 indicate that 1- 70/420 = .83 of the deletions in the A86 chromosomes were photoreactivated, while only 1- 240/260 = .08 of the deletion in the 79B3 chromosomes were photoreactivated.

In conclusion, the data described here constitute an extension of the observation by Bohlender *et al.* (1987) on PR of UV-induced chromatid deletions in hamster X *Xenopus* hybrid cells, and support the notion that PR mechanisms in vertebrate cells do not remove pyrimidine dimers from all vertebrate DNA's with equal efficiency.

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#### A PRELIMINARY SURVEY OF THE COLLEMBOLA OF MAGAZINE MOUNTAIN, LOGAN CO., ARKANSAS

The order Collembola includes small, wingless insects with characteristic abdominal appendages and mouthparts that are enclosed within a gnathal pouch. The first, third and fourth segments of the six-segmented abdomen are modified to form the specialized jumping organs characteristic of the order. Collembola range in size from .25 mm to 1 mm. Development is direct in that newly eclosed individuals differ from adults only in size, body proportions and usually absence of pigment. Collembola molt throughout life, the number of instars ranging from two to 50. They may be found in any habitat from near polar conditions and extreme high altitudes to sea level (Christiansen and Bellinger, The Collembola of North America, North of the Rio Grande, Grinnell College, Grinnell, Iowa, 1981. p. 20). Classification is based almost entirely on external morphology and mouthpart structure.

Little information was available about the order in Arkansas. Previous work in Arkansas has shown 15 species to occur in the state (Christiansen and Bellinger, 1981, pp. 495-1110). There have been lists of collembolan fauna published from 10 other states (Christiansen and Bellinger, 1981). The Tennessee list (Copeland, T.P., A Preliminary List of the Collembola of East Tennessee, Journal of The Tennessee Academy of Science, 35[4] October, 1960, pp. 238-243) recorded 34 genera and 77 species. To further investigate the collembolan fauna in Arkansas, berlese samples were taken from seven study sites representing a diversity of habitats in the Magazine Mountain area from October 1985 through September 1988. The study sites consisted of: north-facing bluffs with limestone outcroppings, sphagnum moss from a boggy area, a dry upland forest trail, rotting leaf litter along a small stream, moss samples, rotting logs, a drainage ravine with deep forest liter, and a hallow, rotting stump. Collembola were collected from the samples, cleared and slide mounted according to the methods outlined by Christiansen and Bellinger (1981) to facilitate examination under oil immersion (1000X). Approximately 1500 slides have been processed and classified from the sites. Findings include members from five families, 28 genera and 59 species. This represents 57 new state records. Voucher collections will be placed in the University of Arkansas Insect Collection. Determinations were made by the authors and by Drs. Kenneth Christiansen, Peter F. Bellinger and Richard J. Snider.

#### SPECIES LIST

Suborder: Arthropleona  
Family: Hypogasturidae

*Anurida (Micranurida) harti* Christiansen and Bellinger  
*Hypogastura (Ceratophysella) armata* (Nicolet)  
*Hypogastura (Ceratophysella) denticulata* (Bagnall)  
*Hypogastura (Ceratophysella) glancei* (Hammer)

Family: Entomobryidae

*Entomobrya (Entomobrya) multifasciata* (Tullberg)  
*Orchesella celsa* Christiansen and Tucker  
*Orchesella villosa* (Linnaeus)  
*Lepidocyrtus pallidus* Reuter  
*Pseudosinella violenta* (Folsom)

*Hypogastura (Hypogastura) lima* Christiansen and Bellinger  
*Hypogastura (Mitchellania) vulgaris* Yosii  
*Hypogastura (Schoetella) albamaculata* Scott  
*Neanura (Crossodonthina) serrata* Folsom  
*Neanura (Neanura) barberi* (Handschin)  
*Neanura (Neanura) muscorum* (Templeton)  
*Paranura anops* Christiansen and Bellinger  
*Paranura caeca* Folsom  
*Paranura colorata* Mills  
*Paranura quadrilobata* Hammer  
*Pseudachortus (Pseudachortus) complexus* (MacGillivray)  
*Pseudachortus (Pseudachortus) sextatalis* Macnama  
*Xenylla welchi* Folsom

Family: Onychiuridae

*Onychiurus (Onychiurus) ramosus* Folsom  
*Onychiurus (Protophoria) armatus* (Tullberg)  
*Onychiurus (Protophoria) pseudoarmatus* Folsom  
*Onychiurus (Protophoria) uenoi* Yosii  
*Onychiurus* n. sp.  
*Tullbergia (Tullbergia) mala* Christiansen and Bellinger

Family: Isotomidae

*Folsomia candida* Willem  
*Folsomia decaxtophalma* Ford  
*Folsomia fimetaria* (Linnaeus)  
*Isotoma subviridis* Folsom  
*Isotoma trispinata* McGillivray  
*Isotoma viridis* Bourlet  
*Isotomurus palustris* Muller  
*Isotomiella minor* Schuffer  
*Proisotoma (Appendisotoma) vesiculata* Folsom  
*Tetracanthella ethelae* complex Wray  
*Tetracanthella bellingeri* Deharveng

*Sinella (Sinella) avita* Christiansen  
*Sinella (Sinella) barri* Christiansen  
*Tomocerus (Pogonognathellus) bidentatus* Folsom  
*Tomocerus (Plutomurus) brevimumronatus* Denis  
*Tomocerus (Pogonognathellus) flavescens* Tullberg  
*Tomocerus (Tomocerine) lamelliferus* Mills  
*Tomocerus (Tomolonus) reductus* (Mills)  
*Tomocerus (Tomocerus) vulgaris* Tullberg  
*Tomocerus (Plutomurus) wilkeyi* Christiansen  
*Willowsia bushi* (Lubbock)  
*Willowsia nigromaculata* (Lubbock)

Suborder: Symphypleona

Family: Sminthuridae

*Dicyrtoma (Ptenothrix) atra* (Linnaeus)  
*Dicyrtoma (Ptenothrix) castanea* Snider  
*Dicyrtoma (Ptenothrix) quadrangularis*, Mills  
*Neosminthurus bakeri*, Snider  
*Sminthurides (Sminthurides) malagreni* (Tullberg)  
*Sminthurides (Sminthurides) welcheseli* Christiansen and Bellinger  
*Sminthurinus henshawi* (Folsom)  
*Sminthurinus* n. sp.  
*Sphyrotheca minnesotensis* (Guthrie)

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REPRODUCTION IN THE WOOD FROG, *RANA SYLVATICA* (ANURA: RANIDAE), FROM ARKANSAS

The reproductive biology of the wood frog, *Rana sylvatica*, has received considerable scrutiny throughout much of its broad range in North America (see review in Davis and Folkerts, 1986). Interestingly, ever since the wood frog was first discovered in Arkansas over 50 years ago (Black, 1933, 1938), very little other than recent new county records (Cline and Tumilson, 1985; Plummer and Godwin, 1979; Robison and Douglas, 1977; Schuier *et al.*, 1972; Trauth *et al.*, 1987; Turnipseed, 1980, 1981) has been published on this species in the state. Within the Ozark Mountains of Arkansas (specifically the Boston Mountains and portions of the Springfield and Salem Plateaus), wood frogs have been found from Washington County in the west to Independence County in the east and from Baxter and Marion counties in the north to Pope County in the south. The Arkansas populations of *R. sylvatica* represent the southwesternmost extent of the species' range in North America; consequently, data on the reproductive biology of *R. sylvatica* in Arkansas can contribute to a better assessment of geographic and intraspecific variability in reproductive parameters (Berven, 1988; Berven and Gill, 1983) in this wide-ranging species. In the present study, we report on the breeding cycle in *R. sylvatica* from northcentral Arkansas.

From early February to late May, 1987 and 1988, breeding activity in *R. sylvatica* was monitored in Stone County; frequent visits to breeding sites in three other counties (Baxter, Marion, and Searcy) were also made. The primary study site consisted of two small farm ponds situated near each other (ca. 50 m apart). The surrounding habitat, located within the Sylamore Ranger District (SRD) of the Ozark-St. Francis National Forest, lies at an elevation of around 380 m and is geomorphically characterized by narrow, rounded ridges and steep, deeply-cut ravines. The forest type consists of a mixture of shortleaf pine and oak-hickory climax communities. We collected egg masses and adult and larval *R. sylvatica* from February to May, 1987, and egg masses and adults in February, 1988. Individual egg masses were placed in containers of 10% formalin, whereas adults were killed in a 20% chloretoe solution and fixed in 10% formalin. Oviductal eggs within expanded ovisacs of females were excised and measured with an ocular micrometer to the nearest 0.01 mm using a dissecting microscope at a magnification of 10X. Ten eggs per ovisac were examined; 10 egg diameters from 10 randomly-selected egg masses were also measured as above. Eggs, larvae and adults were stored in 70% ethanol and deposited in the Arkansas State University Museum of Zoology. Statistical data (means) are accompanied by  $\pm$  two standard errors.

Late winter precipitation coincided with the initiation of immigration to ponds and the so-called explosive breeding activity in *R. sylvatica*. We recorded a peak breeding period from early-to-late February, 1987, and from early February to the first week in March, 1988. The earliest male calling activity and deposition of egg masses were in two isolated SRD forest ponds on 7 February 1987; in 1988, the earliest calling and egg deposition in any pond were 1 February. At the primary study site, the first amplexic pairs were observed in pond #1 (chicken house pond),



## General Notes

and approximately 75 egg masses (mostly in communal ovipositional aggregates) were counted there on 11 February 1987. A 15 cm snowfall had occurred on 3 February, amplexic pairs were first observed in pond #2 (pasture pond). The earliest breeding at the chicken house pond and the pasture pond in 1988 was on 21 February. Amplexic pairs were observed for the last time in both ponds on 26 February 1987 and on 7 March 1988. Hatching of eggs occurred within a week or so after laying at the primary study site, and on 22 March 1987, tadpoles of ca. 22 mm in total length were collected in the pasture pond. This larval size roughly equates to an age of six weeks and is similar to a size-age growth pattern reported for Tennessee populations (Meeks and Nagel, 1973). Emerging froglets were collected around both ponds on 23 May 1987; therefore, the time interval from egg deposition to transformation is about 105 days.

Clutch size was determined by counting all eggs in 35 egg masses and in oviducts (ovisacs) of seven gravid females. Based upon counts from egg masses, clutch size varied from 510 to 1433 ( $\bar{x} = 867.3 \pm 63.5$ ), whereas counts of oviductal eggs ranged from 645 to 1331 ( $\bar{x} = 960.0 \pm 201.9$ ). By placing individual egg mass size into size classes (intervals of 100 eggs), the number of egg masses and clutches were 500 to 600 eggs (2 clutches), 600-700 (5), 700-800 (8), 800-900 (3), 900-1000 (9), 1000-1100 (5), 1100-1200 (2), and greater than 1200 (1). Gravid female *R. sylvatica* ranged from 60 to 70 mm in snout-vent length and, when matched with their clutch complement (in parentheses), were as follows: 60 (645), 60 (888), 62 (716), 65 (1241), 68 (1331), 70 (794), and 70 (1104). Females may oviposit all eggs within a single ovisac at one time (Davis and Folkerts, 1986); therefore, this may account for the variation in oviductal egg number among these females. A chi-square test revealed a significant difference ( $X^2 = 31.9$ ;  $P < 0.05$ ) in the number of oviductal eggs between left and right ovisacs. Although the total number of eggs per female appears to increase with body size, the causative factors attributing to variation in clutch size (e.g., body size, age, environmental parameters, nutritional state, and heredity) in Arkansas wood frogs remain unresolved.

The average clutch sizes (both methods) found for Arkansas *R. sylvatica* were similar to values reported for populations in more northern latitudes. For example, average clutch size (based upon egg mass size) was comparable to values in Virginia ( $\bar{x} = 920$ ; Berven, 1982) and Pennsylvania ( $\bar{x} = 895$ ; Seale, 1982), but was much greater than in the most southern populations in Alabama ( $\bar{x} = 496$ ; Davis and Folkerts, 1986) and Tennessee ( $\bar{x} = 465$ ; Meeks and Nagel, 1973). Moreover, clutch size in Arkansas averaged over 250 eggs more than the average for Missouri populations ( $\bar{x} = 621$ ; as reported for Johnson by Davis and Folkerts, 1986). Recently, Johnson (1987) reported a range (500 to 1000) closer to our findings.

Egg diameters of oviductal eggs in *R. sylvatica* varied from 1.7 to 2.4 mm ( $\bar{x} = 1.92 \pm 0.025$ ;  $n = 100$ ); this average value lies within the overall range of 1.6 to 2.9 mm for this species as summarized by Davis and Folkerts (1986). Diameters of laid eggs with expanded jelly envelopes ranged from 7.0 to 12.6 mm ( $\bar{x} = 9.60 \pm 0.379$ ;  $n = 100$ ) in our study. Arkansas wood frogs laid smaller eggs than in Alabama and Tennessee populations (Davis and Folkerts, 1986; Meeks and Nagel, 1973), but were similar in size to more northern populations (Berven, 1988).

Since the late 1960's, the U.S. Forest Service and the Arkansas Game and Fish Commission have constructed a large number of wildlife ponds in the SRD. Of the 39 ponds monitored in 1987, 74% contained wood frogs and/or eggs. Of 68 different ponds checked in 1987 and 1988, 72% had either frogs or egg masses present. Of 16 different ponds monitored both years, 10 (62.5%) had frogs and/or egg masses.

The ringed salamander, *Ambystoma annulatum*, and *R. sylvatica* were first recorded breeding syntopically during the present study (Trauth *et al.*, 1989). Other species found at these wildlife ponds were *Ambystoma maculatum* (spotted salamater), *Notophthalmus viridescens* (central newt), *Pseudacris crucifer* (spring peeper), and *Rana sphenoccephala* (southern leopard frog). We witnessed interspecific competition for ovipositional sites in several ponds (especially evident between *R. sylvatica* and *A. maculatum*). In many small temporary ponds, these two species deposited their egg masses on top of one another forming several stratigraphic layers. By clustering egg masses, these two species may mutually gain a thermal advantage for developing larvae (Seale, 1982; Waldman and Ryan, 1983); however, this increased fitness via tolerance to cold temperatures may be neglected by the predatory nature of the adult and larval forms of the ecological associates (in particular, *N. viridescens* and *A. maculatum*, respectively; see Walters, 1975).

As reported previously in other populations of *R. sylvatica*, we found many egg masses completely infiltrated by the unicellular green alga, *Oophila amblyostomatis*, a species commonly seen in *A. maculatum* egg masses. The relationship between algae and developing larvae remains mostly conjectural, although several studies have suggested a mutualistic arrangement. Several investigators have shown, in fact, that the alga may actually result in egg mortality. Gilbert (1942) summarized the early literature pertaining to this alga-amphibian egg phenomenon.

In conclusion, reproductive characteristics of *R. sylvatica* in Arkansas generally follow the pattern seen throughout this species' range. Wood frogs are explosive breeders and usually lay eggs in large communal aggregates in woodland ponds during the late winter. At many breeding sites, wood frogs compete with other winter-breeding salamanders and frogs for ovipositional locations within ponds. Hatching occurs rapidly, and transformation requires around 15 weeks, a span of time slightly longer than what is found in northern populations (Berven and Gill, 1983). Because the status of wood frogs in Arkansas has not, as yet, been adequately established (Smith *et al.*, 1984), future studies might consider addressing the interspecific relationships between wood frogs and their ecological associates. Information on interspecific predation could shed some light on survivorship rates and contribute new knowledge on this pond-breeding amphibian of the Ozarks.

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#### PHOTOREACTIVATION OF THE EFFECT OF UV LIGHT ON GAMMA RAY INDUCED CHROMOSOME ABERRATION PRODUCTION IN G1 PHASE *XENOPUS* CELLS

Kulp *et al.* (1985) observed that samples of G1 phase A8W4 *Xenopus* cells exposed to UV (254 nm) fluences (in the range 0-8.0 J/m<sup>2</sup>) shortly before or after being exposed to 200 rads gamma ray exhibited higher frequencies of chromosome deletions and lower frequencies of chromosome exchanges than samples of A8W4 cells exposed to 200 rads gamma ray alone. However, the chromosome-break frequencies (total number of chromosome breaks leading to aberrations/total number of cells scored) observed for cells receiving 200 rads gamma ray plus UV differed little from that for cells receiving only 200 rads gamma ray. The nature of these kinetics coupled with the observation that low fluences of UV produce few (if any) breaks in *Xenopus* G1 phase chromosomes (Griggs and Orr, 1979), and the observation that pyrimidine dimers are among the more prevalent lesions induced by UV (254 nm) in chromosomal DNA (Harm, 1980) suggested the following interpretation: The UV fluences administered had relatively little effect on the chromosome breakage induced by the gamma ray exposures, but did induce pyrimidine dimers in or near the gamma ray-break sites that significantly inhibited rejoining and restitution of broken ends of chromosomes. We describe here our initial test of this interpretation. This test was suggested by the fact that *Xenopus* cells efficiently photoreactivate pyrimidine dimers induced in chromosomal DNA by UV (Griggs and Bender, 1972, 1973; Griggs and Payne, 1981). Specifically, it was reasoned that the interpretation would appear valid if the following two questions could be answered in the affirmative: (1) Do the aberration frequencies (exchange frequency and deletion frequency) exhibited by early G1 phase cells exposed to 200 rads gamma ray + 8.0 J/m<sup>2</sup> UV + appropriate photoreactivating (PR) light fluences lie between the frequencies exhibited by such cells exposed to 200 rads gamma ray and the frequencies exhibited by such cells exposed to 200 rads gamma ray + 8.0 J/m<sup>2</sup> UV? (2) Do the chromosome-break frequencies exhibited by early G1 phase cells exposed to 200 rads gamma ray + 8.0 J/m<sup>2</sup> UV + appropriate PR light fluences lie between the frequency exhibited by such cells exposed to 200 rads gamma ray and the frequency exhibited by such cells exposed to 200 rads gamma ray + 8.0 J/m<sup>2</sup> UV?

The results of experiments performed to answer questions 1 and 2 are displayed in Fig. 1 and Table 1. Conventional techniques for cell culturing (Griggs and Bender, 1972), cell synchronization irradiations (Griggs and Orr, 1979; Cross and Griggs, 1978), preparation of metaphase spreads and aberrational analysis (Wolff, 1961) were employed. In each experiment, the starting point was the preparation of two sets of synchronous cultures of G1 phase cells (sets 1 and 2). Both sets were then irradiated as indicated (Table 1) and set 1 cultures were used to establish a detailed post-irradiation mitotic index (MI) curve. The MI curve described the post-irradiation time range (corresponding to the mitotic peak) for colcemid treatment of the set 2 cultures to obtain appropriate samples of metaphase spreads for aberrational analysis.

The data shown in table 1 indicates a definite pattern of photoreactivation. Comparison of the results of experiments 2 and 3 clearly show that fewer deletions and more exchanges were observed after a PR fluence of 20.0 (J/m<sup>2</sup>/10<sup>3</sup>) than when no PR fluence had been administered. Similarly, results from experiments 4 through 7 lend supporting evidence that increasing fluences of PR between 20-35 (J/m<sup>2</sup>/10<sup>3</sup>) enhanced the rejoining and restitution of the broken ends of chromosomes. These data do indeed answer questions (1) and (2) in the affirmative and, thus, strongly support the interpretation presented by Kulp, *et al.* (1985).

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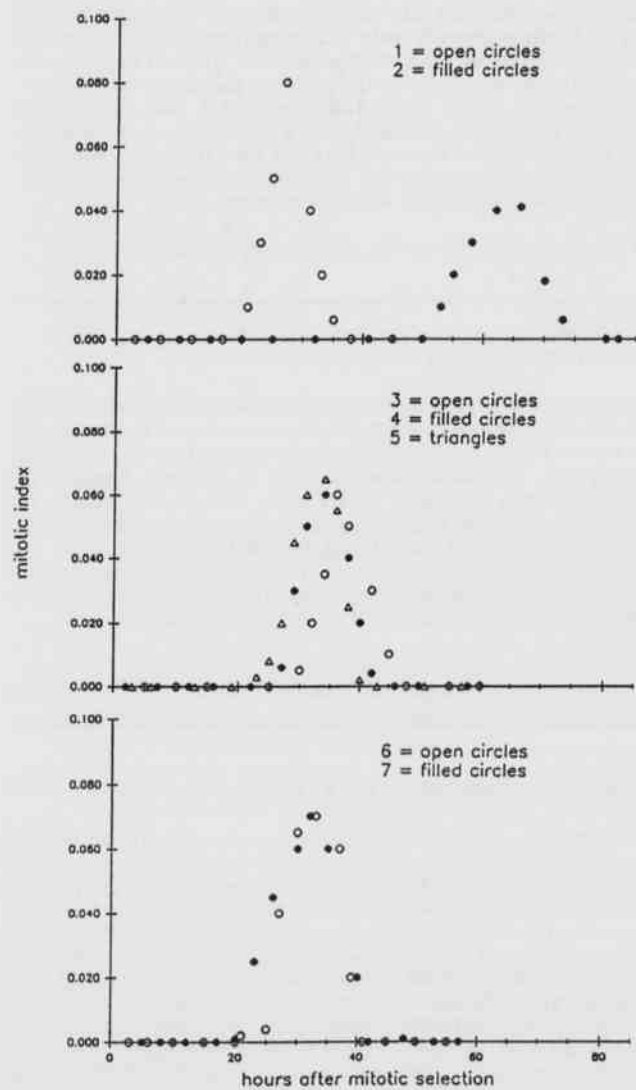


Figure 1. Mitotic index curves determined with sets of A8W4 cells, which were exposed to 200 rads gamma ray in early G1 phase (one hour after mitotic selection) and then exposed to varying UV and PR light fluences as follows: (1) 0.0 J/m<sup>2</sup> UV and 0.0 J/m<sup>2</sup> PR light, control; (2) 8.0 J/m<sup>2</sup> UV and 0.0 J/m<sup>2</sup> PR light; (3) 8.0 J/m<sup>2</sup> UV and 20.0 x 10<sup>3</sup> J/m<sup>2</sup> PR light; (4) 8.0 J/m<sup>2</sup> UV and 23.0 x 10<sup>3</sup> J/m<sup>2</sup> PR; (5) 8.0 J/m<sup>2</sup> UV and 26.0 x 10<sup>3</sup> J/m<sup>2</sup> PR light; (6) 8.0 J/m<sup>2</sup> UV and 30.0 x 10<sup>3</sup> J/m<sup>2</sup> PR light; (7) 8.0 J/m<sup>2</sup> UV and 35.0 x 10<sup>3</sup> J/m<sup>2</sup> PR light. These curves, 1 through 7, are the MI curves for experiments 1 through 7 (of the table), respectively. For each curve the onset of the UV exposure was immediately following the termination of the gamma ray exposure and the onset of the PR exposure was immediately following the termination of the UV exposure. The mitotic index of the cultures of mitotic selection was approximately 0.98.

Table 1. Photoreactivation of the effect of UV on gamma ray-induced aberration production in A8W4 cells. In each experiment synchronous cultures of G1 phase cells were first exposed to 200 rads gamma ray (one hour after mitotic selection) and then exposed to UV and PR fluences as indicated.

| Experiment Number | UV fluence (J/m <sup>2</sup> ) | PR fluence (J/m <sup>2</sup> /10 <sup>3</sup> ) | Cell collection time range (hours after mitotic selection)* | Chromosome type aberrations** | Deletions | Exchanges |
|-------------------|--------------------------------|-------------------------------------------------|-------------------------------------------------------------|-------------------------------|-----------|-----------|
| 1                 | 0.0                            | 0.0                                             | 33-35                                                       | 105                           | 66        |           |
| 2                 | 8.0                            | 0.0                                             | 34-70                                                       | 166                           | 43        |           |
| 3                 | 8.0                            | 20.0                                            | 20-45                                                       | 144                           | 48        |           |
| 4                 | 8.0                            | 23.0                                            | 28-40                                                       | 140                           | 51        |           |
| 5                 | 8.0                            | 26.0                                            | 28-40                                                       | 141                           | 52        |           |
| 6                 | 8.0                            | 30.0                                            | 26-40                                                       | 143                           | 49        |           |
| 7                 | 8.0                            | 35.0                                            | 26-40                                                       | 139                           | 50        |           |

\* Cells were collected for aberrational analysis by colcemid treatments that spanned the indicated time ranges.

\*\* 200 cells were scored in each experiment.

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## FLORISTIC NOTES ON THE ARKANSAS CRUCIFERAE (BRASSICACEAE)

The Cruciferae make up a distinctive group, generally easily recognized by the 4-merous perianth (4 sepals, 4 petals), six tetradynamous stamens (4 long, 2 short) and the 2-carpellate superior pistil that often dehisces so that the septum remains attached to the pedicel. The stamens are sometimes reduced to 4 or 2. The flowers and fruits are commonly arranged in a bractless raceme. The fruits may be short and broad (silicles) or long and narrow (siliques), terete or flattened and with or without a stipe or beak. While the family is generally easily recognized, fruit characters are often required for determination of genera and species. I have worked up a key to the Cruciferae of Arkansas (available for a limited time from the author), including 64 taxa known for the state and 14 taxa reported or suspected for the state, and invite workers in the state and area to use and test the key. In working up the key, I noted the following corrections to the second edition of my *Atlas* (Smith, E.B. 1988. An atlas and annotated list of the vascular plants of Arkansas. Kinko's, 653 West Dickson Street, Fayetteville, Arkansas 72701): (1) The report of *Cardamine flexuosa* With. was based on material of *Sibara virginica* (L.) Rollins, and thus should be deleted from our flora. (2) The varieties of *Descurainia pinnata* (Walt.) Britt., while appearing different in their extremes, intergrade in Arkansas to the extent that their recognition is probably not justifiable.

Additions to the 78 taxa in the key for Arkansas, or suggestions on changes to improve the key would be welcome.

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REDISCOVERY OF *HALESIA DIPTERA* ELLIS (STYRACACEAE) IN ARKANSAS

*Halesia diptera* Ellis, commonly called Snowdrop-Tree or Two-Wing Silverbell is a large shrub or small tree with showy white flowers appearing in early spring before the leaves appear. It is apparently limited to the Coastal Plain from South Carolina to northern Florida and west to east Texas (Little, USDA Misc. Pub. 1342, 1977). The Silverbell has been known from a single collection in Arkansas (Prescott, September 1887, G.W. Letterman s.n., ILL), presumably made in Nevada County (Tucker, G.E., A guide to the woody flora of Arkansas. Unpub. PhD Dissertation, University of Arkansas, Fayetteville, 356 pp. 1976.). That specimen has been verified by Tucker (Ark. Tech. Univ.). Because the species has not been found in Arkansas for the last century, it was excluded from the Arkansas flora (Smith, E.B., An atlas and annotated list of the vascular plants of Arkansas, 2nd Ed. Privately Published by Kinko Press, Fayetteville, Arkansas, 489 pp. 1988).

In September 1983, I collected this plant in southeastern Lafayette County. After identifying the plant as *Halesia diptera*. I forgot about it until September of 1988 after a conversation with Dr. Tucker. I sent the specimen to him and he agreed with my identification. The plant specimen is on hand at Arkansas Tech. University herbarium and will be sent to the herbarium at the University of Arkansas at Fayetteville.

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## A SYNOPSIS OF THE LACCOPHILINAE (COLEOPTERA: DYTISCIDAE) OF ARKANSAS

Dytiscidae, the predaceous diving beetles, is the largest family of aquatic Coleoptera. More than 400 species are estimated to occur in North America (Michael and Matta, 1977). The subfamily Laccophilinae is represented in all major zoogeographical regions of the world, but only one of its three genera, the cosmopolitan *Laccophilus*, occurs in Arkansas. This genus has been revised most recently by Zimmerman (1970). The purposes of this paper are to present the first statewide species list, to delineate geographic distributions, and to define preferred habitats for Arkansas laccophiline species, insofar as present knowledge permits. These species may be identified by using Zimmerman's (1970) key to North American species. Field recognition is enhanced by their habit of springing actively about in the net. The information presented has been synthesized primarily from materials housed in the Aquatic Macroinvertebrate Collection of the Arkansas State University Museum of Zoology (ASUMZ). Additional sources include the museum collections at the University of Arkansas-Fayetteville and -Little Rock and published works.

*Laccophilus proximus proximus* Say was reported as occurring in Arkansas by Zimmerman (1970). This subspecies is our most common form, having been collected in 43 counties and all physiographic provinces (Fig. 1). Young (1954) described it as one of the principal pioneers of newly formed ponds, puddles and other freshwater bodies. Most of the 1,135 specimens examined in this study were collected from lentic systems, including lakes, ponds, swamps, ditches and bogs; however, they were also found in creeks, rivers and springs. Individuals were collected during each month of the year. Guntharp and Harp's (1982) record for *Laccophilus pictus* and that of Harp and Harp (1980) for *L. maculosus* in Crittenden County are actually *L. p. proximus*.

*Laccophilus fasciatus rufus* Melsheimer was reported from two Arkansas sites by Zimmerman (1970). In this study we report 445 individuals from 157 collections in 40 counties (Fig. 2). Young (1954) found *L. f. rufus* most frequently in muddy or silty-bottomed temporary pools formed in roadside ditches or intermittent streams, all associated with well-drained soils. Zimmerman (1960) collected this subspecies from open, unshaded ponds and roadside ditches in clay soils in the Midwest. We found *L. f. rufus* most commonly in creeks and bayous, but it was also found in ponds, lakes, rivers, temporary pools, ditches, springs and rice fields. The occurrence of *L. f. rufus* in such a variety of habitats, in all physiographic provinces, and in all months except December, suggests that it is broadly tolerant of diverse shallow water ecosystems.

*Laccophilus maculosus maculosus* Say was first reported for Arkansas by Pippenger and Harp (1985). A total of 120 individuals was examined from 42 collections in 11 counties (Fig. 3). Zimmerman (1970) listed the geographic range as extending north from near a line running from NE Georgia to SW Nebraska. Previously, only single specimens from coastal South Carolina, SE Alabama and Dallas, Texas, were known to exist south of this line. These occurrences were explained by noting that *L. m. maculosus* adults readily fly and can survive for brief periods in almost any aquatic ecosystem (Zimmerman, 1970). All but one of our records are from the Ozark Plateaus, but rather than reflecting water quality requirements, its state distribution is primarily a reflection of northern Arkansas being the southern limit of its range. Most collections of *L. m. maculosus* (63% of specimens) were from lakes and ponds, but some were occasionally found in creeks, rivers and springs. In Arkansas this subspecies has been found during each month except January and December.

*Laccophilus undatus* Aube is newly reported for Arkansas, and this finding constitutes a major range extension to the SW. A single specimen was collected from a pool of the Saline River downstream of the St Hwy 24 bridge at the Howard-Sevier Co. line on 9 May 1982 (Fig. 3). Zimmerman (1970) reported the range of this species to extend from Bloomington, Indiana, N to Chicago and E to Washington, D.C. and Massachusetts.



It was also noted that this species generally prefers shaded pools of the northeastern deciduous forests and that, in southern Indiana, it has been taken only from slightly acidic slough ponds in the drainage basins of intermittent streams.

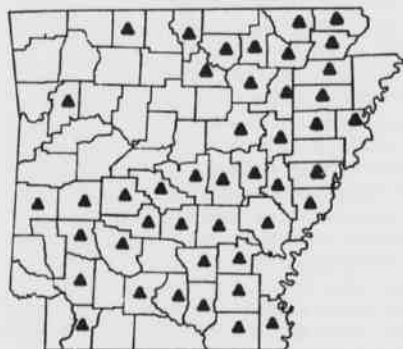


Figure 1. *Laccophilus proximus proximus*



Figure 2. *Laccophilus fasciatus rufus*

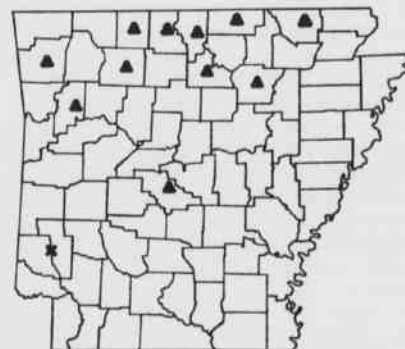


Figure 3. *Laccophilus maculosus maculosus* ▲  
*Laccophilus undatus* x

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JONES, I. C. 1957. The adrenal cortex. Cambridge Univ. Press, London, 316 pp.

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