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Relationships Among Habitat, Cover, and Eastern Cottontails (*Sylvilagus floridanus*) in Kentucky

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ABSTRACT

We examined relationships among habitat, cover, and eastern cottontails (*Sylvilagus floridanus*) on five wildlife management areas in Kentucky in 1989. The pellet plot technique was used to determine habitat-use patterns and preferences, and a vegetation profile board was used to determine foliage cover. Old fields and fencerows contained the greatest foliage cover and were preferred by cottontails year-round. Forested areas and agricultural fields were not high-use areas and were typically avoided.

INTRODUCTION

The eastern cottontail (*Sylvilagus floridanus*), the most widely distributed species of cottontail, is one of the most sought after game animals in America (Chapman and Feldhamer 1982). In Kentucky, the cottontail provides recreation and sport for thousands of hunters each year. However, its numbers have been declining in the state over the last several years (Sole 1989). Changes in land use may be facilitating this decline (Bruna 1952).

Intensive agricultural practices (e.g., overgrazed pastures, large monocultures) typically result in loss of valuable rabbit habitat such as fencerows and other shrubby, so-called waste areas (Bruna 1952). Without areas such as these, food and cover for rabbits may be limiting their numbers (Barbour and Davis 1974). Succession of fields to forests may also reduce quality and quantity of rabbit habitat. Our objective was to examine relationships among habitat, cover, and eastern cottontails to provide information that may be key to conserving cottontails in regions such as Kentucky with declining populations of these animals.

METHODS

We conducted our study on five wildlife management areas (WMAs) operated by the Kentucky Department of Fish and Wildlife Resources: Central Kentucky WMA (Madison County), Clay WMA (Nicholas County), Yellowbanks WMA (Breckinridge County), Taylorsville Lake WMA (Spencer County), and Lloyd WMA (Grant County). We classified areas on each WMA into one of four habitat types: (1) fencerows: shrub- and tree-dominated (> 25% cover) strips of vegetation ranging from 1 m to 30 m wide; (2) forests: areas

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Table 1. Foliage cover and habitat use by eastern cottontails on five wildlife management areas in Kentucky, 1989.

Habitat type	Foliage cover (%)[a]		Cottontail use (%)					
	<1 m	<2 m	Jan	Mar	May	Jul	Sep	Nov
Fencerow	88A[b]	76A	46A	42A	54A	43A	50A	42A
Old field	77A	52B	49A	53A	44A	55A	49A	56A
Agricultural field	—	—	4B	5B	2B	2B	1B	2B
Forest	32B	30C	1B	0B	0B	0B	0B	0B

[a] Foliage cover determined August 1989.
[b] Means in a column followed by the same letter are similar ($P > 0.05$).

of closed hardwood-dominated forest, with tree cover >25%; (3) old fields: habitat ranging from pastures of tall grass to older successional stages containing abundant woody shrubs and small trees, with tree cover ≤25%; and (4) agricultural fields: primarily corn fields, with some winter wheat planted in fall and winter.

Two areas of each habitat type per WMA were randomly selected for study. Habitat use by cottontails was determined using the pellet plot technique (Pietz and Tester 1983). We established and marked with wooden stakes 25 plots, each 1 m^2. Plots were 10 m apart along randomly located transects running through forest, old field, and agricultural field sample areas. In fencerows, transects were located along the midline (total = 50 plots/habitat type/WMA). We counted and removed fecal pellets from plots every 4 to 8 weeks between 20 Nov 1988 and 20 Dec 1989. We tested the null hypotheses that cottontail use did not differ among habitat types, months, and WMAs with a factorial ANOVA followed by a Fisher's LSD test (SYSTAT 1992). We examined habitat preferences using chi-square tests (Neu, Byers, and Peek 1974; SYSTAT 1992) by testing the null hypothesis that cottontail use of habitats was random. All four habitat types were sampled with the same intensity (i.e., 50 plots, each 1 m^2/WMA). If use was random, each habitat type should have received 25% of the total use. We considered cottontail preference of a habitat type if use was greater than expected based on a random habitat-use pattern, and avoidance of a habitat type if use was less than expected based on a random habitat use pattern.

A vegetation profile board was used to determine horizontal foliage cover in each habitat type in August 1989 (Nudds 1977). We estimated the percent of the profile board obstructed by vegetation when viewed from a distance of 10 m, every 25 m along each 250 m transect in each habitat type. Foliage cover was compared among habitat types and WMAs using a factorial ANOVA followed by Fisher's LSD test (SYSTAT 1992).

RESULTS

Cottontail use (Table 1) differed among habitat types ($P<0.05$) but not among months or WMAs ($P>0.05$). Habitat preferences did not differ by month ($P>0.05$). Fencerow and old-field habitats were preferred ($P<0.05$), and forests and agricultural fields were avoided ($P<0.05$).

Foliage cover (Table 1) differed by habitat type ($P<0.01$) but not WMA ($P>0.05$). Foliage cover estimates of agricultural fields were not used because of high variability in cover due to differences in crop rotations and problems associated with frequent disturbance (i.e., harvest and planting activities).

DISCUSSION

The cover that habitats provide is the most influential feature determining use of an area by rabbits (Anderson and Pelton 1976; Swihart and Yahner 1982). Our findings agree with those of Barbour and Davis (1974) and suggest that fencerow and old-field habitats consistently received the most use and were preferred because they had greater foliage cover than forest habitats, at least in summer. The added cover in old fields is important for escape and nesting (Bruna 1952). The additional cover provided by fencerows may have been important as a source of protection, escape routes, and travel corridors (Edwards et al. 1981).

In fall and winter, old fields usually lose much of their value to cottontails, both as a source of food and cover, due to desiccation of herbaceous vegetation and loss of foliage from woody vegetation. Because of this, rab-

bits typically move to forested areas and other areas with abundant woody vegetation that provides adequate cover (Barbour and Davis 1974; Lewis 1972). During our study, however, cottontails continued to prefer old fields and to avoid forested areas even in fall and winter. We believe this may have been due to an adequate amount of cover being provided by old fields, even in fall and winter. In addition, the winter of 1988–1989 was mild, with below average snowfall (USDC-NOAA 1988–1989). Therefore, rabbits may not have needed to move to areas containing more woody vegetation in search of food and cover (Hansen, Bailey, and Siglin 1969).

We suggest that limited use and apparent avoidance of agricultural fields was probably due to the continuous disturbance associated with agricultural practices (Friley 1955). Edwards et al. (1981) reported similar patterns of use and avoidance of agricultural fields by cottontails in Illinois.

We have shown that fencerows and old fields are preferred, year-round cottontail habitats; the creation and maintenance of these areas should be a high management objective. Forests and agricultural fields are not high-use areas but may be seasonally important as sources of food and cover. We suggest that management activities include (1) maintaining and planting of shrubs and trees along fences and field edges to enhance fencerow habitat, (2) disking, mowing, and burning of fields to prevent succession to forest, and (3) coordinating agricultural practices to minimize disturbance of fields and provide additional sources of food.

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Separation of Spawning Habitat in the Sympatric Snubnose Darters *Etheostoma flavum* and *E. simoterum* (Teleostei, Percidae)

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ABSTRACT

Individuals of two snubnose darter species, *Etheostoma flavum* and *E. simoterum*, were observed in Whites Creek, Tennessee, in April 1995 and April 1996. Both species were present and spawning in 1995, but in 1996 the stream had experienced increased siltation and algal growth, and no *E. flavum* individuals were seen. Spawning behaviors of both species were similar to those known for other snubnose darters: *E. flavum* and *E. simoterum* attached their eggs to rock surfaces, and the male followed the female as she searched for appropriate substrate. Behavioral observations in 1995 suggested that these two species utilized different sites for spawning, and that interspecific aggression was not the mechanism by which habitat isolation was obtained. Since most closely related darters have allopatric distributions, studying instances of sympatry such as this may contribute to understanding of distribution patterns of darters as a group.

INTRODUCTION

Theoretical works on species concepts and speciation processes traditionally cite reproductive behavior as a significant isolating mechanism between sympatric species (Mayr 1963; Paterson 1993). Choice of spawning site is one aspect of reproductive behavior that has been proposed as an isolating mechanism in some fishes (lamprologine cichlids, Ribbink et al. 1983; Lake Baikal sculpins, Smith and Todd 1984), and it may be important in some species of darters. Darters are one of the most speciose groups of freshwater fishes in North America, and it is common to find more than 10 species in a single stream. Although most closely related darters are allopatric, some do share overlapping distributions. Several studies have demonstrated the significance of microhabitat segregation in competition among sympatric darters, although reproductive behaviors were not specifically addressed (Greenburg 1988; Kessler and Thorp 1993).

The snubnose darters, members of the subgenus *Nanostoma* (sensu Page 1981, but see Bailey and Etnier 1988) in the genus *Etheostoma,* are comprised of 23 described species, most of them restricted to the southeastern United States (Page and Burr 1991) and most distributed allopatrically. *Etheostoma simoterum,* the snubnose darter, has a wide range throughout the Tennessee and Cumberland river systems where it is often sympatric with two other species of snubnose darters: *Etheostoma duryi,* the black darter, and *E. flavum,* the saffron darter. *Etheostoma flavum* is sympatric with *E. simoterum* throughout much of its range in the lower Cumberland and Duck river systems and in some tributaries of the lower Tennessee River (Etnier and Starnes 1993). Spawning behavior has been described from aquarium observations for *E. simoterum* (Page and Mayden 1981) and from both aquarium and field observations for *E. flavum* (Keevin, Page, and Johnston 1989); both species are classified as egg attachers (Page 1985). Descriptions of both species can be found in Etnier and Starnes (1993). Here I describe the spawning behavior of *E. simoterum* under natural conditions, present the results of observations on spawning habitat separation between *E. simoterum* and *E. flavum* in Whites Creek, Tennessee, and discuss the implications for reproductive isolation between the two species.

MATERIALS AND METHODS

I observed spawning *E. simoterum* and *E. flavum* while snorkeling in Whites Creek at Hwy 41A bridge, Davidson County, Tennessee, on 8, 9, and 16 April 1995, and on 11 April 1996. Observations were made in all available habitat types including silted bedrock pools, gravel/cobble runs and riffles, and algae-covered boulders (rip rap) piled at stream edges; depths ranged from 30 cm to 1 m. In 1995, behaviors of both species were filmed

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on 9 and 16 April using a Sony CCD-FX310 8-mm video camera in a Sony MPK-TRA2 underwater housing. Observations were recorded at the spawning sites and later when I viewed the video tapes. I observed *E. simoterum* at the bridge for a total of 4 hours on 2 separate days, during which time four spawning pairs were followed; additionally, 2 hours of observations including one spawning pair were made over the 2 days in a bedrock pool at a site about 300 m upstream. Three hours of observations on six individuals of *E. flavum* were made on the same 2 days at the bridge. I observed the darters from late morning to mid-afternoon each day. Water temperature during the observations remained around 21° C.

On 11 April 1996 fish were observed in the late morning and early afternoon. The water temperature was 11° C. No darters were filmed, but observations on species present, behaviors of these species, and habitat condition were noted.

RESULTS

Although courtship behavior prior to pair-formation was not observed in either *E. simoterum* or *E. flavum,* the spawning behavior after pair-formation was observed to be identical in both species. The male closely followed the female as she swam over the substrate searching for egg deposition sites. He stayed above and a little behind her with median fins semi-erect; in some cases he left to chase an approaching male. When the female found a potential site for egg-deposition, she visually examined the site, as her body formed a 30–45 degree angle with the substrate surface. She then made one to four sharp jabs at the spot with her snout as the male moved to position himself above her. The head jabs observed were similar to those described for *E. davisoni* by Bart (1992) and for *E. zonale* by Walters (1994) and were a consistent indicator of oviposition, with 100% of all spawns in both species preceded by at least one head jab. Immediately following the head jabs, the female dragged her body over the chosen site as the male hovered closely above her. When the female's genital papilla reached the site, she pressed her papilla tightly against the rock and oviposited, while the male arched his body against her, pressed his caudal peduncle towards the rock, and vibrated, releasing sperm. This spawning behavior is identical not only between *E. simoterum* and *E. flavum* but also among the other species in the subgenus *Nanostoma* that I have observed spawning in the field: *E. barrenense, E. duryi,* and *E. rafinesquei.*

In 1995, *E. simoterum* was the most common fish near the Hwy 41A bridge and the most common darter at the riffle/pool habitat upstream of the bridge. Many individuals were seen in the large cobble raceway near the center of the stream; although individuals of both sexes were observed here, there were more females, and no *E. simoterum* were observed forming pairs or spawning in this area. Fewer *E. simoterum* were present among the large algae-covered boulders piled near the bridge on the north side of the stream; however, there was a greater proportion of males seen here, and the only spawning pairs of *E. simoterum* at the bridge site were located in these piles. I observed pairs attaching eggs to the boulders, and spawning positions ranged from slightly angled near the tops of boulders, to near vertical positions on the sides of boulders, to upside-down under an overhanging boulder. No other darter species were observed in this large-boulder area, although some *E. caeruleum* were seen at its periphery.

In 1995, *E. simoterum* was common at the upstream site near the sides of the stream in areas of algae-covered flat bedrock with some cracks and shelves. There were many individuals in the area, but only one spawning pair was observed. The pair was observed on a bedrock shelf spawning in an algae-covered crevice in a horizontal position. At both sites, individuals spawned on surfaces covered with algae; out of the total of nine spawns observed, eight occurred on large algae-covered boulders or stretches of bedrock in a flowing pool environment at the side of the stream. The other spawn took place on a piece of cobble also covered with algae but located closer to the raceway in faster water.

The six individuals of *E. flavum* observed in 1995 were found only in the cobble raceway above the Hwy 41A bridge. This raceway averaged 60 cm deep; the substrate consisted of gravel and cobble. Two spawning pairs were observed, and two other individuals were seen. In all four spawning bouts the females

chose to attach eggs to pieces of clean rock in the fastest current of the raceway. Three spawns took place on pieces of cobble, while the fourth took place on a side of a rock ledge. Spawning positions were most often at about a 45 degree angle to the substrate surface as females oviposited on the sides of the rocks. *Etheostoma simoterum* and *E. caeruleum* were the two most common fishes in the raceway, but I did not observe individuals of either species spawning there.

There were no interspecific interactions between spawning pairs of *E. simoterum* and *E. flavum* individuals in Whites Creek, but this observation is affected by the demography in the stream; *E. flavum* was not present in the boulders where *E. simoterum* spawned. However, spawning *E. flavum* pairs often encountered *E. simoterum* individuals. Encounters are defined as fishes coming within one body length of each other. Of 11 of the interspecific encounters documented for the *E. flavum* pairs, two were with *E. simoterum* males. In one instance the *E. flavum* individuals ignored an *E. simoterum* male who swam between them; in the other instance the *E. flavum* male swam ahead of the female to approach an *E. simoterum* male who seemed to be feeding in their path. None of the interspecific encounters between single individuals (i.e., not spawning pairs) involved aggression. Of two encounters between *E. flavum* pairs and conspecific males, both involved aggressive interactions; for *E. simoterum* all seven intraspecific encounters involved aggression.

Observations in April 1996 revealed that the habitat in Whites Creek was different from the habitat observed a year earlier. The clean cobble in the faster run where *E. flavum* was seen in 1995 was covered with algae, and the whole area appeared to have experienced more siltation. Both male and female *E. simoterum* were common in the cobble runs, but no *E. flavum* individuals were seen. Large *E. simoterum* males were still common among the algae-covered boulders at the side of the stream, and some *E. simoterum* females were present as well. No spawns were observed, but three pairs of *E. simoterum* were seen: one among the boulders and two in the cobble area at the head of the raceway.

Fish stream associates in 1995 at the bridge site were *Campostoma anomalum, Etheostoma caeruleum, E. crossopterum, E. flabellare, E. smithi, Lepomis cyanellus, L. megalotis, Luxilus chrysocephalus, Micropterus punctulatus,* and *Percina caprodes.* Associates in 1995 at the upstream site were *Campostoma anomalum, Etheostoma blennioides, Lepomis megalotis, Luxilus chrysocephalus, Lythrurus ardens, Micropterus punctulatus,* and *Pimephales notatus.* Observed fish associates in 1996 at the bridge site were *Campostoma anomalum, Catostomus commersoni, Etheostoma caeruleum, E. flabellare, Lepomis cyanellus, L. megalotis, Luxilus chrysocephalus,* and *Micropterus punctulatus.*

DISCUSSION

The spawning behaviors of *E. flavum* and *E. simoterum* observed in Whites Creek are nearly identical to previously published descriptions for these two species (Keevin, Page, and Johnston 1989; Page and Mayden 1981). I found the angle of the spawning position for *E. flavum* to be around 45 degrees; Keevin, Page, and Johnston (1989) reported a near vertical spawning position for aquarium-held fish. These differences may reflect the cobble habitat in which *E. flavum* was found in Whites Creek as opposed to the large flat rocks preferred by aquarium-held Warren Fork fish (Keevin, Page, and Johnston 1989). Males of neither species defended stationary territories; instead, females would roam a large area of substrate searching for oviposition sites, and males defended the females with which they were paired. These observations are similar to those reported in studies of the spawning behaviors of other snubnose darters (Carney and Burr 1989; Keevin, Page, and Johnston 1989; Page and Mayden 1981; Stiles 1974).

Observations of *E. flavum* and *E. simoterum* in Whites Creek in 1995 suggest that in this stream these two snubnose darters utilize different spawning sites in different areas of the stream. Previous studies have shown that under natural conditions, both species preferred flowing-pools with a variety of substrates; in aquaria they preferred to deposit eggs on large stones (Keevin, Page, and Johnston 1989; Page and Mayden 1981). However, Keevin, Page, and Johnston (1989) added that when large rocks are not available, *E. flavum* individuals spawn on a cobble substrate. I found *E. fla-*

vum to be utilizing the clean cobble in the swifter runs, while *E. simoterum* spawning pairs were found among the large, algae-covered boulders in slower current at the side of the stream. These data support the hypothesis that *E. simoterum* individuals in Whites Creek occupy the boulder pool habitat, making it unavailable to *E. flavum* individuals. Etnier and Starnes (1993) stated that *E. simoterum* individuals in sympatry with *E. flavum* are more often found in the swifter riffles. My observations in Whites Creek suggest that while many *E. simoterum* were seen in the faster raceway, this is not the area utilized for spawning. Spawning pairs of *E. simoterum* were found only in the boulder pools, suggesting that this species may use different stream habitats for spawning than for other activities such as feeding. In addition, the presence of *E. simoterum* in the faster water is not a result of interactions with *E. flavum,* as individuals of the latter species were not seen in the pools.

Behavioral observations show that members of the two snubnose darter species rarely interact aggressively with heterospecific individuals, while intraspecific interactions among males usually involve aggression. The greater number of interspecific encounters of *E. flavum* pairs with *E. simoterum* females (nine, versus two with *E. simoterum* males) reflects the demography of the *E. simoterum* population in Whites Creek; females were concentrated in the faster current of the raceway where the *E. flavum* individuals were spawning. Similarly, *E. simoterum* spawning pairs had no interspecific encounters because no *E. flavum* individuals were present in the boulder areas where they spawned. In part because of this relationship to demography, the rarity of direct aggression observed between species does not reject the hypothesis that competition for spawning sites causes *E. flavum* to spawn in the cobble raceway. However, the encounter data do suggest that agonistic behavior is not the means by which habitat isolation is maintained. Other possible mechanisms for maintenance of the habitat segregation observed should be considered, such as more subtle behavioral cues or recognition of male color patterns.

In 1995 there were low numbers of *E. flavum* in this section of Whites Creek. This demographic factor may affect and/or be affected by the patterns of habitat use seen in this species as well as in *E. simoterum.* The rarity of *E. flavum* in this stream increases the likelihood that a given observation of spawning habitat in this species is due to chance and not to substrate choice. However, the spawning substrate used by this species may affect its relative success in this stream if, for example, there is differential success of eggs in the different habitats. In 1996, *E. flavum* individuals were not observed in Whites Creek at Hwy 41A, and there were fewer species of fishes in general. These observations might be explained by the difference in water temperature (21° C in 1995 versus 11° C in 1996), but *E. simoterum* was observed forming pairs in the 11° C water in 1996, so presumably the water was not too cold for darters to be present and active. Or the absence of *E. flavum* might be explained by the increased levels of siltation and algal growth since April 1995; the species spawned on clean cobble in 1995, substrate, which was no longer available in 1996. The absence of *E. flavum* might also be a result of interactions with *E. simoterum*; greater numbers of *E. simoterum* competing for food and lower egg survivorship for *E. flavum* in the faster water are possibilities. At this point, hypotheses concerning which factors might be responsible for the absence of *E. flavum* are speculative.

Etheostoma simoterum is also sympatric with *E. duryi* in the middle Tennessee River system; these two species may also exhibit spawning habitat segregation when sympatric (pers. obs.). Snorkeling observations in April 1995 at Gray Branch (Cypress Creek system, Alabama) revealed that *E. duryi* attached eggs to clean cobble and bedrock at the head of an island, while most *E. simoterum* pairs were spawning on cobble in the edge of a run on one side of the island. These observations are consistent with those reported by Etnier and Starnes (1993). *Etheostoma flavum* and *E. duryi* are occasionally sympatric, particularly in the upper Duck River system as discussed by Etnier and Bailey (1989), but I have not made snorkeling observations at any of these localities.

In most cases, the species of darters found in a given stream are not closely related (i.e., in the same subgenus) and do not exhibit similar reproductive habits such as choice of mi-

crohabitat or spawning substrate. The resulting pattern is that the darter species in most streams coexist without interspecific competition for spawning sites. Field data on *E. simoterum* and *E. flavum* in Whites Creek based on limited observations made during one spawning season suggest that there is some evidence for competition for spawning sites between closely related sympatric darters. If corroborated with experimental data, these results could be significant to studies of the reproductive ecology and distributional history of snubnose and other darter species. For example, if competition is found to be a significant factor in the existence and/or persistence of darter species in a given area today, then its role in the historical distribution of darters could be explored.

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Scientists of Kentucky

Common Names of Vascular Plants Reported by C.S. Rafinesque in an 1819 Descriptive Outline of Four Vegetation Regions of Kentucky

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In the flora of North America, knowledge is scarce on the sources and origins of the common names applied to vascular plants. In botanical literature, however, a large number of studies have been published with the purpose of providing common names of plants for either general or specific regions of the United States. McAtee (1913–1933), in a series of five papers published over a 20-year period, listed 410 common names with the geographic areas in which they were used, and 60 sources of information. His list, consisting mostly of plants of aquatic and marsh habitats, is of names learned during field work for the Biological Survey in the United States Department of Agriculture. McAtee's list, containing an index to the plant names in each of his five papers, provides an excellent working base for obtaining local common names from literature sources published during the first third of the 20th century or earlier.

Constantine Samuel Rafinesque (1783–1840) (Figure 1), the controversial, eccentric, sometimes considered erratic European botanist who traveled in eastern North America during the first third of the 19th century, published articles and books totaling over 900 titles in botany. Many of these publications were on taxonomic botany, including catalogues of floras (Rafinesque 1836, 1836-1838, 1840), geographical floras (Rafinesque 1817b, 1817c, 1819a, 1824), medical floras (Rafinesque 1828, 1830), and studies of genera (Rafinesque 1811, 1820). In his *Medical Flora,* Rafinesque (1828, 1830) provided an English, French, and German name, one or more officinal names (i.e., names used in pharmacy), and one to several "vulgar names" for each species, the last being additional names in English used by members of the public but not preferred for scholarly communication. Otherwise, in these well-known publications, he did not provide common names for the plants listed or described, many of which were designated by him as new taxa. In a few of his papers among this vast output of botanical information, he reported the common names of vascular plants.

Rafinesque traveled and explored for plants in Kentucky during the years 1818 to 1826 and was Professor of Botany and Natural History at Transylvania University in Lexington from 1819 to 1825. During this period he wrote a short article (Rafinesque 1819c; Figure 2) consisting of a descriptive outline of the vascular-plant vegetation in four major regions of the state. This paper was published in 1819 in the second number of the first volume of *The Western Review and Miscellaneous Magazine,* edited by William Gibbs Hunt (Rafinesque 1819c). In addition to providing general remarks, Rafinesque characterized each of the four botanical regions as to geographical location, general topography, kinds of bedrock, and unique features of the vegetation. He also provided a short list of vascular plants that he considered somewhat specific or "peculiar" to each region. Along with each binomial scientific plant name, he added the common name, or in his terminology, the "vulgar" name, noting, however, that "the vulgar names of the plants . . . cannot claim to be generally understood even in [Kentucky], many being merely

Figure 1. Portrait of C.S. Rafinesque by American artist William Birch (1755–1834). After a long succession of private owners, this enamel miniature (2 ¼" diameter) was purchased by Transylvania University, Lexington, Kentucky, in 1938. Photo courtesy of Transylvania University Special Collections.

local or personal. The botanical names are alone to be relied on."

Two years earlier, in his review of the first edition of Amos Eaton's *Manual of Botany for the Northern States* . . . [1817], Rafinesque (1817a) supported the idea of providing the vulgar names in works on local taxonomic botany, and he noted that their inclusion in Eaton's *Manual* was "valuable." He commented that most of them had been taken by Eaton from other cited published sources, rather than being "taken directly from the vulgar," and wrote further that "vulgar names are at all times a valuable appendage to classical synonymy, and indispensable in local botanical writings."

Rafinesque probably prepared the article on vegetation of Kentucky for a popular audience, which would explain his use of the common names of plants, but he reminded his readers that the botanical names were the intended authentic ones to be used for scientific accuracy. Botanists always have and, it is expected, always will recognize that the botanical names should be used in authoritative floristic and vegetational studies. Botanists, however, must also communicate with and make their studies useful to those who work in other fields of study but who are not familiar with Latin binomials. Among these fields are geology, zoology, wildlife and fisheries studies, natural resources management, endangered species preservation, conservation organizations, environmental law, ecological consultation, and other groups with environmental concerns.

The use of common names of plants is of more importance now than it was 25 or 50

MISCELLANY.

BOTANY OF KENTUCKY.

On its principal features, by C. S. RAFINESQUE, *Professor of Botany and Natural History in Transylvania University.*

THE state of Kentucky being situated in the centre of the western country, has a *flora* similar to the generality of the western states and participating in their peculiar features, while it offers in itself a complete specimen of the western botany.

The peculiarities of this botany consist principally in the total want of the maritime and mountain regions, which form such remarkable sections in the local floras of the Atlantic states, and abound with plants peculiar to themselves. Another striking feature in the vegetation of Kentucky and the western states is the propensity which many plants and trees exhibit of growing in a social state, to the almost total exclusion of every other. There are many plants which grow crowded together, all over the United States; such for instance as the grasses, ferns, the *Comptonia*, the *Studfonia*, &c. but they allow many other plants to grow with them; while, in the western country, many extensive spaces of ground are covered with one or a few crowded species, to the exclusion of many others, which are found in their company elsewhere. The plants which may be quoted as a striking instance of this singular fact are not few, among which I shall select the following:

Vernonia prealta,	Iron Weed,
Baptisia cerulea,	Blue Wild Indigo,
Cacalia reniformis,	Kidney Weed,
Stedeoma pulegivides,	Penny-aoyal,
Chenopodium anthelminthicum,	Worm Weed,
Elephantopus scaber,	Elephant's Foot,

Figure 2. First page of C.S. Rafinesque's paper on "Botany of Kentucky[:] on its principal features," which was published in 1819 in volume 1(2) of *The Western Review and Miscellaneous Magazine.*

years ago; it will become more and more prevalent and necessary as time progresses. With increasing frequency, floristic manuals, such as that of Gleason and Cronquist (1991) and the ongoing *Flora of North America North of Mexico,* are providing vernacular names for all or most of the species described therein. The use of common names will demand more accuracy and stability to the extent that nomenclatural guidelines may need to be established in the future. These guidelines may develop in a manner similar to those parameters provided in the *International Code of Botanical Nomenclature.* The correct interpretation of earlier papers in which common names were used requires that the authors' applications of such names be accurately determined. Some names may have been applied in senses different from more recent usage, and some may have fallen into disuse. Efforts to standardize common nomenclature require the selection of appropriate and otherwise acceptable names that will not perpetuate or introduce confusion. Attaining these objectives will require consideration of historic and regional applications of vernacular plant names. For these reasons we bring to the attention of the scientific public the common plant names in Rafinesque's early 19th century publication on the botany of Kentucky.

Rafinesque's paper on the botany of Kentucky is transcribed below. His lists of plant species are annotated, as indicated in the following paragraphs, to permit their interpretation using current botanical and common names. Separate indexes to botanical and common names are provided in Appendix 1.

Rafinesque—owing, he said in the errata, to his absence from town—had no opportunity for proofreading until after the paper had gone to press. Consequently, the original lists of plant names are rife with typographical errors, even among the common names. Rafinesque (1819d) published corrections of a number of these errors in a later issue of the same journal, on page 128. Several others, however, escaped his notice. "Tris" for "Iris," and "Pin week" for "Pin weed," for example, are obvious typographical errors.

In some cases, however, minor alterations presumably do represent Rafinesque's intent. From some of his other publications, it is evident that he preferred a simplified spelling such as *"cerulea"* to the original *"caerulea"* or *"coerulea"* and *"catesbei"* for *"catesbaei,"* and that he frequently made such arbitrary abridgments. Such changes, whether required or proscribed by present rules of nomenclature, are not regarded as new names, and they remain attributed to the original authors. Because of the circumstances discussed above, it appeared best to retain Rafinesque's original format in the lists, which omitted the attribution of the authorship of the botanical names. As the lists appear in the present paper, the botanical names used by Rafinesque are in italic type; the common names, in roman type. (At the left margin we have numbered, in parentheses, each species for indexing purposes.) Indented and in square brackets and smaller type below the names we have added, first, the corrected orthography of the

botanical name used by Rafinesque, in italics if it is not the currently accepted botanical name, with authorship; second, where appropriate and in roman type, the currently accepted botanical name; and third, a common name or names in current use. When the botanical name used (or intended) by Rafinesque remains in use for the species, this name appears in roman type, with the orthography corrected if necessary and the authorship indicated. Curly brackets have been used to indicate the corrections made by Rafinesque (1819d) in the errata. In Appendix 2, notes, mostly on nomenclature, have been added for 14 taxa.

The modern common names we include have generally been obtained from Fernald (1950), Gleason and Cronquist (1991), and the popular field guides by Peterson and McKenny (1968) and Newcomb (1977). The original *Illustrated Flora* by Britton and Brown (1896–1898), which provided common names for nearly every species covered and also recorded many local or otherwise relatively obscure vernacular names, was also consulted.

Rafinesque's paper (Rafinesque 1819c) on the principal features of the botany of Kentucky is as follows:

Botany of Kentucky
On its principal features, by C.S. Rafinesque, *Professor of Botany and Natural History in the Transylvania University.*

The state of Kentucky being situated in the centre of the western country, has a *flora* similar to the generality of the western states and participating in their peculiar features, while it offers in itself a complete specimen of the western botany.

The peculiarities of this botany consist principally in the total want of the maritime and mountains regions, which form such remarkable sections in the local floras of the Atlantic states, and abound with plants peculiar to themselves. Another striking feature in the vegetation of Kentucky and the western states is the propensity which many plants and trees exhibit of growing in a social state, to the almost total exclusion of every other. There are many plants which grow crowded together, all over the United States; such for instance as the grasses, ferns, the *Comptonia,* the *Studfonia* {*Hudsonia*}, &c. but they allow many other plants to grow with them; while, in the western country, many extensive spaces of ground are covered with one or a few crowded species, to the exclusion of many others, which are found in their company elsewhere. The plants which may be quoted as a striking instance of this singular fact are not few, among which I shall select the following:

(1) *Vernonia prealta,* Iron Weed,
[*Vernonia praealta* Michx.; V. gigantea (Walter) Trel. ssp. gigantea; tall ironweed]

(2) *Baptisia cerulea,* Blue Wild Indigo,
[*Baptisia coerulea* Eaton & Wright; B. australis (L.) R.Br. ex W.T. Aiton; blue false-indigo]

(3) *Cacalia reniformis,* Kidney Weed,
[*Cacalia reniformis* Muhl. ex Willd.; *C. muhlenbergii* (Sch. Bip.) Fern; Arnoglossum muehlenbergii (Sch. Bip.) H. Rob.; great Indian-plantain; see Appendix 2, entry 1, for discussion of nomenclature of this species]

(4) *Stedeoma pulegivides* {*Hedeoma pulegioides*}, Penny-aoyal {Penny royal},
[*Hedeoma pulegioides* (L.) Pers.; American pennyroyal, bastard pennyroyal, blue-curls]

(5) *Chenopodium anthelminthicum,* Worm Weed,
[*Chenopodium anthelminticum* L.; C. ambrosioides L. var. anthelminticum (L.) A. Gray; wormseed; see Appendix 2, entry 2, for discussion of nomenclature of this species]

(6) *Elephantopus scaber,* Elephant's Foot,
[*Elephantopus scaber* L.; E. carolinianus Raeusch.; leafy elephant's-foot; see Appendix 2, entry 3, for discussion of nomenclature of this species]

(7) *Gillenia stipulacea,* Indian Physic,
[*Gillenia stipulata* (Muhl. ex Willd.) Nutt.; Porteranthus stipulatus (Muhl. ex Willd.) Britton; American ipecac]

(8) *Miagia arupedinaria* {*Miegia arundinaria*}, Cane, &c.&c.
[*Miegia arundinaria* Raf., nom. nud., presumably intended orthography; Arundinaria gigantea (Walter) Muhl.; giant cane]

I consider the state of Kentucky as divided into four natural sections, or botanical regions, which are all distinguished by some peculiarities in their vegetation. They are:

1. The Fluviatile Region. This includes all the valleys, and bottoms of the large rivers,

such as the Ohio, Mississippi, Tennessee, Cumberland, Kentucky, &c. with their tributary streams. The bottoms of the valleys are formed of an alluvial soil, or the washings from the hills. They are level and often overflowed: while the sides of the valleys are steep, craggy, and composed of limestone, sandstone, or slaty rocks. The following are some of the trees and plants peculiar to this region, and giving a decided character to its vegetation:

(9) *Platanus occidentalis,* Sycamore or Button wood,

[Platanus occidentalis L.; sycamore, buttonwood]

(10) *Hesperis pinnatifida,* Ohio Wall Flower,

[*Hesperis pinnatifida* Michx.; Iodanthus pinnatifidus (Michx.) Steud.; purple rocket]

(11) *Jeffersonia cinata* {*Jeffersonia binata*}, Tavin Weed {Twin leaf},

[*Jeffersonia binata* Barton; J. diphylla (L.) Pers.; twinleaf]

(12) *Capraria multifida,* Sand Ragweed,

[*Capraria multifida* Michx.; Leucospora multifida (Michx.) Nutt.; see Appendix 2, entry 4, for discussion of nomenclature of this species]

(13) *Solanum Carolinianum,* Sand Briar

[An orthographic variant of Solanum carolinense L., possibly preferred and originated by Rafinesque; horse-nettle]

(14) *Lupatorium calutinum* {*Eupatorium coelestinum*}, Sy-weed {Sky weed},

[Eupatorium coelestinum L.; mist-flower]

(15) *Polanina* {*Polanisia*} *graveolens,* Stinking weed,

[*Polanisia graveolens* Raf.; Polanisia dodecandra (L.) DC. ssp. dodecandra; clammy-weed]

(16) *Heliotropium Indicum,* Heliotrope,

[Heliotropium indicum L.; Indian heliotrope, turnsole; see Appendix 2, entry 5, for discussion of nomenclature of this species]

(17) *Catalpium cordata,* Catalpa tree,

[*Catalpium cordifolium* (J.St.-Hil.) Raf.; Catalpa speciosa (Warder ex Barney) Engelm.; northern catalpa; see Appendix 2, entry 6, for discussion of nomenclature of this species]

(18) *Populus angulata,* Cotton tree,

[*Populus angulata* Aiton; P. deltoides Marshall var. deltoides; cottonwood]

(19) *Porcelia tribuba* {*Porcelia triloba*}, Papaw tree,

[*Porcelia triloba* (L.) Pers.; Asimina triloba (L.) Dunal; pawpaw]

(20) *Synandra grandiflora,* Cow mint,

[*Synandra grandiflora* Nutt.; S. hispidula (Michx.) Baill.; see Appendix 2, entry 7, for discussion of nomenclature of this species]

(21) *Nelumbium pentapetalum,* Swamp lily,

[*Nelumbium pentapetalum* (Walter) Willd.; Nelumbo lutea (Willd.) Pers.; American lotus, yellow lotus]; see Appendix 2, entry 8, for discussion of nomenclature of this species]

(22) *Pancratium liviosone* {*Pancratium liriosme*}, Lily,

[*Pancratium liriosme* Raf.; Hymenocallis caroliniana (L.) Herbert; spider-lily; see Appendix 2, entry 9, for discussion of nomenclature of this species]

(23) *Iris crocea,* Red lily,

[*Iris crocea* Raf., nomen nudum; presumably I. fulva Ker Gawl.; copper iris]

(24) *Houstonia fruticosa,* Rock weed,

[*Houstonia fruticosa* Raf., nomen nudum; H. nigricans (Lam.) Fernald var. nigricans; narrow-leaved houstonia; see Appendix 2, entry 10, for discussion of nomenclature of this species]

(25) *Prunus pendula,* Cliff plumb, &c.&c.

[*Prunus pendula* Raf., nomen nudum, non Maxim. nec K.Koch; not definitely identified, probably P. munsoniana W. Wight & Hedrick; wild plum; see Appendix 2, entry 11, for discussion of nomenclature of this species]

These two last are new species from the cliffs of the Kentucky river.

2. The Central Region. It is formed by the limestone tract included between the valley of the Ohio and the hilly ridges or knobs. The ground is slightly broken, very fertile and mostly under cultivation. This section is remarkably poor in the number of botanical species growing spontaneously; I conceive that its flora hardly contains 500 species, including trees, shrubs, and naturalized plants! There are hardly any species peculiar to it; but the following ones, rare elsewhere, are here very common:

(26) *Eupatorium urticefolium,* White nettle,

[*Eupatorium urticifolium* Reichard, "*urticaefolium*"; E. rugosum Houtt.; white snakeroot]

(27) *Pavia muricata,* Prickly Buck-eye,

[*Pavia muricata* Raf., nomen nudum; Aesculus glabra Willd. var. glabra; Ohio buckeye]

(28) *Isanthus ceruleus,* Blue Penny-royal,

[Rafinesque's preferred orthography for *Isanthus coeruleus* Michx.; I. brachiatus (L.) BSP.; False pennyroyal]

(29) *Polymnia uvedalia,* Scented Sun flower,

[Polymnia uvedalia L.; large-flowered leafcup, yellow-flowered leafcup]

(30) *Phlox glaberrima,* Pink, &c.&c.

[Phlox glaberrima L.; smooth phlox]

It is also highly singular that in this region, the woods are open as parks, without shrubs and with very few plants, except grass or some social weeds.

3. The Hilly Region. It contains the hills and ridges which divide the waters of the Kentucky, Green, Licking, Cumberland and Sandy rivers, &c. being spurs from the Cumberland mountains. Those hills are often called knobs, although they have not always the knobby or rounded appearance. The rocks are limestone, or sandstone, or slate. The vegetation approximates exceedingly to that of Virginia and Pennsylvania. On the Cumberland mountain and the highest ridges, I am told that there is a similarity with the Alleghany regions, and that the *Kalmia latifolia,* Common Laurel, and the *Gaultheria procumbens,* Mountain Tea, grow there; but having not yet visited them, I am unable to ascertain whether they ought to form another distinct region, which might be called the mountain region. The hilly region is rich in plants; I shall mention a few of those peculiar to it in Kentucky:

(31) *Iris cristata,* Crested Tris or Flag,

[Iris cristata Aiton; crested iris, crested dwarf iris]

(32) *Stylvianthes* {*Stylosanthes*} *elatior,* Yellow Pea-clover,

[*Stylosanthes elatior* Raf., nomen nudum; S. biflora (L.) Britton et al.; pencil-flower]

(33) *Orchis ciliaris,* Yellow-bunch,

[*Orchis ciliaris* L.; Platanthera ciliaris (L.) Lindl.; orange fringed-orchid, yellow fringed-orchid]

(34) *Juniperus Virginiana,* Red Cedar,

[Juniperus virginiana L.; red cedar]

(35) *Vaccinium album,* Wild Currant,

[*Vaccinium album* Pursh, non L. nec Lam.; V. stamineum L.; deerberry]

(36) *Pinus rigida,* Pitch Pine,

[Pinus rigida Mill.; pitch pine]

(37) *Lechea minor,* Pin week,

[Lechea minor L.; small pinweed]

(38) *Rudbeckia fulgida,* Rough Wort,

[Rudbeckia fulgida Aiton, presumably var. fulgida; orange coneflower, eastern coneflower]

(39) *Gerardia glabrata,* Yellow Wort,

[*Gerardia glabrata* Raf., nomen nudum; not definitely identified, probably Aureolaria laevigata (Raf.) Raf.; Appalachian false-foxglove; see Appendix 2, entry 12, for discussion of nomenclature of this species]

(40) *Asarum Virginicum,* Heart-leaf, &c.&c.

[*Asarum virginicum* L.; Hexastylis virginica (L.) Small; heart-leaf, little brown jugs; see Appendix 2, entry 13, for discussion of identity of this species]

4. The Barren Region, or rather the open region. This has an extensive range in Kentucky, particularly in the western and southern parts of the state. The numerous *barrens* and *licks* compose it, lying scattered and irregularly among the central and hilly regions. The *barrens* are tracts of ground destitute of trees, or with few scattered small ones; but thickly covered with a luxuriant growth of plants; while the licks are almost destitute of them, and those that grow in their immediate neighbourhood are all small, which is owing to their poor, slaty or argillaceous soil. Their vegetation is however similar to that of the *barrens.* Both have a growth of plants very similar to the vegetation of the *prairies* of Ohio, Indiana, and Illinois, and more different from that of the Atlantic states, than the three foregoing regions. The plants peculiar to them are very numerous; I shall mention only a few, among the most remarkable and singular.

(41) *Solidago rigida,* Stiff Golden-rod,

[Solidago rigida L.; stiff goldenrod]

(42) *Polygala polygama,* Nimble weed,

[Polygala polygama Walter; racemed milkwort, bitter milkwort]

(43) *Rudbeckia purpurea,* Purple Sun-flower,

[*Rudbeckia purpurea* L.; Echinacea purpurea (L.) Moench; purple coneflower]

(44) *Ruellia oblongifolia,* Rough Bell,

[*Ruellia oblongifolia* Raf., nomen nudum, Raf. ex Nees, pro syn., non Michx.; R. caroliniensis (J.F. Gmel.) Steud. ssp. ciliosa (Pursh) R.W. Long; hairy ruellia]

(45) *Andropogon arenaceum,* Barren Oats,

[*Andropogon avenaceus* Michx.; Sorghastrum nutans (L.) Nash; Indian grass]

(46) [*Andropogon*] *nutans,* Barren Oats,

[*Andropogon nutans* L.; Sorghastrum nutans (L.) Nash; Indian grass]

(47) *Petalvitemon* {*Petalostemon*} *candidum,* Nimble clover,

[*Petalostemon candidum* (Michx. ex Willd.) Michx.; Dalea candida Michx. ex Willd.; white prairie-clover]

(48) [{*Petalosteum*}] *purpureum,* Nimble clover,

[*Petalostemon purpureum* (Vent.) Rydb.; Dalea purpurea Vent. var. purpurea; purple prairie-clover]

(49) *Silphium therebinthaceum,* Turpentine weed,

[Presumably an intentional abridgement of Silphium terebinthinaceum Jacq.; prairie-dock]

(50) *Silene catesbri* {*Silene catesbei*}, Scarlet Pink,

[*Silene catesbaei* Walter; S. virginica L.; fire-pink]

(51) *Gentiana amarellvides* {*Gentiana amarelloides*}, Yellow Gentian,

[*Gentiana amarelloides* Michx.; Gentianella quinquefolia (L.) Small var. quinquefolia; stiff gentian; see Appendix 2, entry 14, for discussion of nomenclature of this species]

(52) *Buchnera Americana,* Black Wort, &c.&c.

[Buchnera americana L.; blue-hearts]

From the above a faint, but correct idea may be formed of the display and peculiarities of the wide range of vegetation in Kentucky and throughout the western states, wherein the same peculiar divisions or regions may be traced.

The vulgar names of the plants above mentioned are such as I found used in some parts of Kentucky; but they cannot claim to be generally understood even in this state, many being merely local or personal. The botanical names are alone to be relied on, being universal and not liable to mislead.

APPENDIX 1: Indexes to Botanical and Common Names of Plants as Originally Listed and Those Added to Rafinesque's 1819 Paper on the Botany of Kentucky.

A. Botanical or Scientific Names

Aesculus glabra Willd.
  var. glabra (27)
Andropogon arenaceum (45)
Andropogon avenaceus Michx. (45)
Andropogon nutans L. (46)
Arnoglossum muehlenbergii (Sch. Bip.) H. Rob. (3)
Arundinaria gigantea (Walter) Muhl. (8)
Aureolaria laevigata (Raf.) Raf. (39)
Asarum virginicum L. (40)
Asimina triloba (L.) Dunal (19)
Baptisia australis (L.) R. Br. ex W.T. Aiton (2)
Baptisia cerulea (2)
Baptisia coerulea Eaton & Wright (2)
Buchnera americana L. (52)
Cacalia muhlenbergii (Sch. Bip.) Fernald (3)
Cacalia reniformis Muhl. ex Willd. (3)
Capraria multifida Michx. (12)
Catalpa speciosa (Warder ex Barney) Engelm. (17)
Catalpium cordata (17)
Catalpium cordifolium (J. St.-Hil.) Raf. (17)
Chenopodium anthelminthicum L. (5)
Chenopodium ambrosioides L.
  var. anthelminticum (L.) A. Gray (5)
Dalea candida Michx. ex Willd. (47)
Dalea purpurea Vent.
  var. purpurea (48)
Echinacea purpurea (L.) Moench (43)
Elephantopus carolinianus Raeusch. (6)
Elephantopus scaber L. (6)
Eupatorium celestinum (14)
Eupatorium coelestinum L. (14)
Eupatorium rugosum Houtt. (25)
Eupatorium urticefolium (26)
Eupatorium urticifolium Reichard (26)
Gentiana amarelloides Michx. (51)
Gentiana amarellvides (51)
Gentiana quinquefolia (L.) Small
  var. quinquefolia (51)
Gerardia glabrata Raf. (39)
Gillenia stipulacea (7)
Gillenia stipulata (Muhl. ex Willd.) Nutt. (7)
Hedeoma pulegioides (L.) Pers. (4)
Heliotropium indicum L. (16)
Hesperis pinnatifida Michx. (10)
Hexastylis virginica (L.) Small (40)
Houstonia fruticosa Raf. (24)
Houstonia nigricans (Lam.) Fernald
  var. nigricans (24)
Hymenocallis caroliniana (L.) Herbert (22)
Iodanthus pinnatifidus (Michx.) Steud. (10)
Iris cristata Aiton (31)
Iris crocea Raf. (23)

B. Index to Common or Vulgar Names

Little brown jugs (40)
Mist-flower (14)
Narrow-leaved houstonia (24)
Nimble clover (47, 48)
Nimble weed (42)
Northern catalpa (17)
Ohio buckeye (27)
Ohio wall flower (10)
Orange coneflower (38)
Orange fringed-orchid (33)
Pawpaw (19)
Pawpaw tree (19)
Pencil-flower (32)
Penny-aoyal (4)
Penny-royal (4)
Pink (30)
Pin weed (37)
Pitch pine (36)
Prairie-dock (49)
Prickly buck-eye (27)
Purple coneflower (43)
Purple prairie-clover (48)
Purple rocket (10)
Purple sun-flower (43)
Racemed milkwort (42)
Red cedar (34)
Red lily (23)
Rock weed (24)
Rough bell (44)
Rough wort (38)
Sand briar (13)
Sand ragweed (12)
Scarlet pink (50)
Scented sun flower (29)
Sky-weed (14)
Small pinweed (37)
Smooth phlox (30)
Spider-lily (22)
Stiff gentian (51)
Stiff golden-rod (41)
Stiff goldenrod (41)
Stinking weed (15)
Swamp lily (21)
Sycamore (9)
Sy-weed (14)
Tall ironweed (1)
Tavin weed (11)
Turnsole (16)
Turpentine weed (49)
Twinleaf (11)
Twin weed (11)
White nettle (26)
White prairie-clover (47)
White snakeroot (26)
Wild currant (35)
Wild plum (25)
Wormseed (5)
Worm weed (5)
Yellow-bunch (33)
Yellow-flowered leafcup (29)
Yellow fringed-orchid (33)
Yellow gentian (51)
Yellow lotus (21)
Yellow pea-clover (32)
Yellow wort (39)

APPENDIX 2: Notes on the Botanical and Common Names (by J.S.P.)

1. The nomenclature of this species is unsettled at the time of this writing, not only because of differences of opinion as to appropriate generic circumscriptions, but also pending a decision on proposals to reject the name *Cacalia*, or, alternatively, for conserved typification. If *Cacalia* is rejected or is typified otherwise than as noted below, this species will presumably be placed in *Arnoglossum* regardless of whether the genus is circumscribed so as to include *Synosma* Raf. ex Britton & A. Brown; if *Cacalia* is not rejected, but is conserved with *C. atriplicifolia* L. as the type, the correct name for this species will be *Cacalia muehlenbergii* (Sch. Bip.) Fernald.

2. The use of "wormseed" in works by other authors from the same period indicates that "worm weed" probably represents a typographical error rather than a version used in Kentucky during Rafinesque's time.

3. The name *Elephantopus scaber* L. remains the correct name for an accepted species, but not for a species that occurs in Kentucky. *Elephantopus scaber* sensu Michaux has been identified as the species now called *E. carolinianus*.

4. No common name has been located for *Leucospora multifida* in recent references other than the generic name, or the now-obsolete generic name *Conobea*, used as such. If the use of "sand-ragweed" actually persists, it might be revived for wider application, hyphenated because this species is not in the ragweed genus *Ambrosia* (Asteraceae). If, however, this was a coinage that expired with Rafinesque, a name that might seem to indicate a relationship to the true ragweeds would not be ideal.

5. *Heliotropium indicum* L. is now extensively but sporadically adventive or naturalized in the southeastern United States, and, despite Kentucky's inland location, it is not inconceivable that Rafinesque might have found a small population there as early as 1819. However, because no other North American authors mentioned *H. indicum* as a naturalized species until considerably later, and because to the present day *H. indicum* has remained sporadic in its North American occurrences, it hardly seems credible that Rafinesque could have found this species in such abundance that it was among those "giving a decided character to [the] vegetation" of any part of Kentucky. Rafinesque's (1838) later description of the species he had so identified, which he then called *Elopia riparia* Raf., appears to have been derived largely from published descriptions of *H. indicum*, but inevitably one wonders if his concept of the species was actually based on some other boraginaceous species.

6. Rafinesque presumably referred to the *Catalpa* species that is native to Kentucky, viz. *C. speciosa* (Warder ex Barney) Engelm., which he might have seen on his trip to the mouth of the Ohio River in 1818. There seems to be an error in *Index Rafinesquianus* (Merrill 1949) on this point. It is probably appropriate to assume from the similarity of the epithets, as Merrill evidently did, that *Catalpium cordatum* Raf. (Rafinesque 1819c) was intended to be the same as his *C. cordifolium* Raf. (Rafinesque 1819b), published slightly earlier, the second being either a lapsus calami or an intentionally amended orthography for the first. According to Merrill, *Catalpium cordifolium* Raf. was tied nomenclaturally to *Catalpa cordifolia* J. St.-Hil. Merrill apparently confused *C. cordifolia* J. St.-Hil. of 1804 with *C. cordifolia* Moench of 1794, which is a synonym of *C. bignonioides* (Rehder 1949). The illegitimate homonym *Catalpa cordifolia* J. St.-Hil., however, is a synonym of *C. speciosa,* according to Rehder; this fits well with Rafinesque's report of his *Catalpium cordifolium/C. cordatum* from Kentucky.

7. We have located no common name for *Synandra hispidula* in recent literature, except for the generic name used as such. As "cow-mint" does not appear to be used for any other species, it could be considered for this species, which is in the mint family (Lamiaceae).

8. Some recent authors have advocated "lotus-lily" or "water-lotus" for *Nelumbo,* presumably because *Lotus* is the botanical name for a genus in the Fabaceae. With this genus very generally being known simply as "lotus," this illustrates the recurrent question as to what extent standardized English-language names should vary from names actually in common usage. In this case, one might also ask whether "lotus-lily," even if hyphenated, is all that much preferable for standardization, since *Nelumbo* is not in the Liliaceae.

9. The name *Pancratium liriosme* Raf., published 2 years earlier by Rafinesque (1817c), is the basionym of *Hymenocallis liriosme* (Raf.) Shinners. The species thus named is native to Arkansas, Louisiana, Oklahoma, and Texas. According to Shinners (1951), Rafinesque (1817c), in describing *P. liriosme,* "was relying on [Claude C.] Robin's description of plants that the latter had observed wild in Louisiana." The only species of *Hymenocallis* native to Kentucky is *H. caroliniana* (L.) Herb., formerly known as *H. occidentalis* (Leconte) Kunth.

10. No satisfactory common name appears to be available for this species. In popular field guides, the generic name *Houstonia* is used as a common name for species of this genus, except for two species of different aspect that are called "bluets." Britton and Brown (1896–1898) called this species "narrow-leaved houstonia," when it was known botanically *H. angustifolia* Michx., but in the popular field guide by Newcomb (1977), "narrow-leaved houstonia" is used for *H. tenuifolia* Nutt. Complicating the issue, some botanists prefer to include *Houstonia* in *Hedyotis.* The name madderwort has reportedly been applied to *Houstonia,* but this may have been in a context extending to other genera in the Rubiaceae (madder family). "Rock weed" has little to recommend it, having been applied to *Asperula odorata* L. and possibly other species.

11. From the habitat given by Rafinesque—"cliffs of the Kentucky River"—an anonymous reviewer has concluded that Rafinesque's *Prunus pendula* is probably *P. munsoniana* W. Wight & Hedrick, a species that was not otherwise recognized as distinct and given a scientific name until 1911. Under the current rules of botanical nomenclature, because Rafinesque provided no description of his "new species," and because another botanist had used the name *Prunus pendula* for a different species 4 years earlier, this interpretation does not affect the nomenclature of *P. munsoniana.*

12. On the basis of Rafinesque's phytogeographic comments, *Aureolaria laevigata,* "Appalachian false-foxglove," seems the most likely identity of this species, but *A. flava* (L.) Farw., "smooth false-foxglove," cannot be ruled out.

13. Rafinesque's "outline of four vegetation regions of Kentucky" was published before its author visited that portion of Kentucky in which *Hexastylis* species occur; for this reason it has been suggested that his *Asarum virginicum* might not have been any species now referred to *Hexastylis,* the alternative interpretation being that it was actually *A. canadense* L. This seems unlikely because Rafinesque presumably would have been familiar with *A. canadense* under its correct name and because, in all of the literature available prior to 1819 in which *A. virginicum* is described, that species is said to have glabrous, mottled leaves. (Similarly, Rafinesque had not at that time traveled into areas of Kentucky where any species of *Lechea* is native, but one would hardly assume that he had misidentified some other genus as *Lechea.*) Even if Rafinesque's concept of the "Hilly Region" included the Knobs that are east of Lexington but west of the Pottsville escarpment, it seems likely that some of his information, at least in this relatively brief section of the paper, was more or less secondhand, perhaps derived from a combination of accounts from other naturalists, studies of specimens in their herbaria, and extrapolations from his firsthand knowledge of the flora of adjacent states.

14. The name *Gentiana amarelloides* Michx. is a heterotypic synonym of *Gentianella quinquefolia* (L.) Small var. *quinquefolia,* but in Kentucky this species is represented by var. *occidentalis.* From the *Medical Flora,* it is evident that Rafinesque (1828) considered the plants with yellow corollas to be a different species from the more widespread form with purple corollas.

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Some Comments on Constantine Rafinesque's 1819 Description of Botanical Regions in Kentucky

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Perhaps of greatest ecological significance in Rafinesque's (1819a, 1819b) Botany of Kentucky was his recognition of distinct botanical regions or natural sections in the state. His regions and plant associations were not so well defined as those currently recognized (e.g., Braun 1950; Küchler 1964; Quarterman and Powell 1978), but at least they were a first attempt. Meijer (1973) noted that Rafinesque often had good ideas of natural relationships of vegetation as distinguished by its peculiarities.

Rafinesque (1819a) recognized four botanical regions but suggested that a fifth, the Mountain Region, was probable. He had neither collected in nor visited the eastern portions of Kentucky. His entrance into Kentucky was from Pennsylvania via the Ohio River in 1818. From 1819–1825, he was Kentucky's first professional resident botanist, teaching at Transylvania College in Lexington (Meijer 1973). It seems apparent that Rafinesque was familiar with the literature concerning some aspects of natural history on the frontier as detailed by Drake (1815), Filson (1784), Imlay (1797), and Michaux (1904). Kentucky was only 26 years old as a state when Rafinesque arrived in 1818 and only 44 years removed from the establishment of its first permanent settlement.

Rafinesque's botanical regions of Kentucky are listed and described as follows:

1. The Fluviatile Region included the valleys and bottoms of large rivers and their tributaries. Rafinesque especially noted the alluvial deposits but also included the steep-sided gorges bordering many of these streams. His inclusion of the Cumberland and Kentucky rivers might be expected, but his inclusion of the Tennessee River, which prior to 1818 formed the state's western border, and the Mississippi River, which after the 1818 Jackson Purchase became the western border, suggests that Rafinesque was abreast of happenings in the Commonwealth. His recognition of a Fluviatile Region does not fit most modern regional designations; however, Middleton et al. (1926) stated that "the Alluvial land of the Mississippi River bottoms constitutes the fifth distinct area of the state." The Ohio River lowlands was also designated as a distinct geographical region (Burroughs 1926). Cotterill (1917) noted that early maps of Kentucky "seemed a mere network of rivers" (Figure 1).

Rafinesque's list of plants and trees peculiar to this region was limited. He included sycamore (*Platanus occidentalis*) and cottonwood (*Populus deltoides*), which are widely distributed throughout the state, but his inclusion of catalpa (*Catalpa speciosa*) and spiderlily (*Hymenocallis caroliniana*) suggests that he had some knowledge of the western third of the state where these two species are found in lowland forests.

2. The Central Region is the Bluegrass Region, which is bordered by the Knobs. He noted that this fertile limestone area was mostly under cultivation even in 1819. He also recognized a low species diversity including native and naturalized plants. Meijer (1973) pointed to Rafinesque's interest in the flora of introduced weeds and how this invasion coincided with the arrival of white settlers.

Rafinesque's statement that "it is also highly singular that in this region, the woods are open as parks, without shrubs and with very few plants, except grass or some social weeds," is significant. He was describing the blue ash–oak savanna woodland, which was the characteristic vegetation of the Bluegrass region (Bryant 1983; Bryant et al. 1980). His list of species in no way characterizes this region.

3. The Hilly Region included the Knobs as well as extensions of broken ground from the Big Sandy River in the northeast to the Green River in the west. The geologic substrate mentioned by Rafinesque included sandstone, limestone, or slate. He found that the vegetation approximated that of Pennsylvania and Virginia, with which he was familiar. The

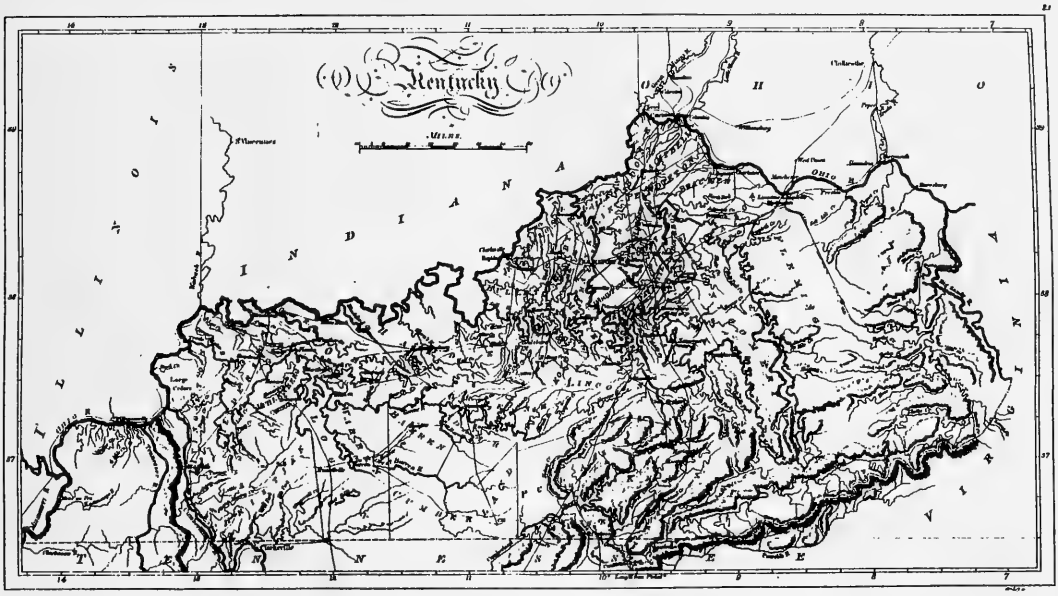

Figure 1. The Samuel Lewis 1814 map of Kentucky showing the principal features. Note the lack of information on the mountains of eastern Kentucky but the detail of the many streams and rivers. After Clark (1979).

plants listed, especially redcedar (*Juniperus virginiana*), pitch pine (*Pinus rigida*), and deerberry (*Vaccinium stamineum*) are found on various substrates in the Knobs Region. Muller and McComb (1983) described the upland vegetation of the Knobs as being dominated by oaks, none of which Rafinesque mentioned.

4. The Mountain Region was included with the Hilly region, but Rafinesque acknowledged that he had not visited this part of the state. He had been told of the similarity of the mountains to the Allegheny region. This region included the Cumberland Mountains and the highest ridges. The presence of mountain laurel (*Kalmia latifolia*) and mountain tea (*Gaultheria procumbens*) and a few of the plants listed for the Hilly Region are inadequate to describe this area. Braun (1950) characterized this portion of eastern Kentucky as the center of the mixed mesophytic forest, the richest forest type in eastern North America.

5. The Barren Region encompassed the open country in the western and southern parts of the state. Rafinesque noted that the vegetation consisted of "a growth of plants very similar to that of the prairies of Ohio, Indiana, and Illinois." He pointed out the sparsity of trees and the presence of licks, which tended to attract grazing animals (e.g., buffalo). Rafinesque's list of plants of this region is perhaps more representative than his lists for the other regions he described. Bluestems (*Andropogon* spp.), Indian grass (*Sorghastrum nutans*), prairie-clovers (*Petalostemon candidum* and *P. purpureum*), prairie-dock (*Silphium terebinthinaceum*), and purple coneflower (*Echinacea purpurea*) are still found in barren remnants (Baskin and Baskin 1978; Braun 1950). Rafinesque was most interested in the Barrens; along with his collaborator, Dr. Charles Short, he collected there extensively (Meijer 1973). Rafinesque's Barren Region is part of the Pennyroyal or Mississippi Plateau.

As can be determined from the above descriptions of Botanical Regions, Rafinesque in 1819 was more familiar with those portions of Kentucky from Lexington westward. Since he travelled to and kept an association with some of the residents of Harmonie (later New Harmony) in southwestern Indiana (Thompson 1898), it seems probable that he botanized on his trips.

In conclusion, it was indeed fortunate that Rafinesque made reference to different botanical regions as being present in Kentucky. He noted some of the plants, although not always

those that might best characterize a community or region. He mentioned topography, geologic substrate, and soils as being of importance in regional differentiations. It is unfortunate that he did not continue his interest and study of plant associations at a time prior to their manipulation and fragmentation by the onrush of settlement.

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Effect of Light on Daily Emergence of Cercariae of the Trematodes *Echinostoma trivolvis* (Echinostomatidae) and *Cephalogonimus vesicaudus* (Cephalogonimidae) from Natural Infections of the Snail *Helisoma trivolvis* (Planorbidae) at Owsley Fork Reservoir, Kentucky

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ABSTRACT

Naturally infected snails, *Helisoma trivolvis*, releasing cercariae of *Echinostoma trivolvis* and *Cephalogonimus vesicaudus*, were collected from Owsley Fork Reservoir in northern Jackson and southern Madison counties, Kentucky, in June or July 1990, 1992, and 1995. Emergence of cercariae from snails was assessed at 26° C for 24 h under the following photoperiod regimens: (1) 12 h light:12 h dark; (2) 12 h dark:12 h light (inverted photoperiod); (3) 24 h light; and (4) 24 h dark. In the split photoperiods, maximum cercarial emergence of *E. trivolvis* and *C. vesicaudus* was significantly greater in the 12 h of light and 12 h of dark, respectively. Photoperiod inversion resulted in a complete reversal in timing of cercarial release for both species. Continuous light or dark for 24 h induced continuous release of cercariae of both species. No significant change was observed in the timing of peak cercarial emergence of *C. vesicaudus* in the 1990, 1992, and 1995 studies using a 12 h light:12 h dark photoperiod, but a marked difference was noted for *E. trivolvis* in 1995 when peak cercarial release was shifted to the dark phase of the 24 h photoperiod. These results are discussed with regard to (1) exogenous vs. endogenous factors affecting cercarial release and (2) the use of cercarial emergence patterns for identifying parasitic strains.

INTRODUCTION

The timing of the emergence of trematode (subclass Digenea) cercariae from their snail host has been linked to activity of the next host in the life cycle in several studies (Lewis, Welsford, and Uglem 1989; Theron 1984), but the physiological triggers associated with cercarial release are poorly understood. It has been suggested that in-vitro cultivation of intramolluscan stages in the absence of snail tissue will be necessary to clarify the exact mechanisms/triggers (Glaudel and Etges 1973). In addition to a possible endogenous circadian rhythm, these triggers may be influenced by a number of environmental factors including temperature, humidity, oxygen, pH, and light (Smyth and Halton 1983). These exogenous factors either act directly on the parasite or are mediated indirectly through the snail host.

That light triggers or inhibits cercarial release from the snail host in many species of digenetic trematodes is well known. Reversal of the 24 h light:dark photoperiod (i.e., photoperiod inversion) often results in complete reversal in the pattern of cercarial release (Asch 1972; Giovannola 1936; Glaudel and Etges 1973; Luttermoser 1955; Oliver 1951; Wagenbach and Alldredge 1974). Such a reversal provides evidence that the pattern of release is not a true circadian rhythm but is mediated by the "rhythm of the snail which is controlled to some extent by illumination" (Asch 1972). However, a number of these studies did not assess whether cercarial emergence ceases to be rhythmic if the light or dark is of a constant magnitude (i.e., continuous light or dark). This criterion must be met to rule out the presence of an endogenous circadian rhythm within the parasite itself (Wagenbach and Alldredge 1974).

Distinct strains of digenetic trematodes have been documented in several studies based on photoperiod and cercarial emergence patterns (Gumble et al. 1957; Riley and Uglem 1995; Theron 1984), but, to our knowledge, shifting patterns of cercarial emergence in a species in the same habitat over time have not been observed. Further studies of this nature might provide (1) insight into use of cercarial emergence patterns as stable biological characteristics for some species of digenetic trematodes and (2) a method for identifying different strains of a species from the same habitat.

Little information is available concerning the effect of light on emergence of cercariae of *Echinostoma trivolvis* (Echinostomatidae)

(Schmidt and Fried 1996), and no studies of this nature have been conducted with cercariae of *Cephalogonimus vesicaudus* (Cephalogonimidae). Both species are parasites of the snail *Helisoma trivolvis* (Planorbidae) at Owsley Fork Reservoir in northern Jackson and southern Madison counties, Kentucky (Rosen et al. 1994). Thus, the objectives of our study were to determine (1) the effect of light on the release of *E. trivolvis* and *C. vesicaudus* cercariae from natural infections of *H. trivolvis* and (2) whether different strains of these species exist in the reservoir based on photoperiod and cercarial emergence patterns assessed over several years.

MATERIALS AND METHODS

Specimens of *H. trivolvis* were collected from Owsley Fork Reservoir in June or July 1990, 1992, and 1995. A brief description of the study site is provided in Rosen et al. (1994). Within 2 h of their collection, snails were placed individually into 50 ml beakers filled with 40 ml of filtered reservoir water, incubated at 26° C with a 12 h light (0700–1859):12 h dark (1900–0659) cycle, and screened for cercarial emergence at 1400, 2030, and 0830 for 1 day. Snails with mature infections (i.e., releasing cercariae of *E. trivolvis* or *C. vesicaudus*) were then immediately acclimated for 24 h at 26° C to one of the following photoperiod regimens: (1) 12 h light (0700–1859):12 h dark (1900–0659), (2) 12 h dark (0700–1859):12 h light (1900–0659), (3) 24 h light, and (4) 24 h dark. Only the 12 h light:12 h dark cycle was used to compare emergence patterns in the 1990, 1992, and 1995 studies.

Following the initial 24 h acclimation period, cercarial counts were made every 2 or 4 hours for 24 h. At each time interval, snails were transferred into new beakers and immediately placed back in their designated photoperiod regimens. The number of cercariae in the original beaker was determined with the aid of a dissecting microscope by direct counts or by averaging ten 0.1 ml aliquots taken from a uniform cercarial suspension that was then adjusted to the number of cercariae/40 ml. The latter technique was necessary when cercarial emergence was copious in a sampling interval.

Cercarial release in the six 2 h light periods and six 2 h dark periods were separately pooled and compared with a Mann-Whitney test to determine if the median cercarial release for the two species was significantly different in the split 24 h photoperiods. In the 12L:12D and 12D:12L analyses, the light time interval consisted of 11.5 h of light and 0.5 h of dark, while the dark interval consisted of 11.5 h of dark and 0.5 h of light due to the times selected to evaluate cercarial emergence. A Kruskal-Wallis test was used to determine if the timing of the mean peak for cercarial release during a 24 h period varied significantly among the years 1990, 1992, and 1995 for *E. trivolvis* and *C. vesicaudus*. A probability of $P < 0.05$ was considered significant for all statistical tests. Means are reported with associated standard errors.

RESULTS

A significant difference (Mann-Whitney test; $P = 0.0233$) was found in the median cercarial release for *E. trivolvis* between the light and dark intervals. An average of 156.7 ± 65.5 and 5.0 ± 2.1 cercariae were released in the light and dark periods, respectively. Similarly, a significant difference (Mann-Whitney test; $P=0.0233$) was found in the mean cercarial release for this species in the inverted photoperiod. An average of 5.0 ± 2.1 and 272.7 ± 41.3 cercariae were released in the dark and light time intervals, respectively. Maximum emergence occurred at 1430 and 0030; little or no emergence was observed during the 2 h intervals of the dark periods (Figures 1a and 1b). Continuous emergence was apparent under conditions of constant light (Figure 1c; range = 12–187 cercariae/2 h interval) and dark (Figure 1d; range = 7–130 cercariae/2h interval).

A significant difference (Mann-Whitney test: $P = .0001$) was found for the median cercarial release of *C. vesicaudus* between the light and dark intervals. An average of 153.8 ± 57.5 and 2021 ± 349.7 cercariae was released in the light and dark periods, respectively. A significant difference (Mann-Whitney test: $P = .0003$) was also found for the median cercarial release in the inverted photoperiod. An average of 1713.6 ± 249.5 and 52.07 ± 135.9 emerged in the dark and light time intervals, respectively. Peak emergence was ob-

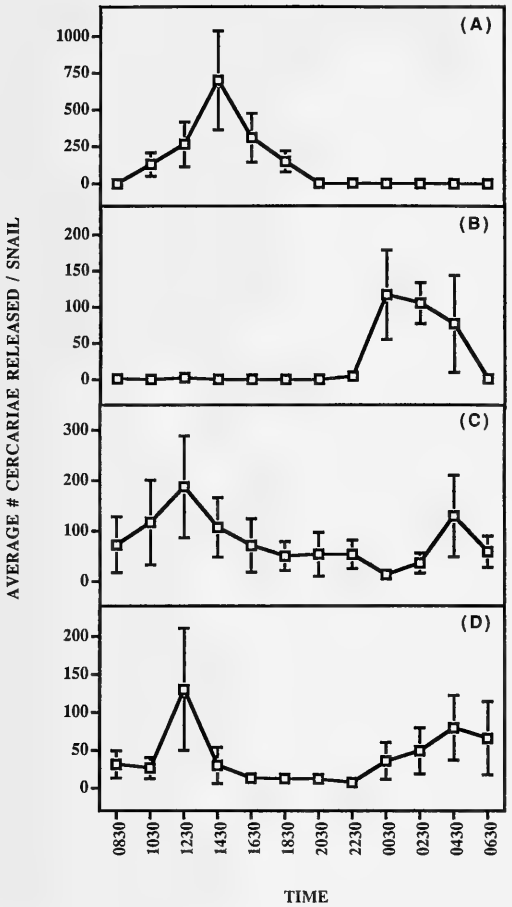

Figure 1. Effect of light on the mean number ± SE of *Echinostoma trivolvis* cercariae released from the snail *Helisoma trivolvis* at 2 h intervals over 24 h at 26° C under the following photoperiods: (a) 12 h light (0700–1859):12 h dark (1900–0659), (b) 12 h dark (0700–1859):12 h light (1900–0659), (c) 24 h light, and (d) 24 h dark. An average of five infected snails was used for each photoperiod.

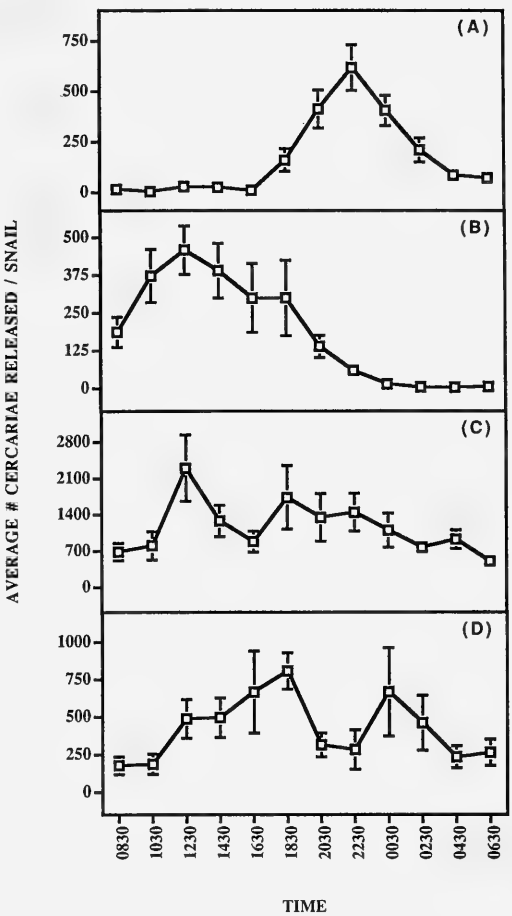

Figure 2. Effect of light on the mean number ± SE of *Cephalogonimus vesicaudus* cercariae released from the snail *Helisoma trivolvis* at 2 h intervals over 24 h at 26° C under the following photoperiods: (a) 12 h light (0700–1859):12 h dark (1900–0659), (b) 12 h dark (0700–1859): 12 h light (1900–0659), (c) 24 h light, and (d) 24 h dark. An average of 15 infected snails was used for each photoperiod.

served at 2230 and 1230 and little or no emergence occurred during the 2 h intervals of the light periods (Figures 2a and 2b). Continuous cercarial emergence was observed under conditions of constant light (Figure 2c; range = 499–2298 cercariae/2 h interval) and dark (Figure 2d; range = 177–808 cercariae/2 h interval).

The long-term study showed that the mean daily time for peak cercarial emergence was significantly different for *E. trivolvis* (Kruskal-Wallis test: P = .0002), but not for *C. vesicaudus* (Kruskal-Wallis test: P=.9482). Peak emergence time for *E. trivolvis* shifted to 2230 (dark period) in 1995 from 1430 (light period) in 1990 and 1992 (Figure 3). By comparison, the peak emergence time of *C. vesicaudus* remained fixed at 2230 (dark period) over the same time (Figure 4).

DISCUSSION

Light significantly triggered the release of *E. trivolvis* cercariae and inhibited the emergence of *C. vesicaudus* cercariae in our 1990 and 1992 studies. Photoperiod inversion was

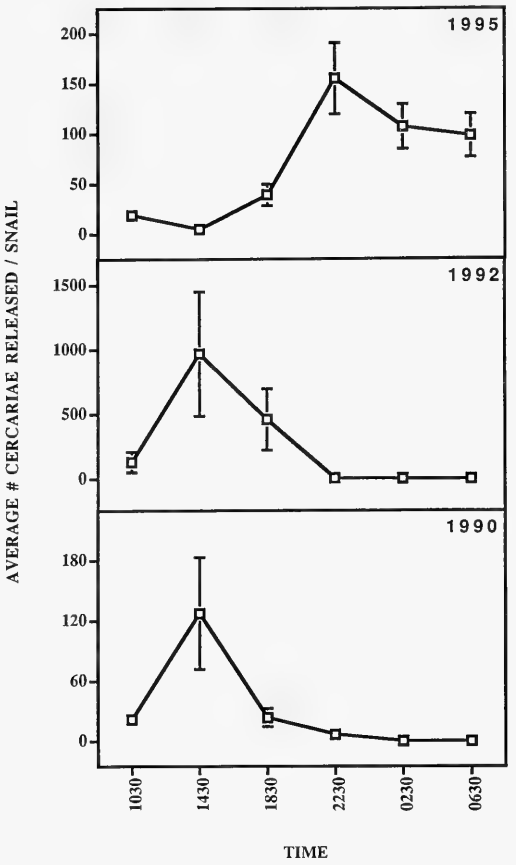

Figure 3. Comparison of the effect of light on the mean number ± SE of *Echinostoma trivolvis* cercariae released from the snail *Helisoma trivolvis* in 1990, 1992, and 1995. Naturally infected snails were collected in June or July from Owsley Fork Reservoir, Kentucky, and assessed at 4 h intervals over 24 h at 26° C under a 12 h light (0700–1859):12 h dark (1900–0659) photoperiod. An average of 10 infected snails was used for each summer/experiment.

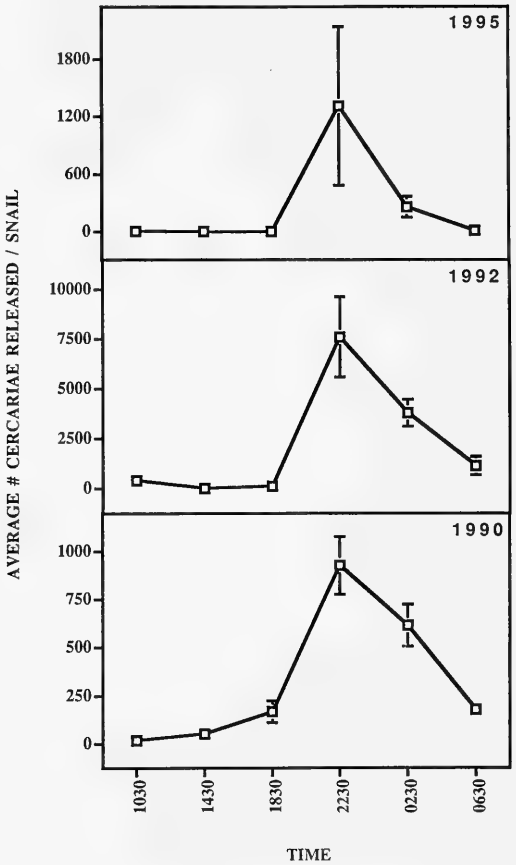

Figure 4. Comparison of the effect of light on the mean number ± SE of *Cephalogonimus vesicaudus* cercariae released from the snail *Helisoma trivolvis* in 1990, 1992, and 1995. Naturally infected snails were collected in June or July from Owsley Fork Reservoir, Kentucky, and assessed at 4 h intervals over 24 h at 26° C under a 12 h light (0700–1859):12 h dark (1900–0659) photoperiod. An average of 10 infected snails was used for each summer/experiment.

accompanied by a complete reversal in the timing of peak cercarial release for both species. This suggested that cercarial emergence of the species is subject to control by the exogenous photoperiod under conditions of constant temperature. By contrast, Schmidt and Fried (1996) found that light vs. dark did not affect the intensity of *E. trivolvis* cercarial emergence. Their study provided no acclimation period for snails to the tested conditions (i.e., snails were maintained in the dark at 12° C and then placed in conditions of light or dark at 28–29° C for an immediate 1 hour evaluation of cercarial emergence). The difference between our results and those of Schmidt and Fried (1996) may be more attributable to experimental design than to possible strain differences of *E. trivolvis*.

Wagenbach and Alldredge (1974) found that cercariae of the digenean *Plagiorchis micracanthos*, which normally emerge during the dark phase of a 12 h light:12 h dark cycle, show a rhythmic emergence in continuous dark. They indicated that this latter phenomenon provided evidence for an innate emergence rhythm, "which is synchronized to photoperiod by the inhibition of light" (Wagen-

bach and Alldredge 1974). Such an "entrainment" of the endogenous rhythm to light:dark cycles has also been suggested for emergence of *Proterometra macrostoma* cercariae, which normally emerge during the dark phase of a light:dark cycle but show peaks of emergence under conditions of continuous light (Lewis, Welsford, and Uglem 1989). The lack of such a distinct rhythm under conditions of continuous light or dark in our study suggests that no circadian rhythm exists for *E. trivolvis* or *C. vesicaudus* in *H. trivolvis,* but longer term studies (i.e., in excess of 24 h) will be required to clarify this.

No change was observed in timing of peak cercarial emergence of *C. vesicaudus* in the 1990, 1992, and 1995 studies, but a significant shift was noted for *E. trivolvis. Cephalogonimus vesicaudus* is an autogenic species that completes its life cycle as an adult in the small intestine of the spiny softshell turtle *Trionyx spiniferus,* which is present at Owsley Fork Reservoir. The timing of peak cercarial release is apparently a stable biological characteristic for the population of this species at this locale. By comparison, mammals (e.g., muskrats) and a variety of migratory waterfowl (e.g., Canadian geese, mallards, etc.) present at Owsley Fork likely serve as definitive hosts for *E. trivolvis* in this habitat. The migratory nature of the avian hosts provides an opportunity for periodic introduction of new strains of *E. trivolvis* into the existing Owsley Fork population of this species. This may be indirectly assessed by observation of new cercarial release patterns appearing in the population over time. In a somewhat similar study, Theron (1984) documented chronobiological variation (i.e., one strain experienced an earlier daily cercarial release than the second strain) in two *Schistosoma mansoni* populations from the same area, but different ecological foci. He linked this variation to the amount of murine host participation in the life cycle. Further experiments determined that the cercarial emergence pattern of *S. mansoni* has a genetic basis that is a consequence of selective pressures exerted by different host species (Theron and Combes 1988). It has also been noted that the response of *Schistosoma japonicum* cercariae to light varies with the geographical strain of the species (Gumble et al. 1957). Both daily and seasonal differences in cercarial emergence have been recorded for eight strains of *P. macrostoma* (Riley and Uglem 1995). Development of these strains has been linked to the species composition of sunfish definitive hosts in a specific geographical region (Riley and Uglem 1995). In the present study, since both potential mammalian and waterfowl hosts of *E. trivolvis* exist in the same "ecological focus," the resulting emergence patterns for this species at Owsley Fork likely represent blending of patterns from several different strains. The changing prevalence of these different strains over time might explain the variation observed in the long-term emergence patterns for *E. trivolvis* cercariae at the reservoir. Molecular techniques recently used to differentiate and establish relationships of species in the genus *Echinostoma* (Morgan and Blair 1995; Petrie, Burg, and Cain 1996; Sloos et al. 1995) may be utilized in future studies to establish genetic differences between these proposed strains of *E. trivolvis.*

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Classifying Free Bieberbach Groups

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ABSTRACT

Let F be a free group on n letters and G be a finite group. To each epimorphism $\in: F \to G$ is associated a free Bieberbach group F/N' where N is the kernel of the epimorphism $\in$ and N' is the commutator subgroup of N. In low dimensions, the free Bieberbach groups are described. In high dimensions, the free Bieberbach groups are described for the case when G is cyclic or dihedral. A theorem is given describing a method for factoring certain free Bieberbach groups as a semi-direct product of a lower dimensional free Bieberbach group and the integral group ring ZG.

INTRODUCTION

Let G be a finite group. A group S is called a crystallographic group (on the point group G) if S contains a finitely generated maximal abelian torsionfree subgroup A of finite index so that $S/A \cong G$ and G acts faithfully by conjugation on A. The rank of A is called the dimension of the crystallographic group. A torsionfree crystallographic group is called a Bieberbach group (Charlap's definition [1986, p.74]). The dimension of the Bieberbach group is given by Schrier's formula $n=|G|(rnk(F)-1)+1$, where F is the free group on k generators with $k=rnk(A)$ (Lyndon and Schupp 1977, p.16).

It is well known that B is an n-dimensional Bieberbach group if and only if $\mathbb{R}^n/B$ is an n-dimensional flat manifold. Let $\langle X \mid R \rangle$ be a presentation of G with finitely many generators, that is, $G \cong F/N$ where F is the free group on X and N is the normal closure of R in F. This may be expressed in terms of the short exact sequence, $1 \to N \to F \to G \to 1$. Let $N'=[N,N]$ be the commutator subgroup of N, $\overline{N}=N/N'$, and $\overline{F}=F/N'$. The sequence above induces a short exact sequence on $\overline{N}$ and $\overline{F}$, $0 \to \overline{N} \to \overline{F} \to G \to 1$. The action by conjugation of G on $\overline{N}$ gives $\overline{N}$ a ZG-module structure. $\overline{N}$ is referred to as the relation module of F/N (Linnell 1981). $\overline{F}$ is known to be a Bieberbach group and will be referred to as a free Bieberbach group (Farkas' definition [1981]). It is noted that of the flat manifolds associated to these free Bieberbach groups, some are orientable and some are non-orientable.

Two free Bieberbach groups $\overline{F}_1$ and $\overline{F}_2$ are isomorphic if there exists a ZG-module isomorphism α and a group isomorphism β such that the following diagram commutes:

$$\begin{array}{ccccccc} 0 & \to & \overline{N}_1 & \to & \overline{F}_1 & \to & G \to 1 \\ & & \alpha\downarrow & & \beta\downarrow & & =\downarrow \\ 0 & \to & \overline{N}_2 & \to & \overline{F}_2 & \to & G \to 1 \end{array}$$

where $\overline{N_1}$ and $\overline{N_2}$ are the relation modules associated to $\overline{F}_1$ and $\overline{F}_2$ respectively.

Since $\overline{N}$ is a ZG-module with free abelian rank equal to n, it is convenient to write $\overline{N}$ as Z^n when we are viewing $\overline{F}$ as an actual crystallographic group. Further, $Aut(\overline{N}) \cong GL(n,Z)$ so the action of G on $\overline{N}$ induces a representation $\varphi: G \to GL(n,Z)$. Since $\overline{F} \cong Z^n \times G$ (as sets), then we may define multiplication on $\overline{F}$ by $(n,g)(m,h)=(n+\varphi(g)m+f(g,h),gh)$, where $n,m \in Z^n$ (thought of as column vectors), $g,h \in G$, and $f \in H^2(G,Z^n)$. The 2-cocycle $f: G \times G \to Z^n$ may be constructed from $i(f(g,h))=s(g)s(h)s(gh)^{-1}$, where $s: G \to \overline{F}$ is a normalized set map and $i: Z^n \to \overline{F}$ is the inclusion map. It is known (Tennant and Turner 1992) that $\overline{F}$ is torsionfree. For the case, $G \neq \{1\}$, this is equivalent to the 2-cocycle $f: G \times G \to Z^n$ being nontrivial.

For a given dimension $n > 0$, there is a free Bieberbach group isomorphic to Z^n where the point group is $G \cong \{1\}$. Note that in this case, the compact flat manifold associated to the free Bieberbach group Z^n is the n-torus, the orbit space $\mathbb{R}^n/Z^n$.

FREE BIEBERBACH GROUPS IN LOW DIMENSIONS

Let G be a finite group. In dimension n = 1, a free Bieberbach group (other than Z with trivial point group) must have the point group $G \cong Z_2$. The compact flat manifold associated to this group is the circle. In dimension n = 2, a free Bieberbach group must have point group $G \cong \{1\}$ and $rnk(F)=2$ so $\overline{F} \cong Z^2$. Therefore, the first case of a Bieberbach group B that is not a free Bieberbach group is defined by the sequence $0 \to Z^2 \to B \to Z_2 \to 1$ where $\mathbb{R}^2/B$ is homeomorphic to the Klein bottle.

In dimension n = 3, a free Bieberbach group (other than Z^3 with trivial point group) must have $G \cong Z_2$ and $rnk(F)=2$. A result of Linnell (1981) states that if two epimorphisms ϵ_1: $F_1 \to G$ and ϵ_2: $F_2 \to G$, with $rnk(F_1)=rnk(F_2)$, are Nielsen equivalent, then their associated free Bieberbach groups are isomorphic. Since any two epimorphisms ϵ_1, ϵ_2: $F(a,b) \to Z_2$ are Nielsen equivalent, consider the epimorphism derived from the presentation of Z_2 given by $\langle a,b \mid a^2, b\rangle$. This presentation and the action of Z_2 on Z^3 induces a monomorphism φ: $Z_2 \to GL(3,Z)$. Since $\det(\varphi(a))=-1$, the flat manifold associated to this free Bieberbach group is non-orientable. The crystallographers refer to this particular manifold (crystal) as belonging to the monoclinic class (Brown et al. 1978).

In dimension n = 4, a free Bieberbach group (other than Z^4 with trivial point group) must have point group $G \cong Z_3$ and $rnk(F)=2$. Since there is only one Nielsen equivalence class of epimorphisms ϵ: $F(a,b) \to Z_3$, there is only one free Bieberbach group with point group $G \cong Z_3$. Since 2 does not divide $|G|=3$, the induced monomorphism φ: $Z_3 \to GL(4,Z)$ must satisfy $\det(\varphi(g))=1$ for all $g \in G$ and so it follows that the flat manifold $\mathbb{R}^4/\overline{F}$ is orientable.

SOME FREE BIEBERBACH GROUPS IN HIGH DIMENSIONS

Let k be a positive integer. If k is odd, there is one free Bieberbach group of dimension n=k+ 1 where the point group is $G \cong Z_k$. The associated flat manifold is orientable by the same reasoning as above. Suppose k is even, G is a point group with $|G|=k$, and ϵ: $F(a,b) \to G$ is an epimorphism. If $G \cong Z_k$ or $G \cong D_k$, the dihedral group with k elements and φ: $G \to GL(k+1,Z)$ is the monomorphism induced by the action of G on Z^n then there exists $g \in G$ with $\text{ord}(g)=2$ such that $\det(\varphi(g))=-1$. It follows that the associated flat manifold is non-orientable. For the point group, $G \cong Z_k$, all epimorphisms, ϵ: $F(a,b) \to Z_k$ are Nielsen equivalent so all free Bieberbach groups with point group $G \cong Z_k$ are isomorphic. On the other hand, there exist k such that not all epimorphisms ϵ: $F(a,b) \to D_k$ are Nielsen equivalent, so we may not apply Linnell's result to conclude that there is only one (k+1)-dimensional free Bieberbach group with point group $G \cong D_k$. Let ϵ: $F_k \to G$ be an epimorphism where F_k is free on k generators. Suppose another generator is added to F_k and mapped to 1 under ϵ to form a new epimorphism ϵ^*: $F_{k+1} \to G$. The following theorem describes how the respective free Bieberbach groups are related.

Theorem

Let ϵ: $F_k \to G$ and ϵ^*: $F_{k+1} \to G$ be epimorphisms such that $\epsilon^*(x_i)=\epsilon(x_i)$, $1 \le i \le k$ and $\epsilon^*(x_{k+1})=1$, then $\overline{F}^*$ is isomorphic to $\overline{F} x_s ZG$ where $\overline{F}$ and $\overline{F}^*$ are the free Bieberbach groups associated to ϵ and ϵ^* respectively and x_s represents a semi-direct product.

Proof

Recall that $\overline{F}$ may be expressed in terms of the short exact sequence

$$0 \to \overline{N} \xrightarrow{\bar{\imath}} \overline{F} \xrightarrow{\bar{\epsilon}} G \to 1.$$

The action by conjugation of G on $\overline{N}$ induces a monomorphism φ: $G \to \text{Aut}(\overline{N})$. Let s: $G \to \overline{F}$ be a normalized set map and define a 2-cocycle f: $G \times G \to \overline{N}$ by $\bar{\imath}(f(g,h))=s(g)s(h)s(gh)^{-1}$. By viewing $\overline{F}$ as setwise equal to $\overline{N} \times G$ we may define multiplication on $\overline{F}$ by $(\bar{n},g)(\bar{m},h)=(\bar{n}+\varphi(g)\bar{m}+f(g,h),gh)$. A theorem of Lyndon (1962) states that $\overline{N}^* \cong \overline{N}/ZG$ as ZG-modules. The isomorphism α: $\overline{N}^* \to \overline{N}/ZG$ induces an isomorphism A: $\text{Aut}(\overline{N}^*) \to \text{Aut}(\overline{N}/ZG)$ and the projection p: $\overline{N}/ZG \to \overline{N}$ induces a projection P: $\text{Aut}(\overline{N}/ZG) \to \text{Aut}(\overline{N})$ so that the action of G on $\overline{N}^*$ induces a monomorphism φ^*: $G \to \text{Aut}(\overline{N}^*)$ that satisfies $P(A(\varphi^*(g)))$. The free Bieberbach group may be expressed in terms of the short exact sequence

$$0 \to \overline{N} \xrightarrow{\bar{i}^*} \overline{F} \xrightarrow{\bar{\epsilon}^*} G \to 1.$$

A set map $s^*: G \to \overline{F}^*$ can be chosen so that the 2-cocycle $f^*: G\times G \to \overline{N}^*$ defined by $\bar{i}^*(f^*(g,h))=s^*(g)s^*(h)s^*(gh)^{-1}$ satisfies $p(\alpha(f^*(g,h)))=f(g,h)$. Define the epimorphism $\bar{\epsilon}: \overline{N}^* \to \overline{N}$ by $\bar{\epsilon}(\overline{n}^*)=p(\alpha(\overline{n}^*))$. Then $0 \to ZG \to \overline{N}^* \to \overline{N} \to 1$ is a short exact sequence of ZG-modules. The group $\overline{F}^*$ is equal to $\overline{N}^*\times G$ (as sets) so define the epimorphism $\hat{\epsilon}: \overline{F}^* \to \overline{F}$ by $\hat{\epsilon}(\overline{n}^*, g)=(\bar{\epsilon}(\overline{n}^*),g)$. The $\ker(\hat{\epsilon})\cong ZG$ and we have the following commutative diagram:

$$\begin{array}{ccccccc}
 & & 0 & & 0 & & \\
 & & \downarrow & & \downarrow & & \\
0 & \to & ZG & \to & ZG & \to & 1 \\
 & & \downarrow & & \downarrow & & \downarrow \\
0 & \to & \overline{N}^* & \to & \overline{F}^* & \to & G \to 1 \\
 & & \downarrow & & \downarrow & & \downarrow \\
0 & \to & \overline{N} & \to & \overline{F} & \to & G \to 1 \\
 & & \downarrow & & \downarrow & & \downarrow \\
 & & 1 & & 1 & & 1
\end{array}$$

Define the normalized set map $\tilde{s}: \overline{N} \to \overline{N}^*$ by $\tilde{s}(\overline{n})=\alpha^{-1}(\overline{n}/\overline{0})$ where $\overline{0}$ is the additive identity in ZG. The set map $\hat{s}: \overline{F} \to \overline{F}^*$ defined by $\hat{s}(\overline{n},g)=(\tilde{s}(\overline{n}),g)$ is then a homomorphism and so it follows that the sequence $0 \to ZG \to \overline{F}^* \to \overline{F} \to 1$ splits and $\overline{F}^*\cong \overline{F}x_sZG$.

REMARK

For the case where $\overline{F}^*\cong \overline{F}x_sZG$, if $\overline{F}$ has dimension n then $\overline{F}^*$ has dimension $n+|G|$ and $\overline{F}^*$ can be viewed as a free Bieberbach group which has a subgroup, $\overline{F}$, that is also a free Bieberbach group. To crystallographers, $\overline{F}^*$ belongs to the class of superspace groups [Janner and Janssen 1979]. Equivalently, $\mathbb{R}^n/\overline{F}$ is an n-dimensional flat manifold sitting inside of the $(n+|g|)$-dimensional flat manifold $\mathbb{R}^{n+|G|}/\overline{F}^*$.

Corollary 1

If $Epi(F_{k+1},G)$ contains only one Nielsen equivalence class and $\in: F_k \to G$ and $\in^*: F_{k+1} \to G$ are epimorphisms then $\overline{F}^*\cong \overline{F}x_sZG$.

As an illustration, the (k+1)-dimensional free Bieberbach group (k odd) with point group $G\cong Z_k$ can be factored as the semi-direct product $\overline{F}\cong Zx_sZ(Z_k)$.

There are certain point groups with the following property (known as the "swap" property [Tennant and Turner 1992]).

Swap Property

Given any two epimorphisms $\in_1,\in_2: F_k \to G$, there exists a finite sequence of epimorphisms $e_1,e_2 \cdots, e_r$ where $e_i: F_{k+1} \to G$ for i odd and $e_i: F_k \to G$ for i even, $1\le i\le r$ such that for each of the pairs of epimorphisms, $(\in_1,e_1)$, (e_i,e_{i+1}) for $1\le i\le r-1$, and $(e_r,\in_2)$, the epimorphism on F_k is isomorphic to the epimorphism on F_{k+1} restricted to some subset (of k basis elements) of F_{k+1}.

Corollary 2

Let G be a finite group, and $\in_1,\in_2: F_k \to G$ be two epimorphisms. If G possesses the swap property then $\overline{F}_1x_sZG\cong \overline{F}_2x_sZG$ where $\overline{F}_1$ and $\overline{F}_2$ are the free Bieberbach groups associated to $\in_1$ and $\in_2$ respectively.

Example

The class of finitely generated abelian groups possesses the swap property (Tennant and Turner 1992). Since point groups must be finite, we are concerned only with the subclass of finite abelian groups.

Corollary 3

If G is a finite abelian group and $\in_1,\in_2: F_k \to G$ are two epimorphisms then $\overline{F}_1x_sZG\cong \overline{F}_2x_sZG$ where $\overline{F}_1$ and $\overline{F}_2$ are the free Bieberbach groups associated to $\in_1$ and $\in_2$ respectively.

When $\overline{F}_1x_sZG\cong \overline{F}_2x_sZG$ is said to be one step stably equivalent to $\overline{F}_2$.

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DISTINGUISHED SCIENTIST AND OUTSTANDING TEACHER AWARDS 1996

Distinguished Scientist Award

Dr. D. Allan Butterfield—Professor of Chemistry and Director of Center for Membrane Sciences, University of Kentucky, Lexington, Kentucky

D. Allan Butterfield received the degree of bachelor of arts with highest distinction from the University of Maine in 1968. After a 3-year high-school teaching position in Zimbabwe (then Rhodesia) he began his doctoral work at Duke University where he received the Ph.D. in physical chemistry in 1974. After a 1-year NIH post-doctoral fellowship in 1974, also at Duke, he joined the Department of Chemistry at the University of Kentucky in fall 1975 as an assistant professor. In 1983 he was promoted to the rank of professor. Since 1986 he has been the director of the University of Kentucky's Center of Membrane Sciences.

Dr. Butterfield has over 140 publications in refereed journals or monographs, an additional 15 publications either in press or submitted for publication, and about 80 published abstracts. He has presented papers at professional meetings throughout the United States and in England, Canada, and Australia. He serves as referee for some 20 journals and organizations. He has been the principal investigator or co-principal investigator of over 20 funded grants valued at nearly $13 million.

Dr. Butterfield's research in the field of membrane science as it pertains to Alzheimer's disease is most impressive. His research allows for a unified understanding of the causes of Alzheimer's disease, contributing to the eventual development of methods for either prevention of the disease or minimization of its effects.

Dr. Butterfield has taught 18 different courses at both the undergraduate and graduate levels and has given invited seminars at universities and colleges. He has directed 17 master's-level and 14 Ph.D.-level students; currently seven graduate students are working under his direction. He has guided 18 undergraduates in NSF sponsored summer research; eight of these students co-authored referreed scientific publications based on their research. Dr. Butterfield has been involved in the placement of about 75 students in graduate and professional positions.

Dr. Butterfield has served on numerous university and department committees. He is a member of several professional organizations and honorary societies, e.g., American Chemical Society, Society of Sigma Xi, Phi Beta Kappa Honorary Society, and Phi Kappa Phi Honorary Society. He serves on the editorial board of *Journal of Membrane Science*. Dr. Butterfield has also been involved with several community organizations, including Habitat for Humanity.

It is obvious that D. Allan Butterfield's research program and teaching are having an immediate positive impact on intellectual growth in the Commonwealth. This in itself is a great accomplishment. However, it is also quite likely that his research, which is providing scientists with a better understanding of the underlying causes of Alzheimer's disease, will lead directly to improvement in the quality of life in the Commonwealth and throughout the world.

Outstanding College/University Science Teacher Award

Dr. Karan Kaul—Professor, Biology Principal Investigator and Program Area Coordinator, KSUCRS, Kentucky State University, Frankfort, Kentucky

Karan Kaul earned the degree of bachelor of science in botany with honors in 1966 and the degree of master of science in botany in 1968 from the University of Delhi. He received a Ph.D. in biology from the University of Kentucky (UK) in 1972. After receiving his doctorate, he remained at UK as a postdoctoral fellow in the Department of Biochemistry until 1977. Dr. Kaul spent 3 years outside Kentucky in research-associate positions before beginning his career at Kentucky State University (KSU) in 1980. Early in his career at KSU most of his time was spent in botanical research. As time passed, however, he began to devote more and more time to teaching. He progressed from assistant professor to associate professor in 1990 and earned the rank of full professor in 1993. Although Dr. Kaul is nationally known as a prominent researcher in botanical and biological studies (with 14 publications since 1991), he has chosen to spend over half of his time in teaching cell biology, vertebrate embryology, histology, principles of biology, botany, and several other biology courses.

Dr. Kaul spends many hours outside the classroom in mentoring students, in coordinating pre-health clinics, and in assisting students in national examinations, sometimes at his own expense. He cares about the education of his students, encouraging their learning through time spent inside and outside the classroom; for this, he has the utmost respect of students and faculty members alike.

Dr. Kaul was listed in *Who's Who Among America's Teachers* this year. The Kentucky Academy of Science is proud to present to Dr. Karan Kaul the 1996 Outstanding University Science Teacher Award.

Outstanding Secondary School Science Teacher Awards

Douglas C. Jenkins—Instructor, Warren Central High School, Bowling Green, Kentucky

Douglas C. Jenkins is co-recipient of the 1996 Kentucky Academy of Science Outstanding Secondary School Science Teacher Award. Mr. Jenkins received the degree of bachelor of science in physics and mathematics from Western Kentucky University (WKU) in 1968. For the next few years, he worked as project engineer for the U.S. Army Night Vision Laboratory in Fort Belvoir, Virginia. During this time, in 1972, he received the Department of Army Civilian Outstanding Performance Award. In 1973 he returned to Kentucky where he worked while continuing his education. He began his career at Warren Central High School in 1976. In 1978, he completed requirements for the degree of master of arts in education from WKU; in 1986 he obtained his Rank I certification.

During his spare time he became a part-time instructor in the Department of Physics and Astronomy at WKU and, more recently, a teacher for the Verbally and Mathematically Precocious Youth Program. At Warren Central he has served as boy's golf and tennis coach. He is an active member in many professional organizations and has served on multiple commissions and task forces. Mr. Jenkins was one of the original planners of the Barren River Imagination Museum of Science, a hands-on science museum that serves surrounding school systems. Through his leadership, most Warren Central High School physics students were involved in preparing and constructing exhibits in 1993. He continues to involve his students in maintaining exhibits and participating in museum programs.

"Doctor J," as Mr. Jenkins is known by students and faculty, has increased interest in science at Warren Central through innovative teaching practices. His teaching incorporates innovative, motivational practices that create opportunities for development of student interest and success. His instruction focuses on learning outcomes. His classes are active and emphasize cooperative and group learning using real-life experiences.

Professional and cooperative, Mr. Jenkins certainly contributes in many ways to the teaching profession. He has long been recognized as an exceptional teacher. In 1983 he received the Presidential Award of Excellence in Science Teaching; in 1986, the Warren County Teacher of the Year Award; and in 1991, the Sallie Mae Teacher Tribute Award.

The Kentucky Academy of Science is proud to present to Douglas C. Jenkins the Outstanding Secondary Science Teacher Award. He is an exemplary representative of the many fine teachers of our Commonwealth.

Janet Yates Ward—Instructor, Reidland High School, Paducah, Kentucky

Janet Yates Ward is co-recipient of the 1996 Kentucky Academy of Science Outstanding Secondary School Science Teacher Award. Ms. Ward received from Murray State University the degree of bachelor of science in 1986, the degree of master of science in biology in 1988, and Rank I certification in 1996. She graduated summa cum laude from her class. In fall 1989 she began her career at Reidland High School. Since that time, she has earned an excellent reputation from students and faculty members alike.

Ms. Ward has made a positive impact on the young people at Reidland. Demanding of her students and creative in her teaching approach, she has a genuine love of science, a love reflected in her attitude. She is instrumental in spurring her students to excellence; many have received awards for their work. As sponsor of the biology competition team, she was instrumental in her students' accomplishments as exhibited by high finishes in competition. One of her students was awarded a full-tuition scholarship to the University of Louisville, and another was invited to present a project at the Kentucky Junior Science and Humanities Symposium sponsored by the University of Louisville. Reidland Science Club, which is sponsored by Ms. Ward, won bronze and silver medals at the State Science Olympiad.

Ms. Ward has been named in *Who's Who Among American Teachers* and has been active on the McCracken County Education Association and National Education Association. She is a member of the Alpha Chi National Honor Society and Kappa Delta Pi Honorary Education Society. In 1994 she was awarded the Golden Apple Achiever Award sponsored by Ashland Inc. Her enthusiasm, her skill at innovation, and her genuine concern for biology education are exemplary. One of her students wrote, "Through Mrs. Ward's biology class, I began to see that science was a great collection of discoveries. I feel I owe to her my utmost gratitude and appreciation for her instruction and ability to convey her love of science, which seems to be contagious!" We hope that many more students will *catch* the love of science from Janet Yates Ward.

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NOTES

Human Myiasis in Kentucky Caused by *Cuterebra* sp. (Diptera: Oestridae).—Species of *Cuterebra* (Diptera: Oestridae) parasitize rodents and rabbits. The rabbit *Cuterebra* can be found also in young dogs and cats (1, 2). Little is known about the host range of this genus in Kentucky. The few documented infestation rates for natural hosts have been quite variable. Over 70% of the trapped specimens of *Peromyscus leucopus*, the white-footed mouse, on Kentucky Lake islands in western Kentucky and Tennessee were infested with *Cuterebra fontinella fontinella* (3). In another study, 36% of *P. leucopus* individuals captured in eastern Tennessee harbored the same oestrid (4). Only three adult specimens of this genus, all identified as *C. fontinella*, are in the entomology collection of the University of Kentucky. No biological information is associated with the material.

The case of myiasis reported herein involved a 19-year-old male. The larva was submitted to the Cooperative Extension Service for identification following its removal from a lesion on the ankle. I determined it to be a late second or early third instar *Cuterebra* sp. The larva was sent to Dr. R. D. Hall, University of Missouri, who confirmed the identification (pers. comm.). Accurate identification of immatures of this group is best accomplished with mature third instars so that cuticular spines and caudal spiracles can be examined (5). Most documented cases of human oestrid myiasis have involved the rabbit-infesting species (6) *C. buccata* and *C. abdominalis*. These parasitize eastern cottontail rabbits and are the two members of this group ranging into Kentucky (R.D. Hall, pers. comm.).

The man answered a set of questions for me that described the general circumstances of the incident at which the infestation might have started and its course. This information is summarized as follows.

The most obvious opportunities for exposure appear to have occurred on four occasions between 5 Sep and 10 Sep 1995 while the man was squirrel hunting in Greenup County, Kentucky. He wore long pants, long socks, and high-topped canvas tennis shoes on all trips. While hunting, he sat at the base of trees in wooded areas for 1 to 2 hours at a time. In most cases he cleared away fallen leaves before sitting on the ground, but occasionally he sat on a fallen tree.

The first indication of a problem was noticed on 14 Sep 1995. He experienced " warm, itching sensation, similar to the feel of a mosquito bite" and discovered a small red bump on skin of the medial surface of an ankle. Two more bumps appeared 2 days later. At that point, the irritation was described as a "gnawing sensation as if the flesh were being torn." Ten days after the initial bump appeared, there were five bumps spaced at about 2-cm intervals along the ankle. At that time, the largest bump was about 2 cm in diameter and protruded about 1.5 cm above the surface of the skin.

Anxious about the increasing size and numbers of lesions, the man visited a physician on 25 Sep 1995. By that time, the largest bump had "come to a head like a boil." The wound was diagnosed as a spider bite; topical application of a hydrocortisone cream was recommended for the area. After returning home, the man squeezed a single bot larva from the boil-like spot. The area itched for about a week after the larva was removed but healed normally.

Following the anxiety associated with the removal of a live maggot from the boil, the young man in this case was relieved to learn the identification of the insect. He remembered that he had occasionally encountered mature bot larvae in subdermal cysts or warbles when skinning squirrels taken on previous hunting trips.

Eggs of *Cuterebra* spp. are laid near burrows or areas frequented by the host (7). Mature eggs can hatch rapidly in response to stimulation of the host, such as an increase in air temperature and CO_2 concentration (8). These maggots can complete a portion of their development in humans (9). Human infestations usually occur when an individual spends some time near a host lair and comes in contact with eggs.

Some human subjects of oestrid myiasis are aware of a "stinging" feeling. This probably occurs as the larvae use their mouth hooks to enter the skin. Penner (10) observed the activity of three *Cuterebra* larvae intentionally placed on his skin. The stinging sensation developed within 9 to 17 minutes and as the larvae entered the skin. Complete penetration took at least 18 minutes. Edema and reddening of the area developed and persisted for 2 weeks even though the larvae were removed immediately after penetration.

The site of infestation, the ankle, is unusual in this Kentucky case. Rice and Douglas (11) reviewed five previously reported cases and documented two new ones. Four were on the face in or around the nose or eyes. The remainder were on the neck or torso. In two of the cases, the individual was outdoors at the time and felt the initial penetration. Baird, Podgore, and Sabrosky (6) summarized the 30 cases of human infestation by *Cuterebra* known to 1982; Baird, Baird, and Sabrosky (5) provided an update 7 years later. All but four infestations were on the torso or head. The infestation site for three was unknown and one was on the scrotum.

In this case from Kentucky, there was a delay of 4 days between the last hunting trip and skin penetration by the maggot. These trips were the most obvious times that this individual could have come in contact with *Cuterebra* oviposition sites. However, a few cases indicate no history or activity that places the person near oviposition sites. In the Kentucky case, an egg may have been picked up and remained on a shoe until development was complete or it was stimulated to hatch.

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Abstracts of Some Papers Presented at the 1996 Annual Meeting of the Kentucky Academy of Science

AGRICULTURAL SCIENCES

Geographic differentiation and allozyme variation in pawpaw (*Asimina triloba*, Annonaceae). HONGWEN HUANG* and DESMOND R. LAYNE, Land-Grant Program, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

The pawpaw (*Asimina triloba*) is the largest tree fruit native to the United States and the only temperate member of the tropical custard apple family. In 1994, Kentucky State University was established as the USDA-ARS-National Clonal Germplasm Repository for *Asimina* spp. Seedling trees from 400 pawpaw accessions representing 70 distinct geographic regions from 17 states are currently being grown at our research farm. In a preliminary study, 32 pawpaw cultivars or advanced selections were assayed in 30 enzyme systems, using an isoelectric focusing polyacrylamide slab gel system of pH 4–9. Twelve enzymes produced high resolution without tissue specificity and were further used for evaluation of allozyme diversity of geographic populations. We discuss the degree of genetic diversity within populations and differentiation between populations as evaluated by the expected heterozygosity (He), the proportion of polymorphic loci (P), the average number of alleles per locus (A), Chi-squared analysis of allele frequency heterogeneity, Nei's standard genetic distance (D), and identity (I). Dendrograms were generated by cluster analysis using the unweighted pair group method to demonstrate the relationships of geographic populations in the 17 states evaluated. The strategy for germplasm conservation and cultivar development through breeding is discussed. Issues related to establishment of a "core" collection are addressed.

Influence of shade and root-zone modification on early growth and development of pawpaw (*Asimina triloba*, Annonaceae) seedlings grown in the greenhouse. DESMOND R. LAYNE, Land-Grant Program, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

This experiment was designed to determine the optimal light level for growing pawpaw (*Asimina triloba*) seedlings in the greenhouse. In addition, we wanted to determine if modifying the root-zone would impact seedling growth and development. We examined the individual and combined effects of shade and root-zone modification on early growth and development of seedlings. Experimental treatments were imposed from sowing until the plants were destructively harvested. The experimental design was a split-plot where blocking was done by position in the greenhouse. The main plot of the experiment was shade. This was accomplished by growing seedlings under a wooden frame covered with shade cloth to reduce incident light intensity received by the plant by 30, 55, 80, or 95%. The control treatment was 0% shade or ambient greenhouse light level. The split plot was root-zone modification. Half of all growing containers were untreated (control) while the other half were painted with Spin-Out™, a commercially available product that stimulates development of a finer root system. There were 40 replicate seedlings per experimental treatment combination per block. Seedling shoot length and unfolded leaf number was recorded 2×/week from seedling emergence until destructive harvest. At harvest, leaf samples were taken for chlorophyll determination. Whole plant leaf area was also determined. Leaves, stems, and tap and lateral roots were separated and dried to determine biomass partitioned to the respective organs. Based on the results of this study, optimal greenhouse growing conditions for pawpaw seedlings have now been refined and are discussed.

Pawpaw (*Asimina triloba*, Annonaceae) morphological development during seed germination and seedling emergence. C.L.H. FINNESETH* and DESMOND R. LAYNE, Land-Grant Program, Atwood Research Facility, Kentucky State University, 40601; R.L. Geneve, Department of Horticulture and Landscape Architecture, University of Kentucky, Lexington, KY 40546.

Stratified pawpaw (*Asimina triloba*) seeds were germinated in vermiculite at 25°C to study embryo development. Seedlings were destructively harvested every 3 days prior to radicle protrusion (day 12) and at 5-day intervals thereafter. At each harvest date, 10 seedlings were randomly chosen for length measurement (mm) and fresh and dry weight (mg) determinations. Initial length of the underdeveloped embryo was less than 2 mm, but by day 70 seedling length increased to more than 350 mm. Twelve days after sowing, simultaneous development of the radicle and cotyledons produced lengths of 3.4 and 3.0 mm, respectively. Neither hypocotyl nor epicotyl was visible at this time. At radicle protrusion, radicle, cotyledon, and hypocotyl lengths were 4.4, 4.0, and 3.2 mm, respectively. Endosperm was the greatest proportion of dry weight (99.1%); radicle, cotyledons, and hypocotyl represented the remainder. The first aboveground indication of germination was hypocotyl hook emergence, which occurred at 27 days. At this time, average seedling measurements (mm length/% dry weight) were 41.7/17.0, 16.7/5.7, and 11.9/1.2 for the radicle, hypocotyl, and cotyledons, respectively. Endosperm dry weight had decreased to 76.1% by this time. Cotyledons reached maximum length at 40 days. At day 45, the development of a discernible epicotyl occurred and the percentage dry weights for the radicle, hypocotyl, cotyledons, epicotyl, and endosperm were 44.4, 12.7, 3.5, 0.4, and 39.0, re-

spectively. At day 50, the seedcoat containing the cotyledons and residual endosperm abscised; average radicle, hypocotyl, and epicotyl measurements were 182.0/93.8, 16.0/6.0, and 7.3/0.2, respectively.

Using flood control reservoirs for paddlefish (*Polyodon spathula*) production. R. ONDERS,* S. D. MIMS, and C. WANG, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Kentucky has a large number of flood-control reservoirs that may be used for paddlefish (*Polyodon spathula*; Polyodontidae) production. Juvenile paddlefish stocked into these reservoirs will feed primarily on zooplankton naturally present in the water. Paddlefish grow rapidly up to 4 kg per year with no feed cost and little management. Paddlefish can be harvested within 2 years for its quality white meat, which is boneless and firm in texture. Potentially this production system also will permit paddlefish to reach sexual maturity and the female fish can be harvested for its roe, which is processed into high value caviar. Roe production will be greatly increased by implementing all-female production technology currently being developed at the Aquaculture Research Center, Kentucky State University. Both the meat and roe of paddlefish will provide an alternative source of income for the landowners, commercial fisherman, and food processors in Kentucky.

BOTANY & MICROBIOLOGY

Cryopreservation of seeds of endangered Ohio wetland plants. LARRY A. GIESMANN,* Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099; VALERIE C. PENCE and NYREE CONARD, Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220.

Cryopreservation of seeds in liquid nitrogen (LN_2) has been shown to be an effective way of preserving germplasm of endangered plant species. With funding from the Robert H. Reakirt Foundation and the L and L Nippert Foundation, the Plant Division of the CREW facility at the Cincinnati Zoo and Botanical Garden has been exploring the feasibility of cryopreserving seeds of endangered Ohio wetland plants. Most wetland species have orthodox seeds and should be candidates for cryopreservation. Seeds of 14 species from nine families of flowering plants were collected from several Ohio wetland sites and tested in the CREW labs. All species are listed by the Ohio Department of Natural Resources as endangered, threatened, or potentially threatened in the state. In our study, four treatments were used for each species with at least 20 seeds in each treatment. In all treatments, seeds were desiccated over silica gel for 7 d. Two groups of seeds were then exposed to LN_2 for 1 hr and rehydrated for 1 d. One group was planted in pots and placed in the greenhouse immediately; seeds in the other group were stratified at 5°C for 3 mo before planting. Seeds in the remaining two treatments were not exposed to LN_2 and served as controls. The seeds of eight species germinated at rates ranging from 3% to 74%. Stratification increased the germination rate in most species. Work continues on 16 additional wetland species collected in the past year.

Fine structure of *Mycena leaiana* (Tricholomataceae) basidiospores. DONALD G. RUCH* and MARK C. ROBBINS, Department of Biology, Ball State University, Muncie, IN 47306.

The basidiospore wall of *Mycena leaiana* is composed of a thin, single, electron transparent layer, which exhibits some variations in thickness. No distinctive surface ornamentation or germ pore is present. The protoplasm is surrounded by a typical membrane, which lacks distinct invaginations. Centrally located nonmembrane-bound lipid droplets comprise the bulk of the protoplasm. Spores are uninucleate. Mitochondria with a few well-delineated plate-like cristae are present. Endoplasmic reticulum is scant. Ribosomes occur regularly attached to the ER and outer mitochondrial membrane, as well as being densely packed throughout the cytoplasm. Various sized single membrane-bound vacuoles containing an electron dense material are present. Microbody-like organelles are readily observed. These microbodies are probably glyoxysomes, since malate synthase assays are positive indicating the presence of the glyoxylate cycle in the spore.

Photomorphogenic control of flower scape elongation in dandelion (*Taraxacum officinale*; Asteraceae). MICHELLE JO LEATHERS and DAVID LOWELL ROBINSON,* Biology Department, Bellarmine College, Louisville, KY 40205.

Dandelion (*Taraxacum officinale*) is a major weed of turfgrass. One reason for this weediness is its ability to produce a large number of seeds disseminated with the assistance of a parachute-like pappus. The flower stalk (scape) aids in this seed dispersal by carrying the seedhead far above the canopy. Elongation of the dandelion scape, therefore, is an important ecophysiological function. Three experimental approaches were taken to study the effect of light on dandelion scape growth. First, we examined scape elongation at ca. 70 different turfgrass sites. Three measurements were taken at each location: the heights of the dandelion flower and seedhead scapes, and the height of the turfgrass surrounding the dandelion plants. We found a significant, positive correlation between the height of the turf and that of the dandelion scapes. We hypothesized that the turf canopy altered the light microenvironment in such a way that it altered scape growth. Our second experiment was done with dandelion scape explants treated with various wavelengths of light. Light inhibited scape growth, with flower scapes being more sensitive than the seedhead scapes. In our third set of experiments we examined whole-plant scape elongation at different wavelengths. Far-red light treatment resulted in longer flower scapes than did treatment with red, blue, or clear filters, indicating a role for phytochrome. Seedhead scapes were less sensitive to light. It appears, there-

fore, that dandelion scape elongation is influenced both by light microenvironment and stage of development.

Taxonomic status of the varieties of Seneca snakeroot, *Polygala senega* (Polygalaceae). AMY E. TRAUTH* and ROBERT F. C. NACZI, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Seneca snakeroot, *Polygala senega* (Polygalaceae), is an uncommon plant ranging throughout most of eastern North America. Two varieties have been described for this species: variety *senega* and variety *latifolia*. The taxonomic merit of these varieties is controversial. In general, both the reproductive and vegetative features of variety *latifolia* are much larger than those of variety *senega*. The goals of this research are (1) to more accurately define taxonomic differences between the varieties and (2) to test the hypothesis that the varieties *senega* and *latifolia* are separate species with morphologic differences not merely induced by ecology. Most of my work on the project has been in the laboratory and field. In the laboratory, univariate and multivariate statistical analysis of 13 vegetative and seven reproductive characters has been completed on 165 specimens from 11 herbaria. Among these morphologic characters are length of the shoot and inflorescence, length/width ratio of the leaves, length of the wing and keel, and size of the seed. Field research involved the gathering of specimens of both varieties and habitat data, including soil samples. Preliminary results suggest significant differences between the measured morphologic characters. These results suggest that the varieties of *P. senega* are better treated as separate species.

GEOGRAPHY

Effects of weather systems on migraine headaches: a pilot study. L. MICHAEL TRAPASSO,* Department of Geography and Geology, Western Kentucky University, Bowling Green, KY 42101; KENNETH EMBRY, Embry Clinic, 1733 Campus Plaza Court, Bowling Green, KY 42101.

Over a period of 9 months in 1992, 15 migraine headache sufferers in the Bowling Green, Kentucky, area were surveyed to document the onset, intensity, and duration of their migraine headache attacks. Eighty-two cases were analyzed with respect to the meteorological conditions of temperature, relative humidity, precipitation, and barometric pressure in effect during the migraine episodes. Graphic analysis consisted of superimposing the migraine attack, represented as a coded line drawn through the time period of the attack, upon the meteorological recording instrument charts. This analysis revealed promising relationships between the simultaneous onset of headaches from several subjects with a drop in barometric pressure, a rise in temperature, and a rise in relative humidity. Statistical analysis, however, failed to find these relationships to be significant. In general it was found that the absence of precipitation and the day of the week maintained the best relationships with the onset of migraine headaches.

HEALTH SCIENCES

Differences in health and diet status: African-American vs. Caucasian rural southern elderly. MARTHA MARLETTE,* SUSAN TEMPLETON, and C. J. LEE, Human Nutrition Research, Kentucky State University, Frankfort, KY 40601.

We surveyed 1554 rural elderly in nine southern states. The sample was 84.2% female, 15.8% male, 37.7% African-American, and 61.7% Caucasian; average age was 72.7. Occurrences of selected diet-related diseases were examined using ANOVA (SPSS). Significant ($p < .05$) differences between the African-American and Caucasian groups were found in the prevalence of diabetes (22.8% vs. 12.9%), hypertension (53.0% vs. 44.1%), osteoporosis (1.8% vs. 10.2%), and atherosclerosis (2.5% vs. 6.1%) and cancer (1.6% vs. 4.7%). Two non-consecutive 24-hour food intakes were analyzed (Nutritionist IV) for average daily nutrient intakes. A Dietary Status Index (DSI), including Dietary Adequacy Status (DAS) and Dietary Moderation Status (DMS) factors, was computed; all dietary differences reported were significant at $p < .05$. African-American participants had a significantly lower DSI than Caucasian participants—a mean of 47.9 vs. 52.4. Though no significant difference for DAS was found, African-Americans did have lower intakes for vitamin E (111% vs. 136% RDA), calcium (65% vs. 73% RDA), and magnesium (68% vs. 82% RDA). African-Americans had a significantly lower mean DMS (49.9 vs. 57.4); though their sodium intake (2100 vs. 2205 mg) was lower, African-Americans had significantly higher cholesterol intakes (327 vs. 228 mg), and more calories from fat (33.1% vs. 32.0%) and saturated fat (12.6% vs. 12.0%). These findings demonstrate the need for nutrition education for African-Americans, especially programs focusing on the relationship between fat intake and health problems.

Health status and dietary knowledge and intakes of southern rural elderly. SUSAN TEMPLETON,* MARTHA MARLETTE, and C. J. LEE, Human Nutrition Research, Kentucky State University, Frankfort, KY 40601.

Rural elders ($n = 1554$) in nine southern states reported arthritis (63%), hypertension (47%), heart (26%), gastrointestinal (18%), diabetes (17%), respiratory (13%), kidney (10%), osteoporosis (7%), atherosclerosis (5%), anemia (5%), and cancer (4%) problems. Smoking status, weight status, and chronic health problems were combined into health scores, range 9–100, mean 77.2; this correlated highly ($r = .3832$, $p < .0001$) with self-reported health status ("poor" to "excellent"). Surveys assessed respondents' dietary knowledge; two non-consecutive 24-hour food intakes were analyzed for nutrient intakes (Nutritionist IV) and food group servings. ANOVA (SPSS) was used to determine significant ($p < .05$) differences among participants with low (LHS), medium (MHS), and high (HHS) health scores. The HHS group had signifi-

cantly greater fiber knowledge (72.6% of responses correct vs. 67.9%, LHS and 66.7%, MHS) and dietary fiber intake (15 grams daily vs. 13, LHS and MHS). The HHS group also had a significantly higher expectation about fruit servings required: 2.3 daily vs. 2.1 for both LHS and MHS. The HHS group consumed significantly more fruit (1.3 servings daily) than the MHS group (1.0) but not the LHS group (1.1); both MHS and HHS groups consumed significantly more grain products (3.9 and 4.1 servings daily, respectively) than the LHS group (3.6). Fat and cholesterol knowledge, fat calorie intake, cholesterol intake, and sodium intake did not differ significantly among the LHS, MHS, and HHS groups. Participants with the best health appear to be those who recognize good fiber sources and include fiber-rich items in their diet.

MATHEMATICS

Polynomial conservation laws of the generalized Emden-Fowler equation. B.D. VUJANOVIC, Faculty of Technical Sciences, University of Novi Sad, 21121 Novi Sad, Serbia; A.M. STRAUSS, Department of Mechanical Engineering, Vanderbilt University, Box 1612 Station B, Nashville, TN 37235; S. E. JONES, College of Engineering, The University of Alabama, Tuscaloosa, AL 35487-0276; PETER P. GILLIS,* Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506.

We consider the polynomial conservation laws of fourth degrees with respect to x of the generalized Emden-Fowler equation $x + (b/t)\,x + \gamma x^{\alpha} t^{k} = 0$. We demonstrate that the existence of conservation laws depends upon the solution of a system of partial differential equations, usually termed the generalized Killing's equations. The general form of the fourth degree conservation laws of the Emden-Fowler equation is given; some concrete examples are discussed.

Surface geometry of the Morehead radio telescope and the optimization of operating parameters. BRIAN M. LEWIS,* CHRISTOPHER L. SMITH,* and BENJAMIN K. MALPHRUS, Morehead Astrophysical Observatory, Morehead, KY 40351.

The operating parameters of the Morehead Radio Telescope (MRT) at Morehead State University (Morehead, KY) affecting its performance are the half power bandwidth, the minimum detectable flux density, and the antenna gain. The MRT is in the process of being resurfaced to improve its radiation pattern and performance characteristics by optimizing the reflector geometry. The surface geometry has a direct effect on the radiation pattern, which determines the operating parameters. To determine these parameters, the precise surface area of the antenna is needed. The purpose of the current research is to determine the surface area and to compare the theoretical parameters before and after the telescope is resurfaced. The empirical and theoretical methods for approximation of the original surface and the new surface are discussed. The empirical method for the old surface is finite in nature and consists of the approximation and summation of planar areas. The empirical methods for finding the new surface is based upon the fact that a curved surface is an altered flat surface. Both theoretical methods involve integral approximations and manipulations. Values from the empirical and theoretical surface areas of the old surfaces are compared to determine the improvements in performance characteristics.

MOLECULAR & CELL BIOLOGY

Alkylation of DNA by strain-induced electrophilic cyclic rings. ELIZABETH M. THOMAS* and ARTHUR CAMMERS-GOODWIN, Department of Chemistry, University of Kentucky, Lexington, KY 40502.

Strain-induced electrophilic cyclic rings should alkylate the minor groove of the DNA fragments containing AAAG sites on the 5′ to 3′ strand. Alkylation of DNA would then inhibit DNA translation, thus proving to be a possible anti-tumor/anti-cancer drug agent. 3-phenylcyclobut-2-en-1-one and 2-propanone-3-(3-phenylcyclobuten-1-diyl) should exothermically alkylate DNA, releasing 2–3 kcal of ring strain in a first chemical step and potentially releasing approximately 27 kcal in a second irreversible step. The research includes the syntheses of these two compounds using zinc in an inert surrounding. DNA (15-mer) incubated with 3-phenylcyclobut-2-en-1-one showed no signs of alkylation by electrophoresis. This result needs to be checked by nmr.

Alzheimer's PS-1 mutation alters calcium homeostasis and sensitizes neurons to death induced by Aβ and trophic factor withdrawal. BILL BARRETT,* QING GUO, and MARK MATTSON, Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40502.

Mutations in the presenilin-1 (PS-1) gene on chromosome 14 are linked to autosomal dominant, early onset, Alzheimer's disease. The amino acid sequence of PS-1 predicts an integral membrane protein with a structure suggesting functions as a receptor or ion channel, or in protein trafficking. PS-1 is localized to the endoplasmic reticulum (ER), and it is shown here that expression of a PS-1 mutation (L286V) in cultured neuronal cells exaggerates Ca^{2+} responses to agonists (carbachol and bradykinin) that induce Ca^{2+} release of the ER. Cells expressing L286V exhibit increased vulnerability to amyloid β-peptide toxicity and apoptosis induced by trophic factor withdrawal. The endangering action of L286V involves oxidative stress and disruption of calcium homeostasis, and antioxidants and calcium channel blockers counteract the adverse consequences of this PS-1 mutation. By perturbing Ca^{2+} homeostasis, PS-1 mutations may sensitize neurons to age-related accumulation of Aβ and reduced trophic support.

Assessment of metal impact in the Robinson Forest creek system using metalliothionein. KEVIN COMPTON,* and CHRISTER HOGSTRAND, Department of Biology, University of Kentucky, Lexington, Ky 40502.

Metallothionein (MT) was chosen and analyzed as a prospect for being a good bioindicator in the Robinson Forest creek system (RFCS), Breathitt County, KY. MT is a low-molecular-weight protein known to be induced by heavy metals such as Cd, Cu, Hg, and Zn, and to also positively correlate with hepatic concentrations of those metals. In the RFCS, recent mining has posed the problem of possible stream contamination. It was, therefore, important to obtain raw data on stream conditions to assist in future monitoring efforts. All water metal concentrations at the six sites sampled were found to be below EPA published guidelines. Four species of fishes were sampled but one prevalent species, the stoneroller (SR) (*Campostoma anomalum*), was found to have the least amount of fluctuation in MT concentrations between members at each site. A significant variance ($p < 0.05$) was also found between two sites when SR MT concentrations were compared. The relative prevalence of the species combined with the lack in fluctuation of their MT concentrations between site members lead to the conclusion that they could be a good species from which MT could be sampled for metal exposure monitoring. Results on possible correlations between hepatic metal and MT concentrations of the species are forthcoming. If the concentrations are shown to positively correlate then it could be concluded that SR's would be a good species from which to sample MT to use as a bioindicator to show increasing metal pollution in the RFCS. The presence of correlations could also possibly indicate a metal of most concern.

Calcium homeostasis neurotoxicity hypothesis: possible evidence through L-type calcium channel density In hippocampal subregions. ALEXANDER COON,* ROSEMARIE BOOZE, and DAVID WALLACE, Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

Alzheimer's Disease (AD) has been diagnosed by measuring neurofibrillary tangle and senile plaque densities. In addition to these pathological markers, considerable neuronal cell death occurs in the brains of patients with AD. Considerable research has focused on determining the mechanism by which selective neuronal cell death occurs. An altered calcium homeostasis is believed to be an integral part of this cell death mechanism. L-type calcium channels are primarily responsible for the movement of Ca^{2+} ions across the neuronal membrane. However, the relationship between channel density and the pathological diagnosis of AD has yet to be fully elucidated. To further examine this relationship we selected [^{3}H]PN200-110, a calcium channel antagonist, to perform radioligand binding experiments. First, rat brain homogenates were used to obtain the channel affinity and B_{max}(density). Second, in vitro receptor autoradiography was performed with [^{3}H]PN200-110 on 25 human hippocampus samples (AD, $n = 15$; control, $n = 10$). Tissue was sectioned to 20 μm using a cryostat and then incubated with 1.0 nM [^{3}H]PN200-110. Non-specific binding was binding in the presence of 1.0 μm Nifedipine. The autoradiograms were then analyzed with computer-aided densiometry to quantify the density of the channels in the hippocampal subregions. Preliminary data suggests that the binding of PN200-110 is specific to the L-type channels. However, the exact relationship between the channel density and the AD pathology is still unclear at this time.

Characterization of AD-like pathology in normal aging. CHRISTOPHER R. BRACKNEY,* LARRY SPARKS, and STEVE SCHEFF, Department of Anatomy and Neurobiology, University of Kentucky, Lexington, Ky 40536.

Alzheimer's disease is characterized by a progressive cognitive deterioration coupled with marked personality changes. The most notable pathological changes associated with Alzheimer's disease are Senile Plaques (SP's) and Neurofibrillary Tangles (NFT's). SP's contain the β-amyloid protein, a 39–42 amino acid peptide fragment derived from a transmembrane amyloid precursor protein (β-APP). NFT's consist of a mass of introcellular argentophilic fibers, which are Paired Helical Filaments (PHF) resulting from abnormally phosphorylated tau protein. The present study assesses which β-APP peptide compromise the β-amyloid immunoreactivity in the brains of nondemented, non-heart disease, individuals 20–80 years of life. By using specific antibodies we will be able to conclusively demonstrate which portion of the β-APP is accumulating in normal aging.

The accumulation of the C-100, a fragment derived from β-APP, precludes the formation and deposition of SP's. The two immediate derivatives of the C-100 fragment are β-A42(43) and β-A40. It has been shown that β-A42(43) is found in greater accumulations then β-40 in Alzheimer's disease. To examine the presence of β-A42(43) and β-A40 in normal aging we used specific antibodies to discrete regions of the β-APP peptide sequence. We found several individuals who had the β-42(43) with lower densities then that of Alzheimer's disease. There were no accumulations of the β-40 in normal aging. It should also be noted there was no correlation between the presence of β-A42(43) and age. These results suggest that the β-APP processing in normal aging is similar to that found in Alzheimer's disease.

Chromosome 17 deletions in a subset of ovarian tumors. LYNN CARRICO* and MAURA PIERETTI, Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington KY 40506.

Ongoing studies in the Pieretti laboratory indicate that different alterations in cancer-controlling genes occur in different histological types of ovarian tumors. The laboratory has already compiled data concerning genetic mutations on 145 tumors. However, in order to statistically correlate this genetic information to patients' survival and recurrence, a larger number of cases needs to be evaluated. For this purpose, 45 additional cases were identified through the Kentucky Cancer Registry, which has a record of clinical, demographic, and follow-up data from Kentucky cancer cases after 1991. These tumors can be stud-

ied for the genetic alterations that characterize the different histological types: genetic deletions of chromosome 17, p53 mutation, and K-ras activation. Genetic deletions of chromosome 17 were studied by polymerase chain reaction of microsatellite markers and were identified as the loss or partial loss of one allelic band.

The tumor tissue was removed from paraffin embedded blocks by a "punching out" method, and the DNA was phenol extracted and ethanol participated. The resultant DNA was then studied for deletions using the previously mentioned method. Preliminary results are available for 13 of the 46 total tumors. Five tumors showed no deletions on chromosome 17. Six tumors showed deletions on both arms of chromosome 17. Two tumors had deletions confined to the short arm of chromosome 17. All genetic deletions were observed in tumors of high grade. Further studies will be conducted with these samples and the resulting data will be evaluated for prognostic significance.

Determination of cytokine production following OVA injection into the eyes of adoptive transfer mice. JASON E. DELONG,* JEROLD W. WOODWARD, RITA EGAN, and RICHARD BLACK, Department of Immunology and Microbiology, University of Kentucky, Lexington, KY 40502.

The eye, along with the central nervous system, has been regarded as an immune-privileged site where normal immune responses such as inflammation are suppressed and antibody production is favored. Anterior chamber-associated immune deviation (ACAID) is a stereotypic systemic response to ocular antigens that involves dendritic cells assisting in trafficking antigens to the spleen where t-cells are activated and an immune response is elicited. The ACAID phenomenon is characterized by a shift in the T-helper 1/T-helper 2 balance of responses toward a dominant Th2 response. This alteration should be evident in the cytokine profiles of mice following immunization with antigen into the eye. Using an adoptive transfer mouse model with an immunoflourescent intracellular cytokine staining procedure, we have been able to detect and analyze individual cytokines secreted by antigen-specific cells. The data attained through this project demonstrates successful isolation and recognition of individual IL-2 and IL-4 cytokines using the staining procedure with flow cytometry. This establishes the intracellular cytokine staining procedure as a useful tool in analyzing the immune response following antigen injection.

Development of a sensing system for antimonite based on genetically engineered bacteria and green fluorescent protein. JANET MERCER,* SRIDHAR RAMANATHAN, and SYLVIA DAUNERT, Department of Chemistry, University of Kentucky, Lexington, KY 40506.

A sensitive and selective sensing system for antimonite based on genetically engineered bacteria will be described. In this system, the selective binding of a bacterial protein to antimonite was coupled with fluorescence. When antimonite enters certain bacteria, it is effluxed out of the bacterial cell by a selective protein pump. The structural proteins ArsA, ArsB, and ArsC form the protein pump. A set of five genes, called the *ars* operon, code for the three structural proteins, in addition to two regulatory proteins, ArsR and ArsD. In the absence of antimonite, the ArsR binds to the promoter region of the *ars* operon inhibiting the expression of the protein pump. ArsR has a highly specific binding site for antimonite. Thus, when antimonite reaches the cytoplasm it binds to ArsR inducing a conformational change, which releases the promoter region of the *ars* operon. This induces the expression of the protein pump, which effluxes the antimonite out of the cell. Bacteria were genetically engineered to introduce the gene of Green Fluorescent Protein (GFP) downstream from the ArsR gene. The concentration of GFP in cells can be monitored easily by checking for fluorescence. Thus, the concentration of the GFP can be related to the concentration of the antimonite in the cell.

Effect of retinioc acid on TIMP and stromelysin in HT 1080 human fibrosarcoma cancer cells. KEITH D. BRICKING* and RAYMOND E. RICHMOND, Department of Biological Sciences, Northern Kentucky University, Nunn Drive, Highland Heights, Ky 41099.

Recent research indicates that stromelysin, a matrix metalloproteinase (MMP) expressed by a large percentage of malignant cancer cells, is capable of degrading extracellular matrix proteins. Matrix degradation is an important first step for cancer cell invasion, which is a precursor of metastasis. Stromelysin may be inhibited by a specific class of naturally occurring polypeptide inhibitors known as tissue inhibitors of metalloproteinases (TIMP). TIMP appears to act by non-covalently binding to stromelysin. Therefore any therapeutic agent affecting cellular expression of stromelysin or TIMP may alter invasion. We examined the hypothesis that retinoic acid (a vitamin A analog believed to have anticancer effects) will increase TIMP expression in a highly metastatic HT 1080 human fibrosarcoma cell line. These alterations will decrease the invasive properties of the cell. Stromelysin and TIMP expression in 10^{-5} M retinoic acid and vehicle control treated cells were detected by polyacrylamide gel electrophoresis and immunoblot analysis. Invasion of HT 1080 cancer cells after addition of retinoic acid and vehicle control has also been analyzed quantitatively using Matrigel Invasion Chambers. Our preliminary data indicate that there was no change in stromelysin expression, an increase in TIMP expression, and a significant decrease in the rate of invasion after the addition of retinoic acid. These results suggest that future antimetastatic agents should focus on TIMP expression as well as matrix metalloproteinases.

Effect on platelet-derived growth factor A-chain gene transcription by *nm23* proteins in the WR-082-01 cell line. MATTHEW SINEX,* BIN LIU, JAMES REID, and DAVID KAETZEL, Department of Pharmacology, University of Kentucky Medical Center, Lexington, KY 40536.

Platelet-derived growth factor (PDGF), a dimeric gly-

coprotein comprised of two disulfide-linked polypeptide chains (A and B), is a powerful mitogen found in normal and malignant cells. This laboratory has previously reported that a 1 kb region of the PDGF A-chain promoter located between nucleotides −1800 and −880 (relative to the transcription start site, +1) exhibits potent transcriptional repression. Within that negative regulatory region (NRR), a 31 bp silencer element (*5′SHS*) was identified at approximately −1400. Screening of a cDNA expression library derived from HeLa cells with a probe derived from the *5′SHS* sequence yielded clones encoding *nm23*, a protein previously implicated in cancer metastasis. Subsequent studies revealed that purified *nm23* binds with high affinity to the *5′SHS* sequence. The purpose of this study was to determine whether *nm23* plays a role in mediating repression of the PDGF A-chain promoter. WR-082-01 cells, which are deficient in *nm23* production, were cotransfected with DNA constructs directing overexpression of the H1 and H2 isoforms of *nm23* and various A-chain promoter fragments. The NRR was inactive in these cells, suggesting that *nm23* was important for its repression activity. Overexpression of *nm23*-H1 and H2 resulted in a general repression (< 2-fold) of all promoter constructs analyzed. While these *nm23* isoforms exerted no additional inhibition upon vectors containing the *5′SHS* element, an additional two-fold repression was conferred by H1 upon vectors containing the entire NRR. These data suggest that *nm23*-H1 mediates repression of A-chain gene transcription, probably via interactions with multiple silencer elements dispersed throughout the NRR.

Effects of nucleotide excision repair mutants on mismatch repair. MARCI ADKINS* and ISABEL MELLON, Department of Pathology, University of Kentucky, Lexington, KY 40502.

We have previously shown that mismatch repair mutants are also deficient in transcription coupled repair. These results are important because they imply a possible correlation between what was previously thought to be distinct DNA repair systems. Present work has been undertaken to further explore the connection between these two repair processes. Since microsatellite instability is a good marker for defective mismatch repair, we are specifically looking for microsatellite instability in Xeroderma Pigmentosum (XP) cell lines. XP patients have deficiencies in one or more nucleotide excision repair genes, which have been named XP-A through XP-G. We have examined XP-A, which is involved in damage recognition, and XP-C, which repairs non-transcribed DNA. Clones of these cell lines have been examined using primers specific for certain dinucleotide repeat sequences in a Polymerase Chain Reaction (PCR). The PCR products are then run on a 6% polyacrylamide gel and viewed by autoradiography. No microsatellite instability has been detected in either one of these cell lines. Therefore, our results deny a connection between mismatch repair and transcription coupled repair.

Electrophysiological studies of the SCN8a voltage-gated sodium channel. BRIAN DELISLE* and JONATHAN SATIN, Department of Physiology, University of Kentucky, Lexington, KY 40502.

SCN8a is a voltage-gated sodium channel isoform expressed in motor neurons. Loss of expression of the alpha subunit in transgenic mice results in Motor Endplate Disease (MED). Transgenic mice often display a phenotype that mimics human disease. Symptoms of MED include progressive paralysis and juvenile death. The alpha subunit of voltage-gated sodium channels consists of four highly conserved domains. Most variability among the isoforms occurs among the one-two linker of the alpha subunits. This region contains functional glycosylation and protein kinase A phosphorylation sites. Utilizing RT-PCR protocol, we cloned the functional sites of SCN8a. Chimeric channels are currently under construction. The process involves ligating digested BR2a, an extensively studied isoform, and cloned SCN8a. The chimeras are expressed in *Xenopus laevis* oocytes. We use the 2-electrode voltage clamp to study the kinetic properties of the expressed ion channels and express the BR2a alpha subunit with and without the beta-1 subunit. Coexpression of the beta-1 subunit with BR2a modulates the rate of current decay and shifts voltage dependance of the channels. All experiments were then performed in the presence of 8-bromo-cAMP, promoting the phosphorylation of the sodium channel. The alpha subunit plus and minus the beta-1 subunit both demonstrate a modest change in current amplitude. We use these experiments to validate a system to study the chimeric channels. The study is being used to extend how coexpression of the beta-1 subunit further modifies the sodium channel's kinetics.

Identification of cytokines responsible for enhancing neonatal immune responses to polysaccharides. NIKOLE L. GILBERT,* RALPH CHEL VARAJAN, and SUBBARAO BONDADA, Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40502.

Polysaccharide antigens elicit a protective immune response in adults but not in neonatal individuals, thereby making neonates especially vulnerable to pathogenic, polysaccharide-bearing bacteria such as *Streptococcus pneumoniae*. Past research has shown that the B cell antibody response, which mediates immune protection in adults, is dependent on the presence of certain cytokines. It was thus hypothesized that the neonatal B cells may be competent to respond to polysaccharide antigens, but fail to do so due to a deficiency of cytokines required to stimulate neonatal B cells. To obtain an adult-like B cell response, murine neonatal splenocytes were cultured in vitro for 4 days with the polysaccharide antigen TNP-Ficoll and various cytokines known to effect B cell activity. The plaque-forming cell-assay technique was used to measure the number of antibody forming cells per culture. Neonatal splenocytes cultured with IL-4 and IL-5 together and IL-1, IL-5, and IL-6 separately resulted in 40–70%

of the adult response, while individual cytokines IL-2, IL-3, IL-4, IL-10, and GM-CSF provided no enhancement of the neonatal immune response. Neonatal splenocytes cultured with a combination of cytokines IL-1 and IL-5 produced 139% of the adult response to TNP-Ficoll. In conclusion, our studies have shown that the neonatal B cells can respond to polysaccharides as well as adult B cells if an adequate mixture of cytokines is provided, supporting the concept that the poor response of neonates to polysaccharides is likely due to a deficiency in cytokine availability.

Identification of genes involved in splicing. ELIZABETH OTTE* and BRIAN RYMOND, Department of Biology, University of Kentucky, Lexington, KY 40506.

The removal of introns from mRNA precursor, a process vital to eukaryotes, is carried out by the spliceosome. This complex enzyme consists of five small nuclear RNA molecules and 50–100 protein subunits. A genetic screen hoping to identify one or more of these spliceosomal proteins was performed. In this screen, a mutant intron was imbedded within the lacZ gene to produce a yeast strain in which production of the enzyme β-galactosidase was dependent upon the proper removal of this intron. Since this fusion gene's intron was poorly excised, this strain was pale blue when grown in the presence of the chromogenic substrate, X-gal. My project was to characterize a set of recombinant plasmids which, when present in 20–50 copies per cell, turned the colonies a much darker blue, apparently having enhanced β-galactosidase expression. It was hypothesized that such genes might suppress the inhibitory effects of the intron mutation and increase pre-mRNA splicing efficiency. The ratio of pre-mRNA to mature mRNA and the level of β-galactosidase in each putative suppressor was assayed. The data obtained indicate that, under the conditions of assay, none of the suppressors enhanced splicing efficiency or enzyme production. I conclude that the enhanced blueness of these strains is due to either an indirect effect, such as enhanced permeability of the X-gal substrate, or to enhancement of lacZ transcription, pre-mRNA splicing, mRNA or protein stability, or enzyme activity when cells are grown on the surface of an agar plate but not when grown (as assayed) in liquid culture.

Innervation of immune tissues and the role of nerve growth factor. MARK PARRISH* and SONIA CARLSON, Department of Anatomy and Neurobiology, University of Kentucky Medical School, Lexington, KY 40536.

The existence of a structural and functional relationship between the nervous and immune systems has been elucidated in detail over the last decade. Immune tissues are innervated by the sympathetic nervous system and the release of neurotransmitter modulates a variety of immune responses. This modulatory relationship is well documented, but the plethora of directional cues guiding these neurons to their immune tissue targets is not well understood. Increasing evidence suggests that a protein, Nerve Growth Factor (NGF), plays a significant role in directing the pattern of peripheral innervation resulting from the dependence of neurons on a target derived supply of this factor. Recent work with a line of transgenic mice, altered to overexpress NGF in skin, has given evidence that this growth factor may also be influential in guiding sympathetic innervation to immune tissues. NGF transgenics show dramatic changes in density and pattern of innervation to spleen and peripheral lymph nodes. To determine if spleens from transgenic mice release a substance that alters neurite outgrowth, sympathetic ganglia were co-cultured with spleen from transgenic and non-transgenic animals in the presence/absence of NGF and outgrowth was measured. Cultured ganglia exhibited growth away from spleen which was reduced when cultured with transgenic tissue or in the presence of NGF suggesting that transgenic spleen retains NGF capable of stimulating outgrowth. However, ganglia paired with transgenic spleen also demonstrated changes in neurite morphology and a reduced total neurite area which we believe may be the result of cytokine production.

Localization of pyrimidine biosynthesis enzymes with fusion proteins. DANA R. WEAVER,* JIANYUAN LUO, and JOHN M. RAWLS, School of Biological Sciences, University of Kentucky, Lexington, KY 40502.

De novo pyrimidine biosynthesis is carried out by six enzymes contained in three proteins in animals: the CAD protein, DHOdehase, and the UMP synthase protein. These proteins are encoded by the *r. dhod.* and *r-l* genes respectively in *Drosophila*. Biochemical cell fractionation studies have shown that the CAD and UMP synthase proteins are cytosolic, whereas DHOdehase is located in mitochondria. We are carrying out studies to determine the distributions of these proteins within cells and among different tissues, using fusion proteins containing the *Aequorea victoria* green fluorescent protein (GFP) and the hemagglutinin epitope (HA). A series of recombinant constructs was created containing the GFP and HA open reading frames fused to the open reading frame of the *dhod* gene. Transgenic animals were produced that contain those fusions and genetic complementation confirmed in vivo function of the transgenes. Patterns of expression of the transgene protein were studied by UV fluorescence microscopy (GFP) and immunocytochemistry (HA epitope) in cells known to abundantly express CAD and DHOdehase: ovaries (i.e., nurse cells) and testes (i.e., elongated spermatids). Results of these experiments will be presented.

Microsatellite instability (MIN) and TGF-β Type II receptor gene mutations in human pancreatic carcinoma. CINDY HARP,* CAROL SWIDERSKI, and JAMES FREEMAN, Department of Surgery, University of Kentucky Chandler Medical Center, Lexington, KY 40536.

Tumor progression is a multi-step process involving an increase in positive growth signals and a decreased response to negative growth regulation. In pancreas cancer

this process often involves the mutational activation of the *ras* cellular oncogene and a concomitant loss in function of tumor suppressor genes. We found that a common characteristic of pancreatic cancers is loss in negative growth regulation by TGF-β. This loss in regulation is often mediated by lack of expression of the TGF-β type II receptor gene (RII), a reported tumor suppressor gene. Microsatellite instability (MIN) has been proposed as a mechanism that targets repetitive sequences in the RII gene for mutation, resulting in loss of RII expression and function. The purpose of this study was to determine the incidence of MIN in pancreas cancer and to determine whether MIN targets the RII gene for mutation. Six loci were analyzed for the occurrence of MIN in pancreas cancer specimens obtained from 16 different patients. The results show that MIN (9 of 16, 56%) and loss of heterozygosity (LOH), (7 of 16, 44%) are common in pancreatic tumors. We further analyzed prospective and retrospective pancreas tumor specimens by ^{32P}-SSCP for RII mutations at regions comprising 2 potential MIN target sites. Mutations at potential MIN sites were detected in only 3 of 21 (14%) specimens all of which were in 5′ region of the gene that included a polyadenine repetitive sequence. RII mRNA was not detectable in 5 of 12 (42%) prospective samples. These studies suggest that there is a greater incidence of MIN in pancreas tumors than reported for other tumor types. MIN, LOH, and loss in RII expression may play a role in the pathology of this disease. However, MIN targeted RII mutations occur in only a small subpopulation of pancreas tumors. Therefore mechanisms other than MIN must account for the lack of RII expression found in many pancreas tumors.

Neuroprotective effects of nicotine in MPTP-treated C57B1/6 mice. ALAN NORTHINGTON* and JAMES R. PAULY, College of Pharmacy, University of Kentucky, Lexington, KY 40536.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a selective loss of CNS dopamine-containing neurons. Epidemiological studies have consistently demonstrated an apparent neuroprotective effect of cigarette smoking on the development of PD, with smokers having approximately half the risk of nonsmokers of developing the disease. Mice injected with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) develop a syndrome resembling human PD with a loss of nigrostriatal dopaminergic neurons. The purpose of the present study was to examine whether nicotine pretreatment reduces the neurotoxic actions of MPTP on striatal dopaminergic neurons. Mice were injected with either saline or nicotine (1.0 mg/kg, sc.) 10 minutes prior to administration of MPTP (40 mg/kg, sc.) or saline. Locomotor activity, motor coordination, and body weight were monitored for 12 days following MPTP treatment. The brains from each treatment group were then used for the evaluation of [^{3}H]-GBR12935 binding to the dopamine transporter and binding to neuronal nicotinic receptor subtypes (using [^{3}H]-cytisine and [^{125}I]-alpha bungarotoxin). Animals treated with saline and MPTP demonstrated significant behavioral toxicity and weight loss; these effects were clearly attenuated in animals pre-treated with nicotine. In the striatum, MPTP caused a significant reduction in the binding of [^{3}H]-GBR12935 and [^{3}H]-cytisine but not [^{125}I]-alpha bungarotoxin. The toxic effects of MPTP on [^{3}H]-GBR12935 and [^{3}H]-cytisine were diminished by nicotine pretreatment. Possible mechanisms of nicotine-induced neuroprotection will be discussed.

Screening for mutations causing failures in the RNA sorting system in spermiogenesis. NATHAN NOVOTNY,* JAN URSO, and JOHN RAWLS, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

In spermatogenesis, post-meiotic differentiation of spermatids is an extensive morphogenetic program that takes place, largely or entirely, in the absence of transcription. It utilizes RNAs synthesized during spermatocyte growth, stored in an inactive form during meiosis, then translated at various times during spermiogenesis. Spermatocytes have an active system that sorts RNAs to be used immediately from those to store for later use; the components of this RNA sorting system are largely unknown. The objective of this research is to identify components of this system among the numerous male-sterile mutations that have been characterized in *Drosophila melanogaster*, by seeking variant expression of *dhod* RNA in mutant flies. A spermatocyte-specific promoter element of the *dhod* gene leads to synthesis of RNA that, mediated by a specific 5′ RNA sequence (*d*TCE), is sequestered for ultimate translation in elongated spermatids. In a primary survey, spermatogenesis stages in over eighty male-sterile strains have been examined for histochemical expression of the *dhod* product dihydroorotate dehydrogenase (DHOdehase). Most strains displayed essentially normal expression patterns for DHOdehase, however a few strains showed aberrant expression patterns that are predicted for mutations of the spermatogenesis gene control system: zero or strongly reduced expression; expression of DHOdehase in early stages (possibly precocious expression). As a secondary survey, mutants showing altered expression of DHOdehase are being tested for expression of an array of *lac*Z reporter transgenes that contain various elements that control *dhod* expression: the *dhod* promoter or the promoter of another spermatocyte-expressed gene; with or without the *d*TCE sequence that controls translational delay. Results of these experiments will be presented.

Site directed mutagenesis of a conserved phenylalanine in cytochrome P450 2E1. ERIC DEDRICK* and TODD PORTER, College of Pharmacy, University of Kentucky, Lexington, KY 40536.

Cytochrome P450 2E1 is an enzyme that catalyzes the oxidation of a wide array of substrates, xenobiotics, and naturally occurring chemicals. The oxidative capabilities of P450s arise from a heme group folded deep within the molecule. Although it is not fully understood how an elec-

tron donated from its reductase partner is able to make the transition from the surface of the P450 molecule to the heme group in the interior, it is thought that a highly conserved phenylalanine at position 429 facilitates this transfer. Previous mutagenesis experiments have suggested that hydrophobic compounds can substitute for this phenylalanine and still provide a functional molecule. Furthermore, because of its structure, proline may fit well at this position, causing a minimal distortion of the P450; notably, a proline has been discovered at this position in a functional P450 enzyme from flax seed. In this experiment, a proline codon was substituted for the phenylalanine codon at this position using polymerase chain reaction. This mutation was then ligated into the expression vector pJL2/3a and expressed in *Escherichia coli*. Once positive mutants have been identified by sequencing, the protein will be characterized in spectrophotometric and catalytic assays.

Stage dependent induced hatching in the muskellunge, *Esox masquinongy*. TAMARA SAPP,* SARAH M. BLANK, and JOHN J. JUST, Department of Biological Sciences, University of Kentucky, Lexington, KY 40506.

The embryos of all aquatic animals are encased in proteinaceous egg cases from which they escape during development. Hatching often involves digestion of the egg case by a hatching enzyme secreted from hatching glands. This study shows that hypoxic environments can induce premature hatching in *Esox masquinongy* 9 days post-fertilization, 5 days before normal hatching. Embryos 9, 10, 11, and 12 days old were subjected to environments with various oxygen pressures by aerating vials containing embryos with oxygen (PO_2 = 760 mm Hg), air (PO_2 = 160 mm Hg), or nitrogen (PO_2 = 0 mm Hg) for 1 hour. At all stages tested, eggs aerated with nitrogen (n = 461) exhibited an increased hatch rate compared to the control embryos treated with air (n = 463), while those treated with oxygen (n = 461) showed a greatly reduced hatch rate. There was a progressive increase in percent hatch with those embryos treated with nitrogen. Percent hatch of 9, 10, and 11 day old embryos after 1 hour of nitrogen treatment was 46%, 90%, and 99% respectively, while 9, 10, and 11 day old control embryos treated with air had hatch rates of 0%, 1%, and 46%. It is believed that hatching can occur only after the development of hatching glands and enzymes. Hatching medium was collected from a mass hatching of *E. masquinongy* embryos and the macromolecules (MW > 10,000) were concentrated via ultrafiltration. Protease activity was demonstrated by digestion of gelatin on photographic film. Work continues to isolate the hatching enzyme and correlate its developmental appearance with the ability to prematurely hatch.

TNF-alpha neuroprotection in opiate-mediated astroglial toxicity. JENN JACKSON,* MARK P. MATTSON, and KURT F. HAUSER, Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

Opiate drugs of abuse liability, such as heroin and morphine, inhibit fetal brain development, in part by disrupting astroglial mitosis and also by inducing astroglial hypertrophy. Although opioids affect growth, the mechanisms by which opioids disrupt neurobehavioral development are not understood. The mu-opioid receptor serves as a primary target of opiate drugs of abuse. In astroglia, mu-opioid receptor stimulation causes increases in intracellular calcium. The resulting elevations in intracellular calcium mediate morphine-induced alterations in astroglial growth. The present study tested the hypothesis that a naturally occurring neuroprotective factor, tumor necrosis factor-alpha (TNF-alpha), will prevent the deleterious effects of opiate drugs by blocking opiate-induced increases in intracellular calcium. Astrocytes were isolated aseptically from the cerebral hemispheres of 1-day-old ICR mice. Astroglial cultures were pretreated with TNF-alpha at least 24 hours prior to the opioid insult to serve a neuroprotective role. Astrocytes were subsequently treated with TNF-alpha in the presence or absence of morphine (1 μM). Intracellular calcium was analyzed by ratiometric analysis of the calcium indicator, fura-2. The study showed TNF-alpha, at concentrations of 10 ng/ml and 100 ng/ml, causes a concentration-dependent reduction in morphine-induced activation of intracellular calcium. These findings suggest that mu-opioid receptor and TNF-alpha signaling pathways converge and have opposing actions in calcium homeostasis. Studies in progress are assessing whether TNF-alpha attenuates morphine-induced alterations in astroglial growth.

Translational regulation of mRNA in human breast cancer. BRAD SEGEBARTH* and STEPHEN ZIMMER, Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40502.

This proposal is based on the hypothesis that increased initiation of messenger RNA (mRNA) translation in human breast cancer cells is a fundamental mechanism mediating increased tumor cell growth and the capacity to invade and metastasize. The eukaryotic initiation factor 4E (eIF-4E) is a 25 kilodalton (kDa) phosphoprotein involved in the recruitment of messenger RNA to the polysomes. This reaction is considered to be the rate-limiting step in the synthesis of cellular proteins, with eIF-4E as the least abundant subunit. Studies show that overexpression of eIF-4E in human breast cancer cells is associated with increased translation, cellular proliferation, transformation, and tumorigenesis. PHAS-1 is a 20 kDa protein found to bind to eIF-4E. The binding seems to be regulated by phosphorylation, with the complex dissociating upon phosphorylation of PHAS-1. About 70% of mRNAs containing extensive 5′-region secondary structure are oncogenes or important growth regulatory genes. One of these, basic fibroblast growth factor (bFGF), is a multifunctional molecule that affects cell growth and induces angiogenesis. Due to the extensive 5′ region secondary structure, bFGF, along with other malignant characteristics, may be disproportionally inhibited in cells overex-

pressing PHAS-1. If this is found to be the case, potential medical utility stems from being able to effectively develop a drug that can affect the PHAS-1/eIF-4E interaction and suppress malignant properties in oncogenic cells.

PHYSICS & ASTRONOMY

Celestial seasons. RICHARD HACKNEY,* KAREN HACKNEY, ROGER SCOTT, CHARLES McGRUDER, MICHAEL CARINI, RICHARD GELDERMAN, SANDRA CLEMENTS, JOHNNY JENNINGS, JOHN NOBLE, and RICO TYLER, Western Kentucky University Astrophysical Observatory, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101.

Many have only a casual acquaintance with (and an inadequate understanding of the *causal* connection between) the seasons and related observable astronomical phenomena. In our introductory astronomy courses, we attempt to engage students' minds through personal observation and interpretation of data to make the connections and to see the astronomical reasons for the seasons. We describe a number of activities suitable for students at various levels to illustrate through student participation the primary astronomical observables related to the cycle of the seasons. We acknowledge funding by the Eisenhower Math and Science Program, the Kentucky PRISM-UG Program, and the NASA Kentucky Space Grant Consortium.

Comet Hale-Bopp: Opportunity for education and developing student involvement. ROGER SCOTT,* KAREN HACKNEY, RICHARD HACKNEY, CHARLES McGRUDER, MICHAEL CARINI, RICHARD GELDERMAN, SANDRA CLEMENTS, and JOHN NOBLE, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101.

Comet Hale-Bopp, already visible with binoculars in the early evening sky, promises to be an easy naked-eye object in March and April 1997. Comets are notorious for being unpredictable in brightness. However, Hale-Bopp seems to be brightening about as expected and may put on a show even better than Hyakutake. Bright comets offer excellent opportunities for student projects and for educational outreach to the general public. They are easily photographed using any 35-mm camera capable of taking a time exposure. We discuss simple student projects and techniques for photographing the comet. Photographs of other bright comets of recent decades are shown as examples. Charts and finding information are provided.

Enigmatic world of extragalactic radio jets. CASEY WATSON,* ILDEFONSO GUILARAN, RYAN CULLER, MONICA DECKARD, RICHARD GELDERMAN, MICHAEL CARINI, JOHN NOBLE, SANDRA CLEMENTS, ROGER SCOTT, KAREN HACKNEY, and RICHARD HACKNEY, Western Kentucky University Astrophysical Observatory and Center for Automated Space Science, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101.

Radio galaxies, quasars, Seyferts, and blazars are all designated by the term Active Galactic Nuclei or AGN. Among the most striking characteristics of AGN are the collimated radio jets, up to millions of light years in length, which emanate from their cores. The radio emission is due to synchrotron radiation, which arises when charged particles from the active nucleus spiral around a magnetic field. However, the precise physical nature of the jets remains undetermined. Discovering the composition of the jets is crucial because that insight will lead to greater understanding of the AGN's central engine. It is the primary goal of this project to make a contribution to current jet theory. Our theoretical work will be complemented by observations, including images from the CCD camera at the WKU Astrophysical Observatory and the Hubble Space Telescope. We thank the NASA Kentucky Space Grant Consortium and the NASA Center for Automated Space Science for support.

Gravitational lensing simulation for the classroom. MARIA FALBO-KENKEL* and JOE LOHRE, Department of Physics and Geology, Northern Kentucky University, Highland Heights, KY 41099.

Gravitational macro-lensing is a celestial phenomenon that can be observed when two or more objects at different distances from the earth lie along the same line of sight in the sky. The observation of this effect, which produces distortions and apparent displacements of the object furthest from earth in the alignment, was listed by Einstein in his famous 1915 paper as one of three important tests of general relativity. There are ways to optically simulate gravitational lensing events. We present an inexpensive and simple method to make a lens that can be used to easily simulate gravitational lensing in the classroom. We discuss two effects that can be studied with the lens: (1) Einstein-ring formation and (2) multiple image formation. This work was supported by funds from the Kentucky Space Grant Consortium.

Morehead radio telescope: design and fabrication of a research instrument for undergraduate faculty and student research in radio frequency astrophysics. BENJAMIN K. MALPHRUS,* BRIAN M. LEWIS, and CHRISTOPHER L. SMITH, Morehead Astrophysical Observatory, Morehead, KY 40351.

Faculty and students of the departments of Physical Sciences and Industrial Education and Technology at Morehead State University (MSU) (Morehead, KY) have designed and assembled the Morehead Radio Telescope (MRT) to provide a research instrument for undergraduate astronomy and physics students and an active laboratory for MSU undergraduate students and faculty in physics, engineering, and computer science and for undergraduate students, faculty, and science teachers throughout Kentucky. Goals of the MRT program are to enhance the curricula in physics, physical science, electronics, and

science education programs by serving to provide (1) a research instrument for investigations in astronomy and astrophysics; (2) an active laboratory in astronomy, physics, electrical engineering, and computer science; and (3) a research instrument and laboratory for science teacher education and inservice programs. The telescope incorporates a modular design in which components may be easily removed for use in laboratory investigations and for student research in design projects. The performance characteristics of the telescope allow a varied and in-depth scientific program. The sensitivity and versatility of the telescope design facilitate the investigation of a wide variety of astrophysically interesting phenomena. The MRT provides hands-on experience in research and instrumentation technology in a cutting-edge science, one that is in the midst of scientific revolution. This presentation provides and overview of the MRT and its current operating status.

NASA Kentucky Space Grant Consortium, Center for Automated Space Science, and EPSCoR opportunities for space-related science, technology, and education. KAREN HACKNEY,* RICHARD HACKNEY, ROGER SCOTT, CHARLES McGRUDER, MICHAEL CARINI, RICHARD GELDERMAN, JOHN NOBLE, SANDRA CLEMENTS, and JOHNNY JENNINGS, Kentucky Space Grant Consortium, NASA EPSCoR Program, and Center for Automated Space Science, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101; MICHAEL BUSBY, Center for Automated Space Science, Center of Excellence in Information Systems Engineering and Management, Tennessee State University, Nashville, TN 37203.

NASA has funded three programs in Kentucky to involve university faculty and students in space-related research, technology, and education. We describe (1) current opportunities in the Kentucky Space Grant Consortium for undergraduate scholarships and graduate fellowships for students in mentored, space-related projects; (2) funding opportunities for development of space-related research projects and activities to support teaching of space science and related disciplines; (3) progress of existing projects and new opportunities in the NASA EPSCoR Program; and (4) special opportunities for minority students in the Center for Automated Space Science.

Use of computers for the enhancement of introductory astronomy for non-science majors. RAYMOND C. McNEIL, Department of Physics and Geology, Northern Kentucky University, Highland Heights, KY 41099.

Within the sciences, introductory laboratory experiences allow students a better understanding of scientific laws and a better appreciation of the scientific method leading to their discovery. At the heart of a laboratory course is the assumption that students will participate in "hands-on" activities that will provide experience in gathering data, analyzing the data to find relationships, and drawing conclusions from the experimental results. Traditionally, introductory astronomy students have been limited in that they generally cannot actively conduct experiments, controlling one or more of the variables, as can students in other sciences. The solution at Northern Kentucky University (NKU) and other institutions has been to utilize laboratory computers to allow students to explore, then investigate in detail, simulations of astronomical phenomena. Observations may be repeated, and in some cases variables changed, just as in the laboratories of other scientific disciplines. The active nature of such experiences can enhance learning and instill a sense of the excitement of scientific discovery. The acquisition and initial use of a dedicated set of computers for the astronomy laboratory at NKU is described. Materials developed by the author or adapted from other sources are discussed, including Contemporary Laboratory Experiences in Astronomy, with copies available upon request. A list of software found to be most promising as the basis for laboratory activities or other types of student involvement is also included, as are future directions for this work. The author gratefully acknowledges the support of the National Science Foundation (Grant #9452219) and NKU.

Optical monitoring of very high redshift quasars. SANDRA CLEMENTS, Western Kentucky University Astrophysical Observatory and Center for Automated Space Science, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101.

The observations and results of a 1-year program to monitor high redshift quasars is reported. Quasars are among the most distant and energetic objects in the universe. Believed to be caused by activity in the centers of galaxies, quasar behavior has been a challenge to understand. After their discovery, monitoring campaigns quickly noted variability in the radiation emitted by some quasars. Subsequent variability studies have uncovered a wealth of information about their underlying physics. However, due to a lack of monitoring data, such studies have yet to be performed on the most remote quasars, those with redshift z greater than 4. Since first discovered in the late 1980s, about 50 of these quasars have been found. Comparing the physical characteristics and behavior of quasars in the very early universe with less remote quasars will provide insight into their evolution. The variability studies required to determine the underlying physics of these objects require monitoring data. To begin to satisfy this requirement, a program to monitor quasars with z greater than 4 was initiated in June 1995. to date, over a dozen objects have been monitored at the University of Florida's Rosemary Hill Observatory, Bronson, FL, and the Western Kentucky University Astrophysical Observatory, Bowling Green, KY.

Photometry with CCDs: Optimizing the signal extraction. RYAN CULLER,* FONSIE GUILARAN, MONICA DECKARD, CASEY WATSON, MICHAEL CARINI, RICHARD GELDERMAN, JOHN NOBLE, RICH-

ARD HACKNEY, KAREN HACKNEY, ROGER SCOTT, and SANDRA CLEMENTS, Western Kentucky University Astrophysical Observatory and Center for Automated Space Science, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101.

The CCD detector has revolutionized astronomical photometry. Photometric precision better than 1% has been achieved for bright sources. Faint sources, such as Active Galactic Nuclei (AGN), can also be studied with photometric precision approaching 1%. This is a significant improvement over the 10% precision achieved with photographic plates and the 5% precision usually achieved with photomultiplier tubes. However, these improvements can be reached only through the proper processing of the CCD image and appropriate extraction of the brightness information from the processed image. We discuss (1) what we have found to be optimal methods for both the processing of CCD images and the extraction of brightness information and (2) application to the measurement of brightness variations in AGN. We acknowledge funding from the NASA Kentucky Space Grant Consortium and the NASA funded Center for Automated Space Science at Western Kentucky University.

PHYSIOLOGY & BIOCHEMISTRY

Effects of ovariectomy and dietary energy restriction on the body weight of Fischer 344 rats. C. BROWN,* Y. ZHANG, C. WANG, and C. J. LEE, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Thirty-two fischer 344 rats were randomly assigned into four groups of eight rats each. Group 1 was sham-operated and fed the control diet (AIN-76A) ad libitum; Group 2 was ovariectomized and fed the control diet at the average intake of Group 1; Group 3 were sham-operated and fed the energy-restricted diet at 60% of the average intake of Group 1; Group 4 were ovariectomized and fed the energy-restricted diet at 60% of the average intake of Group 1. Sixty grams of the energy-restricted diet provided only 60% of the energy content, but equal amounts of protein, minerals, and vitamins of 100 g of the control diet. The experimental period was 8 weeks. There were no significant changes in the body weight of Group 1, whereas the body weight of Group 2 increased by 8% of the initial body weight. Both Group 3 and 4 lost ca. 20% of the initial body weight with no differences between the two groups. These results indicate that ovariectomized rats were more likely to gain weight than the sham-operated rats when fed the control diet and that both ovariectomized and sham-operated rats will lose weight when fed energy-restricted diets.

Effects of ovariectomy surgery on urinary calcium excretion of Fischer 344 rats. D. HAMPTON,* Y. ZHANG, C. WANG, and C. J. LEE, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Sixteen female Fischer 344 rats were fed AIN-76A for 1 week before they were placed in metabolic cages and the urine excretion was completely collected for 3 days. Half of the rats went through ovariectomy with the other half through sham-operation. One week after the operation, rats were placed in metabolic cages with the urine collected for 3 days. Urine samples were analyzed for calcium by atomic absorption spectrophotometry. Dietary intake, thus, dietary calcium intake, was lower after surgery than before surgery. Calcium intake post surgery was lower for ovariectomized than for sham-operated rats. Urinary calcium excretion (mg/day) decreased by 20% for sham-operated rats and by only 5% for ovariectomized rats. These results suggest that surgery decreased calcium absorption from the gut, leading to lower urinary calcium excretion in sham-operated rats; increased calcium mobilization from the bone may be responsible for the higher urinary calcium excretion in ovariectomized rats.

Gentamicin-induced nephrotoxicity in the teleost fish *Astronotus ocellatus* (Cichlidae). W. STEWART CURTSINGER* and HONG Y. YAN, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Aminoglycoside antibiotics (AGs) are effective in treating bacterial infections. The AGs also inflict ototoxic and nephrotoxic effects on treated subjects. Nephrotoxicity of gentamicin sulfate on fish was first described by Yan et al.; edema caused by gentamicin was documented subsequently. We used histological and autoradiographical methods to document temporal course of intake of gentamicin as well as cellular/structural damages caused by the gentamicin to the fish kidney. The experimental subjects, the teleost fish *Astronotus ocellatus*, received one, two, three, or four daily intramuscular injection(s) of 20 mg/kg or 120 mg/kg gentamicin and sacrificed 1 day after the last injection. Fourteen fish also received one injection of ^{3}H-labeled gentamicin (5 μCi/10 gm fish). Two fish as a group were sampled 1, 2, 4, 8, 24, 36, 48 hours after the injection. Kidney tissues were dissected and processed with standard histological procedures. Kodak-NTB3 emulsion was applied to sections with ^{3}H-labeled gentamicin. Exposure was carried out in a light-tight box up to 42 days and developed. Edema was obvious after just one injection. The weight gain increased as fish received more injections in both dosage groups. There was no significant difference in weight gain between two treatments. An indication that 20 mg/kg dosage was sufficient to illicit nephrotoxic damages. The necrosis was evident in the proximal tubular epithelial cells. Autoradiographical results showed the maximal uptake of gentamicin occurred between 36 and 48 hours after injection. The present study shows that fish kidney is a valid model for aminoglycoside nephrotoxicity study. Supported by Deafness Research Foundation, NIH-DC01729, and Howard Hughes Medical Institute.

Potential gentamicin-binding protein(s) in the ear of the cichlid fish *Astronotus ocellatus* (Cichlidae). STEVEN M. RIGDON,* W. STEWART CURTSINGER, BIN-TAO

PAN, and HONG Y. YAN, School of Biological Sciences and Department of Neurosurgery, University of Kentucky, Lexington, KY 40506.

Evidence of aminoglycoside (e.g., gentamicin sulfate) damages to the auditory sensory hair cells of the teleost fish *Astronotus ocellatus* was previously demonstrated. Continuation of the study further showed regeneration of damaged ciliary bundles 10 days after maximal damage. Our preliminary results using both immunocytochemical and autoradiographical methods indicate that the incorporation of gentamicin into hair cells occurs between 36 and 48 hours after intramuscular injection. To better understand the ototoxic and regeneration mechanisms involved, Western blotting was used to identify the presence of potential gentamicin binding protein(s). The fish were injected with gentamicin sulfate (120 mg/kg) and sacrificed either 24 or 48 hours later. Control fish received only saline injection. Three tissues (ear, kidney, and brain) were dissected and processed following standard immuno blotting protocols. Results indicated the presence of potential gentamicin-binding protein(s) in ear and kidney tissues, except tissues from control animals and brain tissues. Higher degree of binding was observed in 48-hr tissues than 24-hr samples. These results corroborated earlier immunocytochemical and autoradiographical findings on temporal uptake sequences of gentamicin. On-going experiments employing both biochemical and molecular techniques are designed to characterize the nature of the potential gentamicin-binding protein(s). It is hoped further understanding of gentamicin-binding protein(s) could lead to the development of prophylactic agent(s) against both ototoxic and nephrotoxic damages caused by aminoglycoside antibiotics. Supported by Deafness Research Foundation, NIH-DC01729 and Howard Hughes Medical Institute.

Relationship between muscle lipid content and body weight of paddlefish (*Polyodon spathula*). X. LOU, T. L. WHITE,* C. WANG, and S. D. MIMS, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Lipid content of fish meat is important to processing and marketing of fish meat. The objective of this study was to determine the muscle composition of paddlefish (Polyodon spathula: Polyodontidae) with various body weight. Twenty-five paddlefish (body weight 4 to 20 kg) were used for this study. Fillets with the red meat trimmed off were prepared and analyzed for moisture, total lipids, protein, and ash content. With increasing body weight, muscle moisture and protein content tended to decrease; muscle lipid content increased with ash content remaining constant. Muscle lipid content increased drastically when the body weight was 10 kg or more. These data will have practical implications for the harvesting of paddlefish and the ultimate use of paddlefish meat.

Skeletal compartmentalization and metabolism of calcium in maturing male and female rats. D. L. DEMOSS* and G. L. WRIGHT, Physiology Department, Marshall University School of Medicine, Huntington, WV 25704.

The ^{3}H-tetracycline (^{3}H-T) bone-labeling procedure was employed to monitor bone resorption from urinary loss curves in male and female rats of various ages. In addition whole body dry skeletal mass and the loss of ^{3}H-T from individual bones was determined. It was found that the dry skeletal mass/body mass ratio of females was significantly greater than for males, indicating the importance of dry skeletal mass in quantitative assessments. The urinary loss of ^{3}H-T was described by a double exponential equation (label loss from bone fluid and calcified skeletal compartments). The results indicate the presence of two distinct and independent exchangeable bone compartments as determined by the ^{3}H-T method. Both compartments decrease in size with age but their label loss activities are different. The label loss activity within the rapidly depleted compartment, which we suggest affects calcium loss from the bone fluid compartment, is "inert" in the sense that it does not change with age or between sexes. Label loss activity in the slowly depleted compartment, which we suggest represents resorption of calcified bone, is increased with aging. Despite a smaller skeletal mass, resorptive activity in this compartment is higher in female rats than in male rats by 24 weeks of age due to a larger compartment size and a maintained higher rate of resorptive activity within the calcified compartment.

Stability of paddlefish (*Polyodon spathula*) meat stored in crushed ice. X. LOU,* C. WANG, Y. XIONG,[1] G. LIU,[1] and S. D. MIMS, Community Research Service, Kentucky State University, Frankfort, Kentucky 40601; [1]Department of Animal Sciences, University of Kentucky, Lexington, KY 40546.

Fillets from six paddlefish (*Polyodon spathula*; Polyodontidae) were cut into seven sections for each fish. The sections were stored in sealed plastic bags covered by crushed ice. One section from each fish was taken on day 0, 1, 2, 3, 5, 7, 10. The samples were analyzed for thiobarbituric acid-reactive substances (TBARS) as an indicator of lipid degradation and protein solubility as an indicator for protein degradation. The data were analyzed with the GLM procedure of SAS and the storage time, fish, and section were the independent variables. There were no significant differences among the sections. There were no significant changes in protein solubility or TBARS during the 10 days of storage. These results indicate that paddlefish meat stored in crushed ice is stable for at least 10 days.

Producing the finest community college graduates. J.G. SHIBER, Division of Biological Sciences, Prestonsburg Community College, Prestonsburg, KY 41653.

At Prestonsburg Community College (Prestonsburg, KY) 998 students completed a questionnaire on how community colleges (CC) can produce the "finest" graduates for the 21st century, i.e., people well prepared for the challenges ahead. Most said that the primary mission of a

CC should be teaching and learning, and that its basic principle should be to provide a broad background so students can further their education and/or enter the job market. Successful graduates, they said, should be versatile, willing to pursue varied career options, and able to integrate disciplines. Nearly all agreed that to achieve this end, the CC should offer courses and programs reflecting basic economy and work force changes, draw upon all parts of society's talent base to train students, and reassess long-standing educational practices, especially in the sciences and engineering. Are these students' expectations being met at their CC? When asked why enrollment is down and what should be done about it, or how the CC can best use its funds or improve its educational mission, the most frequent response was always "Provide more courses/degree programs/career options." Many said that their CC did not offer all the courses their majors required for entry into a university, that better facilities and student services (e.g., daycare and tutoring) were needed, and that more sensitivity to student needs and abilities, with less emphasis on non-academic matters, is essential. Of the 81% planning to attend a university, over half said the most important thing they needed to experience at the CC was improvement of personal discipline (study, note-taking, attendance habits). The results indicate that CC's may have to pay more attention to student academic needs and concerns if they are to produce the "finest" graduates for the 21st century.

Student attitudes toward the study of biology. JOHN R. MORRISON* and RUTH E. BEATTIE, T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

A pre- and post-course attitudinal survey was administered to students enrolled in freshman-level major and non-major biology courses at the University of Kentucky (Lexington, KY). The survey included questions on (1) the perceived importance of biology in everyday life; (2) the image of biology courses; and (3) factors that influenced the student to take a biology course. The surveys were self administered and the resulting data were analyzed using the T-test and mean values. All surveys were administered to students taught by the same instructor. Pre-course data analysis indicated that non-biology-major students had a less positive attitude than biology major students towards the importance of biology in everyday life. Post-course scores showed a significant increase in agreement with the statement that "Everyone should study biology" and that an understanding of biology is important in politics and aids intelligent voting. All test groups agreed strongly with the statement that "Biology involves a lot of memorization." An interest in the subject matter was the primary factor in influencing students to take a biology course. The majority of students surveyed had a positive experience in their high school biology courses.

Student perspectives of field investigations: misconceptions, problems, and educational benefits. TERRY L. DERTING* and JILL H. KRUPER, Department of Biological Sciences, Murray State University, Murray, KY 42071.

Life is the theme of biology, yet all too often it is studied only in artificial laboratory environments. Despite the inherent appeal of the outdoors, the idea of conducting field investigations of biological phenomena is often met with trepidation. We conducted a case study of a field ecology class in which groups of students initiated and completed field investigations. Many of these students had no prior field experience. Throughout the course we monitored the feelings, problems, and successes of each group. Student misconceptions regarding the projects included the necessity of sophisticated knowledge and equipment and the inability of peers to contribute useful input to other groups projects. Problems encountered were difficulty focusing on a specific testable hypothesis, difficulty observing experimental subjects, lack of experimental controls, difficulty drawing conclusions from data, disagreements among group members, and time constraints. Groups whose data did not support their hypotheses, or whose data collection proceeded more slowly, viewed their project as less good than groups whose data supported their hypotheses or who gathered data more quickly. The educational benefits realized included recognition of the importance of flexibility and patience when conducting an investigation, awareness of biases in methodology and data presentation, advantages of working in a group, and recognition of students' abilities to pose and investigate ecological questions. The class consensus was that field investigations are frustrating yet exciting and rewarding. The students agreed unanimously that continual monitoring of each student's progress, thoughts, and feelings was of great benefit.

Traditional vs. computerized education: a student survey. J.G. SHIBER, Division of Biological Sciences, Prestonsburg Community College, Prestonsburg, KY 41653.

A survey of 873 students at Prestonsburg Community College (Prestonsburg, KY) reveals their opinions about learning via computer technology (including TV, videocassettes, Internet) versus the traditional approach of attending live lectures, taking notes, using textbooks, etc. The majority said that they prefer the traditional style of learning and that computer learning is more expensive, less humane, and even less efficient. Most said that computers will be essential in their careers but believe that ultimate success in career and life is best derived from a traditional education, which they define as more well rounded than a computerized one. Teacher-student feedback, the majority agreed, is necessary for quality learning, and professors lecturing in class can give more information, through elaboration, than computers. Most students largely attributed our present social problems and inability to solve them to the fast rate of our technological development. They were almost equally split, however, as to whether or not computer use would eventually cause the demise of society as we know it, or if we can live without

computers. The majority believed that humans are not organically (physically and mentally) evolved enough to be completely dependent on computers. Nor did they see exceptional, near-future benefits from computers. Indeed, the trend towards computerization of everything bothers the majority of those surveyed. Despite their preferences and reservations, however, most students seemed resigned to the inevitability that computerized education will eventually replace the traditional.

Undergraduate course in science ethics for biology and chemistry majors: I. development and content, FRANK H. WILBUR, Department of Biology, Asbury College, Wilmore, KY 40390.

In 1986 selected ethical dilemmas relating to the health professions were incorporated into PHP 201, Introduction to the Health Professions, a course offered at Asbury College (Wilmore, KY) and open to any student interested in exploring career options in health-related fields. In 1990, PHP 201 was dropped in favor of requiring a one-credit-hour course in Science Ethics (BIO 372) for all senior biology and chemistry majors. This course, which is now required of all junior science majors, has been modified each of the 6 years it has been taught. An introduction to ethics and ethical theory, as well as discussions on world views, values, character development, and the prevailing cultural moral climate, precede class exercises focusing on solving ethical situations and dilemmas in scientific research, medicine, and ecology. While the ethical dilemmas presented have varied somewhat from year to year, those dealing with aspects of information disclosure (truth telling and informed consent), allocation of health care, professional conduct in scientific research, and environmental stewardship have been regularly included. Course evaluations submitted by all students enrolled in the course have played a major role in course modifications. The most recent evaluations reveal considerable student satisfaction and enthusiasm for course format and content.

Undergraduate course in science ethics for biology and chemistry majors: II. world view and values, FRANK H. WILBUR, Department of Biology, Asbury College, Wilmore, KY 40390.

In 1990 a one-credit-hour course in Science Ethics (Bio 372) required for all biology and chemistry majors was added to the science curriculum at Asbury College (Wilmore, KY). It readily became apparent that many students taking the course lacked the foundational skills necessary to properly address ethical situations and dilemmas. They had little or no knowledge of basic ethical theory, had not sufficiently formulated their world view, and had not clarified their personal value system. The course, as currently offered, addresses these concerns. Twenty-five percent of the course is devoted to basic ethical theory and assessment of the present cultural climate. Students are presented with examples of different world views. These may include a Biblical world view from the Christian and the Jewish perspective, a postmodern world view, a relativist world view, and/or a humanistic world view. Students are required (1) to formulate and articulate their own world view and to use it as a foundation in the ethical decision-making process, (2) to identify the sources of their moral beliefs, and (3) to articulate and defend their personal value system. Teaching objectives include consciousness-raising and sensitizing; and values analysis, clarifications, and criticism. Student response to this approach, as determined by comments and student evaluations, has been most positive. Also, the ability of students to more intelligently apply appropriate ethical decision-making processes to solving ethical situations and resolving ethical dilemmas has shown marked improvement.

ZOOLOGY & ENTOMOLOGY

Analyses of size and density of *Tectarius muricatus* (Littorinidae) on San Salvador Island, Bahamas. MELINDA L. CRAWFORD,* JACQUELINE M. GRANESE, and RUDOLPH PRINS, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

Samples of *Tectarius muricatus* collected from transects at nine sites on San Salvador Island in the Bahamas were counted and measured with regard to vertical zonation on their respective rocky shores. Analyses were conducted using a two-way ANOVA with no interaction effects. Size differences were found among the different sites. However, the experimental results for *T. muricatus* contradict those of many other littorinids in that no significant vertical size gradient existed in the populations. Densities of the snails within and between the sites were analyzed to determine if vertical gradients existed. No significant differences in density were found among the sites, but a significant differential was exhibited between the lowest two zones of the transects when averaged across all sites. These results are discussed with respect to several possible environmental and physical factors that may have affected the size and density distributions of *T. muricatus*. Quantification of patterns such as distribution, density, and size are necessary in preliminary studies of this virtually unknown littorinid so that its superior physical tolerance may be investigated.

Characterization of snapping action of the alpheid shrimp *Alpheus heterochaelis*. JARED FIALKOW* and HONG Y. YAN, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Alpheus heterochaelis is a alpheid shrimp with a large snapping claw. When this claw is forcibly closed, it results in a loud snap sound. Since underwater sound propagates fast over long distance, it has been hypothesized that the snapping sound may be used for underwater acoustic communication. This work is designed to test the "acoustic communication" hypothesis. Individual shrimp were housed in a rectangular tank and a plastic rod was used to prod to initiate snapping action. A hydrophone was used to record the snapping sound. The recorded sounds were analyzed with a computer-based SIGNAL analysis system. Time, frequency signal, and spectrogram corre-

lations were compared between sounds of different individuals (males, females). Playback of the snapping sound was also performed on 10 shrimp to initiate snapping action. Snapping activity of two encountering individuals under various conditions (light, dark, amputation of antennular and antennal flagellum) was also videotaped and analyzed. Sound similarity analyses showed no significant difference in acoustic signatures made by different animals. Playback of the snapping sound failed to initiate any snapping action. Contacts of antennular or antennal flagellum of two encountering shrimp before snapping occurred were observed in 256 snappings (out of a total of 268). For amputated shrimp physical contact of body parts was required before snapping actions were initiated. The results show that the snapping sound is not used for acoustic communication function. It is likely that the strong underwater jet currents created by the snapping action are used by the shrimp to ward off intruders. The snapping sound produced is the by-product of the snapping action. Supported by Howard Hughes Medical Institute and a University of Kentucky Undergraduate Research Creative Grant.

Comparison of a hybrid (*Heliconia latispatha* × *imbricata*; Heliconiaceae) with its parent species with respect to extrafloral nectaries. C. TONY R. HAMPTON* and THOMAS C. RAMBO, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

As part of on-going studies of the role of extrafloral nectaries on buds of *Heliconia latispatha* and of the insects visiting these nectaries, we studied a hybrid of *H. latispatha* (which has nectaries) and *H. imbricata* (which lacks nectaries). We studied a set of 10 *H. latispatha*, 10 *H. imbricata*, and 8 hybrids, all growing along the edge of the landing strip at Estación Sirena in Parque Nacional de Corcovado, Costa Rica. We recorded the insect visitors to the buds hourly from 0530 to 0930 and from 1330 to 1730, the time of most insect visitation, for 5 days. *Heliconia latispatha* had significantly more insects (primarily ants) on the buds; *H. imbricata* and the hybrid had few to no insects on the buds. The buds of *H. latispatha* and the hybrid are similar in color, so color is not primarily responsible for the attraction of insects. We hypothesize that the insect attraction by *H. latispatha* is chemical, and that the genes for this attraction must be recessive.

Comparison of two species of *Heliconia* (Heliconiaceae) with respect to the presence of extrafloral nectaries. THOMAS C. RAMBO* and C. TONY R. HAMPTON, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

In previous studies we have shown that the platanillo, *Heliconia latispatha*, produces nectar on the outside of bracts of terminal buds in an inflorescence. The nectar, secreted onto the bud's surface, is utilized by a variety of insects, including ants, mosquitoes and other dipterans (Dolichopodidae, Richardidae, Neriidae), crickets, roaches, lygaeid bugs, and wasps. To clarify the function of these extrafloral nectaries we compared the insect visitors to *Heliconia latispatha* with visitors to the buds of *H. imbricata*, a species growing close to *H. latispatha*. We recorded the visitors to 15 buds of each species hourly from 0530 to 0930 and from 1330 to 1730, the time of most insect visitation, for 5 days. *Heliconia imbricata* attracted significantly fewer insects than *H. latispatha*. In fact *H. imbricata* attracted so few insects that we conclude that it does not produce nectar on the buds. We will discuss possible reasons for this difference and their implications for determining the function of the extrafloral nectaries on *H. latispatha*.

Effects of cannibalism on population structure of the marine cave isopod *Bahalana geracei* from Lighthouse Cave, San Salvador Island, Bahamas. JERRY H. CARPENTER and RONALD D. BITNER,* Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Cannibalism by *Bahalana geracei* was observed once in Lighthouse Cave, in July 1995: a 11.0 mm long female was eating a 6.5 mm long female. We performed experiments to investigate factors influencing cannibalism in this troglobitic species. From August to November 1996, two to four specimens were placed together in small plastic bowls, maintained without food in incubators at cave temperature (26°C), and observed daily. We never witnessed cannibalism while it was occurring in the lab; we did find remains of six cannibalized victims (some still alive)—translucent exoskeletons of cannibals revealed remains of victims inside. The following cannibal/victim combinations show the variety of sizes: (1) 3.2 mm second-instar manca (baby)/2.7 mm first-instar manca (sex of mancas could not be determined), (2) 4.2 mm fourth-instar juvenile female/2.7 mm first-instar manca, (3) 8.2 mm female/8.2 mm female slightly damaged in another predator-prey experiment, (4) 14.3 mm female and 7.4 mm female/7.5 mm, (5) 14.3 mm female/7.4 mm female (same individuals as above), and (6) 15.7 mm female and 13.9 mm female/12.4 mm female. These cannibalisms occurred after specimens were together several days, except case #3, which occurred within 1 day. In at least 10 additional cases, two to four specimens of various sizes and sexes were together >30 days without cannibalism. We conclude that cannibalism in *B. geracei* is unpredictable. It is more likely when size difference is large. Cannibalism in *B. geracei* seems less common than in many surface-dwelling arthropods (e.g., spiders and other marine isopods). Cannibalism is reduced by *B. geracei's* starvation resistance and slow metabolism. Nevertheless, cannibalism is probably frequent enough in this long-lived species to significantly affect the population structure.

Female preferences for stimulatory male odors in the prairie vole (*Microtus ochrogaster*). JILL H. KRUPER* and TERRY L. DERTING, Department of Biological Sciences, Murray State University, Murray, KY 42071.

Physical contact with conspecific pheromones or urine of males is necessary for reproductive stimulation of female prairie voles. We investigated whether female prairie voles (*Microtus ochrogaster*) of different ages and reproductive status prefer odors from males who are most likely to stimulate female reproductive activity. We compared odors from intact and castrated adult males because urine from intact males is known to be a more effective stimulant of female reproductive activity than is urine from castrated males. Preferences of females for male odors were determined using a Y-maze olfactometer or nesting apparatus. At 7–10 d post-surgery, young anestrus females significantly preferred the odors of intact males as compared to the odors of castrated males. At 2 months post-surgery, no significant preference for odors of intact or castrated males was exhibited by older anestrus females. Older females in estrus, however, tended to prefer the odor of intact males. Young and old anestrus females showed no significant preference for the odors of castrated or intact males during nesting-preference tests; however, young females in estrus tended to discriminate against the odors of castrated males. Our results indicated that young females, and those in estrus, were most likely to discriminate among male odors, exhibiting less preference for odors from castrated males than intact males. Male odors indicative of reproductive potential may, therefore, play a role in female mate-choice in prairie voles depending upon the age and reproductive status of females.

Impacts of dam construction on densities and distributions of bald eagles, *Haliaeetus leucocephalus*, Accipitridae) along the Ohio River shoreline between Paducah, Kentucky, and Cairo, Illinois. VINCENT EVIN STANFORD* and TERRY L. DERTING, Department of Biology, Murray State University, Murray, KY 42071.

Shoreline densities and distributions of wintering bald eagles (*Haliaeetus leucocephalus*) were compared to examine the impacts of an ongoing lock-and-dam construction project on an Ohio River segment 65 km long between Paducah, KY, and Cairo, IL. Low-altitude flights were conducted once weekly for 10 weeks from mid-December through mid-March in winters of 1987/1988 (pre-dam construction) and 1994/1995 (ongoing dam construction). Densities and distributions of bald eagles per river quarter-mile were recorded during the survey flights. Proportional densities of bald eagles were compared between the two survey periods in a 1- and a 6-mile interval with the site of dam construction centered in each interval. Proportional densities of bald eagles in the 1-mile interval, during the 1994/1995 surveys, were significantly reduced from proportional densities that occurred during the 1987/1988 surveys ($P = 0.0024$). A marginally significant reduction in proportional densities of bald eagles occurred in the 6-mile interval during the 1994/1995 surveys when compared with the 1987/1988 surveys ($P = 0.07$). A marginally significant shift in ¼-mile distributions along the entire segment also occurred between the two survey periods ($P = 0.06$). These results indicated that bald eagles were avoiding the area of dam construction and that overall bald eagle distributions may be shifting away from the area of dam construction. We recommend that further studies be conducted to examine future impacts on bald-eagle densities and distributions as construction proceeds and to determine long-term impacts of dam placement on bald eagles.

Nest-site selection and leaf-nest composition of *Sciurus carolinensis* (Sciuridae) in continuous and isolated woods in western Kentucky. JAMES S. ARMSTRONG* and TERRY L. DERTING, Department of Biological Sciences, Murray State University, Murray KY 42071.

Location and composition of grey squirrel (*Sciurus carolinensis*) leaf-nests were analyzed in three different forest habitats: old (116–125 years) continuous-forest (3 sites, 2 ha each); young (85–92 years) continuous-forest (3 sites, 2 ha each); and isolated woodlot (106 years; 1 site, 3 ha). The isolated woodlot contained a significantly higher number of small (DBH 3–25 cm) and total number of trees than either continuous-forest habitat. No significant differences in vegetational cover existed among habitats. The isolated woodlot had significantly higher nest density (12.5 nests/ha) than the old or young continuous-forest habitats (3.2 ± 0.9 nests/ha and 3.3 ± 0.3 nests/ha, respectively). The density of grey squirrels in the isolated woodlot (13.2 squirrels/ha) was also significantly greater than squirrel densities in old and young continuous-forest habitats (1.9 ± 0.6 and 1.8 ± 1.0 squirrels/ha, respectively). A significant positive correlation existed between leaf-nests/ha and grey squirrels/ha across the seven study sites. Grey squirrels chose significantly fewer small size trees (DBH <20 cm) and significantly more medium size trees (DBH 20–50 cm) for leaf-nest placement than expected based on tree availability. Grey squirrels chose large size trees (DBH > 50 cm) for leaf-nest placement in accordance with tree availability. Tree species selected and the preferred DBH size for nest location were positively correlated in the young continuous-forest and isolated woodlot habitats. The composition of leaf-nests did not differ among habitats although the leaf-nests in the old continuous-forests had the greatest dry masses.

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1. GENERAL

A. Original papers based on research/review in science will be considered for publication in the *Transactions*; at least the first author must be a member of the Academy. Announcements, news, and notes will be included as received.

B. Papers (in triplicate) may be submitted at any time to the editor.

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C. Format/style of papers must conform to practices in recent issues of the *Transactions*, which are, in effect, a style manual. The running head at top right of each page should give name of author(s), a short version of paper title, and page number of total. Do not staple pages together.

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Kohn, J.R. 1993. Pinaceae. Pages 32–50 in J.F. Nadel (ed). Flora of the Black Mountains. University of Northwestern South Dakota Press, Utopia, SD.

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NEWS

KAS Annual Meetings

The 1997 annual meeting of the Kentucky Academy of Science will be held 13–15 November at Morehead State University, Morehead; the 1998 meeting, at Jefferson Community College, Southwest Campus, Louisville.

PUBLICATION

Wildflowers of Mammoth Cave National Park, by Randy Seymour, has just been published. A soft-cover book of 254 pages, it illustrates by excellent color photographs about 400 wildflowers, each with accompanying text. Appendices show flowering times, where in the park the various flowers have been seen, and the number of species noted by Seymour along each of the park's trails. Order from bookstores or directly from The University Press of Kentucky, 663 South Limestone Street, Lexington, KY 40508-4008; ISBN 0-8131-0898-5; price $17.95 (+$3.50 shipping).

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Continuing Decline in the Freshwater Unionid (Bivalvia: Unionidae) Fauna in the Cumberland River Downstream from Cumberland Falls, Kentucky

Ronald R. Cicerello and Ellis L. Laudermilk

Kentucky State Nature Preserves Commission, 801 Schenkel Lane, Frankfort, Kentucky 40601

ABSTRACT

The freshwater unionids in the Cumberland River downstream from Cumberland Falls in southeastern Kentucky were sampled to determine the status of the fauna relative to previous studies. A comparison of collections by Wilson and Clark in 1910–1911, Neel and Allen in 1947–1949, and Stansbery in 1961 revealed the loss of 6 of the 22 species known from the site and reduced abundance in several others. We found only 10 live species, all less abundant than during previous studies, and no shells. The continuing decline in the fauna is attributable to habitat degradation and loss associated with coal mining, general watershed development, and impoundment of the river to create Lake Cumberland.

INTRODUCTION

Long-term monitoring can provide status and trend information needed for conservation of freshwater unionids. To determine changes in the Cumberland River fauna downstream from Cumberland Falls in southeastern Kentucky following the creation of Lake Cumberland, Stansbery (1969) sampled the unionids and compared his findings to those of Wilson and Clark (1914) and Neel and Allen (1964). Wilson and Clark (1914) found 20 species, 10 of which were common or abundant, at this site in 1910–1911 while evaluating the Cumberland River as a source of unionids to replace upper Mississippi River populations overharvested for button manufacturing and pearls. During 1947–1949, just prior to the closure of Wolf Creek Dam and the formation of Lake Cumberland, Neel and Allen (1964) encountered 15 species at the site, 13 of which were common or abundant.

Stansbery (1969) collected below the falls in 1961 and noted that changes in faunal composition prior to Neel and Allen (1964) were attributable to almost 40 years of increased acids from coal mining and washing above the falls. Changes after 1949 may have resulted from continuing impacts from coal mining operations and the influence of Lake Cumberland. By 1947–1949, *Cyclonaias tuberculata, Ellipsaria lineolata, Obliquaria reflexa,* and *Ptychobranchus subtentum* were lost from the site, but *Villosa iris* had been found. Between 1949 and 1961, *Lampsilis cardium* and *Truncilla truncata* were lost, 6 of the 16 taxa found by Stansbery (1969) had decreased in abundance, and the remainder were stable or had increased in number. We resampled this site to determine the present status of the fauna.

STUDY AREA

Cumberland Falls, a 17-m-high barrier to the upstream movement of aquatic organisms (Burr and Warren 1986; McGrain 1966), is located at river km 904.9 in McCreary and Whitley counties in southeastern Kentucky (Figure 1). Land use in the 5120 km^2 watershed (Bower and Jackson 1981) is ca. 84% for-

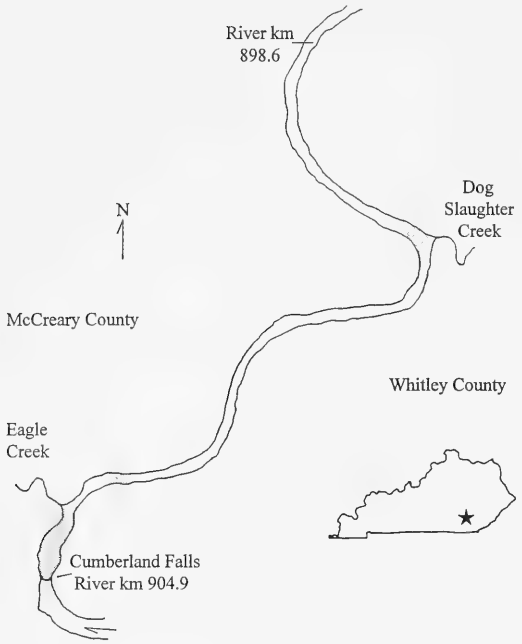

Figure 1. Cumberland River study area downstream from Cumberland Falls, McCreary and Whitley counties, Kentucky. Inset shows study area location in the state. Shaded areas were searched for unionids.

est, 13% agriculture, 2.5% mining, and 0.5% urban and developed areas (Mayes, Sudderth, and Etheredge 1975). Downstream from the falls, the river flows through a forested and boulder-lined gorge and is impounded by the backwaters of Lake Cumberland at approximate river km 898.6, depending on reservoir pool elevation. The Cumberland River was impounded at river km 742 in 1950 to create Lake Cumberland. At low flow, river width ranges from ca. 5 to 24 m in riffles and pools, respectively, and depth exceeds 5 m in pools and 1 m in riffles. Pools and riffles are composed of large boulders, cobbles, and extensive deposits of coal fines and sand intermixed with lesser quantities of gravel, pebbles, coal, and trash in eddies and areas sheltered from swift current. During low flow, huge deposits of sand and coal fines are exposed in the plunge basin below the falls. Mean discharge on the sampling dates was 7.4 m^3/sec, considerably less than the mean of 26.6 m^3/sec for July–September, when streamflow is at or near the annual low, and the mean annual discharge of 89.7 m^3/sec (USGS 1993). Water quality is improving, but the upper Cumberland River continues to be impacted by pollutants associated with coal mining (e.g., acid mine drainage, silt, and metals), domestic waste, highway construction, and poor land use (Harker et al. 1980; Kentucky Division of Water 1994a, 1994b).

MATERIALS AND METHODS

In 1987, 1989, and twice in 1993, we examined a 0.5-km-long river segment from the base of Cumberland Falls to Eagle Creek for unionids (Figure 1). We made additional collections 0.5 km downstream from Eagle Creek and near the mouth of Dog Slaughter Creek to further gauge the status of the fauna. During each visit, one or two persons searched 2 or more hours for shells and live specimens by snorkeling and with waterscopes, and the shoreline was examined for shells. Nomenclature follows Hoeh (1990) and Turgeon et al. (1988).

RESULTS AND DISCUSSION

We found a total of 10 species, a loss of six taxa when compared to Stansbery's (1969) results and an overall 54% reduction in the fauna compared to earlier studies (Table 1). With the exception of *Elliptio crassidens,* all taxa were less abundant than in 1961. Among the taxa lost are *Villosa trabalis,* a USFWS (1994) endangered species, and *Lampsilis ovata, Ptychobranchus subtentum,* and *Villosa lienosa,* which are rare in Kentucky (KSNPC 1996). Others that have declined or have been lost (e.g., *Actinonaias ligamentina, Elliptio dilatata, Lampsilis cardium, Potamilus alatus, Quadrula pustulosa, Tritogonia verrucosa*) have broad habitat requirements (Gordon and Layzer 1989) and often are dominant members of unionid communities (Houslet 1996; Smathers 1990). *Corbicula fluminea,* an introduced exotic bivalve, was uncommon.

Observations by Wilson and Clark (1914) and Neel and Allen (1964) provide anecdotal evidence of the decline in unionid abundance at the falls. Wilson and Clark (1914) reported that unionids were "usually found crowded about the base of large rocks" where they were "easily accessible to their enemies, especially during low water, and many of them are killed by muskrats, raccoons, mink, and occasional otter." Neel and Allen (1964) found

Table 1. Freshwater unionids collected from the Cumberland River downstream from Cumberland Falls, Kentucky, in four studies: 1910–1911, 1947–1949, 1961, 1987–1993.

Species	1910–1911[1]	1947–1949[2]	1961[3]	1987–1993[4]
	Number collected			
Actinonaias ligamentina (Lamarck)	194	A	39	2
Actinonaias pectorosa (Conrad)	73	A	161	>50
Cyclonaias tuberculata (Rafinesque)	P	0	0	0
Ellipsaria lineolata (Rafinesque)	P	0	0	0
Elliptio crassidens (Lamarck)	57	C	(2)	2
Elliptio dilatata (Rafinesque)	122	A	113	7
Lampsilis cardium (Rafinesque)	0	A	0	0
Lampsilis fasciola Rafinesque	16	C	20	0
Lampsilis ovata (Say)[5]	49	0	10	0
Lasmigona costata (Rafinesque)	41	0	1(?)	2
Ligumia recta (Lamarck)	8	A	7	1
Medionidus conradicus (Lea)	P	A	154	0
Obliquaria reflexa Rafinesque	8	0	0	0
Potamilus alatus (Say)	8	C	28	5
Ptychobranchus fasciolaris (Rafinesque)	81	A	35	5
Ptychobranchus subtentum (Say)[5]	P	0	0	0
Quadrula pustulosa (Lea)	49	A	122	10
Tritogonia verrucosa (Rafinesque)	32	A	75	4
Truncilla truncata Rafinesque	16	R	0	0
Villosa iris (Lea)	0	R	27	0
Villosa lienosa (Conrad)[5]	P	0	9	0
Villosa trabalis (Conrad)[5]	41	A	7	0
Total species	20	15	16	10
Total individuals	810	—	810	ca. 88

[1] Wilson and Clark (1914); [2] Neel and Allen (1964); [3] Stansbery (1969); [4] maximum number observed alive on any sampling date during the present study; [5] USFWS (1994) and/or KSNPC (1996) listed species. A = abundant; C = common; R = rare; P = present; 0 = none taken; (2) = subfossil remains only.

"great numbers of mussels, many of which were quite large and very old" in sand pockets among large stones and slabs. We found a concentration of live unionids only in a small area near the mouth of Eagle Creek. No shells deposited by floodwater or left by predators were found along the shoreline.

In the river 0.5 km downstream from Eagle Creek and near the mouth of Dog Slaughter Creek, we found limited numbers (in order of abundance) of only *Actinonaias pectorosa, Tritogonia verrucosa, Actinonaias ligamentina, Elliptio dilatata, Potamilus alatus, Ptychobranchus fasciolaris,* and *Quadrula pustulosa.* These sites are within the river segment extending from "Anvil Shoals," 1.6 km below the falls, to Burnside, Kentucky, that mussel fisherman reported was full of mussels (Wilson and Clark 1914).

The fauna of this last remaining free-flowing segment of the Cumberland River below the falls in southeastern Kentucky is now comprised largely of species with broad environmental tolerances (Dennis 1984; Gordon and Layzer 1989). The change in the fauna is attributable to pollutants associated with coal mining and to the impoundment of the river (Stansbery 1969), and general watershed development (e.g., road building, channelization, urbanization). Recolonization is precluded by Lake Cumberland, which isolates remnant unionid populations in Buck Creek (Schuster, Butler, and Stansbery 1989), the Rockcastle River (Cicerello unpubl. data; Thompson 1985), and the Big South Fork Cumberland River (Bakaletz 1991), and which acts as a barrier to the movement of host fishes. Direct tributaries to the area (e.g., Eagle and Dog Slaughter creeks) do not support unionid populations, and the Cumberland River above Cumberland Falls supports a depauperate fauna comprised of 11 species that generally are uncommon in the basin (Cicerello unpubl. data; Cicerello, Warren, and Schuster 1991).

The unionid community in the river below Cumberland Falls is the last and best remaining vestige of the diverse fauna that historically inhabited the mainstem Cumberland River in southeastern Kentucky. Downstream from Wolf Creek Dam, fluctuating levels of

cold, turbulent water released from Lake Cumberland preclude unionid reproduction, and only *Cumberlandia monodonta* Say and *Cyclonaias tuberculata* persist (Cicerello personal observation; Miller, Rhodes, and Tippit 1984). Lake Cumberland backwaters in the lower Big South Fork Cumberland and Rockcastle rivers, and the mainstem are heavily silted and have yielded *Leptodea fragilis* Rafinesque, *Potamilus ohiensis* Rafinesque, *Pyganodon grandis* Say, and *Utterbackia imbecillis* Say. Only *Potamilus alatus* and *P. ohiensis* inhabit the lower embayed segment of Buck Creek (Schuster, Butler, and Stansbery 1989). Perhaps 20 (31%) of the 65 taxa that historically inhabited the mainstem Cumberland River remain (Cicerello unpubl. data).

Monitoring in the river downstream from Cumberland Falls in the future will provide additional information about the impact of Lake Cumberland and watershed land use on the unionid fauna. However, the survival of Kentucky's remnant Cumberland River unionid community, which includes members of the unique Cumberlandian fauna (Ortmann 1924) (e.g., *Epioblasma brevidens, E. capsaeformis, Ptychobranchus subtentum,* and *Villosa trabalis*) found nowhere else in the state, is dependent upon the protection of Buck Creek, the Rockcastle River, and the Big South Fork Cumberland River.

ACKNOWLEDGMENTS

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Characterization of Tall Fescue Plantlets Regenerated from Cultured Panicle Segments/Anthers for Meiotic, Isozyme, and DNA Changes

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ABSTRACT

Previous research identified four tall fescue (*Festuca arundinacea* Schreb.) genotypes (K8, K16, K25, K27) out of 20 in 'Kentucky 31' that regenerated plantlets from inflorescence culture. The objectives of our study were to determine whether plantlets could be obtained from these same genotypes using anthers as the explant and to characterize the anther-derived plantlets for ploidy, meiotic, isozyme, and DNA changes. Panicle segments of each genotype were cultured on Schenk and Hildebrandt medium with 2.0 or 4.0 mg/liter 2,4-dichlorophenoxyacetic acid in three separate studies. After 2 weeks, florets opened; then anthers were removed from the florets, equally divided among genotypes and hormone treatments, and cultured, 25 anthers per petri plate, on the same medium as the original panicle segment. Variables measured were callus growth, root growth, albino shoot regeneration, green shoot regeneration, and green plantlets transplanted into soil. Significantly more green plantlets regenerated from genotype K25 in two of the three studies. Thirty-two K25 and two K27 regenerants transplanted to the field were hexaploid (2n=6x=42) with normal chromosome pairing and morphologically like non-cultured tall fescue. Isozyme and RAPD profiles were identical to the maternal plants, indicating the regenerants were derived from somatic tissue, rather than being doubled haploids.

INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb., 2n=6x=42) is an open-pollinated, perennial, cool-season pasture and turf grass. Of the ca. 7.0 million acres of grassland in Kentucky, 5.5 million acres have been planted to 'Kentucky 31' tall fescue (Pedersen and Lacefield 1989; Siegel et al. 1984). Kentucky 31 has poor forage quality, and nearly 80% of the tall fescue fields in Kentucky are infected with an endophytic fungus (*Neotyphodium coenophialum* (Morgan-Jones & Gams) Glenn, Bacon, & Hanlin). A diet of infected tall fescue may be detrimental to animals. Efforts at the University of Kentucky to improve the forage quality of tall fescue would be enhanced if non-infected tall fescue plants were identified that could regenerate haploid plantlets dependably from anthers.

Earlier research by Kasperbauer et al. (1980) reported that tall fescue haploids were obtained from inflorescence (panicle) culture; to our knowledge these plants are no longer extant. Using a similar technique, Eizenga and Dahleen (1990) identified four genotypes (plants) from 20 genotypes in non-infected Kentucky 31 tall fescue that regenerated plantlets from inflorescence culture. All 198 inflorescence-derived plantlets were morphologically normal. Meiotic and isozyme analyses of the 95 regenerants showed them to have 42 chromosomes, normal chromosome pairing, and the same isozyme banding patterns as the parent plants. The isozyme banding patterns suggested that the regenerants were derived from somatic tissue.

Bohanec et al. (1995) used differences in RAPD (random amplified polymorphic DNA) profiles among regenerants from onion (*Allium cepa* L.) ovules to identify different ploidy levels. Also, RAPD profiles have been employed to determine the phylogenetic relation-

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ships between species of *Festuca* and *Lolium* (Stammers et al. 1995).

The objectives of our study were (1) to determine whether regeneration would occur from the four tall fescue genotypes identified by Eizenga and Dahleen (1990) using anthers as the explant and (2) to characterize the regenerated plantlets for possible ploidy, meiotic, isozyme, and RAPD variants.

MATERIALS AND METHODS

The four Kentucky 31 tall fescue genotypes (K8, K16, K25, K27) we used were selected from 20 individual plants (genotypes) as regenerating plantlets from cultured tall fescue inflorescences (Eizenga and Dahleen 1990). These genotypes were evaluated in three different studies.

Previously, the procedures used to harvest, decontaminate, and culture the panicles were reported (Eizenga and Dahleen 1990). Cultures of panicle segments were maintained at 25 ± 2° C in the dark for 2 weeks, during which time the florets began to open. After 2 weeks the anthers were removed from the two proximal florets of each spikelet and placed on the same medium used to culture the panicle segments. Twenty-five anthers were placed on each plate. The cultured anthers were maintained at 25 ± 2°C in a 12-hour photoperiod using 40 W cool-white fluorescent lamps (ca. 20 $\mu E/m^2/s$ at the culture surface).

Both the panicle segments and anthers were cultured on Schenk and Hildebrandt basal salt medium (Schenk and Hildebrandt 1976) with 75 g/liter sucrose, 6 g/liter agar, and 2 or 4 mg/liter 2,4-D (2, 4-dichlorophenoxyacetic acid). In the second study (trial II), kinetin (1 mg/liter) was included to determine its effect on plantlet regeneration. A total of 4800, 5400, and 9600 anthers were cultured in the three studies, trial I, trial II, and trial III, respectively; these anthers were equally divided among the genotypes and hormone treatments. The cultured anthers were observed every 3 to 4 weeks for callus, root, albino shoot, and green shoot growth over the next 3 months. This growth was scored as the total number of anthers per plate showing evidence of callus, root, and/or shoot growth. The total number of albino and green plants regenerated from the cultured anthers was recorded. All green plantlets were placed on a growth medium with no auxin and half-strength major salts prior to transfer to Jiffy-7 peat pellets (Jiffy Products, Batavia, Illinois). Subsequently, the green plantlets were transplanted to the field for vernalization and to obtain inflorescences for meiotic analyses.

Data for cultured anthers were analyzed using a 3 × 4 × 2 factorial design with trial, genotype, and 2,4-D concentration (conc.) being the factors. (Each petri plate was a unit of replication.) This analysis showed that the trial × 2,4-D conc., genotype × 2,4-D conc., and trial × genotype × 2,4-D conc. interactions were non-significant. Only the trial × genotype interaction was significant, so the data were reanalyzed with only the trial × genotype interaction included to determine the least-square means. Significance was reported at the 0.05 level using a square root transformation of the least-square means. Similarly, trial II data were analyzed using a 4 × 2 × 2 factorial design with genotype, 2,4-D conc., and kinetin concentration being the factors.

Mitotic analyses followed the procedures of Kasperbauer et al. (1980). Meiotic analyses, pollen stainability, and enzyme assays for acid phosphatase (ACPH), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), and phosphoglucoisomerase (PGI) were according to Dahleen and Eizenga (1990). The alcohol dehydrogenase (ADH) assay was modified according to Eizenga and Dahleen (1990). The chiasma frequency was calculated as follows: × 100.

Genomic DNA was extracted from lyophilized ground leaf tissue with CTAB buffer (0.1 M Tris at pH 7.5, 0.7 M NaCl, 0.01 M EDTA at pH 8.0, 0.14 M β-mercaptoethanol, and 0.03 M mixed alkytrimethylammonium bromide [Saghai-Maroof et al. 1984]), then extracted against 1 volume phenol and 1 volume of chloroform-isoamyl alcohol (24:1), followed by ethanol precipitation and a series of three ethanol rinses (15 mM ammonium acetate in 80%; 70%; 70%). The DNA pellet was dissolved in TE (10 mM Tris at pH 8.0, 1 mM EDTA), proteinase K and 1%(w/v)SDS, and further purified with a phenol-chloroform extraction.

RAPD analysis was modified from Williams et al. (1990) for use on a Perkin-Elmer DNA Thermocycler 480 (PE Applied Biosystems,

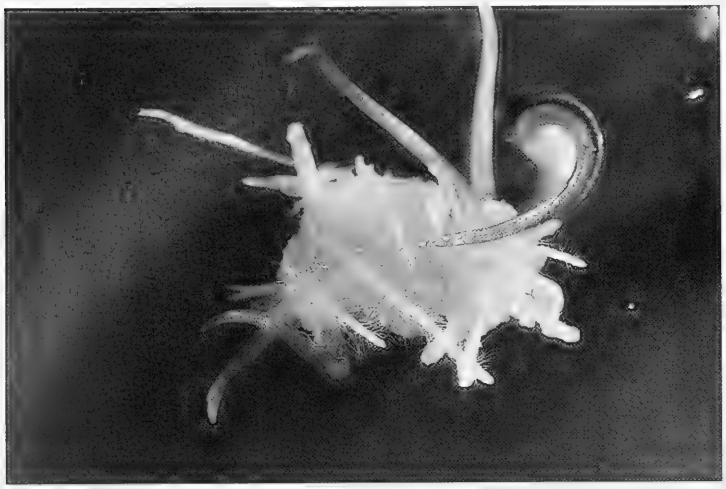

Figure 1. Tall fescue regenerants. Green plantlet regenerating from anther callus. Emerging roots also are visible.

Foster City, CA). The reaction consisted of 25 ng sample DNA, 200 μM of each dNTP (Boehringer Mannheim, Indianapolis, IN), 37.5 ng decamer primer (Operon Technologies, Inc., Alameda, CA), 1.0 U Taq polymerase (Boehringer Mannheim, Indianapolis, IN), and buffer (10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) in 25.0 μL volume. The thermocycler was programmed for six cycles of 94°C for 1 min, 35°C for 1 min, 72°C for 2 min, followed by 35 cycles of 94°C for 1 min, 40°C for 1 min, 72°C for 2 min ending with a 6 min extension period at 72°C. Amplification DNA samples were separated by loading 12 μL of each sample, electrophoresed on 1.2% agarose gels stained with 0.06 μg/μL ethidium bromide, run with 1 × TBE buffer (89 mM Tris-base, 89 mM borate, 2 mL EDTA, pH 8.0) at 100 mV for 2 h, washed, and photographed under UV light. The marker was a 1 Kb DNA Ladder (Gibco-BRL, Gaithersburg, MD).

One hundred twenty-two random decamer primers were initially screened with the maternal plants (K25, K27) to identify those primers that detected different polymorphisms among the maternal plants. The following nine primers identified differences and were selected to screen the entire set of 34 samples: OPE9, OPF10, OPJ15, OPJ19, OPK19, OPT01, OPT20, OPAM18, and OPAM19. Two independent runs were performed for each primer; photographs were scanned for band presence/absence in the maternal plants but band absence/presence in the regenerants.

RESULTS

Both green (Figure 1) and albino plantlets were regenerated from cultured anthers. Forty green plants (4 from K16, 33 from K25, 3 from K27) from three studies were regenerated and transplanted to soil. Thirty-four (32 from K25; 2 from K27) were vernalized in the field and induced to flower. The panicles of the regenerants were morphologically like non-cultured tall fescue.

Statistical analysis of the culture data indicated that the 2,4-D concentrations tested had no significant effect on the number of anthers producing callus, roots, and/or shoots. Similarly, analysis of trial II data indicated that kinetin did not significantly affect the expression of these same variables. The lack of response to kinetin was expected based on other research (Kasperbauer 1990).

Genotypes K16 and K27 gave significantly more callus growth than K8 and K25 in trial I (Table 1). In trial II, significant differences were noted in callus growth with K27 giving the most callus followed by K25 and K16.

In trial I, genotype K16 regenerated significantly more albino shoots (zero to 10 anthers produced shoots per petri plate) than other genotypes. In subsequent studies there were no significant differences among genotypes. The number of anthers per petri plate producing albino shoots ranged from zero to

Table 1. Comparison of tall fescue anther culture response variables (callus growth, root regeneration, albino shoot regeneration, green shoot regeneration, green plantlets transplanted to soil) over three different studies, and four tall fescue genotypes.

Experiment	Genotype	Response variable				
		Callus growth	Root growth	Albino shoots	Green shoots	Green plants
		% anthers responding per 100 anthers				
Trial I	K8	0.25 ab[a]	0.00 a[a]	0.25 bc[a]	0.00 a[a]	0.00 a[a]
	K16	0.67 a	0.33 a	2.17 a	0.33 a	0.33 a
	K25	0.00 b	0.00 a	0.00 c	0.00 a	0.00 a
	K27	0.67 a	0.00 a	0.75 b	0.00 a	0.00 a
Trial II	K16	0.33 b	0.00 a	0.17 a	0.00 b	0.00 b
	K25	0.67 ab	0.00 a	0.00 a	0.44 a	0.67 a
	K27	0.83 a	0.17 a	0.11 a	0.11 ab	0.17 ab
Trial III	K8	0.00 a	0.00 b	0.00 a	0.00 b	0.00 b
	K16	0.04 a	0.00 b	0.00 a	0.00 b	0.00 b
	K25	0.33 a	0.67 a	0.04 a	1.13 a	0.88 a
	K27	0.16 a	0.00 b	0.04 a	0.00 b	0.00 b

[a] Significant at the 0.05 level using a square root transformation of the least-square means.

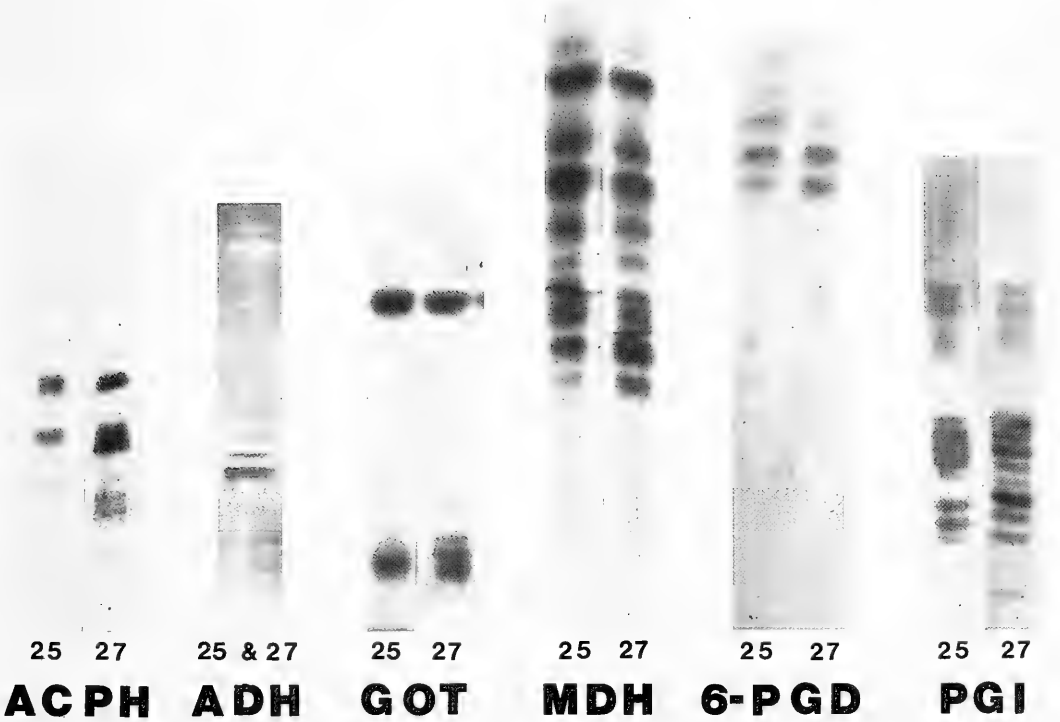

Figure 2. Tall fescue regenerants. Banding patterns of the maternal plants (K25, K27) for ACPH, ADH, GOT, MDH, 6-PGD, and PGI. Banding patterns of the regenerants were identical to those of the maternal plants, indicating that the regenerants were derived from somatic tissue rather than from microspores via spontaneous doubling.

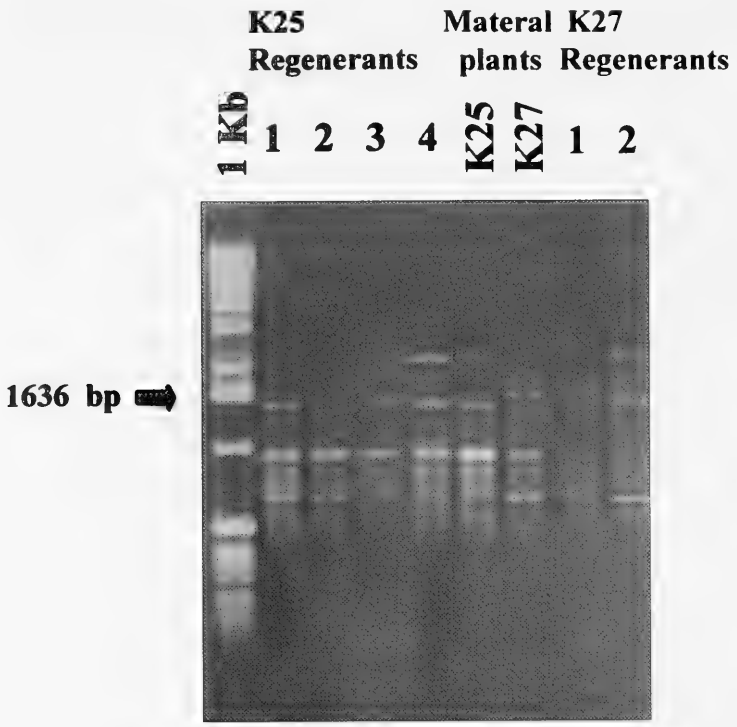

Figure 3. Tall fescue regenerants. RAPD profiles of maternal plants (K25, K27) and their respective regenerants. The identical patterns indicate that the regenerants were derived from somatic tissue rather than from microspores. Genomic DNA was amplified using the primer OPF10. K25 is distinguished from K27 by an additional band at the 1636 bp fragment. The size marker is a 1-Kb DNA ladder (Gibco-BRL).

three. Mitotic analyses of the albino plantlets obtained from the shoots were unsuccessful.

For green shoot regeneration, K16 ranked the highest in trial I, and K25 was the highest in trials II and III. This ranking was the same for green plantlets transplanted to soil and nearly the same for root growth. In trial I, four green plants were regenerated from K16, but all four died when transplanted to soil in the greenhouse. In trial II, 14 of the 15 green plants transplanted to soil survived. Twelve were from K25 and two from K27. In trial III, 20 of the 21 green plantlets regenerated from K25 survived transplanting to soil. One of the 20 plantlets produced some green and white-striped tillers after being transplanted to soil.

All 34 green plantlets that survived transplanting to soil (32 from K25; 2 from K27), had 42 chromosomes and normal chromosome pairing at metaphase I. The overall mean chromosome pairing of the regenerants was 0.17 univalents, 3.03 rod bivalents, and 17.88 ring bivalents with a mean chiasma frequency of 92.4%. The mean pollen stainability of these same plants was 79.3%, which is within the range normally found in tall fescue. These meiotic analyses and pollen stainability were similar to those previously reported for the maternal plants and inflorescence-derived regenerants (Eizenga and Dahleen 1990).

The maternal plants (K25, K27) had different banding patterns for five of the six enzymes tested (Figure 2). The banding patterns were evidence of different alleles being present in the maternal plants for the isozymes ACPH-1, GOT-2, GOT-3, 6-PGD-1 and PGI-2. No variant patterns were identified among the regenerants.

For the nine primers evaluated, differences in band presence/absence were noted in RAPD banding patterns between the maternal plants (K25, K27). Comparing these patterns to the regenerants, no variant patterns, band absence/presence, were found as illustrated with the OPF10 primer (Figure 3). Thus, panicle morphology and meiotic, isozyme, and

RAPD analyses indicated there was no culture-induced variation for these parameters.

DISCUSSION

The fact that isozyme and RAPD banding patterns of the regenerants were the same as the parents implies that regeneration was from somatic tissue, possibly anther wall, rather than from microspores via spontaneous doubling. If regeneration had been from microspores, some of the isozyme phenotypes should have differed from the parents similar to the segregation of isozyme markers observed for PGI-2 and GOT-2 in androgenetic progenies of *Lolium perenne* L. (Hayward et al. 1990). Also, if the plantlets had been doubled haploids (via spontaneous doubling of the chromosomes in the microspore), most likely a maximum of three different alleles would have been present because tall fescue is a hexaploid species. The PGI-2 banding patterns of the K25 and K27 regenerants require four alleles (Eizenga and Cornelius 1991). In addition, the tall fescue haploids obtained by Kasperbauer et al. (1980) had fewer bands for ACPH-1 and PGI-2 than normally found in hexaploid tall fescue (Eizenga, unpublished data). Similarly, regenerants for onion ovules showed differences in RAPD profiles among regenerants of different ploidy levels, but identical profiles were found among regenerants of the same ploidy (Bohanec et al. 1995).

Screening of 764 beet (*Beta vulgaris* L.) regenerants for variant isozyme alleles and 60 regenerants for variant restriction fragments using RFLP (restriction fragment length polymorphisms) analysis suggested a percentage of ca. 0.05% variant isozyme alleles and 0.1% variant restriction fragments (Sabir et al. 1992). Yamamoto et al. (1994) found that RAPD profiles could differentiate 35 rice (*Oryza sativa* L.) varieties, but detection of minor genetic alterations among somaclonal variants or mutants and their maternal varieties was not feasible. These studies suggest that neither isozyme nor random PCR techniques can differentiate among the 34 tall fescue regenerants. An additional limitation is the small sample size and the limited number of primers used.

Two anther culture studies of perennial ryegrass (*Lolium perenne*) genotypes demonstrated a significant genotypic effect on regeneration percentage from microspore-derived embryos (Madsen et al. 1995; Opsahl-Ferstad et al. 1994). Both studies suggested that regenerability was under a relatively simple genetic control. Tall fescue is closely related to perennial ryegrass (Sleper 1985); thus, tissue culture regeneration in tall fescue probably is under a simple genetic control, similar to that described in perennial ryegrass. Genes for increasing regenerability from anther culture and green plant frequency have been mapped to chromosome arms in wheat-rye addition lines (Martinez et al. 1994) and to chromosomal segments in maize (*Zea mays* L.) using RFLP markers (Armstrong et al. 1992).

No significant correlation was found between regenerability from cultured anthers and immature embryos of doubled haploid wheat (*Triticum aestivum* L.) lines, which suggested separate genetic control of regeneration (Agache et al. 1988). Similarly, lack of a significant correlation among regenerability from leaf discs, anthers, and protoplasts was reported in *Solanum phureja* Juz. & Buk. (Taylor and Veilleux 1992). Possibly in tall fescue there exists a separate genetic control for regenerability from somatic tissues and microspores. This would explain why only somatic tissues responded in our study.

By comparison, anther culture of regenerative lines of sorghum (*Sorghum bicolor* (L.) Moench.) and alfalfa (*Medicago sativa* L.) have been unsuccessful in producing haploid plants (Wen et al. 1991). Alternatively, as suggested in the studies of sorghum (Wen et al. 1991), it is possible that the tall fescue albino plantlets produced in this study were haploid.

In conclusion, our research showed that selection for regenerability from cultured inflorescences was a good indicator of regenerability from cultured anthers. The regeneration of green plantlets apparently was from somatic tissues rather than from microspores, although it is possible that the albino plantlets were haploid. Future studies designed to obtain green tall fescue haploids will need to focus on regenerability from microspores and alternate methods of obtaining haploid plants.

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Caddisflies (Insecta: Trichoptera) of the Mainstem of the Kentucky River, Kentucky

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ABSTRACT

Collections of adult caddisflies from six Kentucky River sites were made from 1993 through 1996. Initially, stations 2 and 3 were sampled weekly in 1993–94 to determine phenology and differences in species composition between open-river and lock-and-dam habitats. Notable differences in species composition of hydropsychid caddisflies prompted the addition of stations 1,4,5, and 6 in 1994. Stations 3 and 5 were open-river sites; the remaining stations were at locks and dams. Fifty species representing nine families and 23 genera were collected. Most species belonged to the families Leptoceridae (16), Hydroptilidae(12), and Hydropsychidae (10). Emergences ranged from late April through late October with peak emergences in late July and August. A total of 17 species was found at all stations, *Cheumatopsyche campyla* and *Ceraclea tarsipunctata* being the most common. The open-river stations in comparison to the lock-and-dam sites had distinctly different habitats; this was expressed as differences in species composition. Locks and dams, because of the abundance of hard substrates, provided more habitat for filter feeders, scrapers, and predators.

INTRODUCTION

The collection, evaluation, and monitoring of aquatic communities over periods of time is a tool used to evaluate water quality and to protect aquatic life. However, biological evaluations are of limited value when collections do not represent most or all available habitats. It has become clear that the long-term monitoring (Kentucky Division of Water 1978–1995) of invertebrates from the Kentucky River, using multiplate samplers, does not adequately represent the river's invertebrate community. Some species were missed due to biases of the sampler; other species, because of limitations in larval taxonomy, could be identified only to the generic level. For that reason, light trapping was initiated to collect adult caddisflies, which in most cases can be identified to the species level. Resh's (1975) list of the caddisflies of Kentucky included records for 175 species; of these, only two were recorded from the Kentucky River.

Mozley (1979) pointed out the lack of ecological information regarding benthic macroinvertebrates of large rivers in North America. Prior to that time only a few studies had been conducted on these rivers. Mason et al. (1967) and Fullner (1971) studied and compared the efficiencies of various types of artificial substrate samplers for collection of invertebrates in large rivers. In the Ohio River, Beckett (1982) studied the phenology of *Hydropsyche orris* and Beckett and Miller (1982) investigated colonization rates of larval invertebrates on multiplate samplers in various current velocities. Hall (1982) compared colonization of invertebrates on basket and multiplate samplers in the upper Mississippi River. Beckett et al. (1983) studied distribution of larval macroinvertebrates from four habitat types in the lower Mississippi. Our study is the first to document the caddisfly fauna from one of the larger impounded rivers in Kentucky.

KENTUCKY RIVER DRAINAGE

The North, Middle, and South forks of the Kentucky River originate in the mountains of southeastern Kentucky. Each fork flows in a northwest direction, joining near the town of

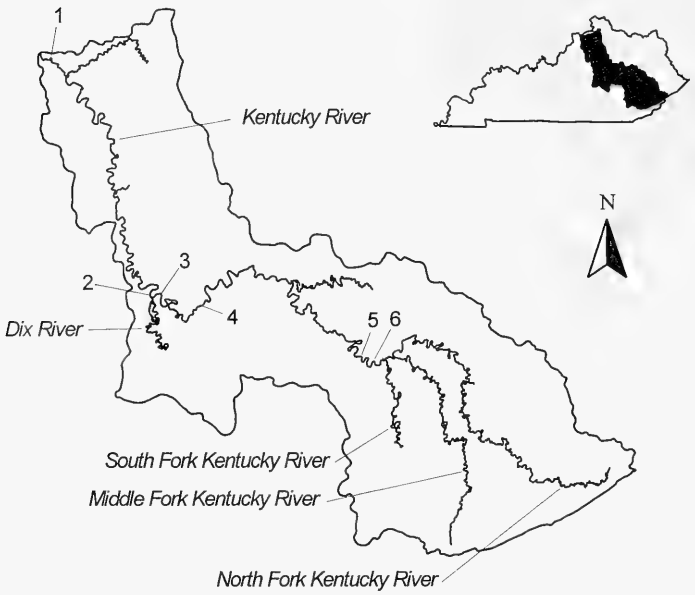


Figure 1. Outline of the Kentucky River drainage area showing sites of collection of Trichoptera from 1993 through 1966. Inset shows area location within Kentucky.

Beattyville, to form the mainstem (Figure 1). From there, the river continues to flow northwest, joining the Ohio River at Carrollton, Kentucky, a distance of 408 km (255 miles) (Martin et. al. 1979). There are 14 locks and dams (L&D) on the mainstem, some of which have been in place for many years. For example, L&D 1 near Carrollton was completed in 1834, L&D 7 at High Bridge in 1895, and L&D 14 at Heidelburg in 1917. Locks 5–14 have been closed for over a decade. The river passes through the Cumberland, Allegheny, and Interior Plateau ecoregions (Omernik 1986). About midway along its course it flows through a part of the Interior Plateau known as the Inner Bluegrass. That segment, called the Palisades, reflects major distinctions in geology and vegetation because of a geologic uplift late in the Paleozoic Era (McFarlan 1943). Sheer limestone cliffs created by the downward cutting of the river are as high as 122 m (400 ft) above the river level, exposing the oldest Ordovician limestone rocks in the state (McFarlan 1943). The absence of other large streams in the Inner Bluegrass and a rapidly growing populace have greatly increased economic and recreational demands on the river.

Table 1. Kentucky River caddisflies. Location of collecting stations along the river mainstem.

Station	Location
1	Carroll Co., Lock and Dam (L&D) 1, at River Mile (RM) 4.0 (River Kilometer [Rkm] 6.4) upstream from Carrollton
2	Jessamine Co., L&D 7, at High Bridge
3	Jessamine Co., at RM 118.6 (Rkm 191), private property 1.0 km upstream from confluence with Dix River
4	Jessamine Co., at RM 157.5 (Rkm 253), downstream from Valley View
5	Estill Co., at RM 221.9 (Rkm 357), 2.4 km upstream from Ravenna
6	Lee Co., at L&D 14, RM 255.0 (Rkm 410), at Heidelberg

Published biological data from the Kentucky River are few and mostly relegated to studies of freshwater mussels that began with Rafinesque (1820). Danglade (1922) and Williams (1974) documented the mussel fauna and reported on commercial potentials of the existing communities for freshwater pearls and buttons. Tolin and King (1986) surveyed the unionid mussels from L&D 4 at Frankfort, downstream to the confluence of the Ohio River. Most recently, Bradfield and Porter (1990) presented a summary of investigations related to surface water quality in the Ken-

Table 2. Trichoptera collected along the Kentucky River mainstem from 1993 through 1996.

Taxa	Station 1	2	3	4	5	6
Glossosomatidae						
Protoptila maculata (Hagen)						X
Hydropsychidae						
Ceratopsyche sparna (Ross)		X				
Cheumatopsyche campyla Ross	X	X	X	X	X	X
Cheumatopsyche minuscula (Banks)		X		X		X
Cheumatopsyche oxa Ross	X					
Cheumatopsyche pasella Ross	X	X	X	X		X
Hydropsyche betteni Ross	X	X		X		X
Hydropsyche frisoni Ross						X
Hydropsyche orris Ross	X	X				
Hydropsyche simulans Ross		X		X		X
Potamyia flava (Hagen)	X	X				
Hydroptilidae						
Hydroptila ajax Ross	X	X	X	X	X	X
Hydroptila angusta Ross	X	X	X		X	X
Hydroptila armata Ross		X	X	X	X	
Hydroptila consimilis Morton	X	X		X		
Hydroptila hamata Morton					X	X
Hydroptila perdita Morton	X	X		X		
Hydroptila waubesiana Betten	X	X	X	X	X	X
Ochrotrichia tarsalis (Hagen)						X
Ochrotrichia xena (Ross)		X				
Orthotrichia aegerfasciella (Chambers)	X	X	X	X	X	X
Orthotrichia cristata Morton			X		X	
Oxyethira pallida (Banks)	X	X	X	X	X	X
Leptoceridae						
Ceraclea ancylus (Vorhies)	X	X				
Ceraclea cancellata (Betten)	X	X	X	X	X	X
Ceraclea flava (Banks)	X	X				
Ceraclea punctata (Banks)		X	X		X	
Ceraclea tarsipunctata (Vorhies)	X	X	X	X	X	X
Ceraclea transversa (Hagen)	X	X	X	X	X	X
Mystacides sepulchralis (Walker)						X
Nectopsyche candida (Hagen)	X	X	X	X	X	X
Nectopsyche exquisita (Walker)	X	X	X	X	X	X
Nectopsyche pavida (Hagen)	X	X	X	X	X	X
Oecetis avara (Banks)						X
Oecetis cinerascens (Hagen)	X	X	X	X	X	X
Oecetis inconspicua (Walker)	X	X	X	X	X	X
Oecetis persimilis (Banks)	X	X	X	X	X	X
Triaenodes ignitus (Walker)	X	X	X	X	X	X
Triaenodes tardus Milne	X	X	X			
Limnephilidae						
Ironoquia punctatissima (Walker)	X					
Pycnopsyche lepida (Hagen)	X					
Philopotamidae						
Chimarra obscura (Walker)	X	X	X	X	X	X
Phyrganeidae						
Agrypnia vestita (Walker)						X
Ptilostomis ocellifera (Walker)	X					
Ptilostomis semifaciata (Say)	X					
Polycentropodidae						
Cyrnellus fraternus (Banks)	X	X	X	X	X	X
Neureclipsis crepuscularis (Walker)			X		X	
Polycentropus cinereus Hagen		X	X		X	
Polycentropus confusus Hagen		X				
Rhyacophilidae						
Rhyacophila lobifera Betten		X				X
TOTAL NUMBER OF SPECIES:	32	36	25	24	24	30

tucky River basin, which included sites in the mainstem.

METHODS AND MATERIALS

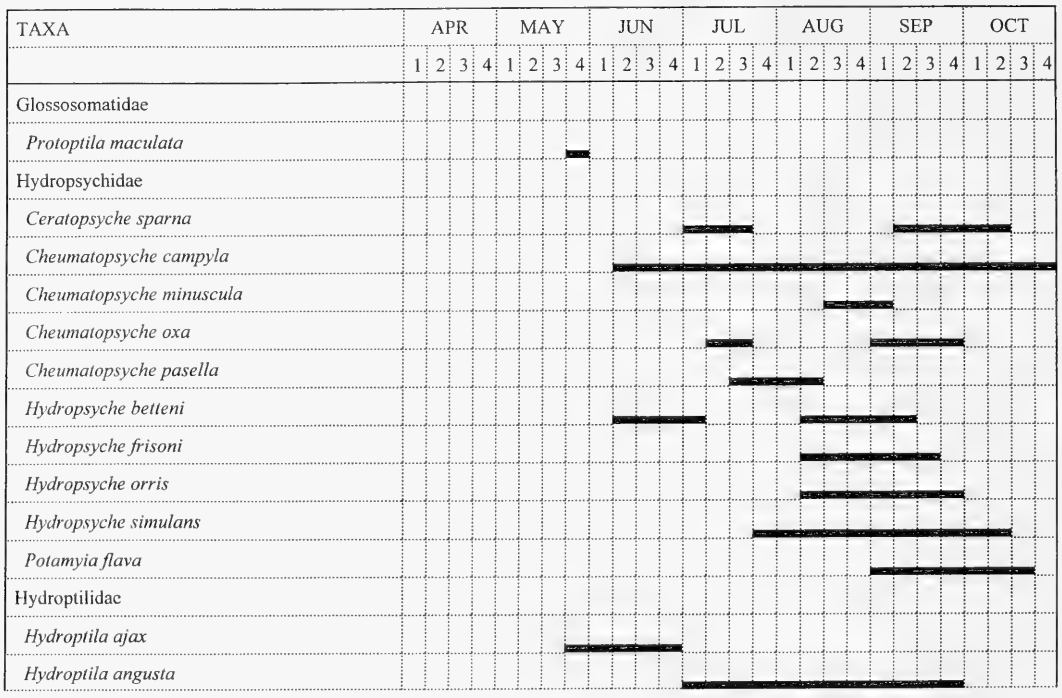
Adult caddisflies were collected from six stations (Figure 1) along the Kentucky River mainstem from 1993 through 1996. Initially, stations 2 and 3 were sampled weekly during 1993–94; stations 1, 4, 5, and 6 were added in 1994. Stations 3 and 5 represented open-river habitats; remaining stations were at L&D. Locations of all stations are shown in Table 1.

Collections were made using a plastic ice chest tray with about 500 ml of 70% ethanol, set at the base of a 12 volt Coleman lantern, equipped with ultra-violet (UV) bulbs. At L&D sites, a lantern was turned on and placed on top of the downstream lock gate mooring wall at dusk; it was turned off an hour after dark. Private docks were used at the open-river sites in the same manner. Species identifications were based mostly on males.

RESULTS AND DISCUSSION

This study began in early April 1993, with weekly collections at stations 2 and 3 (Figure 1). The purpose was to document species composition and emergence periods from different habitat types (i.e., from a L&D site and a typical open-river site). A year of weekly collections showed that species compositions between stations 2 and 3 were similar (Table 1). However, the functional feeding requirements of some species reflected the types and amounts of different microhabitats between the stations. For instance, differences in numbers and species of hydropsychids were notable at the L&D site compared to the open-river site. So stations 1, 4, 5, and 6 were added in 1994 to increase the scope of the study. Sta-

Table 3. Seasonal occurance of trichoptera collected during this study from the Kentucky River Mainstem.



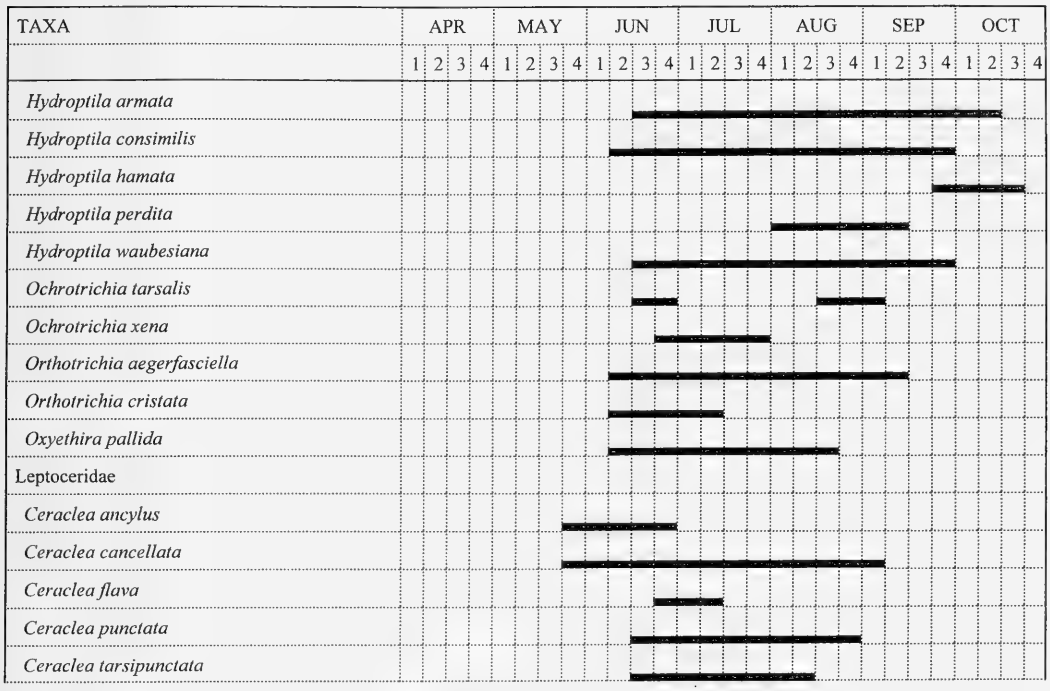
tion 5 was the other open-river site. Filter-feeding species (mostly hydropsychids) from all L&D sites ranged from 6 to 11 species in 1994, compared with 1 and 2 species at open-river sites (Table 1). Station 2 had eight of the 10 hydropsychid species. *Hydropsyche orris* and *Potamyia flava* occurred only at stations 1 and 2; *Cheumatopsyche oxa,* only at station 1; *Ceratopsyche sparna,* only at station 2; and *Hydropsyche frisoni,* only at station 6. Filterer species common to all stations were *Chimarra obscura* and *Cyrnellus fraternus,* though collected in fewer numbers at the open-river sites. Station 2 yielded three of the four species of polycentropodids, with *Polycentropus confusus* occurring only at station 2.

Beckett and Miller (1982) observed that flow velocities contributed to different invertebrate communities becoming established in the Ohio River at locks and dams. In our study of adult caddisflies, it was also evident that different sustained flow velocities were responsible at least in part for the diversity of filterer organisms at the L&D stations and organisms associated with the slower currents at open-river stations.

Hall (1982) found 31 larval taxa in basket samplers and 21 taxa from multiplate samplers in the upper Mississippi River. In comparison, the Kentucky Division of Water's (KDOW), Biological Monitoring Program (BMP) multiplate collections from four mainstem Kentucky River sites (KDOW 1978–1995) ranged from 6 to 31 larval taxa. In our study, adult caddisflies alone ranged from 24 to 36 species at six sites, for a total of 50 species throughout the river (Table 2).

The augmented flow from Dix River (Herrington Lake, hydroelectric turbines) (see Figure 1) is thought to be the reason for the large number of taxa, and especially a large number of filter-feeding species, collected at station 2 (Figure 1). In addition, 14 of 16 leptocerid species were collected there. Because of the occurrences of leptocerid larvae at all stations and habitat types throughout the river, the microhabitat requirements for them are apparently less specific in relation to flow velocities

Table 3. (continued).



or other physical attributes. Although the numbers of individuals collected in open-river sites were much fewer for some species, there were also distributional differences. For example, *Ceraclea ancylus* and *C. flava* were collected only at stations 1 and 2, and *Mystacides sepulchralis* and *Oecetis avara* and were collected only at station 6.

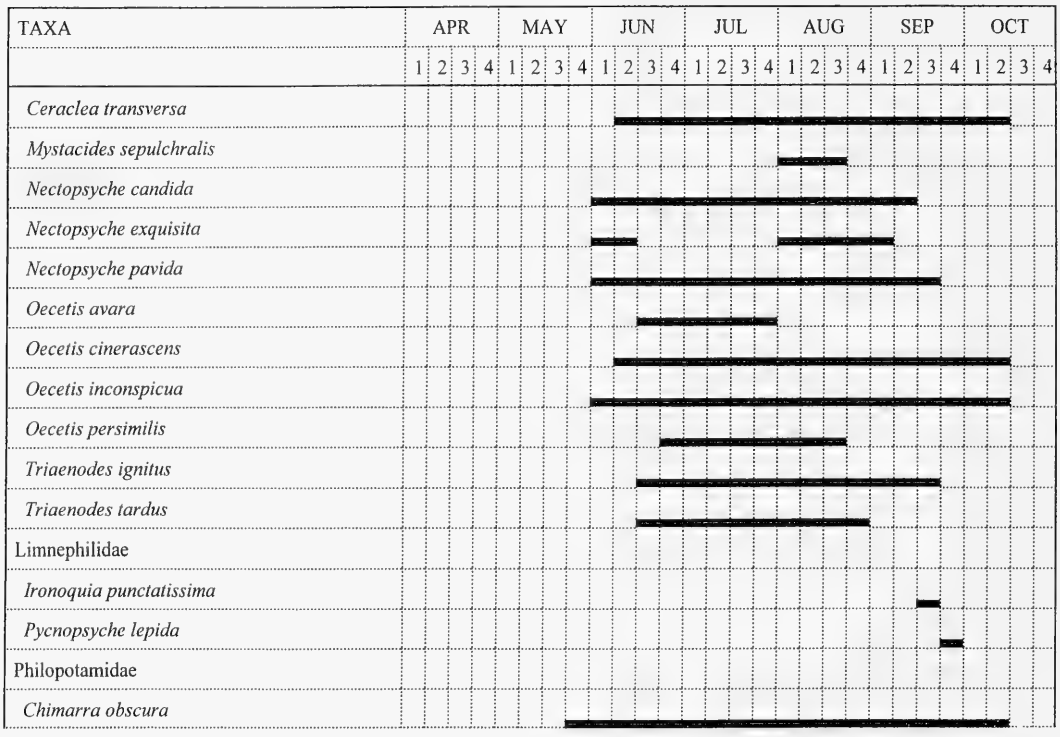
Five of the 12 hydroptilid species were collected from all stations; at station 2 nine of the 12 were common. Most of these species are scrapers during the larval stages, and the L&D provides attachment sites for them as well as for the periphyton (algae and diatoms) they consume. *Ochrotrichia xena* was collected only at station 2; *O. tarsalis,* only at station 6; and *O. cristata,* only at stations 3 and 5.

From the distribution and numbers of hydropsychids, polycentropodids, and certain hydroptilids collected, it is apparent that the L&D sites provided the microhabitats required by these species. These habitats included a variety of flow regimens and firm substrates for needed attachment. In contrast, species such as *Orthotrichia cristata* and *Neureclipsis crepuscularis,* which require slower currents, were more abundant at open-river stations.

It is likely that some of the species collected at light traps flew in from nearby tributary habitats. These included four species from station 1 (*Ironoquia punctatissima, Ptilostomis semifaciata,* and *Ptilostomis ocellifera*), and three from station 6 (*Agrypnia vestita, Protoptila maculata,* and *Rhyacophila lobifera*). These species are often associated with headwater streams.

The flight patterns of all species are shown in Table 3. Most caddisflies exhibit a univoltine life cycle; our data indicate that this is true for all Kentucky River species. It is apparent that two different flight patterns are common among these caddisflies. The first is a single cohort that emerges over a long period of time such as in *Cheumatopsyche campyla* or a short, synchronous emergence period such as in *Protoptila maculata.* The second pattern is shown by species such as *Ochrotrichia tarsalis,* which apparently has two cohorts emerging at different times. In addition, sea-

Table 3. (continued).



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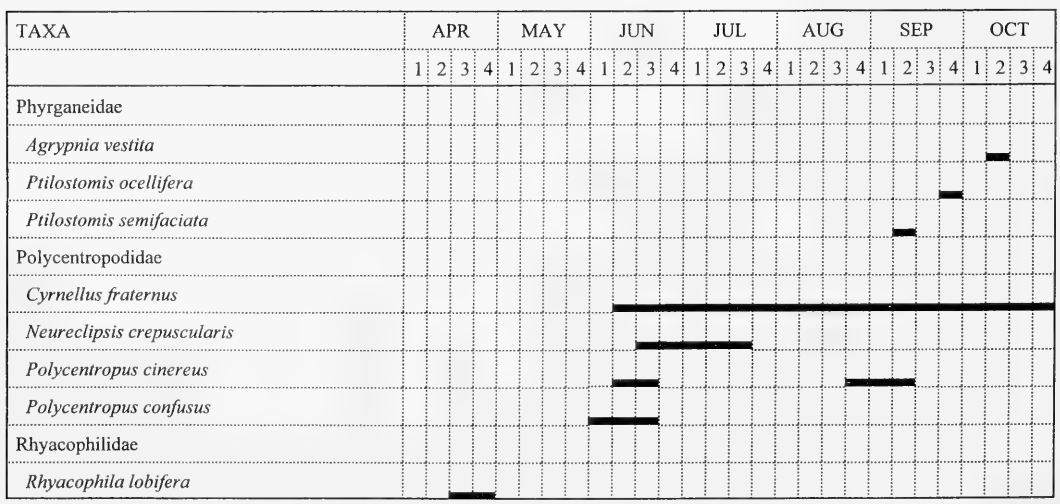

sonal-occurrence data show that while the majority of species emerge throughout the summer some species, e.g., *Rhyacophila lobifera,* have a synchronous emergence period in the early spring and that other species, e.g., *Ironoquia punctatissima* and *Pycnopsyche lepida,* have an emergence period restricted to a very short period in the fall. The family Leptoceridae had the largest number of genera and species active at the same time throughout the river (Table 3).

The most abundant species at all stations and throughout the study was *Cheumatopsyche campyla.* The sex ratio of males to females is often skewed in light trap samples towards females. For example, we collected no males of *Cheumatopsyche oxa. Oecetis inconspicua* and *O. persimilis* were also collected as pharate adults attached to posterior ridges of freshwater clams (Unionidae) in areas below station 2. It is believed that unionids represent an important type of habitat for many large-river insect species in that they provide firm substrates for attachment.

Locks and dams are the largest stationary objects in the Kentucky River. They, in concert with the various flow velocities created by these structures, provide unique habitats for filter feeders, scrapers, and predatory insects in the mainstem where such habitats are often scarce.

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Noteworthy Vascular Plant Discoveries from Kentucky

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ABSTRACT

Nineteen species of rare vascular plants are documented from Kentucky. Two species (*Juncus validus* Coville and *Scirpus [Schoenoplectus] mucronatus* L.) are reported as new to the state; *S. mucronatus* has serious potential as an invasive wetland weed. Three others (*Calopogon tuberosus* (L.) BSP. var. *tuberosus*, *Polygala paucifolia* Willd., and *Rhynchospora macrostachya* Torr. var. *macrostachya*) represent the only known extant Kentucky populations. New county distributional information for 14 other taxa of rare Kentucky plants is also presented.

INTRODUCTION

Kentucky is relatively poorly known floristically. Recent field studies have yielded the state records and other noteworthy collections reported here. Some of these uncommon species are restricted to specific habitats or ecological communities in Kentucky; others are recently naturalized or represent significant range extensions. The rarity of these species in Kentucky is based on the listing of endangered, threatened, special concern, and historical plants and animals of Kentucky (Kentucky State Nature Preserves Commission [KSNPC] 1996) and follows the scientific name where applicable. An asterisk preceding the scientific name indicates a non-indigenous taxon.

NOTEWORTHY SPECIES

Adiantum capillus-veneris L. KSNPC Threatened. LOGAN CO.: Dot 7.5 Quad., wet limestone seeps along Red River, adjacent to KY 102 (near state line), se of Keysburg, rare, 14 Sep 1995, *Libby and Mears 1229* (BEREA, EKY, KNK).

Significance. Cranfill (1980) stated that "no known stations are presently extant" in Kentucky. Southern maidenhair-fern has been collected in seven Kentucky counties (Adair, Boyd, Carter, Powell, Pulaski, Taylor, and Wayne) (Campbell n.d.; Cranfill 1980; McCoy 1938; Medley 1993). In addition, Cranfill (1980) cited a formerly adventive colony fron Nelson County. This collection represents a westward range extension for this epipetric pteridophyte.

Aureolaria patula (Chapman) Pennell. KSNPC Threatened. This species is also a candidate (Category C2) for federal listing (United States Fish and Wildlife Service [USFWS] 1993). ADAIR CO.: Cane Valley 7.5 Quad., along Green River Lake near Holmes, steep, rocky lakeshore, 26 Sep 1995, *Libby 1277* (EKY). BARREN CO.: Meador 7.5 Quad., along Barren River Lake tailwaters near Gaging Station, open riverbank, 4 Oct 1995, *Libby 1302* (EKY). CLINTON CO.: Frogue and Albany 7.5 Quads., along Dale Hollow Lake near Skull Bluff, steep, rocky lakeshore and edge of woods, 20 Sep 1995, *Libby, Bloom, and Evans 1252 & 1253* (EKY). CUMBERLAND CO.: Frogue 7.5 Quad., along Dale Hollow Lake nw of Boys Island, rocky lakeshore, 6 Oct 1995, *Libby 1312* (EKY). GREEN CO.: Summersville 7.5 Quad., along Green River at KY 61/70 bridge, just s of Greensburg, rocky, open woods, 15 Sep 1995, *Libby and Mears 1240* (EKY). TAYLOR CO.: Cane Valley 7.5 Quad., along Green River just below Green River Lake Dam, near KY 55 bridge, steep, forested streambanks, 26 Sep 1995, *Libby 1282* (EKY). WARREN CO.: Bowling Green South 7.5 Quad., along Drakes Creek, ca. 1/4 mile upstream from Old Scottsville Road

bridge, steep, rocky riverbank, 14 Sep 1995, *Libby and Mears 1235* (EKY). WAYNE CO.: Nevelsville 7.5 Quad., along Big South Fork Cumberland River just downstream of confluence with Fanny Hollow, open, limestone bluff, 23 Aug 1995, *Libby 1186* (EKY).

Significance. This species was previously known from only five Kentucky counties (Edmonson, Hart, Logan, McCreary, and Pulaski) (Braun 1941; Campbell n.d.; Evans 1989; Medley 1993). Cumberland false foxglove has now been documented in an additional eight counties (Adair, Barren, Clinton, Cumberland, Green, Taylor, Warren, and Wayne) through extensive field work (Libby 1996). These collections expand the range of *Aureolaria patula* in Kentucky and indicate that this species may not be as rare as previously thought (Kral 1983; Pennell 1935; USFWS 1993).

Baptisia tinctoria (L.) R. Br. KSNPC Threatened. MCCREARY CO.: Sharp Place 7.5 Quad., growing on roadside and oak-pine upland forest margin, w side of KY 167, ca. 0.5 mile n of Kentucky/Tennessee line, 12 Jul 1995, *Libby and Klein 1003* (EKY, KNK). WAYNE CO.: Sharp Place 7.5 Quad., growing on roadside and oak-pine upland forest margin, w side of KY 167, ca. 0.6 mile n of Kentucky/Tennessee line, 12 Jul 1995, *Libby and Klein 1002* (KNK).

Significance. Previously known from Letcher, Harlan, and Bell counties (Campbell n.d.; Medley 1993), yellow wild indigo has now been documented in two additional counties.

Bartonia virginica (L.) BSP. KSNPC Threatened. CLINTON CO.: Albany 7.5 Quad., Pyle's Marsh, 1 mile sse of Abstons Corner, situated e of Old Burkesville Road and w of KY 1590, moss-covered areas around tree bases in swampy area, rare, 15 Jun 1995, *Libby and Bloom 959* (EKY).

Significance. Virginia screwstem had been collected in Calloway, Harlan, Laurel, McCreary, and Whitley counties (Campbell n.d.; Medley 1993). This collection represents an additional county and the first collection of this species from the Highland Rim of Kentucky. The more widespread *B. paniculata* (Michx.) Muhl. var. *paniculata* was found about 10 meters away in the adjacent wet woods. Gillett (1959) found that *B. paniculata* and *B. virginica* are sympatric over a large portion of their ranges and that occasional intermediates occur.

Calopogon tuberosus (L.) BSP. var. *tuberosus.* KSNPC Endangered. LAUREL CO.: Vox 7.5 Quad., powerline right-of-way near Grove Recreation Area, ca. 0.7 road mile ne of large transmission line, diverse assemblage of prairie species, rare, 15 Jun 1992. No collection was made because of the rarity and low relative abundance at the site; photographs are on file at the Kentucky State Nature Preserves Commission, Frankfort, Kentucky.

Significance. This species was formerly reported from 12 counties according to Campbell (n.d.) and Medley (1993). Prior to our report, however, grass pink had not been seen in Kentucky since the early 1980s (Campbell n.d.; Medley 1993).

Carex crawei Dewey. KSNPC Special Concern. WAYNE CO.: Parnell 7.5 Quad., McClown Mountain Glade, ca. 0.5 air mile nw of US 90/KY 858 intersection, xeric limestone glade, locally common, 13 Jun 1995, *Libby and Bloom 937* (EKY, KNK).

Significance. Crawe's sedge was described as "to be looked for" in Kentucky by Beal and Thieret (1986). Since that time, *Carex crawei* is known with certainty only from Bullitt, Grayson, Hardin, Larue, and Nelson counties (Campbell n.d.; Medley 1993). This collection represents a range extension and outlier from known localities in the Bluegrass and the Nashville Basin of Tennessee (Chester et al. 1993). This disjunction is probably due to the unusual occurrence of a flat, xeric limestone glade in this part of the Highland Rim.

Carex stricta Lam. var. *stricta.* CLINTON CO.: Albany 7.5 Quad., Pyle's Marsh, 1 mile sse of Abstons Corner, situated e of Old Burkesville Road and w of KY 1590, forming large tussocks in a wooded swamp, locally abundant, 15 Jun 1995, *Libby and Bloom 960* (EKY, KNK).

Significance. In Kentucky, this species had been collected only in Calloway, Harlan, Hart, McCreary, and Metcalfe counties (Campbell n.d.; Medley 1993). Our collection represents an additional county record for this marsh-adapted sedge. This taxon should be strongly considered as an addition to the endangered, threatened, special concern, and historical plants of Kentucky (KSNPC 1996).

Castanea dentata (Marshall) Borkh.

KSNPC Endangered (reproducing trees only). HARLAN CO.: Louellen 7.5 Quad., Hi-Lewis Pine Savanna, upper s-facing slopes of Hi--Lewis Branch, near Hiram, 24 Jun 1995, *Libby, Libby, Libby, and Evans 988* (BEREA, EKY, KNK).

Significance. This collection of American chestnut in flower, including evidence of last years fruits (hulls only), indicates the presence of sexually reproducing chestnut trees in Kentucky. On a return visit in October 1995 we collected 15 nuts. Some of these were forwarded to J. Durrell (Kentucky Nut Growers Association) to test for reproductive potential. It is significant that 23 trees (usually multistem clumps) were observed in flower at this location. This multistem character suggests that these trees have survived previous chestnut blight [*Cryphonectria parasitica* (Murr.) Barr] (Hicks and Marks 1990).

Cenchrus longispinus (Hack.) Fern. ESTILL CO.: Palmer 7.5 Quad., growing in sandy riverbank downstream from Kentucky River Lock and Dam No. 11, rare, 25 Aug 1994, *Libby and Walker 454* (BEREA, EKY, KNK).

Significance. In Kentucky, this species had previously been reported from Ballard, Fulton, Hickman, Jefferson, and McCracken counties in the Coastal Plain and Bluegrass regions (Medley 1993). Our collection from the Knobs region represents a significant eastward range extension.

Cyperus croceus Vahl. MCCREARY CO.: Barthell 7.5 Quad., growing in wet area along railroad tracks adjacent to Roaring Paunch Creek near Barthell, several clumps, 7 Jul 1995, *Mears and Klein s.n.* (EKY).

Significance. This species was recently reported as new to Kentucky (Madison County) (Mears and Libby 1995); ours is the second collection for the state.

**Cyperus difformis* L. SIMPSON CO.: wet ditch in front of gas station just w of I-65 at exit 6 (KY 100) near Franklin, locally abundant, 14 Sep 1995, *Libby and Mears 1219* (KNK, WKU).

Significance. This naturalized species was recently reported as new to Kentucky (Mears and Libby 1995) from Knott, Floyd, and Pike counties. This collection represents an additional county record and westward range extension. In the United States *Cyperus difformis* is known from Alabama, Florida, Louisiana, Mississippi, North Carolina, Pennsylvania, Tennessee, and Virginia, and also from Nebraska, Texas, and California (Bryson and Carter 1994; Godfrey and Wooten 1979; Lipscomb 1980). Godfrey and Wooten (1979) speculated that the species would likely be found elsewhere in the eastern United States. Holm et al. (1991) ranked *Cyperus difformis* as one of the world's worst weeds. Bryson and Carter (1994) described it as particularly pernicious and noted that it is spreading in the United States especially along major waterways, through introduction at ports-of-entry, and in rice production in California.

Epilobium ciliatum Raf. var. *ciliatum.* MERCER CO.: Wilmore 7.5 Quad., directly below Kentucky River Lock and Dam No. 7, growing in gravel wash, rare, 21 Oct 1994, *Libby and Walker 440* (EKY).

Significance. This is the second report of this taxon from Kentucky. Medley (1993) cited a specimen from McCreary County collected by J. Campbell. We have seen no vouchers of this species from Kentucky. Gleason and Cronquist (1991) described the habitat and nature of this taxon as "wet places, often in unstable habitats, variable [morphology] and often somewhat weedy."

Juncus validus Coville. MCCREARY CO.: Barthell 7.5 Quad., coal mining property ca. 0.5 mile se of KY 791 just n of Blue Heron Scenic Railway, w of Coffee Branch, frequent, 5 Jul 1995, *Mears and Klein s.n.* (EKY). MCCREARY CO.: Bell Farm 7.5 Quad., ca. 0.9 mile s of Pleasant Ridge Church, roadside of KY 1363, wet ditch, 13 Jul 1995, *Libby, Mears, and Klein 1116B* (EKY, KNK); same location, 8 Aug 1996, *Libby and Libby 1403* (BEREA, EKY, KNK). MCCREARY CO.: Barthell 7.5 Quad., ca. 2 miles se of Bald Knob at USFS 575/USFS 6130 junction, USFS 6130 roadside, 19 Jul 1995, *Libby and Mears s.n.* (KNK).

Significance. This species is not reported in Beal and Thieret (1986), Browne and Athey (1992), Campbell (n.d.), or Medley (1993). Our collections document the presence of *Juncus validus* in the Commonwealth.

Lilium superbum L. KSNPC Threatened. LAUREL CO.: growing in edge of swamp along w side of U.S. 25 ca. 1 mile s of intersection with KY 1006, 25 Jun 1993, *Mears*

93-234 (EKY). LAUREL CO.: e of KY 30 between Raccoon and Atlanta, wet woods along Raccoon Creek, 26 Jun 1996, *Libby and Hartowicz 1396* (EKY). MCCREARY CO.: Barthell 7.5 Quad., floodplain along e side of South Fork Cumberland River just n of confluence with Bear Creek, locally frequent, 11 Jul 1995, *Libby, Mears, and Klein 994* (EKY).

Significance. Turk's-cap lily has an odd distribution in Kentucky. According to Campbell (n.d.) and Medley (1993), this species has been collected from Big Black Mountain in Harlan County and also from wet wooded seeps in Calloway, Hickman, and McCracken counties on the Coastal Plain. According to Adams (1981) and Adams and Dress (1982), however, the Coastal Plain specimens must represent *L. michiganense* Farwell. Medley (1993) noted that the Coastal Plain specimens lack the "typical many-flowered inflorescence" of *Lilium superbum,* but the descriptions in Adams (1981), Adams and Dress (1982), and Gleason and Cronquist (1991) allow for variation in number of flowers. Clearly, a thorough taxonomic review of this genus is needed in Kentucky. These represent the only known collections of this species from the Low Hills Section and Cliff Section of the Appalachian Plateau of Kentucky.

**Linum usitatissimum* L. MADISON CO.: Berea 7.5 Quad., growing in tire rut on roadside at the corner of Elm Street and Scaffold Cane Road, a European cultigen, escaped or adventive, 16 Jul 1993, *Libby 343* (BEREA, EKY, KNK).

Significance. This species has been collected in Fulton County from the Coastal Plain. A collection at the University of Kentucky cited by Browne and Athey (1992) and Wharton and Barbour (1991) from Fayette County may be from cultivation (Campbell n.d.; Medley 1993). Our collection represents the second report of cultivated flax naturalized in Kentucky. Grown in southeastern United States until the late 1800s (Strausbaugh and Core 1978), this European cultigen is adventive in fields and roadsides in the United States and southern Canada (Gleason and Cronquist 1991).

Muhlenbergia cuspidata (Torr.) Rydb. KSNPC Threatened. LOGAN CO.: Dot 7.5 Quad., limestone outcrops along Red River, adjacent to KY 102 (near state line), se of Keysburg, several clumps, 14 Sep 1995, *Libby and Mears 1230* (EKY). WAYNE CO.: Mill Springs 7.5 Quad., steep, rocky cliffs at Dry Branch/Lake Cumberland confluence (w side), several large clumps on rock face, 24 Jul 1995, *Libby and Bloom 1136* (EKY).

Significance. In Kentucky, plains muhly had been collected from four counties (Anderson, Estill, Garrard, and Pulaski) (Bryant 1973; Campbell n.d.; Medley 1993). Our collections document its occurrence in two additional counties.

Polygala paucifolia Willd. LAUREL CO.: London SW 7.5 Quad., growing along bluffs on w side of Willie Green Road ca. 2.5 miles n of its intersection with KY 192, 1 May 1993, *Mears 93-181* (BEREA, EKY).

Significance. The report of flowering wintergreen in Gibson (1961) was probably based on McFarland (1942); the voucher for that report was destroyed in the 1948 Norwood Hall fire at the University of Kentucky (Medley 1993). Our collection represents the only known extant population of this taxon in Kentucky. This is another taxon that should be added to the endangered, threatened, special concern, and historical plants of Kentucky (KSNPC 1996).

Rhynchospora macrostachya Torr. var. *macrostachya.* KSNPC Endangered. WAYNE CO.: Parnell 7.5 Quad., Cooley's Meadow, ca. 0.8 air mile ssw of Murl, wet meadow ne of Cooley's tobacco barn and e of Hack Denny's cornfield, rare, 27 Jul 1995, *Libby and Bloom 1160* (BEREA).

Significance. This is the second report of this taxon from Kentucky. The species may be extirpated from Hundred Acre Pond, Hart County (Medley 1993). Our collection may represent the only known extant population of tall beak-rush in the state.

**Scirpus mucronatus* L. [=*Schoenoplectus mucronatus* (L.) Pallas] PULASKI CO.: Ano 7.5 Quad., pond at the end of USFS 807, w of "The Narrows" of the Rockcastle River, 26 Jul 1995, *Mears and Klein s.n.* (BEREA, MICH). PULASKI CO.: same location but found in two additional ponds, possibly originally established from contaminated ornamental water-lilies (*Nymphaea* sp.) planted in largest pond, 10 Oct 1995, *Libby and Mears 1317* (BEREA, EKY, KNK, PH, WIS).

Significance. Scirpus mucronatus has not

been previously reported from Kentucky (Arnold and Beal 1981; Beal and Thieret 1986; Browne and Athey 1992; Campbell n.d.; Medley 1993). This European bulrush is known from very few locations in the eastern United States (S. G. Smith, University of Wisconsin, pers. comm., 21 Oct 1996). Britton and Brown (1970) reported only one location; "a swamp in Delaware County, Pennsylvania" and further stated that *S. mucronatus* is widely distributed in the Old World. Gleason (1952) described the distribution of *S. mucronatus* as "waif on ballast at Camden, New Jersey," but Gleason and Cronquist (1991) did not list this species as occurring within northeastern United States. This species is morphologically very similar to the native *Scirpus purshianus* Fern. (=*Schoenoplectus purshianus* (Fern.) M. T. Strong). *Scirpus mucronatus* can be distinguished from *S. purshianus* by its sharply trigonal culms, trifid styles, and absence of leaf blades. *Scirpus mucronatus* may be established at other ponds and lakes in Pulaski County; based on the relative abundance (dominant or codominant emergent aquatic) at the known sites this bulrush should be considered a pernicious weed. This taxon has been recently placed in the genus *Schoenoplectus* (Bruhl 1995).

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Trans. Ky. Acad. Sci. 58(2):80–84. 1997.

Effects of Acidic Minesoil on Nodulation of the Legume Sericea Lespedeza (*Lespedeza cuneata*; Fabaceae) by *Bradyrhizobium* Nitrogen-fixing Bacteria

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ABSTRACT

Sericea lespedeza (*Lespedeza cuneata* (Dumont) G. Don; Fabaceae) is a N_2-fixing legume used to revegetate minespoils in Kentucky and elsewhere. A greenhouse experiment was conducted (1) to examine the ability of sericea lespedeza to grow and form nodules in acidic minesoil and (2) to compare commercial *Bradyrhizobium* sp. strains with strains isolated from minespoils regarding their abilities to form nodules with lespedeza in acidic minesoil. Minesoil was adjusted to various pH levels with $Al_2(SO_4)_3$ and $CaCO_3$. Although growth of sericea lespedeza was significantly reduced ($P \leq 05$) by soil acidification for some inoculation treatments, the plants were able to grow relatively well under acid conditions even at a pH level of 4.2. Nodulation was significantly ($P \leq 0.05$) suppressed by increasing acidity, particularly in $Al_2(SO_4)_3$-amended minesoil at pH levels of 4.2 and 4.5, which tended to affect nodulation similarly. *Bradyrhizobium* sp. strains responded similarly to acidified minesoil with the exception that the commercial mixture of strains tended to produce more numerous but smaller nodules than local strains isolated from acidic minesoil.

INTRODUCTION

More than 30% of the total energy and over half of the electricity produced in the United States are generated by coal burning plants (U.S. Bureau of the Census 1988). Traditionally, Kentucky has been one of the three leading states in coal mining with more than 700,000 acres of surface-mined land having been disturbed by 4900 mining operations in the Appalachian and Eastern Interior Coal Regions of the state (Office of Surface Mining 1988a, 1988b; U.S. Bureau of the Census 1988). Revegetation of surface-mined land is necessary to reduce stream pollution, control erosion and runoff, restore minesoil productivity, and improve wildlife habitats and for esthetic reasons (Roberts et al. 1988; Vogel 1981).

Minesoil acidity has been considered to be the most important soil fertility factor limiting revegetation of surface-mined land (Carpenter and Hensley 1979; Vogel 1981). Minesoils become acidic due to the oxidation of pyrite, a process occurring when these geologic materials from lower depths are left exposed at the surface after mining. Under acidic conditions high levels of Al, Mn, and H-ions are considered to be the primary sources of toxicity to plants, with Al toxicity generally considered the most important (Foy 1984). Calcium and phosphorus can become deficient in soils with low pH levels and high concentrations of soluble Al.

Nitrogen is normally deficient in newly-deposited minesoils (Reeder and Berg 1977; Ebelhar et al. 1982), and effects of initial N fertilization are short-lived (Carpenter and Hensley 1979). Because continued fertilization is often economically and physically impractical (Bender et al. 1985), plant production becomes dependent on N_2 fixation by legumes (Roberts et al. 1988). One of the major deterrents to achieving desirable revegetation rates on minesites is the slow establishment and growth of N_2-fixing legumes, which are generally less tolerant of acidity than are nonleguminous plants (Bender et al. 1988; Vogel and Berg 1968).

Sericea lespedeza, *Lespedeza cuneata* (Dumont) G. Don (Fabaceae), is a warm-season, perennial, forb-legume that has been used to reclaim surface-mined lands in Kentucky. This species is not planted so frequently as it has been in the past, but it still has a place in reclamation as wildlife cover. Although sericea lespedeza is considered tolerant of soils with pH levels as low as 4.5 (Cline and Senwo 1993; Vogel 1981), the tolerance of its N_2-fixing symbiosis to this pH range is unclear based upon a limited number of qualitative obser-

vations (Hyland 1938; Lyle and Evans 1979; Plass and Vogel 1973; Vogel and Berg 1968).

Bacterial strains commonly used to inoculate lespedeza were developed to fix maximum amounts of N_2 for a number of legume species under more favorable growing conditions than acidic minespoils. Regarding symbiotic N_2 fixation and nodulation, it was reasoned that strains isolated from sericea lespedeza growing in acidic minesoil might be more tolerant of such conditions than commercial strains. This generally proved to be true of *Bradyrhizobium* in pure culture (Cline and Senwo 1994). The objectives of our study were (1) to examine the ability of sericea lespedeza to grow and form nodules in acidic minesoil and (2) to compare commercial strains with strains isolated from acidic minesoils regarding their abilities to form nodules in acidic minesoil.

MATERIALS AND METHODS

We conducted a pot experiment with four acidity treatments and four inoculation treatments using a completely randomized factorial design with eight replications. Inoculation treatments consisted of (1) a noninoculated control, which received inorganic N, (2) a commercially available mixture of *Bradyrhizobium* sp. strains (strain numbers 4122, 3223, 32H1, 150B1, and 176A22 from Liphatech Inc., Milwaukee, WI, USA), and (3, 4) strains 41A and CR1, which were isolated from sericea lespedeza growing on acidic minesoils (Cline and Senwo 1994). Acidity treatments consisted of minesoil collected from an abandoned minesite in Muhlenberg County, Kentucky, with a natural pH level of 5.2 (untreated), minesoil adjusted to pH 6.4 with 1.6 g/kg $CaCO_3$ (Ca-msoil), and minesoil adjusted to pH 4.5 (1Al-msoil) or 4.2 (2Al-msoil) with 4.0 or 5.3 g/kg $Al_2(SO_4)_3(H_2O)_3$, respectively.

Minesoil was collected to a depth of 15 cm, sieved to a particle size <13 mm, and air-dried. A previous study indicated that minesoil collected from this site was N-deficient (Cline and Senwo 1993). The minesoil was supplied with 20, 64, 32, and 5 mg/kg Ca, P, K, and Mo, respectively. Following amendment with $CaCO_3$ or $Al_2(SO_4)_3$, minesoil treatments were equilibrated for 8 weeks at a moisture content of 15 ml H_2O/g soil. The equilibrated minesoil was mixed with river sand (50:50 by volume), and 160 g of the mixture were added to 165 ml conical containers (4 cm d × 21 cm Ray Leach Supercells, Stuewe and Sons, Inc., Corvalis, OR). The mixture was used because lespedeza requires well-aerated soil, and preliminary experiments indicated that the plant did not grow well in pure minesoil due to aeration problems associated with containers. Lespedeza grows well in minesoils under field conditions.

'Serala' sericea lespedeza was grown from seed in a greenhouse for 20 weeks under full sunlight. Additional supplemental light (300 μmol photon/m^2/s photosynthetically active radiation) was provided with halide lamps to provide 16-hour photoperiods. Noninoculated plants received NH_4NO_3 at rates of 50 mg N/kg minesoil after 4 weeks and 25 mg N/kg minesoil after 10 and 15 weeks. All soils were supplied with 5 mg K/kg minesoil and 1 mg Mo/kg minesoil after 10 and 15 weeks. Three milliliters of solution containing a total of 10^8 colony-forming units of *Bradyrhizobium* sp. were added to inoculated plants 2 and 4 weeks after planting. Quantification of colony-forming units was based upon relationships between optical density (600 nm) and colony plate-counts determined for each bacterial strain including individual strains in the commercial mixture.

At harvest, minesoil was removed from roots, and nodules were counted. Nodules and shoots were dried separately at 65° C for 48 hours prior to weighing. Minesoil from each pot was equilibrated with distilled H_2O (0.6 ml H_2O g/soil) for 16 hours and centrifuged at 3000 × g. Supernatants were recentrifuged at 75,000 × g and filtered using Whatman No. 42 filter paper. Final pH values were measured prior to centrifugation. Toxic inorganic Al in the supernatants was determined by the pyrocatechol violet colorimetric method of Kerven et al. (1988a, 1988b); phosphorus was measured in the filtered extracts by the method of Murphy and Riley (1962). Atomic absorption was used to measure Mn, Mg, and Ca.

Significance of main effects and interactions regarding inoculation and acidification treatments were determined by two-way analysis of variance. Significant differences among treatment means were defined by the Least Significance Difference.

Table 1. Mean values of acidity-related parameters (±SE, n = 8) measured in extracts of minesoil acidity treatments used to monitor growth of sericea lespedeza and nodulation by *Bradyrhizobium* nitrogen-fixing bacteria.

Acidity treatment	pH		Al (μM)	Mn (μM)
	Initial	Final		
Ca-msoil	6.41 ± 0.06	5.96 ± 0.06	<5	6 ± 0
Untreated	5.33 ± 0.03	5.15 ± 0.04	12 ± 0	25 ± 1
1 Al-msoil	4.46 ± 0.04	4.54 ± 0.06	85 ± 3	186 ± 10
2 Al-msoil	4.18 ± 0.05	4.24 ± 0.06	178 ± 10	310 ± 17

RESULTS AND DISCUSSION

Soil Parameters

General characteristics of the minesoil have been described by Cline and Senwo (1993); parameters relevant to acidity treatments of the present study are described in Table 1. Values of initial and final pH remained relatively stable throughout the experiment except for Ca-msoil in which pH decreased by 0.4 units but remained near 6.0, an optimal pH for plant and bacterial growth. Concentrations of Mn and toxic Al increased with increasing minesoil acidity. Extract concentrations of P and Ca were ca. 2 μM and 5 mM, respectively, in all treatments except that extracts of untreated minesoil contained only 1 mM Ca. Thus, negative responses of lespedeza or *Bradyrhizobium* to acidified treatments should not be related to P or Ca deficiencies.

Shoot Dry Weights

Shoot dry weights (above-ground plant portions) were generally greatest in Ca-msoil and tended to decrease with increasing acidification (Table 2). However, no chlorosis was observed, and shoot dry weight differences between the 2Al-msoil (most acidic) and Ca-msoil (least acidic) treatments were only significant ($P \leq 0.05$) for strain 41A and the control. The fact that sericea lespedeza was able to produce significant yields in 2Al-msoil (pH 4.2) agrees with the findings of Cline and Senwo (1993) but contrasts with those of Bengtson et al. (1973), who reported negligible lespedeza growth at pH 4.6. In Ca-msoil, dry weight production in the N-fertilized control treatment was highest and significantly ($P \leq 0.05$) greater than production in the commercial and CR1 inoculation treatments. This suggested that sericea lespedeza inoculated with *Bradyrhizobium* probably did not receive sufficient fixed N_2 for maximum growth, even at the most optimal pH level (i.e., Ca-msoil).

Nodulation

Although large numbers of nodules were formed (Table 3), individual nodules tended to be relatively small, as indicated by their low total dry weights (Table 4). No nodules were observed on noninoculated control plants receiving inorganic N. Generally nodule numbers and dry weights from plants grown in Ca-msoil were significantly ($P \leq 0.05$) greater than numbers and weights obtained from the three more acidic soil treatments (Tables 3 and 4). Strain CR1 formed similar numbers of nodules in both untreated minesoil and Ca-msoil, but nodule weight production was sig-

Table 2. Mean values of sericea lespedeza mean shoot dry weight production (g ± SE, n = 8) obtained in minesoil acidity treatments using different strains of *Bradyrhizobium* nitrogen-fixing bacteria. Values in columns followed by different letters were significantly ($P \leq 0.05$) different.

Acidity treatment	Inoculation			
	Commercial	41A	CR1	Control + N
Ca-msoil	2.94 ± 0.17a	3.26 ± 0.18a	2.98 ± 0.16ab	3.53 ± 0.23a
Untreated	2.96 ± 0.14a	2.92 ± 0.14ab	3.30 ± 0.24a	2.98 ± 0.40b
1 Al-msoil	2.69 ± 0.14a	2.73 ± 0.17bc	2.63 ± 0.08b	2.26 ± 0.24c
2 Al-msoil	2.67 ± 0.17a	2.52 ± 0.14c	2.87 ± 0.16ab	2.41 ± 0.11c

Table 3. Mean values of nodule numbers (±SE, n = 8) obtained for sericea lespedeza grown in minesoil acidity treatments using different strains of *Bradyrhizobium* nitrogen-fixing bacteria. Values in columns followed by different letters were significantly ($P \leq 0.05$) different.

Acidity treatment	Inoculation			
	Commercial	41A	CR1	Control + N
Ca-msoil	53.0 ± 6.9a	71.9 ± 18.3a	52.4 ± 8.3a	0.0a
Untreated	29.2 ± 3.7b	42.3 ± 6.5b	52.0 ± 11.5a	0.0a
1 Al-msoil	35.7 ± 5.9b	27.9 ± 7.0bc	24.2 ± 4.0b	0.0a
2 Al-msoil	36.7 ± 4.0b	26.8 ± 4.6c	29.4 ± 3.5b	0.0a

nificantly ($P \leq 0.05$) higher in Ca-msoil (Table 3). Conversely, the commercial mixture of strains and strain 41A produced similar nodule dry weights in untreated minesoil and Ca-msoil but formed significantly ($P \leq 0.05$) greater numbers of nodules in Ca-msoil (Table 4). Thus, although nodulation was generally decreased in untreated minesoil compared to Ca-msoil, the response among strains differed regarding nodule numbers and size.

In the more acidic 1Al-msoil and 2Al-msoil treatments, nodule dry weight production was significantly ($P \leq 0.05$) suppressed compared to other treatments (Table 4). However, nodulation in these acidic treatments did occur, agreeing with Hyland (1938) who observed significant nodulation from pH 4.1 to 4.8. For each inoculation treatment there were no significant differences in nodule dry weights between 1Al-msoil and 2Al-msoil acidification treatments, indicating that nodule dry weight production was suppressed similarly by both treatments (pH 4.5 and 4.2, respectively). Compared to nodulation at the more optimal pH levels, nodulation in the more acidic treatments was suppressed less than that reported by Cline and Senwo (1993). For both 1Al-msoil and 2Al-msoil treatments, no significant differences were detected among nodule dry weights obtained from the three inoculated treatments, suggesting that the strains were equally tolerant of the acid conditions. However, in these treatments nodule numbers for the commercial mixture of strains tended to be significantly ($P \leq 0.05$) greater than numbers obtained for strains 41A and CR1, indicating that the commercial strains tended to form more but smaller nodules under these acidic conditions. This could be important since larger nodules would be expected to contain greater amounts of nitrogenase per gram of nodule due to surface area/volume relationships. In previous pure culture experiments, strains 41A and CR1 appeared to be more tolerant of acidic soil factors than commercial strains (Cline and Senwo 1994), whereas here they responded similarly in terms of nodule dry weight production.

SUMMARY

Although growth of sericea lespedeza was significantly reduced ($P \leq 0.05$) by acidification in some inoculation treatments, the plants were able to grow relatively well under the acidic conditions even in 2Al-msoil at a pH level of 4.2. Compared to Ca-msoil, nodulation was significantly ($P \leq 0.05$) suppressed by the more acidic treatments, particularly 1Al-

Table 4. Mean nodule dry weight production (mg ± SE, n = 8) obtained for sericea lespedeza grown in minesoil acidity treatments using different strains of *Bradyrhizobium* nitrogen-fixing bacteria. Values in columns followed by different letters were significantly ($P \leq 0.05$) different.

Acidity treatment	Inoculation			
	Commercial	41A	CR1	Control + N
Ca-msoil	13.9 ± 2.0a	26.1 ± 5.6a	21.6 ± 2.2a	0.0a
Untreated	13.5 ± 1.9a	23.3 ± 3.1a	14.2 ± 2.3b	0.0a
1 Al-msoil	7.2 ± 1.3b	8.0 ± 1.4b	9.4 ± 1.1c	0.0a
2 Al-msoil	8.9 ± 1.3b	10.0 ± 1.2b	9.3 ± 1.0c	0.0a

msoil and 2Al-msoil, which appeared to suppress nodulation similarly for individual inoculation treatments. In acidified minesoil, nodulation was similar among *Bradyrhizobium* inoculation treatments with the exception that the commercial mixture of strains tended to produce more numerous but smaller nodules than local strains isolated from acidic mine spoils.

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Structure and Composition of Three Swamp Forests on the Mississippi Alluvial Plain of Kentucky's Jackson Purchase Region

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ABSTRACT

Three swamp forests on the Mississippi alluvial plain of the Jackson Purchase Region of Kentucky are described. One forest was dominated by *Taxodium distichum*, one by *Nyssa aquatica*, and one by *T. distichum* and *N. aquatica*. In terms of basal areas and densities, these forests compared favorably to other little disturbed swamps in the southeastern United States. Basal areas of 56.5, 72.8, 84.6 m^2/ha were two to three times greater than most mature upland forests of Kentucky. These swamps are remnants of a once more abundant wetland system on the Mississippi alluvial plain.

INTRODUCTION

Bottomland forests, like other wetland types in Kentucky and the southeastern United States, have undergone great reductions in area and changes in composition (Sharitz and Mitsch 1993). Today, only 20% of Kentucky's originally estimated 650,000 ha of wetlands remain; only 20% of those remnants are forested (KEQC 1992). The majority of forest remnants are found in the Jackson Purchase Region, especially on the Mississippi alluvial plain in Ballard, Carlisle, Fulton, and Hickman counties (KNPC 1986). Other remnants occur eastward along the Ohio, Green, and Tradewater rivers of the Western Kentucky Coalfields Region (Braun 1950; Mitsch et al. 1983).

Bottomland forests, hardwoods, and deepwater swamps were first reported from the Jackson Purchase in William O. Henderson's 1820 General Land Office Survey (Bryant and Martin 1988); however, since that time little basic information on these vegetation types has been added. Aside from a few early timber surveys (DeFriese 1880, 1884; Loughridge 1888); county floras (e.g., Grubbs and Fuller 1991); a floristic list for Murphy's Pond, a swamp in Hickman County (Adams et al. 1968); and a report on nutrient dynamics for that same swamp (King et al. 1989), the literature is limited. Here and elsewhere on the Mississippi alluvial plain, few quantitative studies of bottomland forests have been reported (Mitsch et al. 1979). Specifically, information is scarce on the relative abundance of species, structural features, gradient analysis, and diversity relations (Robertson et al. 1978). In an attempt to fill in some of the information gaps, Bryant (1991) analyzed and compared several bottomland remnants in the Jackson Purchase. Additionally, forest vegetation of the loess bluffs, which form an eastern border along the Mississippi alluvial plain, was reported (Bryant 1993). Forested wetlands of the Western Kentucky Coalfield have recently been the subjects of ecological analysis (Benson 1986; Cardamone 1985; Hill 1983; Mitsch et al. 1983, 1991; Taylor 1985) and may be better known.

The purpose of this paper is to describe and quantitatively compare stand characteristics for some deepwater swamp communities in which baldcypress (*Taxodium distichum*) and/or water tupelo (*Nyssa aquatica*) are major contributors. Comparisons to other swamp and bottomland forests are made, especially as they relate to providing answers to the forest structure and community composition.

THE ENVIRONMENT

The Mississippi alluvial plain is a division of the larger Coastal Plain Province (Fenneman 1938). In western Kentucky, it is the broad historic and recent floodplain of the Mississippi and Ohio river systems. Most of the alluvium that covers the floodplain has been deposited since the Pleistocene. The Mississippi alluvial plain's aggraded surface is marked by river meanders and oxbow lakes, natural levees and backswamp areas, and shallow sloughs

and marshes (Powers 1966). In their natural state the low gradient streams that flowed through the bottomlands were sluggish and widely meandering, carrying a large sediment load.

As a result of microtopographical differences on the floodplain, a mosaic of forested swamps and bottomland hardwoods occurs across the bottomlands. Flooding frequency, hydroperiod, and depth of flooding are factors that work with the microtopography to sort out the communities. The forested swamps occur in long, narrow, often curving sloughs, oxbow lakes, bayous, and stream margins. Levees, channelization, and drainage may have modified flood plains and altered modern flooding regimes.

Stands of baldcypress and water tupelo usually have a long hydroperiod; however, a fall drydown of water often occurs (Wharton et al. 1982). Soils are generally saturated for most of the year.

METHODS

Efforts were made to locate swamp forests that showed few signs of recent human disturbance (e.g., stumps). I sampled three swamps during the fall drydown period in October 1989. At that time it was possible to walk or wade into the interior of each swamp. Two of the swamps were in Ballard County (Oscar Bottoms area and Fish Lake area) and one was in Carlisle County (Back Slough area). All were in recognized wetland priority areas (KNPC 1986). Circular plots (0.04 ha), spaced at 30 m intervals, were located through the interior of each swamp. No samples were taken near the swamp edge. The number of plots sampled per swamp (or the size of the area sampled) was to equal or exceed that sampled by Anderson and White (1970) in their study of a baldcypress swamp in southern Illinois. Based on species area curves the number of plots taken per stand was sufficient. Six plots were taken at Fish Lake, 10 at Back Slough, and 12 at Oscar Bottoms.

All trees ≥10 cm at 1.4 m above the ground (dbh) were measured in each plot. Because of the swollen buttresses of baldcypress, Anderson and White (1970) proposed measuring these trees at 2.1 m to make the basal areas more comparable to those in other forest communities. They found a linear relationship between baldcypress diameters at 1.4 m (normal dbh) and 2.1 m. The 2.1 m dbh was about 90% ($Y = .90X - 2.9$) of the 1.4 m dbh. All 1.4 m dbh values for baldcypress were converted to the 2.1 m values based on the regression formula of Anderson and White (1970), and basal areas for both heights were determined. Water tupelo has swollen bases also, but no diameter conversions were attempted for that species.

Tree data were analyzed to relative density (RD), relative dominance (RDo), importance value (IV), and importance percentage (IP). IP, determined as IV/2, was used for comparisons to 100. Density (trees/ha), basal area (m^2/ha), species diversity (H′), and evenness (J) were determined for each swamp forest. Species diversity was calculated using the Shannon-Weaver function:

$$H' = -\sum_{i=1}^{s} (p_i)(\log_2 p_i)$$

where s is the number of species, and p_i is the proportion of the total sample belonging to the ith species (Shannon and Weaver 1949). The similarity coefficient (C; Bray and Curtis 1957) was used to compare the swamps of this sample and to compare these swamps to others in the literature. The equation for calculating similarity is $C = (2w)/(a + b)$, where a equals the sum of the IVs or IPs for Swamp One, b equals the sum of the IVs or IPs for Swamp Two, and w is the sum of the lower values for the species that occur in both samples. The equitability (evenness) of the stand (J) was calculated using a method based on the Shannon-Wiener function (H′): $J = H'/Hmax$, where Hmax is the diversity under maximum equitability ($H' = \log_2 S$), and S is the number of species in the sample.

RESULTS

According to the dominant species, the swamps sampled were classified as baldcypress (Back Slough), water tupelo (Fish Lake), and baldcypress-water tupelo (Oscar Bottoms). In terms of composition and stand structure (IP), the three swamps showed low degrees of similarity, C = <2% to >42% range. Similarity was lowest between the baldcypress and the

Table 1. Number (N), relative density (RD), relative dominance (RDo), importance value (IV), and importance percentage (IP) for Black Slough, Carlisle County, Kentucky.

	N	RD	RDo	IV	IP
Taxodium distichum	171	92.93	99.05	191.98	95.99
Planera aquatica	9	4.89	0.30	5.19	2.60
Fraxinus profunda	2	1.09	0.07	1.16	0.58
Salix nigra	1	0.54	0.54	1.08	0.54
Forestiera acuminata	1	0.54	0.03	0.57	0.29
Total	184	99.99	99.99	199.98	100.00

water tupelo communities, which were nearly monospecific stands.

Of the five tree species in the baldcypress swamp (Table 1), baldcypress accounted for 93% of the density and >99% of the basal area. There were 454.5 trees/ha with a basal area of 116.5 m^2/ha. The converted basal area (Anderson and White 1970) was 72.8 m^2/ha. Because of the near monospecific nature of this stand, diversity ($H' = 0.46$) and evenness ($J = 19.99$) were low. Baldcypress knees were abundant, and fallen logs were occasional on the floor of this swamp, but no standing dead trees were observed.

Cephalanthus occidentalis (buttonbush) was the most abundant shrub in the swamp. Herbs that occurred on baldcypress buttresses, fallen logs, and drydown soils included *Bidens discoidea, Boehmeria cylindrica, Hypericum walteri, Lycopus rubellus, Cuscuta* sp., and *Saururus cernuus.* Anderson and White (1970) reported a similar but more extensive list of herbs in southern Illinois. Because the sampling timeframe was limited for my study, little effort was made to thoroughly characterize the herb layer of this and the other swamps. Heineke (1987) presented a more complete listing of plants from the middle portion of the Mississippi alluvial plain.

The water tupelo swamp contained only three tree species (Table 2). Water tupelo contributed 95% of the density and >94% of the stand basal area. There were 526.9 trees/ha. Basal area was 85.3 m^2/ha; converted basal area was 84.6 m^2/ha. Species diversity ($H' = 0.36$) and evenness ($J = 22.71$) were low in this nearly pure stand. Buttonbush was the only commonly encountered shrub.

Twelve tree species were present in the baldcypress-water tupelo swamp (Table 3). Baldcypress and water tupelo accounted for 70% of the 69.9 m^2/ha basal area. The conversion value was 56.5 m^2/ha. There were 403.4 tress/ha; however, baldcypress and water tupelo collectively accounted for only 35% of the total density.

Other species, *Quercus lyrata* (overcup oak), *Fraxinus pennsylvanica* (green ash), and *Acer saccharinum* (silver maple) were major associates, especially in regard to their combined density contributions. The other seven tree species reflected the hydric nature of the site; their presence assured a higher diversity ($H' = 2.84$) relative to the other two swamps. The contributions of the several species were reflected by a greater evenness ($J = 79.3$). The hydroperiod may differ from that of the other two swamps sampled. Barry (1980) found that, with decreases in flooding depth and duration, more codominants appear.

Average density for the three swamps sampled was 461.6 trees/ha; average basal area was 90.6 m^2/ha; and average converted basal area was 71.3 m^2/ha. Those values are comparable to other little disturbed swamp forests in the southeastern United States (e.g., An-

Table 2. Number (N), relative density (RD), relative dominance (RDo), importance value (IV) and importance percentage (IP) for the Fish Lake Swamp Forest, Ballard County, Kentucky.

	N	RD	RDo	IV	IP
Nyssa aquatica	121	94.53	94.30	188.83	94.42
Taxodium distichum	3	2.34	1.56	3.90	1.95
Quercus lyrata	4	3.14	4.13	7.26	3.63
Total	128	100.00	99.99	199.99	100.00

Table 3. Number (N), relative density (RD), relative dominance (RDo), importance value (IV), and importance percentage (IP) for the Oscar Bottoms Swamp Forest, Ballard County, Kentucky.

	N	RD	RDo	IV	IP
Taxodium distichum	41	20.92	40.39	61.31	30.66
Nyssa aquatica	28	14.29	29.61	43.90	21.95
Quercus lyrata	45	22.96	11.19	34.15	17.08
Acer saccharinum	33	16.84	5.79	22.63	11.32
Fraxinus pennsylvanica	25	12.76	6.43	19.19	9.60
Ulmus americana	8	4.08	0.40	4.48	2.24
Populus heterophylla	6	3.06	1.32	4.38	2.19
Populus deltoides	2	1.02	3.22	4.24	2.12
Planera aquatica	4	2.04	0.12	2.16	1.08
Platanus occidentalis	2	1.02	0.92	1.94	0.97
Salix nigra	1	0.51	0.57	1.08	0.54
Forestiera acuminata	1	0.51	0.03	0.54	0.27
Total	196	100.01	99.99	200.00	100.02

derson and White 1970; Penfound 1952; Robertson 1987; Schlesinger 1978).

Size class distributions for baldcypress and water tupelo followed bell-curves, suggesting even-aged stands (Figures 1, 2). No trees were cored for age determinations; however, rough age estimates were obtained by applying average diameter growth rate (3.25 mm/yr) for baldcypress at Hovey Lake, Indiana (Kozel et al. 1989) to baldcypress trees at Oscar Bottoms and Back Slough. Maximum ages were 322 yr and 416 yr, respectively. Age estimates using Mattoon's (1915) "rule of thumb" that 12 inches (30.5 cm) in diameter equals 100 years gave similar, but somewhat greater ages. Results of either estimate add support that these are long-established stands.

DISCUSSION

Baldcypress and water tupelo, either singly or in association, are clearly the dominant tree species at the hydric end of the moisture gradient on the Mississippi alluvial plain in Kentucky's Jackson Purchase Region. Deepwater

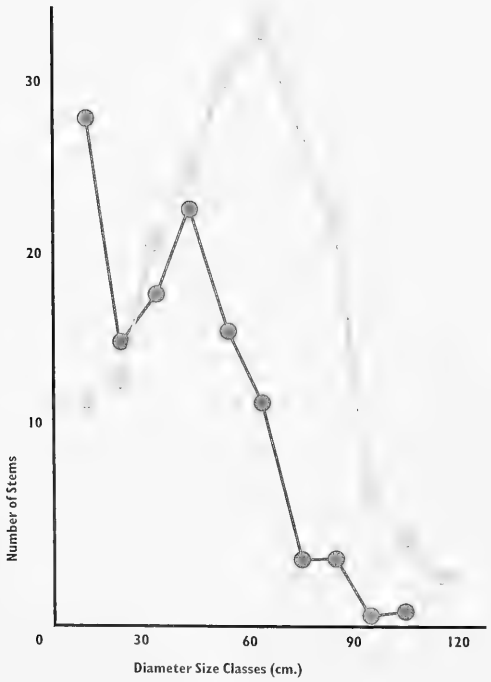

Figure 1. Size-class distributions for baldcypress (*Taxodium distichum*) at Back Slough and for water tupelo (*Nyssa aquatica*) at Fish Lake in the Jackson Purchase Region of Kentucky. Gray data points = baldcypress; black data points = water tupelo.

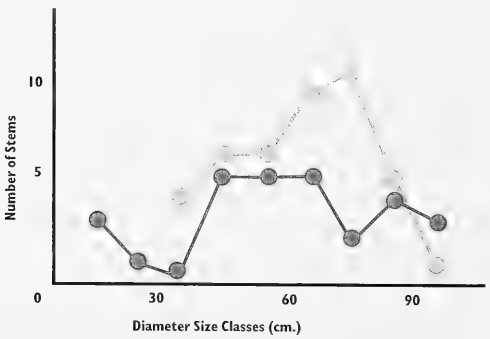

Figure 2. Size-class distribution for all trees of baldcypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*) in the Oscar Bottoms Swamp, Ballard County, Kentucky. Gray data points = baldcypress; black data points = water tupelo.

swamps are covered by water throughout most of the year (Penfound 1952). Basal areas (m^2/ha) and densities (trees/ha) for the three swamps sampled compared favorably to other southeastern United States swamp forests (e.g., Schlesinger 1978), but basal areas greatly exceeded those reported for the Western Coalfield Region (Mitsch et al. 1991). Swamp forests tend to have significantly greater stand densities (Nelson 1997) and basal areas than most regional upland forests including such highly productive forests as the coves of the Smoky Mountains (Whittaker 1966). Basal areas, converted or not, are two to three times greater than those of mature upland forests in eastern North America (Held and Winstead 1975; Martin 1992) (Table 4). Tree species diversities are low (Robertson 1987). Mitsch et al. (1991) noted that complexity indices of cypress-dominated systems were low.

Braun (1950) observed that bottomlands from Louisiana northward to southern Illinois show a great floristic uniformity. An ecological comparison of the swamps in my study was made to a swamp in Louisiana (Conner and Day 1970) and one in southern Illinois (Anderson and White 1970). Maximum similarity values were only 58% among those locations. Bryant (1991) reported a 51% similarity between a bottomland hardwood stand in Kentucky and one in Louisiana. Those similarities are rather low and indicate some differences in composition but especially in the contributions (IVs or IPs) of species in common between sampling sites along this north-south gradient. Other differences may reflect the location of sampling plots. Anderson and White (1970) reported red maple (*Acer rubrum*) from a southern Illinois swamp, but in western Kentucky red maple was a tree of swamp margins, not of their interiors (Bryant, unpublished data). Guthrie (1989) also noted the apparent absence of water tupelo from Reelfoot Lake, Tennessee, a swamp just south of the Jackson Purchase sites. In regard to hardwoods, Bryant (1991) reported higher importances for pecan (*Carya illinoinensis*), other hickories, and oaks in Kentucky than in bottomlands further south. Braun (1950) may be correct in regard to floristic similarity, but vegetationally there appears to be a greater variability in the bottomlands.

Even-aged, densely stocked monospecific stands of baldcypress or water tupelo appear to be most common in deep water (Anderson and White 1970; Johnson and Shropshire 1983). Monospecific stands of water tupelo have been attributed to past logging and removal of baldcypress (Robertson 1987). That was not confirmed in my study, although stumps were present in areas adjacent to sampling sites. Water depth and hydroperiod play extremely important roles in determining species composition on the alluvial plain. Baldcypress-water tupelo forests may support recruitment of mixed bottomland species following drought or draining (Sharitz and Mitsch 1993). Swamp sites with shallower and more

Table 4. Basal areas (m^2/ha) for selected upland and lowland forests in Kentucky for comparison to swamps of the Jackson Purchase Region of Kentucky.

Lilley Cornett Woods (Martin 1975)	
Sugar maple-Basswood-Tulip poplar	30.0 m^2/ha
Hemlock	42.4 m^2/ha
Chestnut oak	29.1 m^2/ha
Beech-White oak	28.8 m^2/ha
Lilley Cornett Woods (Muller 1982)	
Old growth composite	27.8 m^2/ha
Bonayer Forest (Bougher and Winstead 1974)	
Oak forest	31.9 m^2/ha
Boone County Cliffs Nature Preserve (Bryant 1978)	
Total forest	27.2 m^2/ha
Lloyd Wildlife Preserve Forest (Bryant 1985)	
Mixed forest	35.5 m^2/ha
Dinsmore Woods Nature Preserve (Held and Winstead 1976)	
Oak-ash-maple forest	28.1 m^2/ha
Myers Woods (Held 1980)	
Beech forest	37.1 m^2/ha
Greenwood Forest (Chester et al. 1995)	
Wet woods	37.8 m^2/ha
Upper Bottoms (Bryant 1991)	
Sugarberry forest	35.6 m^2/ha
Martin Creek (Martin 1983)	
Southern red oak	29.2 m^2/ha
Cypress Swamp (Mitsch et al. 1991)	
Stagnant swamp	35.9 m^2/ha
Semi-permanent flooded	32.7 m^2/ha

variable water depths may reflect this but areas where flooding is relatively constant are usually dominated by baldcypress (e.g., Back Slough) or water tupelo (e.g., Fish Lake). The low numbers of standing dead trees at the three swamps suggests that their compositions are relatively stable and not undergoing compositional shifts as was found in the Western Kentucky Coalfield (Mitsch et al. 1991).

Estimated ages of selected trees in the swamps indicate that they are remnants of long-established swamp systems. Baldcypress trees with >1.m dbh were not unusual, but none as large as the 2.3 m dbh reported by DeFriese (1880) were found.

In their study of bottomland forests at Horseshoe Lake, southern Illinois, Robertson et al. (1978) found increased dominance, lower equitability, and decrease in number of species of intermediate importance from the mesic to the wet segment of the moisture gradient. They attributed that reduction in diversity to increased flooding and poorer drainage. Wharton et al. (1982) referred to this as an anaerobic gradient. Robertson et al. (1978) stated, "The complex gradient acts as a filter which effectively screens a number of species. This filtering action continues as flooding increases, until swamp forests dominated by *T. distichum* and *N. aquatica* prevail." That apparently is also the situation on the Mississippi alluvial plain in the Jackson Purchase Region.

Ewell and Odum (1984) stated, "Understanding wetland ecosystems is a major goal of contemporary ecological sciences." Wetlands continue to be lost in Kentucky. It has taken over 175 years for baldcypress and water tupelo swamp forests to be described for the Jackson Purchase Region. More research on Kentucky's diminishing wetlands is needed so that the roles these valuable ecosystems play in the land-water interface may be better understood.

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Planning a Trip

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ABSTRACT

The problem of minimizing the driving time for a trip is considered. A hypothetical trip from Louisville to Memphis is used as an illustration. A mathematical model is constructed and a solution technique is devised and implemented.

DESCRIPTION OF THE PROBLEM

We consider a hypothetical trip from Louisville to Memphis. We suppose the driver wishes to depart no earlier than 1100 and desires to arrive between 1745 and 1945, say, to attend a meeting or presentation scheduled for 2000. The driver wishes to select a departure time that minimizes total travel time. We assume the driver must travel 20 miles through Louisville at a speed of 55 mph, except for lunch hour from 1200 to 1300, when speed is restricted to 45 mph, and for "rush hour" from 1600 to 1800, when speed is held to 30 mph. The motorist can then drive at 65 mph over the next 150 miles to Nashville, except for a 5-mile stretch near Elizabethtown, Kentucky, on which the speed is limited to 40 mph from 1600 to 1700 due, say, to traffic from a plant closing. The driver then travels 25 miles through the Nashville area, with the same restrictions as the Louisville area. There follows a 155-mile stretch of highway on which the motorist can proceed at 65 mph, except for a 15-mile long construction area beginning 40 miles outside of Nashville. In this area, the speed is restricted to 20 mph until 1700 after which speeds are held to 55 mph. Finally, the driver must proceed 20 miles through the Memphis area, with the same speed limits as in Louisville and Nashville.

Suppose we establish a departure time of 1200. Consider Figure 1. At that departure time we are restricted to 45 mph because of lunch traffic. At 1227 we have travelled for 20 miles and reached the outskirts of Louisville, at which point we can travel at 65 mph for a 150-mile stretch. We then hit the Nashville area at 1445 where we must slow to 55 mph. Continuing in this fashion, we obtain the results in Table 1.

RESULTS

Wishing to obtain the starting time resulting in the shortest travel time, we select possible starting times spaced at regular intervals and calculate the travel time for each. Proceeding as above for each of these times would be prohibitively time-consuming. Therefore, we have written a computer program that calculates the arrival time and travel time for each of the possible starting times. (See Section 3 for details.) We thus generate the results in Table 2. The output is restricted to those trips for which the arrival times satisfy the given constraints. Thus we see that the optimal departure time is 1300.

THEORY AND IMPLEMENTATION

We now consider the general problem of minimizing the driving time for a trip. One has a set of acceptable departure times $S = \{t_0, t_1, t_2, \ldots\}$, where $t_0 < t_1 < t_2 < \ldots$ (For example, S might be {1500, 1530, 1600 ...}.) One would also typically have an earliest desired arrival time T_1 and a latest desired arrival time T_2. Of course, we require $t_0 < T_1 < T_2$. One also has constraints on speed. These involve not only the posted limits but traffic congestion caused by factors such as rush hour in a large town or restricted speed limits due to school zones or construction. Note that these factors are typically dependent on both time and position. Using these considerations, we can construct an ordinary differential equation $x'(t) = f(t, x(t))$, where t is the time after departure and $x(t)$ is the distance travelled at time t. Finally, we let D be the travel distance. We assume that the acceleration/deceleration times between speed zones are negligible. We also assume that the driver travels at the maximum speed possible at all times. We note that

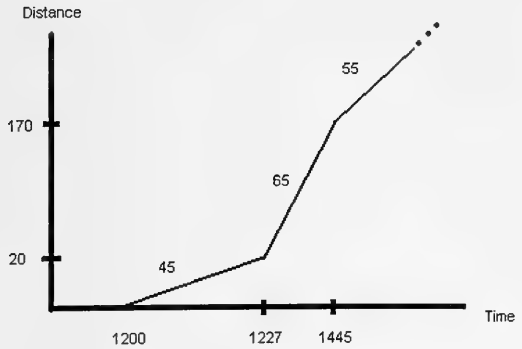

Figure 1. Planning a trip. Illustration of the trip starting at noon.

these assumptions force f to be discontinuous. Removal of these restrictions is discussed in the last section. Letting $\bar{t}$ represent the departure time and T the arrival time, we thus obtain the following model:

Choose $\bar{t}$ so that $T - \bar{t}$ is minimized subject to

$$x'(t) = f(t, x(t)),\ t \in [\bar{t}, T],$$
$$x(\bar{t}) = 0,$$
$$x(T) = D,$$
$$T_1 \leq T \leq T_2,$$
$$\bar{t} \in S,\ \bar{t} < T.$$

Since f is discontinuous, a differentiable solution may be impossible to find. For example, consider

$$x'(t) = \begin{cases} 0, & \text{if } 0 \leq t < 1 \\ 1, & \text{if } 1 \leq t \leq 2, \end{cases} \qquad t \in [0, 2]$$
$$x(0) = 0.$$

One is tempted to propose

$$x(t) = \begin{cases} 0, & \text{if } 0 \leq t \leq 1 \\ t - 1, & \text{if } 1 \leq t \leq 2 \end{cases}$$

as a solution; however, $x'(1)$ does not exist and hence x does not satisfy the differential equation. As a result, we must modify the classical definition of solution.

Definition 1: x: $[\bar{t}, T] \to R$ is a *solution* to

$$x'(t) = f(t, x(t)),\ t \in [\bar{t}, T]$$
$$x(\bar{t}) = x_0$$

Table 1. Planning a trip. Calculation of the trip time for the trip starting at 1200.

Time of day	Distance travelled
1227	20
1445	170
1512	195
1549	235
1634	250
1806	350
1828	370

if and only if i) x is continuous on $[\bar{t},T]$, ii) $x'(t) = f(t,x(t))$ for all but a finite subset of $[\bar{t},T]$, iii) $x(\bar{t}) = x_0$.

Actually the definition of solution that is standard for discontinuous differential equations involves Lebesgue measure theory; the interested reader can consult Chapter 2 in (Coddington and Levinson 1955). Our velocity constraint function f is assumed to satisfy the following.

Definition 2: $f : [t_0, T_2) \times [0,D) \to R$ is *blockwise-constant* if and only if there exists a finite collection of disjoint sets $A_i = \{(t, x) : a_i \leq t < b_i, c_i \leq x < d_i\}$ such that $\cup A_i = [t_0, T_2) \times [0,D)$ and $f|_{Ai}$ is constant for each i.

Example 1: Consider f: $[0, 1) \times [0, 30) \to R$ defined by

$$f(t, x) = \begin{cases} 15 & \text{if } \frac{1}{4} \leq t < \frac{1}{2},\ 5 \leq x < 6 \\ 35 & \text{elsewhere} \end{cases}$$

This f could model a 30-mile stretch of highway on which the speed limit is 35, except for

Table 2. Planning a trip. Output from travel problem code.

Starting time	Arrival time	Travel time
1112	1754	6 hr 42 min
1124	1803	6 hr 39 min
1136	1810	6 hr 34 min
1148	1817	6 hr 29 min
1200	1828	6 hr 28 min
1212	1840	6 hr 28 min
1224	1852	6 hr 28 min
1236	1858	6 hr 22 min
1248	1901	6 hr 13 min
1300	1907	6 hr 7 min
1312	1923	6 hr 11 min
1324	1942	6 hr 18 min

OPTIMAL TRIP LENGTH = 6 hr 7 min

a school zone from mile 5 to mile 6 on which the speed limit is 15 from $t = ¼$ to $t = ½$.

Before we consider numerical approaches, we must ensure that our model is well-posed.

Theorem 1: Let $f : [t_0, T_2) \times [0,D) \to R$ be blockwise-constant and positive-valued. Let $\bar{t} \in S$, $\bar{t} < T_2$. Then, one (and only one) of the following must hold:

i) There exists $T \in (t_0, T_2]$, $x : [\bar{t}, T] \to R$ such that x is a solution to $x'(t) = f(t, x(t))$ on $[\bar{t},T]$, $x(\bar{t}) = 0$ and $x(T) = D$, or

ii) there exists $x : (\bar{t},T_2] \to R$ such that x is a solution to $x'(t) = f(t,x(t))$ on $[\bar{t},T_2]$, $x(\bar{t}) = 0$ and $x(T_2) < D$.

The proof of Theorem 1 is straightforward and is left to the reader.

Theorem 2: Let $f : [t_0, T_2) \times [0,D) \to R$ be blockwise-constant and positive-valued. Let $\bar{t} \in S$, $\bar{t} < T_2$. Suppose x is a function satisfying one of the two conclusions of Theorem 1. Let $\bar{x}$ be continuous, $\bar{x}'(t) = f(t,\bar{x}(t))$ for all but a finite subset of the domain of $\bar{x}$ and assume there exists some $\hat{t}$ such that $x(\hat{t}) = \bar{x}(\hat{t})$. Then, $x(t) = \bar{x}(t)$ for all t in the intersection of the domains of x and $\bar{x}$.

Proof. *Claim 1:* x and $\bar{x}$ agree to the right of $\hat{t}$, that is, if $\hat{t} \in [\bar{t},\bar{T})$, where $\bar{T}$ represents the right-hand endpoint of the intersection of the domains of x and $\bar{x}$, then $x(t) = \bar{x}(t)$ for all $t \in [\hat{t},\bar{T}]$.

Proof of Claim 1: Suppose not. Then, without loss of generality, there exists some $\tau \in (\hat{t},\bar{T}]$ such that $\bar{x}(\tau) > x(\tau)$. Let $t^* = \sup\{t \in [\hat{t}, \tau) \mid x(t) = \bar{x}(t)\}$. Since x and $\bar{x}$ are continuous, we have $x(t^*) = \bar{x}(t^*)$ and $\bar{x}(t) > x(t)$ for all $t \in (t^*,\tau]$. There exists some $\hat{\tau} \in (t^*,\tau)$ such that $(t,x(t))$ and $(t,\bar{x}(t)) \in$ interior of some A_i for all $t \in (t^*,\hat{\tau})$. Let k_i represent the constant value of f on A_i. Then, for $t \in (t^*,\hat{\tau})$, we have $x(t) = x(t^*) + (t - t^*)k_i = \bar{x}(t^*) + (t - t^*)k_i = \bar{x}(t)$, a contradiction.

Claim 2: x and $\bar{x}$ agree to the left of $\hat{t}$, that is, if $\hat{t} \in (\bar{t},\bar{T}]$, where $\bar{T}$ represents the right-hand endpoint of the intersection of the domains of x and $\bar{x}$, then $x(t) = \bar{x}(t)$ for all $t \in [\bar{t}, \hat{t}]$.

Proof of Claim 2: Analogous to the proof of Claim 1.

Corollary 1: The function x guaranteed by Theorem 1 is unique.

As a result of Theorems 1 and 2, for each $\bar{t} \in S, \bar{t} < T_2$, the numerical procedure has one and only one solution to approximate.

For each of the starting times, the numerical method employed in this study is an adaptive version of Euler's method. Given an initial value problem (IVP)

$$\begin{cases} x'(t) = f(t, x), & a \le t \le b, \\ x(a) = \alpha, \end{cases}$$

Euler's method, discussed in any elementary numerical analysis textbook (see, for example, (Burden and Faires 1997)) generates an approximate solution to the IVP at a set of discrete points τ_j, where $a = \tau_0 < \tau_1 < \ldots < \tau_N = b$, by approximating the solution $x(t)$ locally by the first two terms in its Taylor series. Euler's method produces an approximation u_j to the exact solution $x(\tau_j)$ at the mesh points according to the iteration

$$\begin{cases} u_0 = \alpha, \\ u_j = u_{j-1} + h_j f(\tau_{j-1}, u_{j-1}), \\ \qquad j = 1, 2, \ldots, N, \end{cases}$$

where $h_j = \tau_j - \tau_{j-1}$. Euler's method is in fact *exact* if the true solution $x(t)$ is linear throughout the interval $[a,b]$. It is an immediate extension of this fact that Euler's method will also produce the exact answer if the exact solution is merely continuous and *piecewise* linear with respect to the mesh $\{\tau_j\}_{j=0}^{N}$(that is, the only changes in slope occur on some subset of the set of mesh points $\{\tau_j\}_{j=0}^{N}$). In our travel problem, the function $f(t,x)$ is blockwise constant, and therefore the exact solution $x(t)$ is continuous and piecewise linear. As a consequence, it is possible to obtain the exact solution (aside from roundoff errors) from the use of Euler's method, so long as suitable adjustments are made. For example, a given time step may need to be modified to avoid overshooting a block.

SUMMARY

We have considered the problem of minimizing the driving time of a trip by modeling the problem with a discontinuous differential equation. Although it is straightforward (yet tedious) to solve the differential equation by hand, efficient solution of the minimization problem may be accomplished through repeated application of Euler's method on a

computer. We finally consider a few ideas for further work. One could include the fact that a car has maximum acceleration and deceleration, say, by adding the constraint $x''(t) \in \{-d,0,c\}$ or, $x''(t) \in [-d,c]$, where c,d are positive constants. Thus, one would need to consider differential inclusions rather than differential equations. One would now require $x'(t) \in [0,f(t,x(t))]$ rather than $x'(t) = f(t,x(t))$, since with acceleration constraints one may be unable to always drive at the speed limit. The subject of differential inclusions is not an easy one to get into; a well-known resource is (Aubin and Cellina 1984). Also, one may wish to consider problems in which the driver may have multiple routes from which to choose. An application of graph theory may be necessary. This problem can be extended further by attempting to choose the ideal route and departure time given certain driver preferences. For example, the driver may be interested in the best route and departure time to get from A to B, but may wish to pass through C on the way. The reader is invited to use his or her imagination to add even more twists along these lines. Finally, we mention that it would be worthwhile to consider shapes other than rectangles to subdivide the domain of f. For example, consider a school zone that drops the speed limit from 35 mph to 15 mph at 1530. In the blockwise-constant model, the driver who enters the school zone at 1529 at 35 mph will immediately realize at 1530 that he or she should slow to 15 mph as long as he or she remains in the school zone. Realistically, the warning that the speed limit has changed from 35 mph to 15 mph may only be given at the start of the school zone. In such a case, the driver mentioned above would continue at 35 mph rather than 15 mph, having no warning of the speed limit change. The reader may convince himself or herself that the proper shape in the tx-plane for this f is a trapezoid or a triangle.

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NOTES

Blue Catfish (*Ictalurus furcatus*; Ictaluridae) Predation on the Zebra Mussel in the Ohio River near Paducah, Kentucky.—Blue catfish (*Ictalurus furcatus*) were examined to determine whether they are predators of the exotic zebra mussel (*Dreissena polymorpha*) in the Ohio River. Predation on zebra mussels by diving ducks, crayfish, and several fish species other than blue catfish has been described (1, 2, 3, 4). Cyprinids such as the roach (*Rutilis rutilis*), bull chub (*Nocomis raneyi*), and common carp (*Cyprinus carpio*) are predators of zebra mussels (5, 6, 7). Other fishes with molariform pharyngeal teeth or chewing pads—e.g., freshwater drum (*Aplodinotus grunniens*), red-ear sunfish (*Lepomis microlophus*), and smallmouth buffalo (*Ictiobus bubalus*)—are considered predators of the mussels in North America (4).

A common species in large rivers of southeastern United States is the blue catfish. Blue catfish lack pharyngeal teeth but do feed on molluscs (8). Members of the Ictaluridae (catfish family) can feed on bivalves by swallowing them whole. French (1993) noted a zebra mussel with valves intact in a brown bullhead (*Ameiurus nebulosus*). On 1 Oct 1995 a commercial fisherman on the Ohio River told us that he observed zebra mussel shells in the stomachs of blue catfish. To confirm this observation we chose to examine blue catfish from a fish market that processed fish from the Ohio River.

Blue catfish were sampled on 4, 6, and 7 Nov 1995 from Three Rivers Fish Market in Paducah, Kentucky. The sample consisted of 150 individuals with lengths ranging from 25 to 50 cm. Esophagus, stomach, and intestine were removed from each fish and examined for the presence of zebra mussels. The fish had been caught in the Ohio River near Paducah between Smithland Dam and Dam 52; exact site information was not available from the commercial fishermen. A total of 1473 bivalves was recovered from 27 of the 150 catfish. Three species of mollusks were present: Asian clam (*Corbicula fluminea*), fawnsfoot (*Truncilla donaciformis*), and zebra mussel.

Blue catfish with lengths of 28 to 33 cm were found to contain the majority of bivalves and consisted of 58% (87) of the sample. Of the 87 stomachs, only 31.0% (27) of individuals of this size had fed on bivalves. Eighteen stomachs (66.7%) contained only Asian clams; six (22.2%) contained zebra mussels. One stomach that had zebra mussels also had three fawnsfoot mussels with lengths of 7, 11, and 12 mm. Three (11.1%) blue catfish with lengths of 29, 30, and 32 cm had both Asian clams and zebra mussels. This size grouping of blue catfish had 72.6% (1040) of the total number of Asian clams and 94.6% (35) of the total number of zebra mussels. In the three species of bivalves the hinge was intact, the shells were whole, and some visceral tissue was present. A total of 1433 Asian clams was found with lengths between 1 and 12 mm. Asian clam lengths of 3 to 7 mm comprised 92.9% (1331) of the total number of Asian clams found in the stomachs. Thirty-seven zebra mussels were collected with lengths between 3 and 15 mm. Zebra mussel lengths of 3 to 7 mm comprised 78.4% (29) of the total number of zebra mussels found in stomachs. Our observations on zebra mussels in the Ohio River at Dam 52 provide a length range of 3 to 24 mm with the most common lengths of 12 to 20 mm.

Our study confirms that blue catfish do feed on zebra mussels. The degree of predation and effects of this predation on the Ohio River zebra mussel population are unknown. However, our findings suggest that there may be a size selection (3–7 mm) occurring when blue catfish feed on zebra mussels and Asian clams. Also, there appears to be a size range for blue catfish that feed on bivalves. Considering the high numbers of blue catfish in large rivers and reservoirs in southeastern United States and the impact of the zebra mussel invasion, we recommend more extensive research toward assessing predation by blue catfish on zebra mussels and efforts toward distinguishing whether predation is incidental or selective.

We thank Hancock Biological Station of Murray State University for laboratory facilities and equipment.

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Rare and Extirpated Plants and Animals of Kentucky: 1997 Update.—The Kentucky State Nature Preserves Commission (KSNPC) published a list of rare and extirpated plants and animals of Kentucky in 1996 (1). The list, developed with assistance from many scientific authorities, was based on distributional and ecological data available as of 1 Mar 1995. KSNPC (1) committed to update the list annually

so that decision makers would have current information on rare species in Kentucky. Herein we update the 1996 list based on data available through 31 Dec 1996.

The methods and status categories used herein follow KSNPC (1), with the following exceptions. The United States Fish and Wildlife Service (USFWS) (2) discontinued use of status review Category 2 (C2) and Category 3 (3A) to reduce confusion and to clarify that these species were not candidates for listing as endangered or threatened. The USFWS (2) now maintains a list of only those taxa that are candidates for listing as endangered or threatened (C, which supersedes category C1), and taxa proposed for listing as endangered or threatened (PE and PT). Those species, including three taxa subsequently listed as endangered by the USFWS (3), are listed in Table 1 with other species whose conservation status is being changed. Changes in nomenclature and additions to the list are presented in Tables 2 and 3, respectively. Common names are used only when there is a nomenclature change from KSNPC (1), to identify undescribed species, and when a species is added to the list. Sources for plant names are Gleason and Cronquist (4), McAtee (5), and Wilbur (6). Sources consulted for animal names are as follows: gastropods—Hubricht (7) and Turgeon et al. (8); insects—Barr (9), Garrison (10), and The Dragonfly Society of the Americas (11); fishes—Robbins et al. (12) and Warren, Burr, and Grady (13); and birds—American Ornithologists' Union (14). We welcome any questions or comments about this update or KSNPC (1).

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Table 1. Conservation status changes for rare and extirpated Kentucky plants and animals.

	KSNPC[1]		US[2]	
	Old	New	Old	New
Plants				
Aureolaria patula	T	S	—	—
Berchemia scandens	E	T	—	—
Elodea nuttallii	H	T	—	—
Halesia tetraptera	E	T	—	—
Lonicera dioica var. *orientalis*	H	E	—	—
Trepocarpus aethusae	E	T	—	—
Triplasis purpurea	E	H	—	—
Viburnum lentago	H	delist	—	—
Snails				
Anguispira rugoderma	S	T	—	—
Mussels				
Alasmidonta atropurpurea	—	—	PE	E
Epioblasma brevidens	—	—	PE	E
Epioblasma capsaeformis	—	—	PE	E
Crustaceans				
Cambarus ornatus	S	delist	—	—
Orconectes inermis	T	S	—	—
Insects				
Pseudanophthalmus catoryctos	T	E	—	—
Pseudanophthalmus pholeter	T	E	—	—
Fishes				
Ammocrypta clara	X	E	—	—
Etheostoma cinereum	T	S	—	—
Etheostoma tippecanoe	S	delist	—	—
Macrhybopsis gelida	—	—	C1	C
Macrhybopsis meeki	—	—	C1	C
Percina evides	S	delist	—	—
Birds				
Aquila chrysaetos	X	delist	—	—

[1] S = Special Concern, T = Threatened, E = Endangered, H = Historic, X = Extirpated or Extinct.
[2] PE = Proposed Endangered, E = Endangered, C = Candidate for Listing, C1 = Candidate for Listing.

Table 2. Nomenclature changes for rare and extirpated Kentucky plants and animals.

Old name	New name
Plants	
Carex atlantica ssp. *capillacea* Howe sedge	Prickly bog sedge
Gentiana alba	*Gentiana flavida*
Halesia tetrapera var. *tetraptera*	*Halesia tetraptera*
Heracleum maximum	*Heracleum lanatum*
Insects	
Pseudanophthalmus abditus A cave beetle	Concealed cave beetle
Pseudanophthalmus hypolithos Stone-dwelling cave beetle	Ashcamp cave beetle
Pseudanophthalmus inexpectatus A cave beetle	Surprising cave beetle
Pseudanophthalmus scholasticus Schoolhouse cave beetle	Scholarly cave beetle
Fishes	
Etheostoma clarum	*Ammocrypta clara*
Etheostoma pellucidum	*Ammocrypta pellucida*
Etheostoma vivax	*Ammocrypta vivax*
Notropis sp. Palezone shiner	*Notropis albizonatus*
Birds	
Elanoides forficatus forficatus American swallow-tailed kite	Swallow-tailed kite
Casmerodius albus	*Ardea alba*

University of Louisville; D. Dourson and J.R. MacGregor, United States Forest Service; J.J. Lewis; L.E. Kornman, B.D. Laflin, and K.W. Prather, Kentucky Department of Fish and Wildlife Resources; T.L. Poulson, University of Illinois-Chicago; S.P. Rice, Kentucky Transportation Cabinet; J. Slapcinsky, Field Museum of Natural History; C.A. Taylor, Illinois Natural History Survey; and K.J. Tennessen.

Table 3. Additions to the list of rare and extirpated Kentucky plants and animals.

	KSNPC[1] status	US[2] status
Plants		
Viburnum molle Missouri arrow-wood	T	—
Snails		
Helicodiscus notius specus A snail	T	—
Helicodiscus punctatellus Punctate coil	S	—
Insects		
Celithemis verna Double-ringed pennant	S	—
Fishes		
Etheostoma percnurum Duskytail darter	E	E

[1] S = Special Concern, T = Threatened, E = Endangered.
[2] E = Endangered.

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***Epilobium brachycarpum* (Onagraceae) in Kentucky.**—While collecting plants in a railroad yard in northern Kentucky, we noted, among *Hypericum perforatum* and *Potentilla recta,* a number of non-flowering individuals of a plant we did not recognize. We eventually concluded that it was probably *Epilobium brachycarpum* C. Presl (*E. paniculatum* Nutt.) (Figure 1), a xerophytic annual new to the flora of Kentucky. Our conclusion was proved correct when, later, the plants came into flower. In the conterminous U.S., the plant was known east of the Mississippi only from Minnesota and Wisconsin. Further west, it occurs in the Dakotas and from Montana south to New Mexico and west to the Pacific; in Canada it is known from Quebec to British Columbia (International Vascular Plant Database, Biota of North America Program, North Carolina Botanical Garden, University of North Carolina, Chapel Hill, NC 27599).

The species is distinctive even in non-flowering condition, with (1) its elongate leaves that often have fascicles of smaller leaves in their axils and are folded up along the midrib (V-shaped), sharply acute, and entire to obscurely toothed, the teeth low, gland-tipped, and mostly 1 to 4 mm apart; and (2) its cracking and exfoliating epidermis at the base of the stem in older plants. Its flowers have pink petals about 6 mm long that are lobed to about the middle; the pollen is shed as monads, not as tetrads as in most species of *Epilobium* (1). Our plants are glabrous. Although infraspecific taxa have been described for this polymorphic species, none of these was recognized by Raven (1).

We cite here a voucher specimen for the presence of *E. brachycarpum* in Kentucky: KENTUCKY. Kenton Co., in gravelly soil along tracks in railroad yard along Licking River ca. ⅓ mile s of I-275 bridge, ca. 2 miles s of Latonia, 23 Jul 1997, *Thieret and Allen 60239* (KNK, MO).

Figure 1. *Epilobium brachycarpum.* Photocopy of pressed specimens, × ⅓.

LITERATURE CITED. (1) Raven, P.H. 1976. Generic and sectional delimitation in Onagraceae, tribe Epilobieae. Ann. Missouri Bot. Gard. 63:326–340.—**John T. Kartesz,** Biota of North America Program, North Carolina Botanical Garden, University of North Carolina, Chapel Hill, NC 27599; **Peter Allen** and **John W. Thieret,** Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Horned-Pondweed, *Zannichellia palustris* (Zannichelliaceae) from Northern and Central Kentucky.—*Zannichellia,* a genus of aquatic monocots of the family Zannichelliaceae, is represented in North America by a single species, *Z. palustris* L. (Figure 1). Beal and Thieret (1) located no Kentucky voucher specimens for the species but indicated that it is "possibly to be expected" in the state. They also noted that a population of this species formerly occurred just across the Ohio River from northern Kentucky in Hamilton County, Ohio, but was destroyed by expansion of the city of Cincinnati. In 1987 Patricia Haragan found the species in Fayette County but did not report the find. Two years later Webb and Chester (2) published Kentucky records of *Zannichellia* for Calloway, Lyon, Marshall, and Trigg counties (Kentucky Lake).

In May 1994, while looking for plants along a proposed

Figure 1. *Zannichellia palustris.* Plant (×¼); lower right, achenes (×6). Drawing by John Myers; used by permission of *Flora of North America.*

right-of-way for the reconstruction of KY 17, near Independence, Kenton County, Kentucky, we found horned-pondweed in a pond with *Eleocharis obtusa* and *Lemna minor*; in May 1995 we found *Zannichellia* at two sites in Bourbon County (in a roadside pool along US 68, ca. 0.5 miles northeast of Paris, and in a pond north of Millersburg); and in July 1996 we discovered a population in a pond off KY 356, ca. 2 miles east of Cynthiana, Harrison County. The documented Kentucky distribution of this

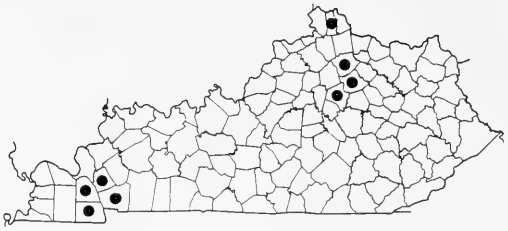

Figure 2. *Zanichellia palustris.* Documented county distribution in Kentucky.

species now includes the northern and central parts of the state (Figure 2). Additional field work will probably yield more Kentucky records.

Our voucher specimens are as follows: KENTUCKY. Bourbon Co., US 68, ca. 0.5 miles ne of Paris, roadside pool, 22 May 1995, *Meade 2169* (MOKY, Morehead State University Herbarium); Bourbon Co., n end of Millersburg, farm pond, 23 May 1995, *Meade 2229* (MOKY, Morehead State University Herbarium); Fayette Co., 8 miles e of Lexington, plants covering spring-fed farm pond, 11 Jun 1987, *Haragan 1528* (Univ. Ky. Coll. Agric. Herbarium; KNK); Harrison Co., off KY 356, ca. 2 miles e of Cynthiana, farm pond, 3 Jul 1996, *Meade 2284* (MOKY, Morehead State University Herbarium); Kenton Co., nr. Independence, farm pond, 23 May 1994, *Meade 1511* (MOKY, Morehead State University Herbarium).

We acknowledge aid from Kathy Stewart and Nancy Hopkins.

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