Transactions of the Kansas Academy of Science Contents of Volume 96 (1993)

Numbers 1-2

SYMPOSIUM IN GRAVITATIONAL BIOLOGY

- The Second Annual Symposium of the NASA Specialized Center of Research and Training (NSCORT) in Gravitational Biology, p. 1-6. Brian S. Spooner. See <u>Abstract</u>
- Association of Actin with Alpha Crystallins, p. 7-12. S. Gopalakrishnan, D. Boyle, and L. Takemoto. See <u>Abstract</u>
- Cytoskeleton-Amyloplast Interactions in Sweet Clover, p. 13-19. James A. Guikema, Emmanuel Hilaire, and William R. Odom. See <u>Abstract</u>
- Preliminary Observations on the Effects of Vectoro-Averaged Gravity on the Embryonic and Larval Development of the Gastropod Mollusk, *Ilyanassa obsoleta* Stimpson, p. 20-27. Gary W. Conrad, Andy P. Stephens and Abigail H. Conrad. See <u>Abstract</u>
- Detergent Solubilization of the EGF Receptor from A431 Cells, p. 28-34. Raman Dayanidhi and David A. Rintoul. See <u>Abstract</u>
- Identification of Amino Acid Sequences in the Polyomavirus Capsid Proteins that Serve as Nuclear Localization Signals, p. 35-39. D. Chang, J.I. Haynes, Jr., J. N. Brady, and Richard A. Consigli. See <u>Abstract</u>
- The Use of the Tyrosine Phosphatase Antagonist Orthovanadate in the Study of a Cell Proliferation Inhibitor, p. 40-45. Daniel J. Enebo, Gabrielle Hanek, Heideh K. Fattaey and Terry C. Johnson. See <u>Abstract</u>
- Embryonic Lung Morphogenesis in Organ Culture: Experimental Evidence for a Proteoglycan Function in the Extracellular Matrix, p. 46-55. Brian S. Spooner, Kenneth E. Bassett and Brian S. Spooner, Jr. See Abstract
- Extracellular Matrix and Growth Factors in Branching Morphogenesis, p. 56-61. Patricia Hardman and Brian S. Spooner. See <u>Abstract</u>
- Preliminary Observations on the Effects of Selenate on the Development of the Embryonic Skate, *Raja eglanteria*, p. 62-68. Gary W. Conrad, Carl A. Luer, Avelina Q. Paulsen and James L. Funderburgh. See <u>Abstract</u>
- The Use of Centrifugation to Study Early *Drosophila* Embryogenesis, p. 69-73. Michael K. Abbott. See <u>Abstract</u>
- Ground-Based Experiments Complement Microgravity Flight Opportunities in the Investigation of the Effects of Space Flight on the Immune Response: Is Protein Kinase C Gravity Sensitive?, p. 74-79. Stephen K. Chapes, Keith M. Woods and Jason W. Armstrong. See <u>Abstract</u>

- Space Station Freedom: A Unique Laboratory for Gravitational Biology Research, p. 80-86. Robert W. Phillips and Keith L. Cowing. See <u>Abstract</u>
- The Role of Bioregenerative Life-Support Systems in a Manned Future in Space, p. 87-92. Cary A. Mitchell. Director, NSCORT in Bioregenerative Life Support, Purdue University, West Lafayette, Indiana.

TECHNICAL PAPERS

- Breeding Colonies and Foraging Habitats of Herons in Sedgwick County, Kansas, p. 94-102. Alan D. Maccarone. See <u>Abstract</u>
- An Annotated List of the Herpetofauna of the F.B. and Rena G. Ross Natural History Reservation, p. 103-113. Brian E. Viets. See <u>Abstract</u>
- Assessment of Restored Streamflow on Fish Communities in the Arkansas River of Southwestern Kansas, p. 114-130. Mark E. Eberle, Guy W. Ernsting, Joseph R. Tomelleri and Shelley L. Wells. See <u>Abstract</u>
- Hamilton *Fossil-Lagerstätte* (Upper Pennsylvanian, Greenwood County, Kansas): Internal Stratigraphy and Addition to the Microfossil Assemblage, p. 131-139. Christopher R. Cunningham. See <u>Abstract</u>
- An Approximate Molecular Orbital Model for the Hydrogen Bond Donor Acidity of the Alkanols, p. 140-147. Orland W. Kolling. See <u>Abstract</u>
- Additional Specimens of the Western Cottonmouth (*Agkistrodon piscivorus leucostoma*, Reptilia: Squamata) from Kansas, p. 148-151. Eric M. Rundquist. See <u>Abstract</u>
- A New Species of *Myxidium* (Myxozoa: Myxidiidae) from the Gall Bladders of Anuran Amphibians from Peru, p. 152-157. Mary Ellen Gray. See <u>Abstract</u>

Errata

• A Reanalysis of The Influence of Sex Ratio on Dispersal in the Prairie Vole, *Microtus ochrogaster*, p. 158. A.K. Sanders and M.S. Gaines.

Numbers 3-4

- Ecological Research, Conservation and Management--an Editorial, p. 159-160. Robert P. McIntosh.
- Kansas Climate with Global Warming: Agricultural and Other Economic Impacts, p. 161-166. Wayne M. Wendland. See <u>Abstract</u>
- The Use of Selenium (IV) Oxide to Oxidize Aromatic Methyl Groups, p. 167-180. Eric L. Trump and Marilyn Xiaohong Zhou. See <u>Abstract</u>

- *Wetapolystoma almae* n. gen., n. sp. (Monogenea: Polystomatidae) Parasite of *Bufo typhonius* (Linnaeus, 1758) (Amphibia: Bufonidae) from Tropical Peru, p. 181-185. Mary Ellen Gray. See <u>Abstract</u>
- Age Structure and Reproductive Activity in a Kansas Beaver Population, p. 186-195. Debra K. Welch and Robert J. Robel. See <u>Abstract</u>
- Mortality Profiles of *Mammuthus imperator* (Leidy) Sheridan County, Nebraska, p. 196-203. H.E. LaGarry-Guyon and M.R. Myers. See <u>Abstract</u>
- Cottonwood Site-Index Growth Curves, p. 204-212. Wayne A. Geyer and Keith D. Lynch. See <u>Abstract</u>
- Relative Abundance of Snakes in Kansas, p. 213-224. Henry S. Fitch. See <u>Abstract</u>

Short Notes

- A Case of Colonial Breeding in the Green-backed Heron, p. 225-226. Alan D. Maccarone and Robert Gress.
- *Phoxinus erythrogaster* (Cypriniformes: Cyprinidae) Range Extension in Kansas, p. 227-228. Linda Fuselier and David Edds.

Memorial

• William J. Argersinger, 1918-1992, p. 229-230.

Abstracts

• The Second Annual Symposium of the NASA Specialized Center of Research and Training (NSCORT) in Gravitational Biology. Brian S. Spooner. NSCORT, Division of Biology, Kansas State University, Manhattan, Kansas 66506

The second annual meeting of the NSCORT in Gravitational Biology was held at Kansas State University on September 29-October 1, 1992. Symposium presentations at the meeting included ones on basic gravitational cellular and developmental biology, spaceflight hardware for biological studies, studies on Space Shuttle, and special talks on Space Station Freedom and on life support systems.

• Association of Actin with Alpha Crystallins. S. Gopalakrishnan, D. Boyle, and L. Takemoto. Division of Biology, NSCORT, Kansas State University, Manhattan, Kansas 66506.

The alpha crystallins are cytosolic proteins that co-localize and co-purify with actin-containing microfilaments. Affinity column chromatography employing both covalently-coupled actin or alpha crystallin was used to demonstrate specific and saturable binding of actin with alpha crystallin. This conclusion was confirmed by direct visualization of alpha aggregates bound to actin polymerized in vitro. The significance of this interaction in relation to the functional properties of these two polypeptides will be discussed.

• Cytoskeleton-Amyloplast Interactions in Sweet Clover. James A. Guikema, Emmanuel Hilaire, and William R. Odom. Division of Biology, Kansas State University, Ackert Hall, Manhattan, Kansas 66506-4901.

The distribution of organelles within columella cells of sweet clover was examined by transmission electron microscopy following growth under static or clinorotating conditions. A developmentally conditioned polarity was observed, with a proximal location of the nucleus and a distal accumulation of the endoplasmic reticulum. This polarity was insensitive to clinorotation. In contrast, clinorotation altered the location of amyloplasts. Application of cytoskeletal poisons (colchicine, cytochalasin D, taxol, and phalloidin), especially during clinorotation, had interesting effects on the maintenance of columella cell polarity, with a profound effect on the extent, location, and structure of the endoplasmic reticulum. The site of cytoskeletal interactions with sedimenting amyloplasts is thought to be the amyloplast envelope. An envelope fraction, having over 17 polypeptides, was isolated using immobilized antibody technology, and will provide a means of assessing the role of specific peptides in cytoskeleton/amyloplast interactions.

 Preliminary Observations on the Effects of Vectoro-Averaged Gravity on the Embryonic and Larval Development of the Gastropod Mollusk, *Ilyanassa obsoleta* Stimpson. Gary W. Conrad,^{1,2,4} Andy P. Stephens,^{1,3} and Abigail H. Conrad.^{1,2}

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Fertilized eggs of *Ilyanassa obsoleta* Stimpson were collected immediately after their deposition in egg capsules. Unopened egg capsules then were affixed to glass slides, and incubated either statically (controls) or on a clinostat (experimentals). After incubation for 9-14 days, hatching occurred sooner and

in a higher percentage of clinostated capsules than in controls. Embryos that hatched while undergoing clinostat incubation were abnormal in morphology, whereas other embryos present in non-hatched capsules in the same tubes appeared normal, as did embryos in the control tubes. Although the results are compatible with a conclusion that vector-averaged gravity in the experimental tubes caused the altered development, some other aspects of how the incubations were done may have contributed to the differences between the control and experimental results.

• Detergent Solubilization of the EGF Receptor from A431 Cells. Raman Dayanidhi and David A. Rintoul. Division of Biology, NSCORT, Kansas State University, Manhattan, Kansas 66506-4901.

Functional reconstitution of purified preparations of human epidermal growth factor receptor (EGFR) requires dissociation of the protein from its plasma membrane lipid environment. Solubilization of membrane proteins in this manner requires the use of detergents, which are known to disrupt plasma membrane lipid/protein interactions. We have investigated to the ability of three nonionic detergents to solubilize the human EGFR selectively, and have also analyzed the effect of these various treatments on the intrinsic tyrosyl kinase activity of the receptor. The nonionic detergent known as n-octyl glucoside (n-octyl β-D-glucopyranoside) was found to give the best combination of selectivity, yield, and maintenance of enzymatic activity of the human EGFR.

 Identification of Amino Acid Sequences in the Polyomavirus Capsid Proteins that Serve as Nuclear Localization Signals. D. Chang,¹ J.I. Haynes, Jr.,¹ J. N. Brady,² and Richard A. Consigli.¹

¹Division of Biology, NSCORT, Section of Virology and Oncology, Kansas State University, Manhattan, Kansas 66506. ²Laboratory of Molecular Virology, National Cancer Institute, Bethesda, Maryland 20892.

The molecular mechanism participating in the transport of newly synthesized proteins from the cytoplasm to the nucleus in mammalian cells is poorly understood. Recently, the nuclear localization signal sequences (NLS) of many nuclear proteins have been identified, and most have been found to be composed of a highly basic amino acid stretch. A genetic "subtractive" and a biochemical "additive" approach were used in our studies to identify the NLS's of the polyomavirus structural capsid proteins. An NLS was identified at the N-terminus (Ala¹-Pro-*Lys-Arg-Lys*-Ser-Gly-Val-Ser-Lys-Cys¹¹) of the major

capsid protein VP1 and at the C-terminus (Glu³⁰⁷-Glu-Asp-Gly-Pro-Glu-*Lys-Lys-Arg-Arg-Leu*³¹⁸) of the VP2/VP3 minor capsid proteins.

• The Use of the Tyrosine Phosphatase Antagonist Orthovanadate in the Study of a Cell Proliferation Inhibitor. Daniel J. Enebo, Gabrielle Hanek, Heideh K. Fattaey and Terry C. Johnson. Division of Biology, NSCORT, Kansas State University, Manhattan, Kansas 66506.

Incubation of murine fibroblasts with orthovanadate, a global tyrosine phosphatase inhibitor, was shown to confer a "pseudo-transformed" phenotype with regard to cell morphology and growth characteristics. This alteration was manifested by both an increasing refractile appearance of the cells, consistent with many transformed cell lines, as well as an increase in maximum cell density was attained. Despite the abrogation of cellular tyrosine phosphatase activity, orthovanadate-treated cells remained sensitive to the biological activity of a naturally occurring sialoglycopeptide (SGP) cell surface proliferation inhibitor. The results indicated that tyrosine phosphatase activity, inhibited by orthovanadate, was not involved in the signal transduction pathway of the SGP.

• Embryonic Lung Morphogenesis in Organ Culture: Experimental Evidence for a Proteoglycan Function in the Extracellular Matrix. Brian S. Spooner, Kenneth E. Bassett and Brian S. Spooner, Jr. NSCORT in Gravitational Biology, Division of Biology, Kansas State University, Manhattan, Kansas 66506.

The lung rudiment, isolated from mid-gestation (11 day) mouse embryos, can undergo morphogenesis in organ culture. Observation of living rudiments, in culture, reveals both growth and ongoing bronchiolar branching activity. To detect proteoglycan (PG) biosynthesis, and deposition in the extracellular matrix, were metabolically labeled with radioactive sulfate, then fixed, embedded, sectioned and processed for autoradiography. The sulfated glycosaminoglycan (GAG) types, composing the carbohydrate component of the proteoglycans, were evaluated by selective GAG degradative approaches that showed chondroitin sulfate PG principally associated with the interstitial matrix, and haparan sulfate PG principally associated with the basement membrane. Experiments using the proteoglycan biosynthesis disrupter, βxyloside, suggest that when chondroitin sulfate PG deposition into the ECM is perturbed, branching morphogenesis is compromised. • Extracellular Matrix and Growth Factors in Branching Morphogenesis. Patricia Hardman and Brian S. Spooner. Division of Biology, NSCORT, Kansas State University, Manhattan, Kansas 66506.

The unifying hypothesis of the NSCORT in gravitational biology postulates that the ECM and growth factors are key interrelated components of a macromolecular regulatory system. The ECM is known to be important in growth and branching morphogenesis of embryonic organs. Growth factors have been detected in the developing embryo, and often the pattern of localization is associated with areas undergoing epithelial-mesenchymal interactions. Causal relationships between these components may be of fundamental importance in control of branching morphogenesis.

• Preliminary Observations on the Effects of Selenate on the Development of the Embryonic Skate, *Raja eglanteria*. Gary W. Conrad,^{1,2} Carl A. Luer,³ Avelina Q. Paulsen² and James L. Funderburgh.²

¹Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672. ²Division of Biology, NSCORT, Kansas State University, Manhattan, Kansas 66506.

³Mote Marine Laboratory, 1600 Thompson Parkway, Sarasota, Florida 34236.

Morphogenesis of the clearnose skate, *Raja eglanteria*, was not significantly inhibited as a result of 7 days of exposure to 1-2 mM selenate in the sea water during Days 59-69 of embryonic development (hatching would normally have occurred at 82±4 days of incubation). Although corneal transparency appeared normal in the eye, preliminary measurements of the thickness of Bowman's layer of the cornea suggested that it was significantly thinner in the corneas of embryos exposed to 1-2 mM selenate. Selenate is an ion reported to inhibit sulfation of glycosaminoglycans in connective tissue.

• The Use of Centrifugation to Study Early *Drosophila* Embryogenesis. Michael K. Abbott. Division of Biology, NSCORT, Kansas State University, Manhattan, Kansas 66506.

By the end of 10th nuclear cycle, the somatic nuclei of the *Drosophila* embryo have migrated to the periphery of the egg. Centrifugation of embryos did not result in the displacement of these nuclei, since cytoskeletal elements anchor them to the cortex. But, mild centrifugal forces displace the centrally located, nascent yolk nuclei. If this increased sensitivity to hypergravity occurs before the beginning of nuclear differentiation during cycle 8, when the nascent yolk

and somatic nuclei physically separate, then it would mark the earliest functional difference between these two lineages.

• Ground-Based Experiments Complement Microgravity Flight Opportunities in the Investigation of the Effects of Space Flight on the Immune Response: Is Protein Kinase C Gravity Sensitive? Stephen K. Chapes, Keith M. Woods and Jason W. Armstrong. Division of Biology, NSCORT, Kansas State University, Manhattan, Kansas 66506.

This manuscript briefly reviews ground-based and flight experiments, discusses how those experiments complement each other, and details how those experiments lead us to speculate about the gravity-sensitive nature of protein kinase C.

• Space Station Freedom: A Unique Laboratory for Gravitational Biology Research. Robert W. Phillips¹ and Keith L. Cowing.²

¹Chief Scientist, Space Station Freedom and ²Manager, Pressurized Payload Accomodations, Space Station Freedom, Office of Space Systems Development, NASA Headquarters, Washington, DC 20546.

The advent of Space Station Freedom (SSF) will provide a permanent laboratory in space with unparalleled opportunities to perform biological research. As with any spacecraft there will also be limitations. It is our intent to describe this space laboratory and present a picture of how scientists will conduct research in this unique environment we call space. SSF is an international venture which will continue to serve as a model for other peaceful international efforts. It is hoped that as the human race moves out from this planet back to the moon and then on to Mars that SSF can serve as a successful example of how things can and should be done.

• Breeding Colonies and Foraging Habitats of Herons in Sedgwick County, Kansas. Alan D. Maccarone. Department of Biology, Friends University, Wichita, Kansas 67213.

Populations of wading birds in Sedgwick County, Kansas, were studied in 1991 to determine the size and species composition of active colonies, and to document the locations of major foraging areas. Two colonies were active that year: a rural one of 60-70 pairs of Great Blue Herons (*Ardea herodius*), and a larger colony in Wichita, which contained 530 nests of five species. Local breeding populations of wading birds are declining. Apparent high levels of mortality were evident in the mixed-species colony, with more than 160 eggs

and 60 dead chicks found on the ground and in nests. Egg predation by American Crows (*Corvus brachyrhynchos*) also was documented. Interspecific differences were noted in the compass directions of foraging flights taken by birds from the mixed-species colony, and in the types of habitats used. Wading birds were observed at more than 100 foraging sites.

• An Annotated List of the Herpetofauna of the F.B. and Rena G. Ross Natural History Reservation. Brian E. Viets. Division of Biological Sciences, Emporia State University, Emporia, Kansas 66801.

A two-year study of the herpetofauna of the F.B. and Rena G. Ross Natural History Reservation was conducted. A list of the herpetofauna of the Reservation was prepared, and notes were made on the natural histories of these animals. Field observations by the author, as well as recorded field observations in the Reservation files, were used to compile the herpetofauna list. In all, 10 amphibian species and 27 reptilian species were found to occur, consisting of 2 salamanders, 8 frogs, 4 turtles, 6 lizards, and 17 snakes.

• Assessment of Restored Streamflow on Fish Communities in the Arkansas River of Southwestern Kansas. Mark E. Eberle, Guy W. Ernsting, Joseph R. Tomelleri and Shelley L. Wells. Natural Science Research Associates, 409 East Eleventh Street, Hays, Kansas 67601-3505.

In 1987, the Arkansas River flowed throughout its course in southwestern Kansas during much of the year. Streamflow was reduced or absent in many segments of the stream during the summer of 1988, which has been the typical case in recent years. Quantitative surveys of the ichthyofauna were conducted in 1987 and 1988 to assess the initial impacts of temporarily restored streamflow on the fishes. A total of 25 species of fishes was collected during this study, compared to 22 species reported in earlier surveys. None of the four previously reported taxa protected in Kansas was collected, but six distributional records within Kansas and increased populations of several species of fishes were documented in 1987.

 Hamilton Fossil-Lagerstätte (Upper Pennsylvanian, Greenwood County, Kansas): Internal Stratigraphy and Addition to the Microfossil Assemblage. Christopher R. Cunningham. Kansas Geological Survey and Museum of Natural History, University of Kansas, Lawrence, Kansas 66045-2454.

Three localized laminated limestones occurring near the top of the Hamilton paleochannel sequence (Virgilian) contain the well-known Hamilton fossil

assemblage, a thanatocoenosis of terrestrial, marine, nonmarine, and euryhaline forms including plants, invertebrates ,and vertebrates exhibiting "skin preservation." Dissolution or disaggregation and sieving of the three limestones and associated limestone and mudstone units, the Hamilton *Fossil-Lagerstätte* proper, has produced the following additions to the Hamilton quarry (main quarry) assemblage: 1) the ostracodes *Amphissites, Geisina, Gutschickia?, Pseudobythocypris,* and *Whipplella*?, 2) two foraminiferids, an encrusting form and *Globivalvulina,* and 3) the shark *Xenacanthus.* Additional taxa are well-known fossils abundant in rocks of this age. As in the situtation of previously identified taxa from the Hamilton *Fossil-Lagerstätte,* the listed forms lived in a range of paleoenvironments from freshwater (*Whippella*?) to marine (e.g., *Globivalvulina*), reflecting the coastal, and probably estuarine, setting of the Hamilton beds.

• An Approximate Molecular Orbital Model for the Hydrogen Bond Donor Acidity of the Alkanols. Orland W. Kolling. Natural Science Division, Southwestern College, Winfield, Kansas 67156.

For the lower alcohols, the tendency to serve as hydrogen bond donors (HBD) toward solutes is commonly expressed through various semiempirical scales incorporating polarity and acidity parameters. In this report a simplified model for such HBD acidity is proposed which is based upon molecular orbital perturbation theory. Two first-order perturbation terms are included in the Hamiltonian which carry over to the total energy function for the highest occupied molecular orbital (HOMO) of the alkanol. Quantitative tests using dipolarity and HBD acidity data support the two-term formulation of the Hamiltonian.

 Additional Specimens of the Western Cottonmouth (*Agkistrodon piscivorus leucostoma*, Reptilia: Squamata) from Kansas. Eric M. Rundquist. Department of Herpetology, Sedgwick County Zoo and Botanical Garden, 5555 Zoo Boulevard, Wichita, Kansas 67212. James Triplett. Department of Biology, Pittsburg State University, Pittsburg, Kansas 66762.

The collection of two Western Cottonmouths (*Agkistrodon piscivorus leucostoma*) from Cherokee County in 1991 is reported. The history of this taxon in Kansas is discussed and previous records are discredited. The collection of the latest specimens confirms the presence of this taxon in extreme east-central Cherokee County.

• A New Species of *Myxidium* (Myxozoa: Myxidiidae) from the Gall Bladders of Anuran Amphibians from Peru. Mary Ellen Gray. Department of Systematics and Ecology and Museum of Natural History, University of Kansas, Lawrence, KS 66045-2454.

Myxidium typhonius, a myxosporidian protozoan, is described from the gall bladders of 13 species of anuran amphibians in Peru, Deapartamento de Madre de Dios, Provincia Tambopata, Cusco Amazónico, Rio Madre de Dios, ca. 15 km E Puerto Maldonado. The ellipsoidal spores of the parasite are distinguished from those of other myxidia infecting anurans on the basis of overall size, nature of the sutural line, number of longitudinal ridges, number and features of transverse ridges, polar capsule size, coils in polar filament, and identical left and right sides. Each spore of *M. typhonius* has a longitudinal suture extending pole to pole, 2 longitudinal ridges, and 9 or 10 transverse ridges. Two nuclei are located between the 2 polar capsules and each polar filament has 4 or 5 turns (coils). Average measurements (and ranges) in microns of 325 spores from the frontal view are: spore length 10.9 (9.8-12.2); spore width 7.2 (5.7-8.9); polar capsule length 3.8 (3.3-4.9); polar capsule width 3.6 (3.3-5.0). Myxidium typhonius appears to be a common parasite of the gall bladder of anurans from Cusco Amazonico, Peru, with an average prevalence of 85.5%.

• Kansas Climate with Global Warming: Agricultural and Other Economic Impacts. Wayne M. Wendland. Illinois State Water Survey and Department of Geography, University of Illinois, Champaign, Illinois 61820.

Increasing levels of atmospheric carbon dioxide suggest increasing temperatures over the earth, all other causes of climate being constant. This paper reviews changes to Kansas climate perceived by the mid-21st century. Annual temperatures are expected to warm by some 9°F, with precipitation remaining about the same as today, although perhaps distributed somewhat differently during the year. The warming would cut heating degree days by about 40%, increase cooling degree days by an additional 170% (changing costs accordingly), and increase growing degree days by about 50%. The latter change implies a faster growth rate for field crops, but also implies increased moisture stress. A corn yield model suggests that the warmer climate would decrease corn yields in eastern Kansas by about 19%.

• The Use of Selenium (IV) Oxide to Oxidize Aromatic Methyl Groups. Eric L. Trump and Marilyn Xiaohong Zhou. Department of Chemistry, Emporia State University, Emporia, Kansas 66801.

In this series of experiments, the effect of selenium (IV) oxide (SeO_2) on aromatic methyl groups was investigated. The products obtained when 2-

picoline, 4-picoline and 8-methylquinoline were oxidized by selenium dioxide were 2-pyridine carboxylic acid, 4-pyridine carboxylic acid and 8-quinoline aldehyde, respectively. The yield corresponding to each product above was 50%, 77%, and 49%, respectively. Other compounds, such as 2, 6-lutidine, 2-methylquinoline and 4-methylquinoline were also studied. The products were 2, 6-pyridine dicarboxylic acid, 2-quinoline aldehyde and 4-quinoline aldehyde. Additionally, the compounds which did not react with SeO₂ were toluene, 2-nitrotoluene, 2, 4-dinitrotoluene, 2-chlorotoluene and 4-chlorotoluene. The mechanism for reactions involving selenium (IV) oxide is discussed in detail. The products were analyzed qualitatively by using FTIR, NMR, and other physical and chemical methods.

 Wetapolystoma almae n. gen., n. sp. (Monogenea: Polystomatidae) Parasite of *Bufo typhonius* (Linnaeus, 1758) (Amphibia: Bufonidae) from Tropical Peru. Mary Ellen Gray. Museum of Natural History and Department of Systematics and Ecology, University of Kansas, Lawrence, Kansas 66045-24540.

Wetapolystoma almae n. gen., n. sp. (Monogenea: Polystomatidae) is described from the urinary bladder of *Bufo typhonius* (Linnaeus, 1758) collected in Cusco Amazonico, Peru. Its large terminal muscular oral sucker, voluminous uterus, and long ovary with a straight fore-end distinguish it from the other 3 polystomatid genera (*Polystoma, Mesopolystoma,* and *Riojatrema*) known to inhabit South American amphibians. The opistphaptor is wider than the body and armed with one large pair of anchors with multiple striations and scleritized accessory pieces concentrated at the base of the hook. There are eight hooks surrounding the genital pore. The long and coiled uterus is filled with many eggs, exhibiting ovoviviparous reproduction, the perfect adaptation to a terrestrial host, a toad. *Wetapolystoma almae* is the perfect polystome described from *B. typhonius* from Peru and the second polystomatid genus from *B. typhonius* from South America. It is the second polystomatid genus and species from Peru and the fourth genus known to occur in South American amphibians.

• Age Structure and Reproductive Activity in a Kansas Beaver Population. Debra K. Welch and Robert J. Robel. Division of Biology, Kansas State University, Manhattan, Kansas 66506-4901. Lloyd B. Fox. Kansas Department of Wildlife and Parks, Emporia, Kansas 66801-1525.

Age structure and reproductive activity of beavers in Kansas were determined by examining carcasses collected from trappers. Kits and yearlings comprised 49% of the sample, and none of these were reproductively active. The mean number of embryos and/or placental scars in the uteri of female beavers older than yearlings was 2.9, and the litter size varied with age. Data produced by this study indicated that the beaver population sampled was not overexploited.

• Mortality Profiles of *Mammuthus imperator* (Leidy) Sheridan County, Nebraska. H.E. LaGarry-Guyon and M.R. Myers. University of Nebraska State Museum and Department of Geology, University of Nebraska, Lincoln, Nebraska 68588-0514.

Mortality profiles of *Mammuthus imperator* (Leidy) were derived from samples of isolated and socketed teeth recovered from the Rushville (Sh-3) and Gordon (Sh-5) Quarries of Sheridan County, Nebraska. The Sh-3 mortality profile (MNI=11) is dominated by adult and old individuals and closely corresponds to selective mortality of *Loxodonta africana* after extended periods of drought. The Sh-5 mortality profile (MNI=7) corresponds to mortality profiles that reproduce a stable age distribution of East African *L. americana*, and time-averaged assemblages and mass death events of other North American mammoth samples.

• **Cottonwood Site-Index Growth Curves.** Wayne A. Geyer and Keith D. Lynch. Division of Forestry, Kansas State University, Manhattan, Kansas 66506.

Site-index curves are presented for evaluating the growth potential of cottonwood growing in native timber stands in Kansas. Curves for both eastern and western portions of the state were developed to reflect climatic influences.

• **Relative Abundance of Snakes in Kansas.** Henry S. Fitch. Fitch Natural History Reservation, 2060 E 1600 Road, Lawrence, Kansas 66044-9460.

Forty-four samples of the Kansas ophifauna from localities well distributed over the state total 33,117 snakes of 34 species. *Diadophis punctatus* proved to be by far the most prevalent species. Others that are both wide-spread and numerous are *Coluber constrictor, Thamnophis sirtalis, Pituophis catenifer* and *Nerodia sipedon. Thamnophis radix, Elaphe emoryi, Agkistrodon contortrix, Lampropeltis triangulum, Nerodia rhombifera,* and *Heterodon nasicus* also are well represented in the state. However, a group of diminutive species of secretive and/or fossorial habits, including (besides *Diadophis punctatus*) *Carphophis amoenus, Sonora semiannulata, Storeria dekayi, Tantilla gracilis, T. nigriceps* and *Tropidoclonion lineatum,* all predators on invertebrates, were found to attain much higher densities (scores or even hundreds per hectare) than the larger kinds that prey on vertebrates.