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Pistillate clump of eastern mistletoe (*Phoradendron leucarpum*) in a Bradford pear tree (*Pyrus calleryana*), a new host tree for the eastern United States, in Berea, Kentucky. Photograph by Ralph L. Thompson. See article on Page 3 of this issue.

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Eastern Mistletoe (*Phoradendron leucarpum*, Viscaceae) in the City of Berea, Kentucky: a High Incidence of Infestation and Eight New Host Species for Kentucky

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ABSTRACT

A survey of eastern mistletoe (*Phoradendron leucarpum*, Viscaceae) occurrence in infested trees and shrubs within the city limits of Berea, Kentucky, was conducted from February to April 2007. *Phoradendron leucarpum* was found in 2320 host plants among 21 trees species (two introduced exotics, one hybrid, 18 native) and one naturalized shrub. The predominate host tree observed in Berea was *Prunus serotina* with 920 trees followed by *Juglans nigra* with 534 trees, *Acer saccharinum* with 257 trees, and *Ulmus americana* with 187 trees. Eight new eastern mistletoe-infested hosts documented for Kentucky were *Betula populifolia*, *Crataegus* × *lavallei*, *Lonicera maackii*, *Prunus munsoniana*, *Pyrus calleryana*, *Quercus imbricaria*, *Q. palustris*, and *Ulmus pumila*. Five of these host plants, *Betula populifolia*, *Crataegus* × *lavallei*, *Lonicera maackii*, *Prunus munsoniana*, and *Pyrus calleryana*, were new host records within the continental United States. Berea had the highest incidence of eastern mistletoe and the greatest number of host species for any city of its size in Kentucky.

KEY WORDS: Berea, host plants, Kentucky, mistletoe infestation, *Phoradendron leucarpum*, Viscaceae

INTRODUCTION

A survey of eastern mistletoe (*Phoradendron leucarpum*, Viscaceae) occurrence in definitive host trees and shrubs within the city limits of Berea, Kentucky, was conducted

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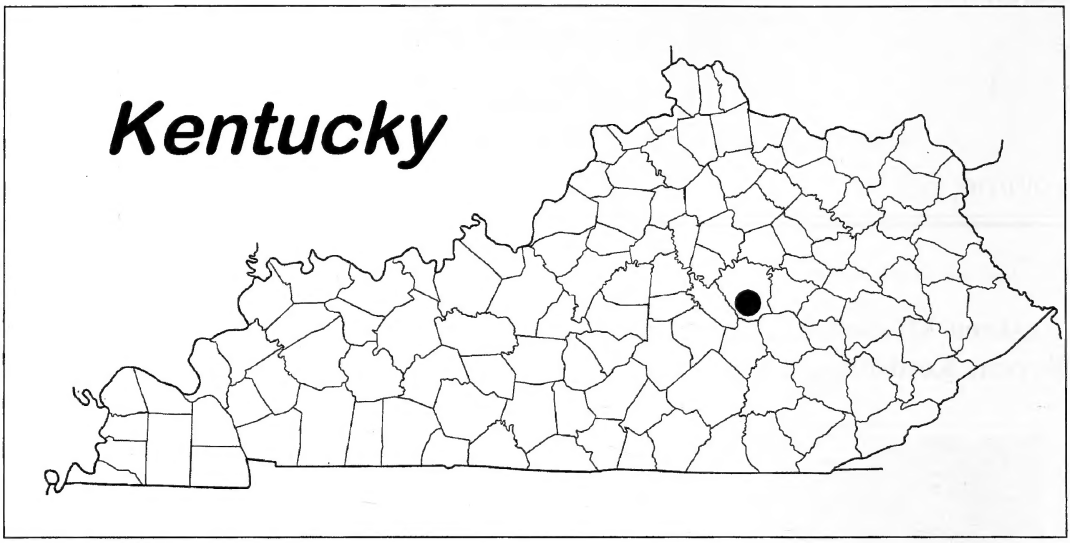

Figure 1. Map of Kentucky showing location of Berea in southern Madison County.

from February to April 2007. Our study in Berea completes the eastern mistletoe (a.k.a., American mistletoe or oak mistletoe) inventory for Madison County. In 1992, the Lexington-Blue Grass Army Depot in Madison County was surveyed (Thompson 1992). The city of Richmond and the remainder of the county were inventoried during the winters of 1988, 1990, 1995, and 1996. Previously published mistletoe county studies in Kentucky are Rockcastle County (Thompson and Noe 2003), Garrard County (Thompson and Poindexter 2005), and Robertson County (Thompson 2005).

Phoradendron leucarpum (Raf.) Reveal & M.C. Johnston is an epiphytic, evergreen hemiparasitic shrub of many deciduous tree species in the Eastern Deciduous Forest (sensu Braun 1950). Eastern mistletoe (hereafter mistletoe) is characterized by simple, decussate, coriaceous succulent leaves, inconspicuous spikes of small staminate and pistillate flowers on separate plants (dioecious), and viscid, pearl-white, globose berries each with a single seed (Kuijt 1982, 2003) (see overleaf Page 2 of this issue). Avians are the primarily agents of dispersal for mistletoe fruits.

Kuijt (2003) mapped the distributional range of eastern mistletoe from Oklahoma, Arkansas, and Tennessee to southern Missouri, Illinois, Indiana, and Ohio, throughout Kentucky and West Virginia to Maryland and New Jersey southward through all the Atlantic and Gulf Coastal States westward to eastern Texas. In his monograph of *Phoradendron*, Kuijt (2003) referred to eastern mistletoe within the eastern United States as *Phoradendron serotinum* (Raf.) M.C. Johnston ssp. *serotinum*. Nevertheless, we maintain *Phoradendron leucarpum* (Raf.) Reveal & M.C. Johnston for current nomenclature. The combination was approved by the Committee for Spermatophyta (Brummitt 1988) and subsequently was named by Reveal and Johnston (1989).

THE STUDY SITE

Berea is a small city measuring 4101.2 ha or 24.4 km^2, with a population of nearly 13,000 people (Paul S. Schrader, Berea Land Surveyor, pers. comm., 7 Aug 2007). Berea is situated in southern Madison County of east-central Kentucky within latitude 37°34′37″N and longitude 84°17′37″W (Figure 1). The major human population resides in older established neighborhoods, more recent suburbs, and other developed areas primarily in the south-central portion of Berea (Figure 2). The largest institution is Berea College, a liberal arts undergraduate work college of 1500 students that owns a substantial portion of the city land including 270 ha of Berea College agricultural farm land.

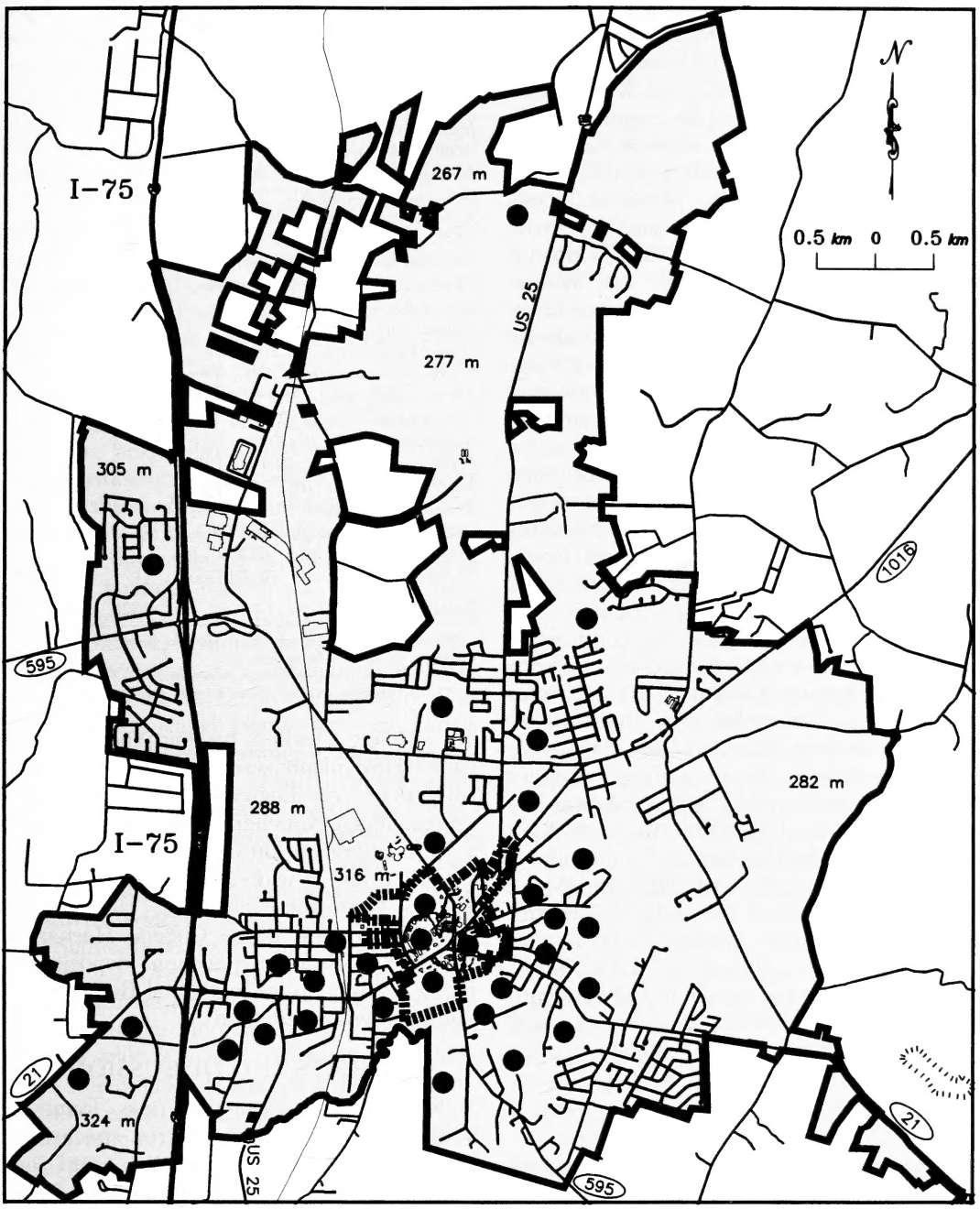

Figure 2. Berea, GIS-Survey Department, City of Berea, Kentucky, adapted from Schrader (2006). Berea city limits are delineated by a dark outline. Boundary of Berea College indicated by dashed line. Each solid black circle designates 70 individual eastern mistletoe host occurrences.

Elevations within the city of Berea range from 267.0 m at the Silver Creek floodplain in northern Berea to 324.0 m in the Dogwood Heights subdivision (Figure 2). Berea lies within two Kentucky ecoregions. The Knobs-Norman Upland occupies the major portion of the dome-shaped "knobs" or rounded mountains of south, east, west, and central Berea along the Berea Ridge. This narrow knob ridge possesses a significant amount of geo-

logical, topographical, and ecological diversity. The Outer Bluegrass at the northern portion of Berea is characterized by broad rolling hills and open flatlands (Woods et al. 2002).

The complex geology of Berea ranges from Ordovician limestone and shale in the Outer Bluegrass to Silurian mudstone and dolomite and Devonian shale within uplands of the city of Berea proper. There are also Quaternary alluvial and terrace deposits along floodplains of Brushy Fork, Silver Creek, and Walnut Meadow Branch (Weir 1967). Residual forest soils are comprised of two major soil associations in the area. The Colyer-Weikert-Captina Association is found around the populated Berea Ridge proper, and the Lawrence-Mercer-Robertson Association is found on the wide flats, rolling hills, and broad ridges to the north (Newton et al. 1973). Deciduous forest vegetation of southern Madison County consists of Western Mesophytic Forest (Braun 1950) and Oak-Hickory Forest (Küchler 1964; Woods et al. 2002).

The climate of Kentucky is continental, temperate, humid mesothermal usually with warm to hot summers and mild to cold winter temperatures (Trewartha and Horn 1980). Kentucky climatic data for 1971-2000 were derived from the Berea College weather station. Mean annual precipitation is 120.2 cm with the highest in May at 13.4 cm and the lowest in October at 7.8 cm, and a mean annual snowfall of 29.7 cm. Mean annual temperature of Berea is 13.6°C with January the coldest month at 1.5°C and August the warmest month at 24.3°C. Mean length of the growing season is 194 days with median first frost, October 22, and median last frost, April 10 (Kentucky Climate Center 2006).

MATERIALS AND METHODS

We surveyed all the paved and unpaved streets in populated Berea by foot and the residential subdivisions and roadsides in rural portions of the city by vehicle to observe *Phoradendron leucarpum*. Our map reference for all the streets and city limits was the GIS Survey Map of Berea, Kentucky by Schrader (2006). We used binoculars to spot and identify mistletoe from trees with any visible signs of infestation or malformation (i.e., branch and trunk clusters or clumps, cankerous swellings, leafless brooms, and limb dieback). Host plants were recorded by species with notes on degree of infestation, and representative vouchers were collected with a 12 m fiberglass linesman pole. A mistletoe specimen and a winter twig from each host species were then mounted, labeled, and deposited in the Berea College Herbarium (BEREA). Mistletoe specimens deposited in BEREA from earlier incidental Berea collections also were examined.

Table 1. Host specificity of *Phoradendron leucarpum* within city boundary of Berea, Kentucky.

Tree/shrub species	Total	Percentage
Prunus serotina [*RLT 07-24*]	920	39.66
Juglans nigra [*RLT 07-05*]	534	23.02
Acer saccharinum [*RLT 07-06*]	257	11.08
Ulmus americana [*RLT 07-23*]	187	8.06
Celtis occidentalis [*RLT 07-26*]	113	4.87
Robinia pseudoacacia [*RLT 07-10*]	85	3.66
Acer saccharum [*RLT 07-27*]	58	2.50
Fraxinus americana [*RLT 07-20*]	53	2.29
Gleditsia triacanthos [*RLT 07-09*]	32	1.38
Acer rubrum [*RLT 07-08*]	19	0.82
**Ulmus pumila* [*RLT 07-02*]	14	0.60
**Quercus palustris* [*RLT 07-19*]	13	0.56
***Lonicera maackii* [*RLT 07-30*]	11	0.47
***Pyrus calleryana* [*RLT 07-21*]	6	0.26
**Quercus imbricaria* [*RLT 07-14*]	4	0.17
Betula nigra [*RLT 07-40*]	4	0.17
***Betula populifolia* [*RLT 07-12*]	3	0.13
Maclura pomifera [*RLT 07-29*]	2	0.09
Diospyros virginiana [*RLT 07-01*]	2	0.09
Liquidambar styraciflua [*RLT 07-11*]	1	0.04
***Crataegus* × *lavallei* [*RLT 07-16*]	1	0.04
***Prunus munsoniana* [*RLT 07-33*]	1	0.04
Totals 22	2320	100.00

Representative voucher specimen numbers from the most recent collections are included in brackets.

* Kentucky new host trees for eastern mistletoe.

** United States record host species for eastern mistletoe.

RESULTS AND DISCUSSION

Phoradendron leucarpum was found in 2320 host trees among 21 tree species (18 native, two introduced exotics, and one hybrid) and one naturalized exotic shrub within the city limits of Berea (Table 1). The predominate mistletoe-infested tree species observed in Berea was wild black cherry (*Prunus serotina* Ehrh.) with 920 trees (39.66%). The next most important host trees were black walnut (*Juglans nigra* L.) with 534 (23.02%), silver maple (*Acer saccharinum* L.) with 257 (11.08%), and American elm (*Ulmus americana* L.) with 187 (8.06%). These four

host tree species accounted for 1898 of 2320 host trees (81.82%) among the 22 host species.

Studies in surrounding areas also document wild black cherry and black walnut as the two most numerous host trees in Garrard County (Thompson and Poindexter 2005), in Rockcastle County (Thompson and Noe 2003), and in the Lexington-Blue Grass Army Depot in Madison County (Thompson 1992). Eastern mistletoe is considerably more prevalent in the southern and central portions of Berea in older established neighborhoods and developed areas with a complex of woodlots and street patterns, than in recent subdivisions and more rural city limit environs (Figure 1). Native trees with mistletoe-infestation in Berea are most often found in upland open-canopy woodlots, forest edges, fields, fence rows, pastures, roadsides, street borders, and yards. Some of the trees that regularly occur outside of the actual city street and residential patterns include wild black cherry, black walnut, American elm, black locust (*Robinia pseudoacacia* L.), thorny honey locust (*Gleditsia triacanthos* L.), common hackberry (*Celtis occidentalis* L.), white ash (*Fraxinus americana* L.), sugar maple (*Acer saccharum* Marshall), and Osage orange (*Maclura pomifera* (Raf.) Schneid.). Planted trees along residential street margins, yards, and roadsides that frequently become mistletoe-infested include black walnut, silver maple, American elm, sugar maple, thornless honey locust (*Gleditisia triacanthos* L. var. *inermis* (L.) C.K. Schneid), white ash, and red maple (*Acer rubrum* L.). Mistletoe-infested hosts entirely restricted to residential Berea street margins and yards were Siberian elm (*Ulmus pumila* L.), pin oak (*Quercus palustris* Münchh.), Amur honeysuckle [*Lonicera maackii* (Rupr.) Maxim.], exotic Bradford pear (*Pyrus calleryana* Descne.), shingle oak (*Quercus imbricaria* Michx.), river birch (*Betula nigra* L.), gray birch (*B. populifolia* Marshall), common persimmon (*Diospyros virginiana* L.), sweetgum (*Liquidambar styraciflua* L.), Lavalle hawthorn (*Crataegus* × *lavallei* Herincq ex Lavallee), and wild goose plum (*Prunus munsoniana* W. Wight & Hedrick).

With regard to overall mistletoe infestation in host plants, visible signs ranged from one cluster to over 150 clusters. Among the 21 host species, the majority had light infestation (1–10 clusters) or moderate infestation (11–30 clusters). Host trees only lightly infested included black locust, red maple, Amur honeysuckle, shingle oak, river birch, gray birch, Osage orange, common persimmon, sweetgum, Lavelle hawthorn, and wild goose plum. The four shingle oaks had one cluster each. A single cluster was observed in sweetgum and wild goose plum and one Lavalle hawthorn had two clumps. Trees species with only moderate infestation included common hackberry, sugar maple, white ash, honey locust, and Bradford pear. Heavily infested trees (31–100 clusters) included certain black walnut, wild black cherry, American elm, Siberian elm, pin oak, and silver maple. A few mature black walnut and wild black cherry trees were extensively infested (100–150+ clusters). Although eastern mistletoe is an intriguing hemiparasitic plant, the outcome of heavy and extensive infestation can be very detrimental, and over several years, such trees will succumb to this infestation and eventually die. Actual tree mortality was observed in several older black walnut and wild black cherry trees with numerous large cankerous swellings, limb dieback, leafless brooms, and bare branches.

Prior to this survey, nine incidental mistletoe specimens with accompanying host twigs had been collected within Berea city limits: *Crataegus* × *lavallei* [*Poindexter 04-01*], 23 Mar 2004; *Gleditsia tricanthos* var. *inermis* [*RLT 88-3090*], 2 Dec 1988; *Lonicera maackii* [*Abbott 4604*], 26 Nov 1992; *Prunus serotina* [*RLT 88-24*], 16 Jan 1988; *Pyrus calleryana* [*RLT 03-1227*], 8 Dec 2003; *Quercus imbricaria* [*RLT 90-21*], 11 Jan 1990; *Quercus palustris* [*RLT 89-06*], 2 Jan 1989; *Robinia pseudoacacia* [*RLT 97-310*], 14 May 1997; and *Ulmus pumila* [*RLT 92-307*], 30 Jan 1992.

Seven new definitive host trees and one exotic host shrub were documented for Kentucky from this survey and previous collections. The state records were based on a thorough search of Kentucky herbaria, a literature review (Reed and Reed 1951; Thompson 1992, 2005; Kuijt 2003; Thompson and Noe 2003; Thompson and Poindexter 2005), and mistletoe surveys in all of the 120 Kentucky counties over the last twenty years by the first author. The seven new host tree records documented for Kentucky are gray

birch, Lavalle hawthorn, wild goose plum, exotic Bradford pear, shingle oak, pin oak, and exotic Siberian elm. The eastern Asian naturalized shrub, Amur honeysuckle, is an additional new host plant record (Table 1). Within the total distributional range of eastern mistletoe in the United States, Amur honeysuckle, gray birch, Lavalle hawthorn, wild goose plum, and Bradford pear, were not listed as host tree records for *Phoradendron leucarpum* based on the monograph of Kuijt (2003) [sensu *Phoradendron serotinum* subsp. *serotinum*], the Tennessee mistletoe host tree surveys by Hemmerly and his students (Ferguson and Hemmerly 1976; Rucker and Hemmerly 1976; McKinney and Hemmerly 1977; Brown and Hemmerly 1979; Hemmerly et al. 1979; Anderson and Mundy 1980; Henderson and Pekala 1980; Hemmerly 1981; Sadler and Hemmerly 1984; Hemmerly et al. 1987), nor any other mistletoe inventories (James 1958; Éleuterius 1976; Cole and Hemmerly 1981; Spooner 1983). Consequently, these five host species are treated as new distribution records for *Phoradendron leucarpum* in the United States.

Berea has the highest incidence of eastern mistletoe-infested trees and the highest number of host species for any urban area of its size in Kentucky. Indeed, based on extensive and intensive searches of the entire state of Kentucky, Berea has more mistletoe-infested trees (2320) and a higher number of host species (21 trees and one shrub) than most Kentucky counties or portions of several counties together, i.e., Garrard County with 1740 trees from 12 host tree species (Thompson and Poindexter 2005), Robertson County with only 45 trees of seven host tree species (Thompson 2005), and Land Between The Lakes National Recreation Area (parts of Lyon and Trigg counties, Kentucky, and Stewart County, Tennessee) with 358 trees from 12 host tree species (Thompson and McKinney 1990). Of published eastern mistletoe surveys in Kentucky, only Rockcastle County has a greater occurrence of mistletoe-infested trees (3502) but only from 15 host tree species (Thompson and Noe 2003). In comparison, Reed and Reed (1951) listed only 24 mistletoe-infested tree species in a host distribution survey of the entire state of Kentucky. Berea also has more mistletoe-infested trees than any of the Tennessee studies by Hemmerly and his students (cited above).

The massive eastern mistletoe infestation and host species diversity in Berea are a consequence of several ecological, geological, topographic, climatic, and genetic factors. Berea has great diversity in geological substrate and forest soils, which support a complex forest vegetation with accessible trees for potential infestation. A high density of susceptible host trees is available from native volunteering trees in various habitats as well as numerous yard-dwelling cultivated taxa. The most commonly infested host trees are also some of the most numerous canopy trees in Berea (e.g., wild black cherry, black walnut, silver maple, American elm, and common hackberry). Mistletoe-infested trees are usually in close proximity to each other, facilitating greater seed dispersal, which most likely contributes to an increased aggregated (clumped) mistletoe distribution within the city. In addition, an abundance of older and taller mature host trees are present that typically form open canopy crowns with better insulation for mistletoe success and provide greater infestation opportunities from avian vectors over time. A topographic feature of Berea is the presence of open upland terrain in the rolling hills, upper slopes, and ridges that frequently supports disturbed forested habitats. Open upland terrain has been found to be a significant parameter in other eastern mistletoe studies in Kentucky (Thompson 1992; Thompson and Noe 2003; Thompson 2005; Thompson and Poindexter 2005).

Certain avian vectors of eastern mistletoe are allured to trees in urban cities and towns (McKinney and Hemmerly 1977; Cole and Hemmerly 1981; Thompson and Noe 2003; Thompson and Poindexter 2005) for protective perches, roosts, and feeding purposes. Birds are the agents of dispersal for the initial incidence of mistletoe from ingesting the berries, wiping their bills, dislodging fruits, and defecating on branches. Birds, often in large flocks, are attracted to the large numbers of mature, older, and taller canopy trees on the higher upland elevations in southern Berea, which reinforces the aggregated mistletoe distribution pattern. The preeminent bird vector observed in mistle-

toe-infested trees in Berea is the gregarious European starling (*Sturnus vulgaris* L.). Twelve bird species readily associated with mistletoe were identified in contiguous Rockcastle County, Kentucky (Thompson and Noe 2003).

Eastern mistletoe has been copious during the rather mild winter temperatures in Berea for the last several years, and mild winter temperatures enable the evergreen mistletoe to survive and proliferate. Low winter temperatures have been proven to be one of the limiting factors for the distribution and success of eastern mistletoe in Berea and elsewhere. Based on personal observations of the first author, much of the eastern mistletoe was killed back to the endophytic haustorial root system in the host trees when cold harsh winters occurred in Berea during the middle 1980s and middle 1990s; but, mistletoe subsequently recovered and flourished during the warmer winters that follow. Spooner (1983) found the main reason for the limited northern extension of eastern mistletoe into southern Ohio was because of mild January temperatures. Deam (1924) and Garmen (1913) remarked on the mortality of eastern mistletoe due to low temperatures in Indiana and Kentucky, respectively. Thompson (2005) reported low temperatures as a limiting factor in the sparseness of eastern mistletoe in Robertson County, Kentucky.

The city of Berea was a sampling site for *Phoradendron leucarpum* in a physiological ecology and population genetics dissertation study by Panvini (1991). Leaf samples were taken from a population of 32 mistletoe-infested trees from nine host tree species. Other mistletoe populations for leaf sampling were from Alabama, Mississippi, North Carolina, and Tennessee. Panvini (1991) presented electrophoresis and statistical data that genetic variation (host races) exists among-and-within populations of eastern mistletoe. Her study supports the concept that mistletoe infestation is correlated with definitive host tree species in different geographical regions. This evidence may well be another important factor applicable to the high prevalence of eastern mistletoe in Berea. Further genetic studies of *Phoradendron leucarpum* are needed to better clarify relationships between host races, demography, incidence of infestation, and susceptibility to infestation in host species.

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Role of CTLA-4, IL-18 and IL-10 on the Induction of Low Dose Oral Tolerance

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ABSTRACT

Oral Tolerance is the temporary loss of systemic immunological responsiveness to a specific soluble antigen after ingestion of that antigen. Results from our lab and others indicated that CTLA-4 and lack of IL-12 played a role in the induction of low dose oral tolerance at the Th1 cell level. Previous literature suggested that IL-18 also played a role in preventing oral tolerance induction while the cytokine IL-10 had been shown to be a factor contributing to suppressed immune responses. To determine the role of CTLA-4 in conjunction with either IL-18 or IL-10 in low dose oral tolerance induction, anti-CTLA-4 mAb and either IL-18 or anti-IL-10 mAb were administered concurrently to mice fed either ovalbumin (OVA) or water. Results showed that the PLN cell proliferation of mice treated with anti-CTLA-4 mAb and IL-18 remained significantly suppressed compared with water-fed controls, while a partial abrogation of suppressed IL-4 and IFN-γ levels were observed. In contrast, mice treated with anti-CTLA-4 mAb and anti-IL-10 mAb exhibited a reversal of PLN cell proliferation and IL-4 suppression; however, IFN-γ levels remained suppressed. Results suggest that IL-10, IL-18 and CTLA-4 play roles in the induction of oral tolerance at the cell proliferation and cytokine level.

KEY WORDS: oral, tolerance, anti-CTLA-4, IL-10, IL-18

INTRODUCTION

Soluble antigen that is delivered orally often results in suppressed immune responses to subsequent immunogenic delivery of that same antigen. The development of this phenomenon, known as oral tolerance, may have the evolutionary advantage of preventing hypersensitivity reactions to ingested food proteins (Kagnoff 1987; Strobel and Mowat 1998). In addition, oral tolerance may aid in reducing the risk of inducing autoimmune reactions via cross-reactivity of ingested proteins with self-antigens (Kagnoff 1987). While oral tolerance has been observed and studied for years, the mechanism responsible for inducing this suppressed immune state remains to be fully elucidated.

Results from previous studies suggested that suppression of T cell responses might be due to an interaction of B7 on the antigen presenting cell (APC) with CTLA-4 on the T cell, rather than with the co-stimulatory molecule, CD28 (Perez et al. 1997). Studies had shown that tolerance induced by oral administration of antigen was at least partially prevented by *in vivo* administration of anti-CTLA-4 mAb, as indicated by decreased antibody secretion, lymphocyte proliferation, and cytokine secretion (Samoilova et al. 1998). Further studies by our laboratory and others had shown that suppression of IFN-γ levels and IgG2a in tolerized mice could be completely prevented if the pro-inflammatory cytokine IL-12 was administered concurrently with anti-CTLA-4 mAb at the time of oral tolerance induction (Van Parijs et al. 1997; Barone et al. 2002). These results were consistent with those of others suggesting that decreased levels of IL-12 must be present during oral tolerance induction to prevent the development of a Th1 cell response (Claessen et al. 1996; Marth et al. 1996; Karpus et al. 1998).

Recently it has been suggested that the cytokine IL-18, in combination with IL-12, may play a role in preventing the induction of oral tolerance at the Th1 cell level (Eaton et al. 2003). IL-18, produced by macrophage cells, keratinocytes, intestinal epithelial cells, adrenal cortex cells, and osteoblasts (Conti et al. 1997; Matsui et al. 1997; Stoll et al. 1997; Torigoe et al. 1997) is similar to IL-12 in that it primes the immune response and enhances IFN-γ production in various cells including natural killer cells and most T cells (Okamura

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et al. 1995; Ushio et al. 1996; Xu et al. 1998). In addition, IL-18 is believed to up-regulate IL-12R, induce transcription of IFN-γ and, in combination with IL-12, upregulate CD80 expression on dendritic cells (Walker et al. 1999; Chang et al. 2000; Eaton et al. 2003).

In contrast with IL-18, IL-10 is an anti-inflammatory cytokine produced by a number of cells, including T cells, macrophages and dendritic cells (Moore et al. 1993). It is thought to exert its effects by acting on the APC, primarily by down-regulating molecules that are involved in T cell co-stimulation, including CD80 expression and IL-12 production (Moore et al. 1993). Results from recent studies support the hypothesis that IL-10 plays a critical role in the induction of oral tolerance. Mice administered antigen orally exhibited increased levels of IL-10 compared with water-fed control mice (Chen et al. 1997; Gonnella et al. 1998; Marth et al. 2000), and administration of IL-10 at the time of feeding enhanced tolerance (Slavin et al. 2001). However, administration of anti-IL-10 mAb has been effective at inhibiting suppression in some but not other oral tolerance experimental models (Aroeira et al. 1995; Rizzo et al. 1999), and suppression in IL-$10^{-/-}$ mice has been variable in response to fed antigen (Aroeira et al. 1995). These results suggest that IL-10 alone is not responsible for all of the phenomena observed in oral tolerance induction.

Given our previous studies indicating that anti-CTLA-4 mAb in combination with IL-12 was capable of preventing suppression of Th1 cell responses, it was of interest to determine if either IL-18 or anti-IL-10 mAb, in combination with anti-CTLA-4 mAb, would also be able to prevent the induction of low dose oral tolerance. To assess this, mice were treated *in vivo* with anti-CLTA-4 mAb and either anti-IL-10 mAb or IL-18 at the time of oral administration of OVA; subsequently, mice were assessed for their ability to generate immune responses. Our results indicate that treatment with anti-CTLA-4 mAb and IL-18 is able to partially reverse tolerance with respect to suppression of both IL-4 and IFN-γ levels, while treatment with anti-CTLA-4 mAb and anti-IL-10 mAb is capable of preventing oral tolerance induction at the proliferative and IL-4 levels.

MATERIALS AND METHODS

Mice

Female 6-to 8-week old BALB/c mice were obtained from Harlan-Sprague Dawley (Indianapolis, IN) and were housed in the animal facility at Thomas More College in accordance with guidelines outlined by the American Association for Laboratory Animal Care.

Antigen, Antibodies and Cytokines

Chicken egg albumin (OVA), Grade V, was obtained from Sigma Chemical Co. (St. Louis, MO). Rat IgG was purchased from Sigma Chemical Co. Anti-CTLA-4 mAb was obtained from culture supernatants of hybridoma UC10-4F10-11 cells (ATCC, Rockville, MD). Hybridomas were cultured in 1% Nutridoma-SP serum-free medium (Boehringer Mannheim, Indianapolis, IN), and the secreted monoclonal antibodies were partially purified by ammonium sulfate precipitation. The antibody preparation was then dialyzed against PBS. Sample purity and antibody concentration were determined by SDS-PAGE analysis using rat IgG (Sigma Chemical Co.) as a standard. Murine rIL-18 was obtained from MBL International Corporation (Woburn, MA). Anti-IL-10 (JES2A5) was obtained from Pharmingen Division of BD Biosciences (San Diego, CA).

In Vivo Antibody and Cytokine Treatment of Mice

Mice were injected i.p. with 120 μg/mouse of anti-CTLA-4 mAb, 150 μg of rat IgG Ab, 0.5 μg of IL-18 and 40 μg of anti-IL-10 mAb. Mice were treated on days 0, +1, and +2.

Induction of Tolerance, Immunization, and Collection of Tissue Samples

Mice were orally tolerized by feeding 1 mg of OVA/mouse for 3 consecutive days; antigen was delivered in 0.5 ml of water by gastric intubation. Mice were fed on days 0, +1, and +2. Ten days after the last feeding (day +12), all mice were immunized in the foot pad and tail base with OVA (10 μg/mouse) emulsified in IFA. Eleven days after immunization, mice were sacrificed by cervical dislocation; popliteal lymph nodes (PLN) were then removed and single cell suspensions prepared.

Cells were cultured in supplemented RPMI 1640 (Gibco BRL) containing 5% heat inactivated FCS, 25 mM Hepes, 1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 5×10^{-5} M β2-mercaptoethanol, Pen/Step (100 units of penicillin, and 100 mg of streptomycin) and 5 mg/ml gentomycin (cRPMI). Cultures were maintained at 37°C in humidified 5% CO_2 atmosphere.

In Vitro Lymphocyte Proliferative Responses

Cells were aliquoted at 5×10^5 cells/well in 96-well flat-bottom plates along with OVA at final concentrations of 1000 μg/ml (Barone et al. 1998; Barone et al. 2002). All cultures were then incubated at 37°C in humidified 5% CO_2 for 48 h. Proliferative responses were assayed using the BrdU cell proliferation colorimetric kit (Boerhinger-Mannheim), per manufacturer's recommendations. Briefly, BrdU was added to cultures, and the cells were incubated for 8 h at 37°C; BrdU was then removed by centrifugation. After air-drying for 1 h, fixdent solution was added to each well and cells were incubated for an additional 30 min at room temperature (RT). Fixdent was removed and anti-BrdU mAb was added; cells were incubated for 1 h at RT. Plates were then washed three times and substrate (TMB) was added for an additional 30 min. Absorbance was measured at a wavelength of 450 ηm using an automated microplate reader (BioTek Instruments, Inc.). Addition of 100 μg/ml of an unrelated antigen, BSA, was added to control wells to assess non-specific proliferation.

Cytokine Analysis

Cells were aliquoted at 4×10^6 cells/well in 24-well flat-bottom plates along with either cRPMI alone or 1000 μg/ml OVA; total volume of 2 ml (Barone et al. 1998; Barone et al. 2002). Cells were then incubated at 37°C in humidified 5% CO_2 for 48 h. Supernatants were harvested and stored at −70°C until assayed. IL-4 and IFNγ production was quantified using murine IL-4 and IFNγ ELISA sets, respectively (BD Biosciences – Pharmingen, San Diego, CA). All ELISAs were carried out per the manufacturer's recommendation. Briefly, coating antibody was added to 96 well plates for 24 h at 4°C and then washed. Plates were blocked for 1 h at RT using BSA buffer. Next, standards and samples were added and allowed to incubate for 2 hr at RT. After washing, biotinylated detecting antibody plus horseradish peroxidase conjugate was added for 1 h at RT. Plates were again washed, and TMB substrate was added for 30 min; the reaction was terminated by the addition of 2N sulfuric acid solution. Absorbance was measured on an automated microplate reader set at 450 ηm. The following ranges of concentrations were used to generate standard curves: IL-4 (7.8 pg/ml to 500 pg/ml) and IFN-γ (31.3 pg/ml to 2000 pg/ml). The concentration of experimental samples was determined using the KC Junior computer software program (BioTek Instruments, Inc.).

Statistical Analysis

For proliferation and cytokine assays, pooled samples for each group were assayed in either quadruplicate (proliferation) or duplicate (cytokine) and expressed as OD or pg/ml ± standard deviation. Statistical significance of differences among experimental values was determined using Student's *t*-test for multiple comparisons. Values of $P \leq 0.05$ were considered significant. All experiments were repeated at least once.

RESULTS

Cell Proliferation Responses: Effect of Anti-CTLA-4 mAb, IL-18 and/or anti-IL-10 mAb Treatment

Initial experiments were performed to assess the role of CTLA-4, IL-18 and IL-10 in the suppressed cellular proliferation response of mice fed low doses of OVA. As expected, control (rat IgG-treated) mice fed low doses of OVA exhibited decreased levels of proliferation compared with water-fed mice. In agreement with earlier findings from our lab (Barone et al. 2002), treatment with anti-CTLA-4 mAb alone during feeding of antigen failed to prevent this suppression (Figure 1). The suppression index (SI) for PLN from rat-IgG-treated, OVA-fed mice was 0.75, while that of anti-CTLA-4 mAb–treated, OVA-fed mice was 0.66. Suppression indices were calculated by dividing values of OVA-fed mice by those of the corresponding water-fed mice.

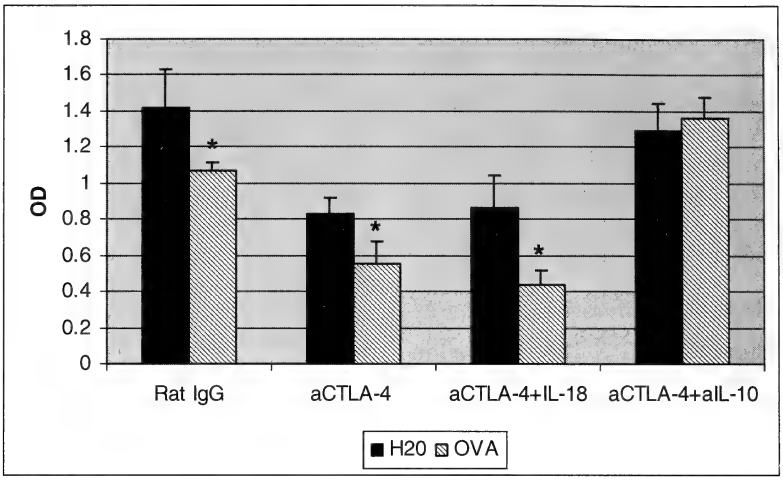

Figure 1. Effect of *in vivo* anti-CTLA-4 mAb, IL-18 and anti-IL-10 mAb administration on cell proliferation in orally tolerized mice. Mice were treated *in vivo* as indicated and fed either water (black bars) or OVA (grey bars). Ten days after feeding, mice were immunized with OVA in the foot pad and tail base and sacrificed 11 days later. PLN cells were removed and assessed for proliferation (see Material and Methods section). The error bars represent standard deviations calculated from quadruplicate samples. Asterisks indicate Student *t*-test values that significantly differ ($P \leq 0.05$) from water-fed controls.

As seen in Figure 1, the co-administration of IL-18 along with anti-CTLA-4 mAb failed to reverse suppression of cellular proliferation. However, suppression of proliferation was abrogated in PLN's from orally tolerized mice treated with both anti-CTLA-4 mAb and anti-IL-10 mAb. The SI for PLN from anti-CTLA-4 mAb and IL-18-treated, OVA-fed mice was 0.52, while that of anti-CTLA-4 mAb and anti-IL10 mAb –treated, OVA-fed mice was 1.05.

Cytokine Responses: Effect of anti-CTLA-4 mAb, IL-18 and/or anti-IL-10 mAb Treatment

The effect of co-administration of CTLA-4 mAb with either IL-18 or anti-IL-10 mAb on the cytokine response was assessed. Results showed that PLN from both rat IgG and anti-CTLA-4 mAb OVA-fed mice exhibited reduced IL-4 (Table 1) and IFN-γ (Table 2) levels compared with water-fed mice. Interestingly, mice treated with anti-CTLA-4 mAb and IL-18 exhibited a partial abrogation of tolerance with respect to both PLN IL-4 (Table 1) and IFN-γ (Table 2) levels, although both of these cytokines continued to remain significantly suppressed compared to water-fed control mice (SI of 0.86 and 0.72, respectively). Treatment with IL-18 alone did not result in increased IL-4 or IFN-γ levels (data not shown). In contrast, IL-4 levels in PLN from OVA-fed mice treated with anti-CTLA-4 mAb and anti-IL-10 mAb exhibited a complete abrogation of suppression and were no longer inhibited compared with water-fed control mice (Table 1; SI of 0.97). IFN-γ levels remained significantly suppressed in anti-CTLA-4 mAb and anti-IL-10 mAb-treated OVA-fed mice.

DISCUSSION

The mechanisms underlying the induction of low dose oral tolerance have been long studied and appear to be due to a multifaceted process. The precise procedure utilized can vary and may include deletion and anergy of antigen-specific CD4 cells as well as the production (or lack of production) of various cytokines. The growing consensus among scientists in this field is that low dose oral tolerance is most likely a combination of these various mechanisms (Garside and Mowat 1997; Barone et al. 1998).

Previous research on oral tolerance induction has implicated interaction of B7 on the APC with CTLA-4, a molecule that is expressed on the surface of T cells 24-72 hr post-activation (June et al. 1994) and binds to B7 with 20- to 50-fold higher affinity than does CD28 (June et al. 1990). Once bound,

Table 1. Effect of anti-CTLA-4 mAb, anti-IL-10 mAb and/or IL-18 on IL-4 secretion in PLN.

Treatment	Fed	In Vitro Stimulus	[IL-4] (pg/mL)	IL-4 SI[1]
Rat IgG	H_2O	1000 μg OVA	26.92 ± 0.77	
Rat IgG	OVA	1000 μg OVA	7.81 ± 1.34	0.29*
Rat IgG	H_2O	Medium	0.87 ± 1.50	
Rat IgG	OVA	Medium	0.0 ± 0.0	0
Anti-CTLA-4	H_2O	1000 μg OVA	120.30 ± 13.71	
Anti-CTLA-4	OVA	1000 μg OVA	34.19 ± 7.46	0.28*
Anti-CTLA-4	H_2O	Medium	21.07 ± 4.44	
Anti-CTLA-4	OVA	Medium	2.02 ± 1.07	0.10*
Anti-CTLA-4/anti-IL-10	H_2O	1000 μg OVA	57.25 ± 4.67	
Anti-CTLA-4/anti-IL-10	OVA	1000 μg OVA	55.44 ± 9.17	0.97
Anti-CTLA-4/anti-IL-10	H_2O	Medium	8.43 ± 3.44	
Anti-CTLA-4/anti-IL-10	OVA	Medium	5.43 ± 1.64	0.64*
Anti-CTLA-4/IL-18	H_2O	1000 μg OVA	51.57 ± 7.89	
Anti-CTLA-4/IL-18	OVA	1000 μg OVA	44.43 ± 10.26	0.86*
Anti-CTLA-4/IL-18	H_2O	Medium	18.11 ± 6.82	
Anti-CTLA-4/IL-18	OVA	Medium	3.50 ± 1.10	0.19*

[1] Suppression indices were calculated by dividing values of OVA-fed mice by those of the corresponding water-fed mice.
* Student *t*-test values differ significantly ($P \leq 0.05$) from water-fed controls

CTLA-4 is thought to deliver a negative or down-regulatory signal (Walunas et al. 1994, 1996; Krummel and Allison 1995) manifested by inhibited IL-2 receptor expression (Walunas 1996) and restriction of normal cell cycle progression (Krummel and Allison 1996). Various laboratories, including our own, have shown that blocking this molecule with anti-CTLA-4 mAb can play a role in preventing the induction of oral tolerance. However, for complete reversal of Th1 cell suppression, IL-12 needed to be administered concurrently with anti-CTLA-4 mAb at the time of feeding (Barone et al. 2002). It was proposed that restoration of this response was due to blocking of CTLA-4, thus allowing B7-CD28 interaction to occur. In addition, the presence of IL-12 may have increased IL-2R expression on T cells, thus promoting the proliferative response, as well as allowing for the differentiation of Th0 cells into IFN-γ producing Th1 cells.

Recently, studies have shown that suppression of IFN-γ and DTH responses could be prevented in orally tolerized animals that were treated with both IL-12 and IL-18 at the time

Table 2. Effect of anti-CTLA-4 mAb, anti-IL-10 mAb and/or IL-18 on IFN-γ secretion in PLN.

Treatment	Fed	In Vitro Stimulus	[IFNγ] (pg/mL)	IFNγ SI[1]
Rat IgG	H_2O	1000 μg OVA	4911.25 ± 42.77	
Rat IgG	OVA	1000 μg OVA	813.89 ± 66.19	0.17*
Rat IgG	H_2O	Medium	1243.62 ± 87.83	
Rat IgG	OVA	Medium	5.67 ± 9.82	0.00*
Anti-CTLA-4	H_2O	1000 μg OVA	4951.04 ± 47.11	
Anti-CTLA-4	OVA	1000 μg OVA	481.77 ± 50.53	0.10*
Anti-CTLA-4	H_2O	Medium	1280.76 ± 82.58	
Anti-CTLA-4	OVA	Medium	55.22 ± 31.35	0.04*
Anti-CTLA-4/anti-IL-10	H_2O	1000 μg OVA	9344.96 ± 62.20	
Anti-CTLA-4/anti-IL-10	OVA	1000 μg OVA	187.73 ± 62.20	0.02*
Anti-CTLA-4/anti-IL-10	H_2O	Medium	47.77 ± 28.64	
Anti-CTLA-4/anti-IL-10	OVA	Medium	0 ± 0	0.00*
Anti-CTLA-4/IL-18	H_2O	1000 μg OVA	4517.05 ± 230.04	
Anti-CTLA-4/IL-18	OVA	1000 μg OVA	3245.88 ± 502.65	0.72*
Anti-CTLA-4/IL-18	H_2O	Medium	924.24 ± 34.43	
Anti-CTLA-4/IL-18	OVA	Medium	514.66 ± 28.71	0.56*

[1] Suppression indices were calculated by dividing values of OVA fed mice by those of the corresponding water
* Student *t*-test values differ significantly ($P \leq 0.05$) from waterfed controls

of feeding (Eaton et al. 2003). Results from this study indicate that the presence of these two cytokines allowed for the production of Th0 cells and subsequent differentiation into Th1 cells, perhaps due to the ability of IL-18 in combination with IL-12 to up-regulate B7 (CD80) expression on APC and the ability of IL-12 to induce the differentiation of Th0 cells into Th1 cells. Experiments were thus performed in our laboratory to assess the ability of anti-CTLA-4 mAb and IL-18 to prevent the induction of Th1 oral tolerance. Our data indicated that this treatment did not reverse suppression of PLN proliferation in OVA-fed mice. This may have been due to the inability of IL-18 treatment alone to increase B7 expression (Eaton et al., 2003) and thus allow for optimal B7-CD28 interaction and full T cell activation to occur. However, this same treatment did result in a partial reversal of both Th1 (IFN-γ) and Th2 (IL-4) cytokine levels in orally tolerized mice.

It is possible that despite our inability to detect a restoration in the proliferative response of tolerized, anti-CTLA-4 mAb/IL-18 treated mice, levels of B7-CD28 interaction were sufficient to permit some, albeit minimal, Th0 cell development. That some activation may have occurred is supported by results from Samoilova et al. (1998) who showed that anti-CTLA-4 mAb treatment alone was sufficient to prevent suppression of the proliferative response in a high dose oral tolerance model. In our model, the presence of IL-18 in tolerized mice may have permitted the differentiation of Th0 cells into Th1 cells (and thus IFN-γ production) by increasing their sensitivity to endogenous IL-12 via up-regulation of IL-12 receptors. It should be noted that it is unlikely that the increase in IFN-γ detected was due to a direct stimulation by IL-18 of NK cells or other innate cells, because treatment with IL-18 alone resulted in continued suppression of IFN-γ levels in OVA-fed mice (SI of 0.1; data not shown). With respect to the partial reversal of suppressed IL-4 levels observed, recent studies indicate that IL-18 has the potential to induce Th2 cytokines in addition to its well documented ability to generate Th1 cytokines (Rodriguez-Galan et al., 2005). Alternatively, Th2 cell cytokines could have been produced if the putative default pathway (i.e., in the absence of IL-12) was utilized, with at least some Th0 cells differentiating into Th2 cells (Sadick et al. 1990; Patton et al. 2001).

Results from a number of studies support the hypothesis that IL-10 plays a critical role in the induction of oral tolerance (Chen et al. 1997; Gonnella et al. 1998; Marth et al. 2000), including the down-regulation of B7 expression on the surface of APC's (Moore et al. 1993). Lower levels of B7 on APC's would thus favor engagement with the high affinity CTLA-4 molecule on the T cell (resulting in suppression) rather than CD28 (resulting in activation). Therefore, it was of interest to see if low dose oral tolerance induction could be prevented if anti-CTLA-4mAb and anti-IL-10 mAb were co-administered to mice at the time of feeding. Our results indicated that mice treated in this manner no longer exhibited suppressed proliferative responses, perhaps due to the blocking of CTLA-4 along with the up-regulation of B7 on APC's; such results might allow for sufficient B7-CD28 binding to promote T cell activation. In addition, tolerized mice treated with both anti-CTLA-4 mAb and anti-IL-IL-10 mAb produced levels of IL-4 similar to that of non-tolerized mice, while IFN-γ levels remained suppressed. Feeding has been shown to result in lower IL-12 levels (Marth et al 1996; Karpus et al. 1998) and our treatment did not entail or allow for the production of IL-12, a necessary component for Th1 differentiation. In addition, unlike treatment with IL-18, anti-IL-10 mAb treatment has not been shown to increase IL-12R, thus negating the possibility that Th0 cells might be more sensitive to low levels of endogenous IL-12. However, the restoration of the IL-4 response in treated, tolerized mice might be explained if the activated, proliferating T cells differentiated primarily into Th2 cells. As mentioned previously, IL-18 has been shown to induce the differentiation of Th2 cells (Rodriguez-Galan et al., 2005) and a number of studies have shown that in the absence of IL-12, T cells will differentiate into Th2 cells via a "default pathway" (Sadick et al. 1990; Patton et al. 2001). Alternatively, requisite but minimal secondary signal interaction (i.e., B7-CD28) could result in a decreased state of dendritic cell activation, a situation that has been shown to favor Th2

over Th1 development (Chaussabel et al. 2003; Reis e Sousa, 2004). It also should be noted that the increase in IL-4 levels in tolerized, treated mice could be due to cytokine secretion by Th0 or other non-T cells and is an area of future investigation.

Interest in discovering the underlying mechanism of specific immune suppression has intensified recently due to the therapeutic potential it holds for the treatment of certain autoimmune diseases (Faria and Weiner 2006). Additional studies assessing CTLA-4, IL-12 and IL-10 utilization will help to further clarify the complicated process of oral tolerance induction.

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The Status of Science Safety in Kentucky Secondary Schools

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ABSTRACT

Our study sought to answer two questions: what is the status of safety in Kentucky school science programs, and can pragmatic tools be developed to address identified science safety needs? Between the spring of 2003 and the fall of 2004, state agency representatives worked with science safety researchers to assess the status of science safety in Kentucky schools and then create the *Total Science Safety System CD-Kentucky Edition* that contained all applicable federal and state laws, codes, and professional standards. In addition the CD included safety audits, chemical databases, safety videos, and strategic weblinks. This report concentrates on findings about science laboratory safety at the secondary level gathered from teacher surveys representing approximately 54 Kentucky school districts. Among the most significant findings was that teachers from the participating schools did not know or consistently observe many standard safety procedures. Furthermore, most participating science teachers generally were not aware of applicable science safety laws, codes, and standards.

KEY WORDS: Science safety, Kentucky, facilities, equipment, procedures

INTRODUCTION

In recent years there has been much inquiry surrounding safety conditions in science settings throughout the United States. Safety experts note that creating a safe science classroom need not be a daunting task if teachers have the right tools and knowledge (The Science Teacher 2002). Efforts are being undertaken in several states to ascertain the knowledge levels of teachers and provide them with educational safety solutions. Earlier studies (Gerlovich et al. 1997, 1998, 2001) indicated that few teachers were aware of their legal and professional responsibilities for safety. Additionally, a study in Iowa (Gerlovich et al. 1998) indicated that poor facilities and equipment combined with inadequate understanding of legal and professional obligations were associated with increased numbers of accidents and lawsuits. A 2002 national study, conducted electronically through the National Science Teachers Association, corroborated these results (Science Teacher 2003).

On the basis of these data, Karen Kidwell, State Science Consultant for the Kentucky Department of Education, contacted Dr. Jack A. Gerlovich, Professor of Science Education/Safety at Drake University, with the objectives beginning to examine the status of science safety in Kentucky schools and then to providing tools to address the safety needs. This report presents a summary of the project, including status of facilities and equipment and understanding of applicable laws, codes, and professional standards as they relate to safety.

METHODS AND MATERIALS

In spring 2003, a statewide science safety advisory committee was created, including

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people from the Kentucky Department of Natural Resources, Kentucky Department of Education (Facilities Consultant, Science Consultant), Kentucky State Fire Marshal's Office, Kentucky Occupational Safety and Health Administration (OSHA), and the Kentucky Department of Health. National expert on science safety Dr. Jack Gerlovich and technical advisor Dennis McElroy met with this advisory group to introduce questionnaire/survey tools, workshop agenda models, and science safety CD-ROM models from other states that might form the basis for a Kentucky model.

Each committee member researched safety measures or guidelines required by their respective state agency and shared the information with the committee. The committee prioritized the safety issues, and survey questionnaires were developed reflecting the information. The ultimate purpose of the questionnaire was to assess the safety status of Kentucky elementary and secondary school science facilities, equipment, procedures, and teacher understanding of legal and ethical obligations.

The committee determined that a combination of professional development sessions and a CD-ROM would be the best approach to take in efforts to inform and improve school science safety issues. Content for the professional development/CD was confirmed with the safety advisory committee, and a final version of *Total Science Safety System-The Kentucky Edition* CD (2003) was created. The CD was to serve as a comprehensive resource for professional development and for safety audit purposes in Kentucky school science programs. From November 2003 through November 2004, Gerlovich and McElroy conducted several full day workshops for teachers from across the Commonwealth. These professionals represented several geographic regions of the state. Participating school and district personnel were required to complete the survey questionnaires prior to attending training sessions on science safety. The workshops were designed to enable the attending science educators to effectively use The Total Science Safety System-Kentucky Edition CD to work towards the evolution and maintenance of a safe teaching/learning environment by

- establishing district science safety policies,
- performing science safety audits that enable the identification and correction of safety hazards,
- complying with Kentucky laws, and codes and professional standards,
- making peers, administrators, and students aware of science safety issues, and
- managing chemicals, cradle to grave (purchase to disposal), via accepted safety procedures.

RESULTS AND DISCUSSION

One important part of the project was the use of the surveys to establish baseline information about secondary level science facilities, equipment, and the understanding of laws, codes, and standards. The information collected represented a vital step in moving Kentucky science educators forward by focusing on their own facilities, equipment, and understanding of safety responsibilities.

Approximately 120 secondary teachers participated in the training sessions (Table 1A). Due to administrative problems (surveys not passed to teachers by administrators, surveys not returned), some participants did not return their response forms. The number of correctly completed and useable surveys ranged from 97–102. The returned surveys provided a valuable first step towards understanding the status of science safety in Kentucky secondary schools. It enabled participants to target needed changes in improving their own facilities, equipment, and understanding their safety responsibilities, while providing baseline data for the state project. It is anticipated that additional information will be secured through on-going surveys and workshops, as well as follow up surveys with baseline participants to assess actual use of the CD tool to ensure school/district safety.

It should be noted that 94% of the schools represented in this study were public institutions. This might be an area that should be probed further for unique concerns of private schools; however, it was beyond the scope of this study.

Facilities (Table 1A)

Responses to the "type of building" question indicated that slightly more than 71% of

the respondents represented high schools, with 29% being from middle schools or middle/high school combinations.

Lab age can pose serious challenges to safety. Older lab facilities can impose limitations to newly mandated federal and state codes (Gelrovich and Parsa 2002). Just over 36% of the labs of the responding teachers were less than ten years of age, while 62% were constructed more than 11 years ago. The 33% that were older than 20 years might raise concerns relative to their ability to meet some of the more contemporary safety laws, codes, and professional standards. It also appeared that the majority of labs (68%) and lab/classroom combination facilities (65%) were significantly below the size needed to accommodate students. National recommendations suggest between 45 and 60 ft^2 per student depending on the design. The Kentucky Plan Review Checklist for School Building Projects (2004) document states that each science classroom/lab shall have 1625 ft^2 and stand-up workstations for 28 students. In order to satisfy these criteria, either enrollments must be reduced or the curriculum must be limited to activities that have few potential hazards. In addition, and perhaps more importantly, teachers were unaware of essential information relative to facilities, making it difficult, if not impossible, to identify and initiate safety essentials for themselves and their students.

Brennan (1970) found that class enrollment and laboratory space had a significant relationship to laboratory accidents. The larger the class enrollment and the smaller the laboratory space, the greater the frequency of accidents. Eliminating overcrowding is the one change that will most quickly and strategically effect safety in science laboratories. Overcrowding in laboratories and combination laboratory/classrooms is a serious problem in many Kentucky schools represented in this project. Teachers must ensure that administrators are aware of these issues and that all attempts have been made to protect themselves and students in the substandard settings.

Lab total square footage can be critical to the safety of teachers and students (Gerlovich et al. 1998, 2003). It is generally recommended by the science teaching profession that class enrollment should be limited to 24 students (Beihl et al. 1999). In addition, separate labs should have a minimum of 45 ft^2 per student of open floor space in science labs (1080 ft^2 and a class enrollment not to exceed 24 students) (Biehl et al. 1999). The survey indicated that nearly 72% of the labs represented had less than 1000 ft^2, with approximately 46% having less than 750 ft^2.

Combination lab/classroom settings present special safety concerns to teachers and students. Approximately 67% of the responding teachers indicated that in lab/classroom settings, the total square footage was less than 1000 ft^2. The NSTA recommends 60 ft^2 per student (1440 ft^2 total for 24 students) in such settings (Biehl et al. 1999).

Chemical fume hoods are essential pieces of safety equipment for many laboratory investigations; however, it is critical that they be tested regularly. The Occupational Safety and Health Administration (OSHA) recommends fume hood testing every 90 days (OSHA 1990). The Kentucky survey revealed that nearly 63% of respondents had either never tested their hood or had not done so in more than two years. By contrast, only 14% of the respondents had tested this equipment within the last year. This is especially dangerous because of the faith teachers put in such equipment for protecting themselves and their students from such a wide variety of chemicals. With regard to fume hood testing, the American Conference of Governmental Industrial Hygienists (ACGIH) stated in 1982 that the optimum face velocity for general safe hood operation was 60–100 fpm, with 60 fpm as a working minimum. For most operations, the 60 fpm speed is sufficient for secondary school science labs.

When the participants were queried about the type of hood they had, the responses varied considerably. The most common type was an exhaust hood. Thirty-two percent responded that they had no hoods; however, they really did need one to support their curriculum. These teachers may wish to investigate the incorporation of the new ductless fume hoods that are self-monitoring, self-contained, and, in many cases better options for school labs. In addition, the ductless hoods are portable and can be rolled wherever best used. Ductless fume hoods generally meet all applicable hood standards

Table 1. Kentucky Science Safety Pre-Training Survey: the following are responses to the cumulative totals (Nov. 2003, May 2004, and Nov. 2004) from responses to the survey sent to all invitees in preparation for the Kentucky custom statewide science safety-training programs.

Item	Response	Total	N
A. Facilities			
1. What is your type of school?	A) Public	94	100
	B) Private	6	
2. What type of building characterizes your school?	A) Middle School	17	101
	B) High School	71	
	C) Combination	12	
	D) Other	1 = university	
3. How old is your lab facility?	A) 10 yrs.	36	102
	B) 11–20 yrs.	29	
	C) 21–30 yrs.	13	
	D) 30+ yrs	20	
	Blank	4	
4. How many square feet of floor space do you have in your lab?	A) 00–749	42	97
	B) 750–999	24	
	C) 1000–1499	19	
	D) More than 1450	7	
	Blank	5	
5. If you have a lab/classroom-combined area, How many square feet of floor space does it provide?	A) 00–749	25	79
	B) 50–999	26	
	C) 100–1450	20	
	D) More than 1450	5	
	N/A	3	
6. When was the last time you tested your fume hood with proper instrumentation?	A) Never	38	102
	B) Within the past 90 days	14	
	C) More than 2 years ago	24	
	D) No hood	23	
	Other	3	
6. How many turnovers of air per hour can your lab ventilation system provide?	A) 3	4	100
	B) 6	1	
	C) More than 10	1	
	D) I Don't know	88	
	No Vent/Blank	6	
7. IF your chemistry lab is greater than 500 ft^2, how many exits does it have with outward opening doors?	A) 1	30	101
	B) 2	47	
	C) More than 2	14	
	D) Not Applicable	7	
	E) Other	3	
B. Equipment			
1. Are all lab electrical outlets near water faucets GFI/GFCI protected?	A) Yes	52	100
	B) No	30	
	C) I Don't know	18	
2. How many ABC Tri-class fire extinguishers do you have in your lab?	A) 0	6	100
	B) 1	60	
	C) 2 or more	31	
	D) I don't know	3	
3. How many functioning eyewash stations do you have in your lab capable of delivering aerated, running water for 15 minutes?	A) 0	15	96
	B) 1	68	
	C) 2 or more	13	
4. Do you have OSHA "approved" safety goggles for use in your labs?	A) Yes	83	96
	B) No	2	
	C) I don't know	11	
5. What type of hood do you have in your science lab?	A) None- not needed	18	100
	B) Exhaust type	63	
	C) Ductless type	0	
	D) None, however, I do need one	14	
		5 = other	

Table 1. Continued.

Item	Response	Total	N
C. Procedures			
1. How recently have you received science safety training?	A) Within 2 years	19	93
	B) Within 5 years	23	
	C) Longer than 5 years	51	
2. Under what conditions do you allow students to wear contact lenses when working with caustic chemicals?	A) Never	33	92
	B) With safety glasses	35	
	C With non-vented goggle	23	
	D) With a face shield	1	
3. Do you require student safety contracts of all science lab students?	A) Yes	73	95
	B) No	22	
4. How do you store chemicals?	A) Alphabetical Order	0	97
	B) Compatible families	88	
	C) Other	3	
	D) I don't know	6	
5. Do you administer student safety tests to your students each semester?	A) Yes	56	95
	B) No	39	
D. Understanding Laws, Codes, Standards			
1. What organization recommends minimal floor space/student in science labs?	A) NSTA	11	98
	B) OSHA	35	
	C) KY Education Code	7	
	D) I don't know	45	
2. What organization(s) recommend(s) lowering of science enrollments in science labs for special needs students?	A) EPA	0	99
	B) ANSI	2	
	C) NSELA	11	
	D) I don't know	86	
3. What organization(s) recommends teacher: student ratio for field trips?	A) NSTA	13	95
	B) OSHA	4	
	C) NSELA	5	
	D) I don't know	73	
4. In Kentucky, specific Eye Protective Equipment legislation for science students appears where?	A) KY OSHA	35	98
	B) KY Education Code	6	
	C) KY Fire Codes	1	
	D) I don't know	56	
5. In Kentucky, the use of "sharps" is addressed by what organization?	A) KY OSHA	27	98
	B) KY Education Code	1	
	C) KY Fire Code	0	
	D) I don't know	70	
6. In deciding teacher negligence in student injury cases it is important that teachers can show that they performed these three duties:	A) Instruct, Supervise, Maintain	48	96
	B) Practice, Maintain, Report	14	
	C) Teach, Test, Verify	7	
	D) I don't know	27	

and typically monitor themselves for performance.

Room ventilation can be viewed as an extension of the fume hood component and also as a lead in to the lab exits component (Biehl et al. 1999). It is recommended by the National Fire Protection Association (NFPA 1991) that room air in science labs be turned over 4–12 times per hour depending on the types of activities being performed. It was noted that 88% of the Kentucky respondents were completely unaware of the air turnover rates in their room.

The National Fire Protection Association (NFPA 1991) codes 45 and 10 adopted in many communities spell out the lab exit requirements for lab facilities. NFPA 45 requires that labs have two exits not greater than 50 ft. distance from any point in the lab if they 1) contain explosion hazards that could block them, 2) are Class A labs (hazardous materials that present significant fire hazards), 3) are larger than 500 ft^2, 4) are Class B labs (moderate fire hazard), 5) are Class C labs (low fire hazard) and exceed 1000 ft^2 in work area, 6) have a lab fume hood located near a primary lab exit, or 7) contain a compressed gas cylinder larger than lecture bottles containing a flammable or cryogenic gas with a NFPA Health Rating of 3 or 4.

Approximately 67% of responding teacher labs had two or more lab exits with outward opening doors. Most applicable federal and state agencies, as well as professional science/education organizations (Biehl et al. 1999; Kentucky Dept. of Education 2004) require or strongly suggest two exits, with outward opening doors for science labs. Thus, the 30% of the reporting labs that do not have exits with outward opening doors create a serious problem for the expeditious exiting of labs during emergencies.

In Bush v. Oscoda Area Schools (1981), a teacher and principal were held to have potential liability even though the school district was protected by the doctrine of sovereign immunity. In this case, a student was burned when methanol caught fire during a science experiment that was being conducted in a mathematics classroom. The classroom was being used due to a shortage of space in the science labs. The room did not have adequate storage for essential equipment nor was it properly ventilated, equipped with fire extinguisher(s), or fire blankets. The student was returning her extinguished alcohol burner to the counter in the back of the room when she noticed a burner that was still lit. When she attempted to extinguish it, the burner exploded, causing serious burns. The fire eventually was extinguished using the fire extinguisher located at the opposite end of the room. Negligence was cited for the failure to supervise, failure to instruct as to dangers, and failure to provide safety equipment and space. Although the school district was immune from suit, the principal and teacher were found negligent for allowing a science activity to occur in a room not designed for that purpose and for lacking of essential safety equipment. In Science Classroom Safety and the Law, Ryan (2001) reviewed the implications of this case for principals and teachers: "School administrators take note of this case because it has important implications in overcrowded facilities. This case stands for the proposition that principals can be held liable for injury if they schedule classes improperly or hold them in inappropriate facilities. Thus, for teachers working in poor facilities and overcrowded conditions, it is imperative that the school district be placed on 'actual notice' of the existence of conditions constituting a threat to the safety of students and teachers."

Equipment (Table 1B)

One of the most significant safety shortcomings relative to equipment (Table 1B), is that so many (48%) of the teachers responding had no Ground Fault Interrupter (GFI)/Ground Fault Circuit Interrupter (FCI) protected electrical outlets or did not know what they were. This was especially discouraging because of the danger involved and the ease of correcting the problem. Ground fault interrupters (GFI) or ground fault circuit interrupters (GFCI) are examples of simple, yet very strategic items (Kaufman 1995). While nearly 52% of the responding teachers' labs had GFI/GFCI's, approximately 48% either lacked this equipment or the teachers do not know what they are. The lack of FCI/GFI may have been due in part to the age of the buildings.

Fire extinguishers are essential equipment items for science lab settings. Slightly more than 91% of the responding teacher's labs had at least one appropriate fire extinguisher. Only 9% of the labs either lacked a fire extinguisher, or the teacher did not know if they had the appropriate type. All science laboratories need at least one ABC tri-class fire extinguisher.

Eyewash stations are required safety equipment for science labs. According to OSHA these are vital to safety (OSHA 1990). Teachers must assure that these are readily available whenever working with chemicals or materials that could damage eyes. Eighty-four percent of responding teachers indicated that they had at least one piece of this equipment; however, 16% of the responding teachers had no such essential safety equipment.

The NSTA Guide to Facilities (Biehl et al. 1999) provides extensive information concerning essential equipment for general students and for meeting Americans with Disabilities Act (ADA) requirements. The American National Standards Institute (ANSI 1993) Z358.1 code describes the essential safety role of this equipment. The OSHA (1990) standard, 29 CFR 1910.151(c), parallels the requirements of ANSI Z358, requiring emergency eye/face wash stations and drench showers. In addition, The Kentucky Building Plan Review Guide provides information

concerning facilities and some of the essential equipment.

When asked if they had OSHA Code approved goggles 86% of the responding science teachers reported that they had the essential equipment. The fact that 14% either did not have the equipment or were unaware if they did is disconcerting considering the widespread need for protection for almost all science activities. The equipment is simple to implement and maintain with students. So much unnecessary damage can be done to students' eyes in a short time without the proper, approved eye protective equipment being worn by students and modeled by teachers.

Safety Procedures (Table 1C)

It often is assumed that teachers have received safety training for all of the essential duties that they are asked to perform. The data suggest that the assumption is incorrect. Approximately 55% of the responding teachers had not received any type of science safety training in the past five years. The statistic is of concern given the recent proliferation of codes and standards and for society's propensity to sue for personal injuries to themselves or their children. Teachers must stay current in their field with regards to safety for themselves and their students.

Wearing contact lenses is increasingly common among teachers and adolescents. When contact lenses are worn in science labs, the "potential" for injury can increase (Gerlovich et al. 2002, 2003). Teachers should know which students are wearing contacts and be prepared to address their emergency medical needs relative to the science activities being performed. When asked "under what conditions do you allow students in your science labs to wear contact lenses?" only 25% responded that they allow the wearing of contact lenses with non-vented cover goggles. By contrast, 75% percent of the responding teachers indicated that either they never allow contact lenses to be worn in labs or if so only with safety glasses or a face-shield.

The most succinct position on this issue was rendered in 1994 when the OSHA published its Personal Protective Equipment (PPE) for General Industry Standard in 29 CFR 1910; Final Rule (Federal Register 1990). Part of the preamble stated

> "OSHA believes that contact lenses do not pose additional hazards to the wearer and has determined that additional regulation addressing the use of contact lenses is unnecessary. The Agency wants to make it clear, however, that contact lenses are not eye protective devices. If eye hazards are present, appropriate eye-protection must be worn instead of, or in conjunction with, contact lenses."

At the 2000 American Chemical Society (ACS) national meeting in Dallas, Texas, the Committee on Chemical Health and Safety reinforced this position when it issued the following statement (Segal 2002):

> "In many workplaces where hazardous chemicals are used or handled, the wearing of contact lenses is prohibited or discouraged. A good number of these prohibitions are traceable to earlier statements in this book (Safety in Academic Laboratories, 6th Ed., ACS Committee on Chemical Safety: Washington, DC, 1995) that were based on rumors and perceived risks. A careful study of the literature by knowledgeable consultants has refuted these risks. Recent studies and experience have suggested that, in fact, contact lenses do not increase the risks but can actually minimize injury in many situations. Because of the ever-increasing use of contact lenses and the benefits they provide, the American Chemical Society Committee on Chemical Safety, having studied and reviewed the issue, is of the consensus that contact lenses can be worn in most work environments provided the same approved eye protection is worn as required of other workers in the area. Clearly, the type of eye protection needed depends upon the circumstances. It should be stressed that contact lenses, by themselves, do not provide adequate protection in any environment in which the chance of an accidental splash with the Personal Protective Equipment exists (29 CFR 1910.132 and 133), and ANSI Z87.1a-1991 should always be worn in such situations."

From this legislation (Federal Register 1990) and professional standards (Biehl et al. 1999) it does appear that teachers and students may safely wear contact lenses in science labs as long as they wear the proper ANSI approved safety equipment over them.

One of the simplest and most effective means for a science teacher to communicate essential safety issues to students is through the utilization of Student Safety Contracts. Nearly 77% of responding teachers indicated

that they require contracts from their students; however, 23% of responding teachers did not require this simple tool. It is very cheap insurance because the guidelines are spelled out, and students sign the document. In many states students between 7 and 14 years of age can be held accountable for their actions. However, there is often a 'refutable presumption' that they are incapable of contributory negligence. With sufficient evidence concerning their intelligence, maturity, and the circumstances of the incident, this presumption can be rebutted and the court may find them negligent (Ryan 2001).

Student safety 'exams' can be powerful tools for assessing student perceptions of their safety understanding (Gerlovich et al. 2001, 2002). Fifty-nine percent of the responding teachers indicated that they incorporated safety assessments; however, 41% did not. These can be powerful resources when the teacher attempts to verify the importance of safety for their students and can serve as evidence that the students were properly instructed and informed of safety procedures.

The nature of the chemicals incorporated into science lessons is another significant issue for teachers, administrators, and insurance companies (Gerlovich et al. 2003). The Chemical management/storage system also raises numerous safety questions. When asked about their preferred Chemical Storage System, teacher responses were clearly segregated with nearly 91% preferring the chemical families system, while 9% of the teachers were unsure of what system was used in their own storage areas, or they used something entirely different.

Teacher Understanding Of Laws, Codes and Professional Standards (Table 1D)

It was quite disturbing to note that 46% of respondents indicated that they did not know the minimum floor space standards/requirements per student in science labs (Gerlovich et al 1998), yet they were living within these parameters with students on a daily basis. Science settings are innately hazardous, given the nature of the materials utilized to probe into the phenomena of our natural world. This situation can be even more complicated when student-centered investigations are combined with a wide range of student abilities and dispositions towards rules designed to protect themselves and their peers. Limiting class size to guidelines of the profession and following fire and building codes can help assure a safe positive learning experience for everyone.

With respect to minimal enrollment requirements for special needs students in science labs, an overwhelming 89% of the responding teachers were unaware of laws, codes or standards developed to protect these students in science settings. This is a critical standard for all science teachers to meet because many special needs students will be unaware of the actual dangers they may be facing during the science investigations, or they may be unable to avoid them due to their special needs (Gerlovich et al. 1997, 1998, 2001, 2002, 2003).

Equally disconcerting was that 77% of the responding secondary level teachers were unaware of guidelines implemented to establish teacher:student ratios while on field trips. Given the frequency with which field trips occur at this level, a low percentage is of correct teacher responses is a serious concern. Controlling and protecting students in stimulating environments away from the familiarity of the school setting can be a challenge if strict rules are not enforced. One teacher to 10 students has been proven to enhance a teachers' ability to keep students safe on field trips off school grounds (Biehl et al. 1997).

When science teachers were asked where one might find specific Eye Protection equipment legislation applicable to schools and students in their state, over 94% could not identify the source of this requirement. The finding brings into question whether state legislation is being enforced in its general intent to best protect all science teachers (and likewise, their students). Basic requirements state that appropriate eye protective equipment (meeting ANSI Z87 standard for burn and breakthrough) should be worn whenever the potential exists for an eye injury (Gerlovich et al. 2002, 2003, Biehl et al. 1999). Such equipment is generally identified by a Z87 symbol on the faceplate and/or the molding.

The next question asked what organization addressed the use of "sharps" in Kentucky schools. Sharps are instruments used by medical personnel and science teachers to assist in puncturing the skin and a blood vessel to draw

blood. They might also include scalpels and pins used in dissections and even such sharp objects as glass slides, etc. Again only 29% of the responding teachers knew of the existence of any applicable Kentucky legislation.

The last question addressed the issue of Teacher Negligence in student injury cases. Fifty percent of the respondents were unaware of the three duties that they must satisfy to avoid negligence suits and to assure reasonable and prudent protection for their students. Basically, teachers must provide students with instruction appropriate for their mental abilities for the lesson being undertaken, adequate supervision (teacher:student ratio of 1:24 in science settings and 1:10 on field trips, with adjustments for students requiring special attention), and proper maintenance of the setting (proper safety equipment, procedures, etc.).

The most significant message gleaned from this section is the importance of practicing the three teacher duties for avoiding accidents and negligence allegations. Most responding teachers were aware that they must instruct properly, supervise adequately, and maintain the teaching/learning environment in order to protect themselves and their students from "foreseeable" injuries.

Teachers are not expected to be superhuman in anticipating hazards. Rather, they simply need to be "reasonable and prudent" in their judgment. They need only have the foresight of a person with their level of training, years of experience and professionalism. They also must keep themselves informed of the expected standards for safety within the science teaching profession (Biehl et al. 1999). For example, science teachers should be reasonably expected to (1) develop lesson plans and student lab reports that contain appropriate safety cautions, rehearsals, etc., (2) check the environment to assure that it is not overcrowded and that at least two emergency exits are accessible to all students and easy to reach, and (3) check all safety equipment items and verify that they are accessible to all students, that all essential equipment functions properly, and that students know where the items are and how and when to use them.

If, despite all precautions, an accident were to happen, the courts and parents generally would be forced to explore other options for the cause. Some states have adopted what is called contributory negligence, in which a plaintiff cannot recover for damages if their negligence in some way contributed to their injury. One important caveat is age of students. Generally speaking, the younger the student, the more difficult it is to prove contributory negligence.

SUMMARY

The 2003–2004 Kentucky Science Safety Project attempted to gauge the status of safety in secondary and elementary schools throughout the state and begin to provide professional development and resources to improve safe practices. Approximately 54 Kentucky school districts were represented in the survey and initial professional development. Results from the survey report focused on the status of facilities, equipment, and teacher knowledge and procedures related to teaching science safely. Although the results of this study indicated some areas of concern, there also were some very positive findings. In the area of facilities and equipment, a number of buildings were new, included two lab exits to facilitate quick exit, had GFI/GFCI protected electrical outlets, adequate numbers of fire extinguishers, eyewash units, and safety goggles for students. With respect to teacher procedures, most teachers required safety contracts with their students, properly stored their chemicals, and administered safety tests to students.

Because the teachers participating in this science safety program were self-selected, represented a variety of middle and high school class sizes, and came from all geographic locations of the state of Kentucky, it was assumed that they were generally reflective of science teachers across the state. Although we recognize that the sample size was small, the objective of this project was to assess the status of safety in Kentucky school science programs and provide tools for addressing them. Findings point to some significant safety concerns relative to facilities, primarily focusing on inadequate size to accommodate the laws and standards for student enrollments and the lack of regular testing of equipment. Teachers from the participating schools did not consistently

observe most standard safety procedures. In addition, most participating science teachers were not aware of many of the applicable science safety laws, codes, and standards.

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Annotated List of the Subfamilies Donaciinae and Criocerinae (Coleoptera: Chrysomelidae)

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ABSTRACT

An examination of leaf beetle specimens (Coleoptera: Chrysomelidae) in the five largest beetle collections in Kentucky, recent inventory work in state nature preserves, and a review of the literature revealed four species of the subfamily Donaciinae and eleven species of Criocerinae present in Kentucky, six of which are new state records. Distribution maps and label data are presented for fifteen Kentucky species of the subfamilies Donaciinae and Criocerinae, including spatial (state and Kentucky county records), temporal (years and months of collection in Kentucky), and plant association information. The following species are reported from Kentucky for the first time: *Donacia limonia* Schaeffer, *Crioceris duodecimpunctata* (Linnaeus), *Neolema cordata* White, *N. ovalis* White, *Oulema collaris* (Say), and *O. sayi* (Crotch).
KEY WORDS: Kentucky, leaf beetles, Chrysomelidae, biodiversity, new state records

INTRODUCTION

This is the second in a series intended to present a synopsis of the historical collection data on leaf beetles (Coleoptera: Chrysomelidae) from the major Coleoptera collections in Kentucky and augment these data with new information gained from recent monitoring in state preserves and other locations. The first paper presented information on the subfamily Cassidinae (Barney et al. 2007), and this paper will address the subfamilies Donaciinae and Criocerinae.

The subfamily Donaciinae has 54 species in five genera reported from America north of Mexico (Riley et al. 2002). Askevold (1988, 1991) reviewed the North American species of the genera *Neochaemonia* and *Plateumaris*, and Marx (1957) treated the genus *Donacia* to which Askevold (1987a, 1987b) added significant corrections. Three species of Donaciinae have been listed previously from Kentucky, two by Riley et al. (2003), and a third by both Schaeffer (1925) and Marx (1957). The closely related subfamily Criocerinae is a relatively small group of leaf beetles with 46 species in five genera in America north of Mexico (Riley et al. 2002). A recent revision of the subfamily (White 1993) that entailed an examination of well over 10,000 specimens from collections in the United States and Canada listed only three species from Kentucky: *Lema trivittata trivittata* Say, *Neolema sexpunctata* (Olivier), and *Oulema melanopus* (Linnaeus).

The purpose of this study was to present the historical and current knowledge of the distribution, abundance, and plant associations of donaciine and criocerine leaf beetles in Kentucky.

MATERIALS AND METHODS

To establish a historical perspective, leaf beetle specimens from the major insect collections in Kentucky (and from collections located in other states known to contain Kentucky specimens) were examined, re-identified, and their label data recorded. The following collections were studied: the University of Kentucky Insect Collection (UKIC, Lexington, KY) that contains the Charles V.

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Covell, Jr. Collection (emeritus professor of the University of Louisville); the private collection of Robert J. Barney (RJBC, Frankfort, KY) that comprises two time periods of collecting in Kentucky, 1976–1984 and 2004–2007; the private collection of Charles Wright, the Kentucky Beetles Project Collection (CWC, Frankfort, KY) that was established in 1991 in an effort to document Coleoptera within the state; the Western Kentucky University Collection (WKUC, Bowling Green, KY); the Brigham Young University Collection (BYUC, Provo, UT); the Charles Dury Collection at the Cincinnati Museum of Natural History (CMNH, Cincinnati, OH); and the Kentucky State University Insect Collection (KSUC, Frankfort, KY) that houses the specimens generated by the Kentucky Leaf Beetle Biodiversity Project.

We currently are conducting extensive collecting in many grass-dominated barrens and rock outcrop (glade) communities known for possessing uncommon plants and plant associations (Jones 2005). These sites are primarily in state nature preserves that have never been surveyed for plant-feeding beetles. Most specimens were collected by the senior author within five state nature preserves in 2004–2007: Crooked Creek Barrens (Lewis County) and Blue Licks Battlefield (Robertson County) in northeastern Kentucky, Eastview Barrens (Hardin County) and Thompson Creek Glades (LaRue County) in central Kentucky, and Raymond Athey Barrens (Logan County) in western Kentucky.

For each donaciine and criocerine species documented for Kentucky, the following data are presented: state-level distribution in the United States (from Riley et al. 2003), Kentucky county records, abundance by year and month in Kentucky, and specimens per collection. When present on specimen labels, other pertinent information, such as the method of collection and plant association information, is presented in the "Comments" section for each species. This information provides the opportunity to determine, from a historical perspective, abundance, seasonality, and distribution. One should note that plant collection records taken from specimen labels are notoriously inaccurate and may not reflect true host plants (Clark et al. 2004).

RESULTS

According to the "Catalog of Leaf Beetles of America North of Mexico" (Riley et al. 2003), there are 42 species of Donaciinae and 19 species of Criocerinae recorded in at least one of the seven states contiguous to Kentucky, thus establishing a "ballpark" estimate for the state. However, in that work only nine species were listed from Kentucky. An examination of 19 donaciine and 195 criocerine leaf beetle specimens from the major collections in the state and others known to contain Kentucky specimens revealed 14 species, including eight of the nine recorded in Riley et al. (2003), plus six new state records (Table 1). A fifteenth species, *Donacia liebecki* Schaeffer, was reported from Kentucky by both Schaeffer (1925) and Marx (1957).

The state collection at the University of Kentucky (UKIC) contains a total of 96 criocerine leaf beetles representing eight species, including three of the new state records reported herein. This collection contains the oldest in-state specimen records for Kentucky leaf beetles, with collection dates as early as 1889. The CWC collection has five specimens representing four species, including one of the new state records reported herein. The WKUC collection has four specimens representing two species. Recent collecting in state nature preserves (the KSUC collection) has produced twelve specimens of four species but no new state records. The RJBC collection contains 74 specimens representing seven species from Kentucky, including one of the new state records reported herein. An examination of the BYUC revealed four specimens representing three species. No criocerine leaf beetles were found in the historical Dury Collection (CMNH), which comprises approximately 75,000 specimens primarily collected between 1871 and 1931 in the Cincinnati/northern Kentucky area (Vulinec and Davis 1984). Only 19 donaciine leaf beetle specimens collected from Kentucky were found in the following collections: 3-BYU, 1-CMNH, 10-CWC, 3-KSUC and 2-RJBC.

DONACIINAE

Plateumaris metallica (Ahrens) (Figure 1A)

Kentucky Counties: Hardin, Laurel, Letcher, Lewis, McCreary, Menifee, Whitley

Table 1. List of Donaciinae and Criocerinae (Coleoptera: Chrysomelidae) recorded from Kentucky with number of Kentucky specimens examined, number of Kentucky county records, range of years of collection in Kentucky, and new state records.

Subfamily Donaciinae	
Tribe Plateumarini	
Plateumaris metallica (Ahrens)	16 specimens: 7 counties, 1983–2005
Tribe Donaciini	
Donacia fulgens LeConte	1 specimen: 1 county, 1994
Donacia liebecki Schaeffer	State-level literature record only
Donacia limonia Schaeffer	2 specimens: 1 county, 1988 (new state record)
Subfamily Criocerinae	
Tribe Criocerini	
Crioceris asparagi (Linnaeus)	35 specimens: 8 counties, 1937–2006
Crioceris duodecimpunctata (Linnaeus)	25 specimens: 5 counties, 1937–2006 (new state record)
Tribe Lemini	
Lema daturaphila Kogan & Goeden	43 specimens: 9 counties, 1889–2007
Lema trivittata trivittata Say	4 specimens: 4 counties, 1947–1970
Neolema cordata R. White	6 specimens: 1 county, 1959–1967 (new state record)
Neolema ovalis R. White	1 specimen: 1 county, 1940 (new state record)
Neolema sexpunctata (Olivier)	49 specimens: 5 counties, 1945–2006
Oulema collaris (Say)	17 specimens: 2 counties, 1992–2007 (new state record)
Oulema melanopus (Linnaeus)	8 specimens: 6 counties, 1974–2005
Oulema palustris (Blatchley)	5 specimens: 4 counties, 1995–2007
Oulema sayi (Crotch)	2 specimens: 2 counties, 1979–2006 (new state record)

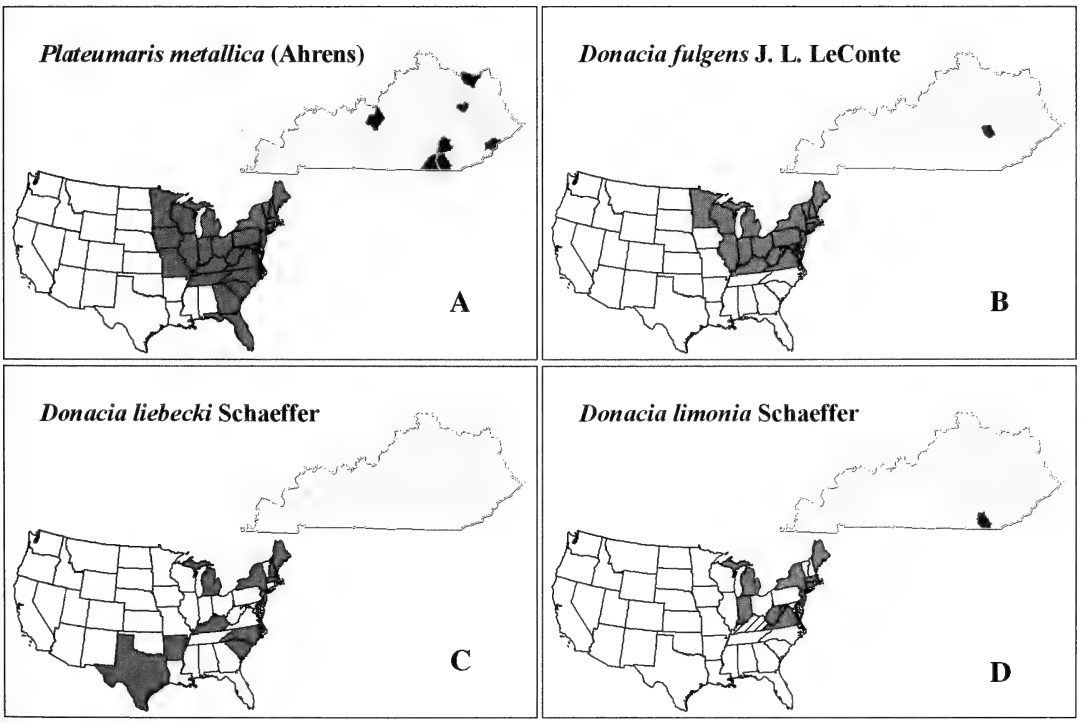

Figure 1. The known distribution of Donaciinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Years: 1983 (2), 1992 (2), 1995 (2), 1996 (4), 2005 (5)

Months: May (14), June (1)

Abundance: 16 specimens: 1-CMNH, 10-CWC, 3-KSUC, 2-RJBC

Comments: The single specimen in the Dury Collection (CMNH) had only "Ky." on the label, therefore no year or month is recorded for this specimen.

Donacia fulgens J. L. LeConte (Figure 1B)

Kentucky County: Jackson

Year: 1994 (1)

Month: May (1)

Abundance: 1 specimen: 1-BYU

Comments: This species has been associated with *Sparganium* (Clark et al. 2004) and belongs to a complex of very similar species that are confidently identified only by the aedeagal characters presented by Askevold (1987a). Other species in the complex, such as *D. subtilis* Kunze, almost certainly occur in Kentucky.

Donacia liebecki Schaeffer (Figure 1C)

Comments: No specimens from Kentucky were seen during this study. Schaeffer (1925) cited one specimen labeled "Ky" from the O. Dietz collection, and Marx (1957) reported one female specimen from Kentucky without further label data.

Donacia limonia Schaeffer (Figure 1D)

Kentucky County: Whitley

Year: 1988 (2)

Month: May (2)

Abundance: 2 specimens: 2-BYU (new state record) (Figure 1D)

Comments: This species has been reported from *Carex* and *Scirpus* (Clark et al. 2004).

CRIOCERINAE

Crioceris asparagi (Linnaeus) (Figure 2A)

Kentucky Counties: Fayette, Franklin, Jefferson, Jessamine, Logan, Russell, Warren, Woodford

Years: 1937 (4), 1945 (8), 1963 (1), 1967 (2), 1992 (1), 1994 (2), 1997 (1), 2005 (11), 2006 (5)

Months: April (8), May (8), June (8), July (9), August (2)

Abundance: 35 specimens: 2-CWC, 2-KSUC, 14-RJBC, 14-UKIC, 3-WKUC

Comments: The common name for this established immigrant to North America is the asparagus beetle. Label data note collection from asparagus, *Asparagus* sp., and by Malaise trap.

Crioceris duodecimpunctata (Linnaeus) (Figure 2B)

Kentucky Counties: Fayette, Franklin, Jefferson, Jessamine, Russell

Years: 1937 (3), 1944 (5), 1951 (3), 1953 (5), 1954 (1), 1971 (1), 1987 (2), 1994 (3), 2005 (1), 2006 (1)

Months: April (1), May (15), July (3), August (4), October (2)

Abundance: 25 specimens: 4-RJBC, 21-UKIC (new state record) (Figure 2B)

Comments: The common name for this established immigrant to North America is the spotted asparagus beetle. Label data note collection from asparagus, *Asparagus* sp., and by Malaise trap.

Lema daturaphila Kogan & Goeden (Figure 2C)

Kentucky Counties: Fayette, Franklin, Hardin, Hart, LaRue, Lewis, Logan, Rowan, Wayne

Years: 1889 (1), 1892 (5), 1895 (3), 1901 (1), 1910 (3), 1914 (1), 1917 (1), 1939 (1), 1947 (1), 1954 (1), 1970 (1), 1971 (1), 1972 (1), 1984 (1), 1985 (1), 1987 (4), 1995 (1), 2005 (5), 2006 (8), 2007 (2)

Months: April (3), May (17), June (12), July (7), August (2), September (1), October (1)

Abundance: 43 specimens: 1-BYU, 6-KSUC, 15-RJBC, 21-UKIC

Comments: UKIC labels record associations with black nightshade, *Solanum americanum* P. Mill.; *Solanum* spp.; and alfalfa, *Medicago sativa* L. Collection by Malaise trap and at lamp was also documented. Host plants for this species are members of Solanaceae (Clark et al. 2004).

Lema trivittata trivittata Say (Figure 2D)

Kentucky Counties: Campbell, Christian, Fayette, Pulaski, Trigg, Warren

Years: 1947 (1), 1956 (1), 1967 (1), 1970 (1)

Months: March (1), May (2), June (1)

Abundance: 4 specimens: 4-UKIC

Comments: Two Kentucky counties are cited by White (1993). Host plants for this

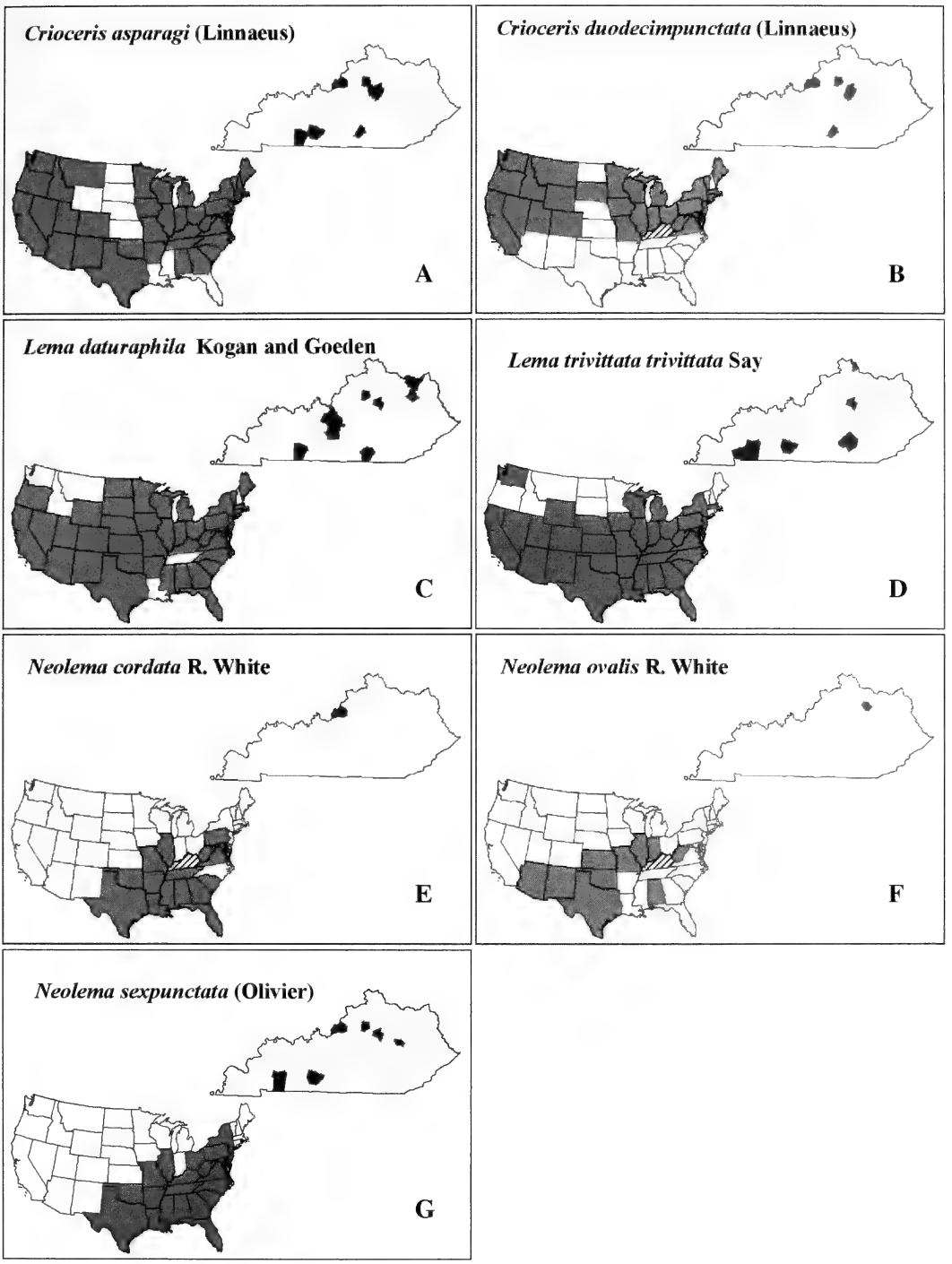

Figure 2. The known distribution of Criocerinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

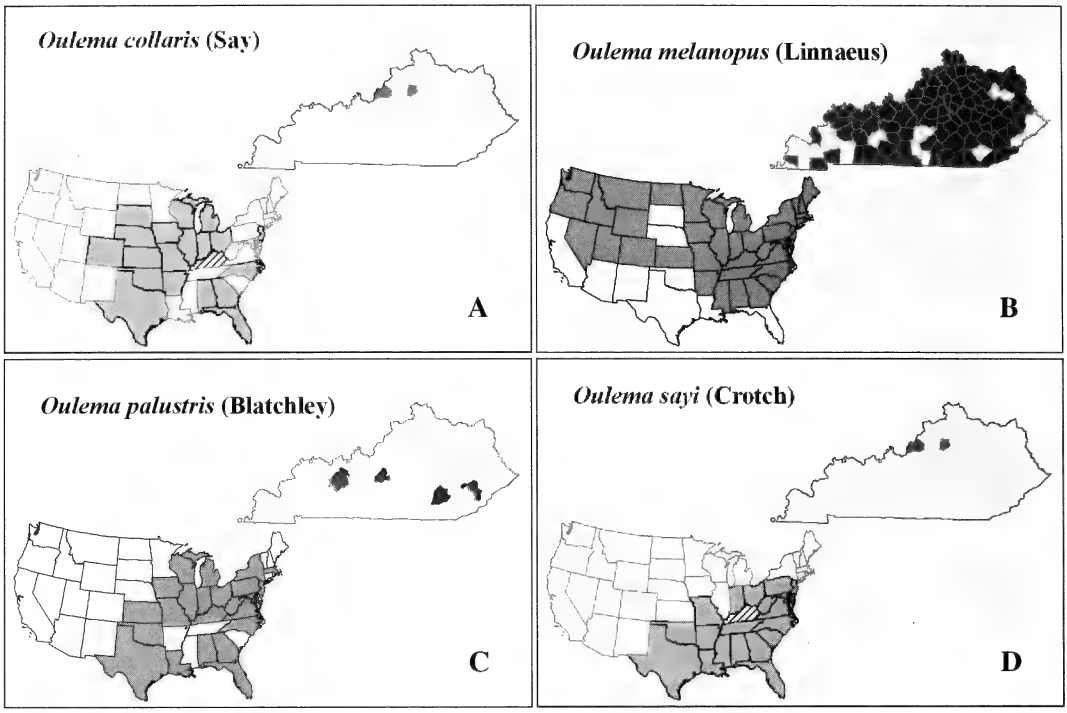

Figure 3. The known distribution of Criocerinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

species are members of Solanaceae (Clark et al. 2004).

Neolema cordata R. White (Figure 2E)

Kentucky County: Jefferson
Years: 1959 (5), 1967 (1)
Months: April (1), May (5)
Abundance: 6 specimens: 6-UKIC (new state record) (Figure 2E)
Comments: This species has been recorded in association with Commelinaceae (Clark et al. 2004).

Neolema ovalis R. White (Figure 2F)

Kentucky County: Nicholas
Year: 1940 (1)
Month: May (1)
Abundance: 1 specimen: 1-UKIC (new state record) (Figure 2F)
Comments: This species has been recorded in association with Commelinaceae (Clark et al. 2004).

Neolema sexpunctata (Olivier) (Figure 2G)

Kentucky Counties: Christian, Fayette, Franklin, Jefferson, Powell, Warren
Years: 1945 (1), 1950 (8), 1952 (13), 1959 (4), 1993 (1), 2003 (1), 2006 (21)
Months: May (5), July (10), August (34)
Abundance: 49 specimens: 1-CWC, 21-RJBC, 26-UKIC, 1-WKUC
Comments: A label from an UKIC specimen documents rearing on *Commelina communis* L. One Kentucky county is cited by White (1993). This species has been recorded in association with Commelinaceae (Clark et al. 2004).

Oulema collaris (Say) (Figure 3A)

Kentucky Counties: Franklin, Jefferson
Years: 1992 (1), 2005 (5), 2006 (10), 2007 (1)
Months: April (6), May (4), June (7)
Abundance: 17 specimens: 1-CWC, 16-RJBC (new state record) (Figure 3A)
Comments: Label data document collection from *Tradescantia subaspera* Ker Gawl. and *Tradescantia* spp. This species has been recorded in association with Commelinaceae (Clark et al. 2004).

Oulema melanopus (Linnaeus) (Figure 3B)

Kentucky Counties: Adair, Allen, Anderson, Barren, Bath, Bell, Boone, Bourbon, Boyd,

Boyle, Bracken, Breathitt, Breckinridge, Bullitt, Calloway, Campbell, Carroll, Carter, Casey, Clark, Clay, Clinton, Daviess, Edmonson, Estill, Fayette, Fleming, Floyd, Franklin, Gallatin, Garrard, Grant, Grayson, Greenup, Hancock, Hardin, Harlan, Harrison, Hart, Henderson, Henry, Hickman, Hopkins, Jackson, Jefferson, Jessamine, Johnson, Kenton, Knott, LaRue, Laurel, Lawrence, Lee, Leslie, Lewis, Lincoln, Livingston, Logan, McCracken, McCreary, McLean, Madison, Magoffin, Marion, Martin, Mason, Meade, Menifee, Mercer, Monroe, Montgomery, Morgan, Muhlenberg, Nelson, Nicholas, Ohio, Oldham, Owen, Owsley, Pendleton, Perry, Powell, Pulaski, Robertson, Rockcastle, Russell, Scott, Shelby, Simpson, Spencer, Todd, Trigg, Trimble, Union, Warren, Washington, Wayne, Webster, Whitley, Wolfe, Woodford

Years: 1974 (3), 1984 (2), 1990 (1), 2004 (1), 2005 (1)

Months: April (3), May (1), June (4)

Abundance: 8 specimens: 1-BYU, 2-KSUC, 2-RJBC, 3-UKIC

Comments: The common name for this established immigrant to North America is the cereal leaf beetle. Eleven Kentucky counties are cited by White (1993) and a published map compiled from pest surveys during 1960 to1994 provided 83 additional counties (Johnson 1993). This species has been recorded in association with Poaceae (Clark et al. 2004).

Oulema palustris (Blatchley) (Figure 3C)

Kentucky Counties: LaRue, Laurel, Ohio, Perry

Years: 1995 (1), 2005 (2), 2006 (1), 2007 (1)

Months: April (1), May (3), July (1)

Abundance: 5 specimens: 2-BYU, 1-CWC, 2-KSUC

Comments: This species has been recorded in association with *Cirsium* and *Tradescantia* spp. (Clark et al. 2004).

Oulema sayi (Crotch) (Figure 3D)

Kentucky Counties: Franklin, Jefferson

Years: 1979 (1), 2006 (1)

Months: May (1), August (1)

Abundance: 2 specimens: 2-RJBC (new state record) (Figure 3D)

Comments: This species has been recorded in association with Commelinaceae (Clark et al. 2004).

DISCUSSION

We believe the data presented here are the most complete representation of the donaciine and criocerine leaf beetles known from Kentucky. The large number of new state records (6 of 15, or 40%) reflects a historical lack of leaf beetle collecting in Kentucky. Certainly additional species of Donaciinae, a group of beetles that is usually not collected by routine sweeping, will be found in Kentucky. Notably absent in our tally are the wide-ranging eastern *Donacia* species associated with waterlily and various waterlily-like genera (*Brasenia*, *Nelumbo*, *Nuphar* and *Nymphaea*).

Three of the eleven Criocerinae recorded from Kentucky are economically important species that were accidentally introduced to the United States from Europe: *C. asparagi*, *C. duodecimpunctata* and *O. melanopus*. Both *Crioceris* species are pests on asparagus, and *O. melanopus* feeds on major cereal crops such as oats, wheat and barley (White 1993), and other grasses. A fourth accidentally introduced criocerine, *Lilioceris lilii* (Scopoli), has not been found in Kentucky.

Only four species of Donaciinae and Criocerinae were found during extensive collecting within state nature preserves. Three specimens of the donaciine, *P. metallica*, were collected within two state nature preserves by sweeping vegetation next to a small stream and pond. The relatively abundant *L. daturaphila* was collected in four preserves. *Oulema melanopus*, the introduced cereal leaf beetle, was found at Jim Scudder State Nature Preserve and Raymond Athey Barrens State Nature Preserve. Two specimens of *O. palustris* were caught by sweeping at Thompson Creek Glades State Nature Preserve. However, six species were discovered while gardening and collecting on the author's (RJB) property in rural Franklin County: *C. asparagi*, *C. duodecimpunctata*, *L. daturaphila*, *N. sexpunctata*, *O. collaris*, and *O. sayi*.

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The Effects of Stream Channelization and Restoration on Mammal Species and Habitat in Riparian Corridors

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ABSTRACT

We investigated differences in habitat characteristics, mammal richness, and mammal activity among three stream types: channelized, unchannelized, and restored. Riparian plant species richness was significantly higher at the unchannelized stream than at the other two streams. The Kentucky Macroinvertebrate Bioassessment Index was highest at the unchannelized stream and much lower at the restored stream than the other two streams. Small mammal activity did not differ among stream types. Richness of terrestrial mammals, as determined by motion-sensitive cameras, did not differ significantly among streams. Track plot surveys showed, however, that unchannelized and restored streams had significantly higher terrestrial mammal species richness than the channelized stream. Likewise, track transect surveys conducted in snow, showed that species richness at the unchannelized stream was significantly higher than the channelized stream. There was some indication that bat activity was affected by an interaction between month and stream type. Results suggested that channelization and restoration affect habitat characteristics, mammal richness, and mammal activity; however, restored streams have potential to recover many of the characteristics of an unchannelized stream.

KEY WORDS: Channelization, invertebrate, mammal, plant, stream restoration

INTRODUCTION

Riparian habitats are critical to aquatic, amphibious, and terrestrial species and often harbor very different communities of organisms compared with uplands (Sabo et al. 2005). Strips of riparian habitat also provide potential movement corridors for many taxa to disperse among habitat fragments (Beier and Noss 1998; Tewksbury et al. 2003). Stream channelization alters riparian habitat through straightening, deepening, and widening of an existing stream or by digging a ditch through a braided river or wetland. Channelization reduces the diversity and abundance of taxa that live in a riparian corridor by reducing habitat heterogeneity, altering flood frequency patterns, and allowing human development of floodplain habitat (Simpson et al. 1982; Brookes 1988). Therefore, channelization has the potential to negatively affect the ability of riparian habitats to add diversity to the landscape and to function as dispersal corridors.

Most stream channelization studies have focused on in-stream habitats and organisms (e.g., Adams and Maughan 1986; Negishi et al. 2002). The effects of stream channelization on terrestrial vertebrates have not been researched extensively; but small mammal species diversity and abundance can be greatly reduced by channelization, affecting species with specific habitat requirements more than habitat generalists (Possardt and Dodge 1978). Channelization reduces the abundance of furbearers, especially semi-aquatic species such as American beaver (*Castor canadensis* Kuhl) (Gray and Arner 1977; Barclay 1980). Few, if any, studies have examined the effects of channelization on bat

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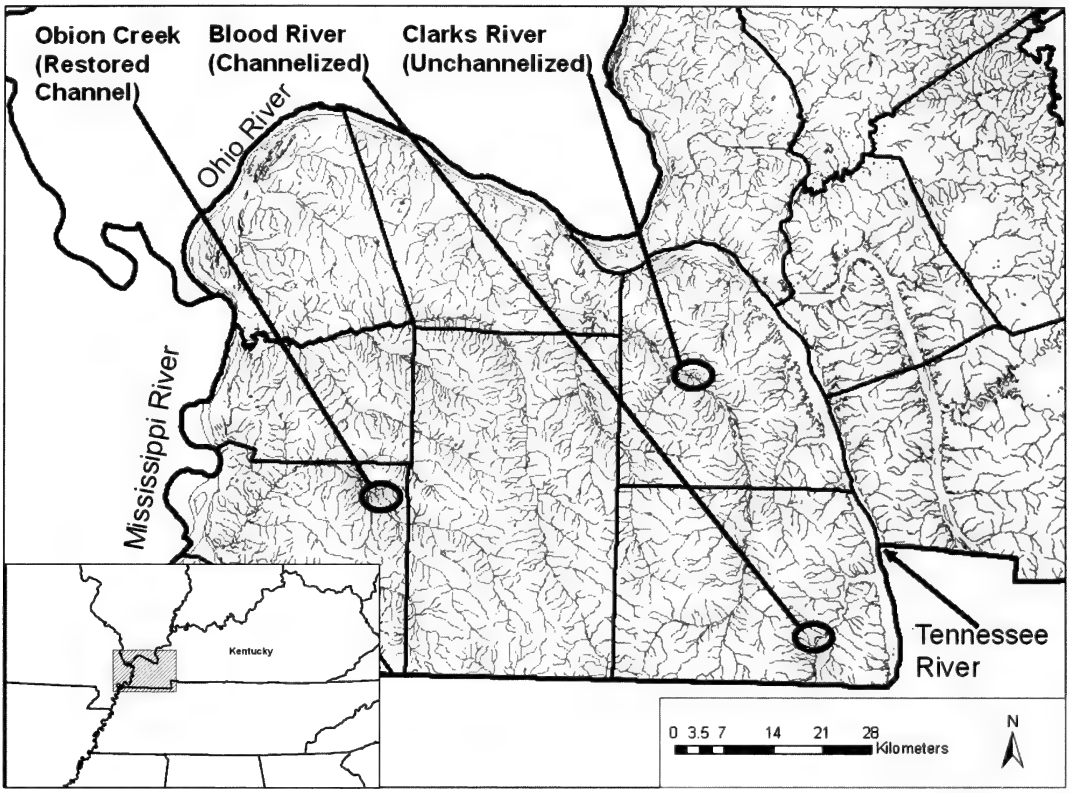

Figure 1. Location of three study streams in the Jackson Purchase area of western Kentucky, USA.

species; however, the reduced insect diversity associated with stream channelization (Adams and Maughan 1986; Negishi et al. 2002) suggests that channelization might affect stream corridor use.

Some streams currently are being restored to a meandering state with flood regimes more typical of an unchannelized stream (Roni et al. 2002; Moerke and Lamberti 2004); however, little is known about the effectiveness of channel restoration in restoring ecosystem function or the possible negative results of channel restoration. Channelized streams eventually revert to a semi-natural state, but the process may take many decades (Shankman 1996). Although channel restoration can be carried out relatively quickly, it is not completely understood whether the biological management value of stream channel restoration is worth the associated disturbance.

Examinations of the long term effects of channelization and the effects of restoration are needed to make the best possible management decisions for riparian habitats. In addition, insight gained from stream channel restoration studies can be broadly applicable to other types of ecological restoration projects (e.g., prairie restoration, mine reclamation, and wetland restoration). We investigated the effects of stream channelization and channel restoration on mammal species richness and activity, stream invertebrates, and riparian habitat characteristics in at three sites western Kentucky.

MATERIALS AND METHODS

Study Sites

Our research was conducted at three 4th–5th order stream sites in the Jackson Purchase area of western Kentucky, United States: one channelized stream, one restored channel stream, and one unchannelized stream (Figure 1). All three streams were low gradient, silt-bottomed, and typical of the coastal plain of the United States. Each stream, except for a portion of the restored stream, was bordered by a width of at least 100 m of bottomland

hardwood and bald cypress (*Taxodium distichum* L., Rich) forest for the lengths of the study areas. Forests abutting all three stream corridors had been managed for wildlife habitat, and adjacent land use at all three streams consisted of a mixture of agriculture and forested conservation areas. A 1 km section, beginning 100 m downstream of a road was sampled at each stream. All sample points were chosen randomly within the 1-km sections.

The unchannelized stream reach was located within the Clarks River National Wildlife Refuge along the East Fork of the Clarks River, which is a 5th-order stream with a drainage area of approximately 692 km^2 above the reach (Hydrology of Kentucky GIS 2004). The Clarks River is a tributary of the Tennessee River. It is unchannelized for most of its length, although there are areas where meanders have been cut off by human-made channels. The reach sampled had no channels.

The channelized stream reach was located within the Blood River Wildlife Management Area where Blood River is a 4th-order stream with a drainage area of approximately 158 km^2 above the area studied (Hydrology of Kentucky GIS 2004). It has been a highly channelized tributary of the Tennessee River for several decades.

The restored stream was located within the Obion Creek Wildlife Management Area where Obion Creek is a 5th-order stream with a drainage area of 297 km^2 (Hydrology of Kentucky GIS 2004). Downstream of the restored segments is unchannelized stream that flows into the Mississippi River. Obion Creek was channelized in the 1930's. In subsequent years, the channel filled with sediment and debris until its capacity to hold water was diminished, resulting in widespread flooding and timber die-off (Jackson Purchase RC&D Foundation 2005). In summer 2003, two sections of meandering stream channel were excavated through the efforts of the Obion Creek Watershed Conservancy District Board of Directors to link two pre-channelization remnant segments of Obion Creek. The study area included 400 m of excavated channel and 600 m of remnant channel downstream. Forest clearing and earthworks resulting from channel restoration were confined to the north side of the stream and created a strip of cleared land that was approximately 20 m wide. The remnant channel portion of the study area and south side of the excavated channel consisted of continuous bottomland forest species that were similar to those at the other two study streams.

Data Collection

Physical attributes of the streams. During the summer of 2005, physical aspects of each stream were measured at ten points every 100 m on each stream bank: bank height (±0.25 m), bank angle (as determined with a clinometer; ±10°; Suunto PM-5/2 PC, Finland), and bank width (as determined with a laser range finder; Nikon Laser 440, U.S.A.). To measure flooding, we examined the height of debris piles and the percent of tracking tubes (see small mammal sample methods) that contained silt after two separate rain/flood events.

Vegetation Sampling. We measured vegetation characteristics of each riparian zone at the 20 points used for sampling physical attributes. Canopy cover was measured with a spherical densiometer (model-C: Robert E. Lemmon, Forest Densiometers, Bartlesville, Oklahoma, U.S.A.) in four directions: facing toward the stream, downstream, away from the stream, and upstream. Four measurements were then averaged for each point. Average vertical cover was measured in four directions by visually estimating the percent coverage of each zone of a vertical cover board (0–0.125, 0.125–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.25, 1.25–1.5, 1.5–1.75, 1.75–2, and 2–2.25 m above ground level). We recorded the species and dbh (±1 cm) of the four trees nearest to the sample point (no minimum dbh). We also recorded presence of plant species (herbaceous, vine, shrub) within a 1-m radius of the sample point. Palaeontological Statistics (PAST) software version 1.38 was used to calculate Shannon H^1 diversity indices for each stream (Hammer et al. 2001).

Macroinvertebrate Sampling. During mid-March 2006 we sampled macroinvertebrates at the three streams in order to compare in-stream habitat quality. Five randomly chosen points were sampled by collecting five jab samples with D-frame nets at each point. Samples were taken primarily from undercut banks, organic detritus near a bank, and at

the base of woody debris in a channel. All macroinvertebrates collected at a point were separated from debris in a sorting tray and preserved in 95% ethanol. Organisms were later identified to the lowest practicable level, which was usually genus. The protocols used for collecting and analyzing invertebrate data came from the Kentucky Macroinvertebrate Bioassessment Index (KMBI) (Pond et al. 2003).

Mammal Sampling. All mammal sampling was conducted during four seasons (spring 2005-winter 2005/06) unless otherwise noted. Sample points were randomly chosen using aerial photography and ArcView GIS 3.3 and then located on the ground using a GPS (Garmin Ique 3600, accuracy 3–7 m; Garmin International, Olathe, Kansas, U.S.A) and aerial photographs. All sampling except for snow track transects and bat surveys was conducted at 10 points along each stream reach.

Small Mammals. Tracking tubes were constructed as suggested by Nams and Gillis (2003). Each tube consisted of a 30 cm length of polyvinyl chloride (PVC) pipe that acted as a waterproof housing for a strip of white copy paper. Half of the tubes were constructed of 3.2 cm pipe and half were of 5.1 cm pipe. The larger tubes were made in an attempt to record the tracks of rodents larger than those that Nams and Gillis (2003) recorded. Tubes were staked to the ground with u-shaped pieces of #9 wire to prevent loss during flood events. Strips of copy paper, used as a tracking medium, had a 50 mm square of clear packing tape applied to the middle. We smeared an ink made from a 1:2.5 weight ratio of graphite powder to mineral oil onto the square of tape. Ink was added in the field, directly before loading papers into the tubes. Tubes were left loaded with paper and un-baited for 10 days during each sampling period.

The 10 day, un-baited protocol was chosen because field trials showed that longer periods of time and/or the use of bait yielded tube success of 90% or higher at all sites. According to Nams and Gillis (2003), differences in small mammal density might not show up when all sampling areas have high rates of tube success because 90% of the tubes in one area could have been visited immediately, while 90% of the tubes in another area could have been visited over a longer period of time. The protocol used in our research generally resulted in visitation rates between 30–50% at the least-visited sites. Refraining from the use of bait also prevented animals from obliterating their tracks by repeatedly traveling through the tubes.

A 50-m transect containing five tubes, at 10 m intervals, was placed parallel to a stream beginning at each randomly chosen point. The 50 tubes at a study site included 25 tubes of each diameter. The placement of each diameter of tube was selected randomly. Tubes were located at tree bases or near fallen logs.

After collecting papers from the tubes, those that had tracks were scanned and the images saved for analysis. We used a reference collection of tracks from local species to identify tracks as those of a mouse (e.g., golden mouse, *Ochrotomys nuttallii* Harlan; deer mice, *Peromyscus* spp.; house mouse, *Mus musculus* L.; eastern harvest mouse, *Reithrodontomys humilis* Audubon and Bachman), vole (e.g., woodland vole, *Microtus pinetorum* Le Conte; prairie vole, *M. ochragaster* Wagner), or shrew (e.g., southern short-tailed shrew, *Blarina carolinensis* Bachman; northern short-tailed shrew, *B. brevicauda* Say). We used each set of five tubes as a sample point (10 points per stream) and calculated the number of tubes that had tracks divided by the number of functional tubes to produce indices of small mammal activity. Some tracking tube papers were not useful because they were pulled out by animals or eaten by insects during the 10 day period.

A bat detector (ANABAT II bat detector, Titley Electronics, Ballina, New South Wales, Australia) connected to a laptop running Anabat 6.3 g recording and analysis software was used during the summer of 2005 to sample bat activity at all three streams. Each stream was visited three times during June through September with sampling beginning at dusk. We sampled on nights when the moon was less than half full or the sky was overcast, and all three streams were monitored on nights with approximately the same temperature during each month (always above 13°C) (Indiana Bat Recovery Team 1999). Each of five, randomly chosen, sample points within a stream was visited for 30 min before moving to the next point. When bats were not

heard, we constantly pointed the bat detector upstream, downstream, and overhead in order to locate areas of possible activity. Johnson et al. (2002) found that active monitoring resulted in more observations and the detection of more species than passive sampling (i.e., fixed location of bat detector). An index of bat use, given by the number of minutes with bat activity at each point, was calculated for each sample point (Miller 2001).

Track Surveys. At each stream, 10, 3 × 0.5-m plots were checked for mammal signs once during each season. The 10 plots were located at sections of muddy bank that were nearest to 10 randomly chosen sample points. All three study areas were sampled on the same day to minimize differences in tracking substrate due to weather. Species were identified using sign identification guides (Murie 1974; Halfpenny and Biesiot 1986; Stokes and Stokes 1986; Rezendes 1999; Elbroch 2003). No scent attractant was used at tracking plots to avoid influencing animal movements. Data were recorded using an integrated GPS/Personal Digital Assistant (Garmin Ique 3600) running CyberTracker 2.77 software (Liebenberg 1996). We used the number of species whose tracks were observed at each plot in species richness comparisons.

Snow Tracking. During December 2004 and February 2006 snow tracking transects were completed at all three streams. During both years, all three streams were visited on the same day in order to minimize the effects of snow melt and time since snowfall on the number of tracks observed. Five, 20-m transects were located perpendicular to the stream at 100 m intervals and on each bank for a total of 10 transects per stream. We used the number of species whose tracks were observed on each transect in species richness comparisons.

Motion-triggered Cameras. Each season, three motion-sensitive cameras (spring and summer: PhotoHunter, Trailtimer, St. Paul, Minnesota, U.S.A; TrailMAC, Trailsense Engineering, Middletown, Delaware, U.S.A; and Snapshot Sniper, Snapshot Sniper LLC, Duncan, Oklahoma, U.S.A.; fall and winter: three Snapshot Sniper cameras) were placed at three randomly chosen points. Cameras were located approximately 1–3 m from the stream and oriented towards game trails. Bait was placed within the trigger area of each camera. Baiting was necessary due to the low number of available cameras and the small trigger area of motion-sensitive cameras. All cameras were left at each stream for five consecutive nights during each season. We used the number of species recorded by each camera in species richness comparisons.

Data Analysis

Unless otherwise mentioned in individual technique sections, SAS (2002–2003 SAS Institute Inc.) was used for all analyses. Analysis of variance (ANOVA) was used to compare measurements made at the three stream types. For analysis of vertical cover, stream type and vertical cover zone were the independent variables for two-way ANOVAs. For indices of mammal activity and for species richness analyses, stream and sample period were the independent variables for two-way ANOVAs. If data did not meet the assumptions of ANOVA (i.e., percent canopy cover, bank height, plant richness, vole activity, shrew activity, and track plot richness), then a nonparametric one-way ANOVA (Kruskal-Wallis) was used with stream as the independent variable. Tukey's honest significant difference (HSD) was used for post-hoc comparisons of ANOVAs, and Fisher's least significant difference (LSD) was used for post-hoc comparisons of nonparametric ANOVAs. Statistical significance was established at $P \leq 0.05$ ($\alpha = 0.05$). Data are presented as means ± 1 standard error (SE).

RESULTS

Physical Attributes

Mean bank height differed significantly among all three streams (Kruskal-Wallis, $P < 0.0001$; LSD $P < 0.05$). The unchannelized stream had the highest mean bank height, and the restored stream had the lowest. Mean bank angle was significantly steeper at the channelized stream than the restored stream (Kruskal-Wallis, $P = 0.0019$; LSD $P < 0.05$). Mean width differed significantly among all three streams (Kruskal-Wallis, $P < 0.0001$; LSD $P < 0.05$). The unchannelized stream was the widest and the restored stream the narrowest (Table 1).

Table 1. Summary of selected vegetative and physical characteristics at each stream. Means (±1 SE), within a row that have the same letter are not significantly different from each other (Kruskal-Wallis, $P < 0.05$; LSD $P < 0.05$).

Variable	Stream Type		
	Channelized	Unchannelized	Restored
Bank Height (m)	1.96 ± 0.07 (c)	4.75 ± 0.28(a)	3.05 ± 0.28 (b)
Bank Angle (degrees)	76.25 ± 4.23 (a)	61.25 ± 4.99 (ab)	48.50 ± 6.28 (b)
Bank Width (m)	18.40 ± 1.00 (b)	22.80 ± 0.78 (a)	12.80 ± 0.65 (c)
*Drainage Area (km^2)	158	692	297
*% Track Tube Flooding	0.00 ± 0.00	27.18 ± 4.70	74.38 ± 5.60
*Plant Diversity (Shannon-Weiner Index)	1.18	1.27	1.42
Plant Richness	4.55 ± 0.39 (b)	6.10 ± 0.31 (a)	3.65 ± 0.45 (b)
% Canopy Cover	95.33 ± 1.25 (b)	99.75 ± 0.26 (a)	74.65 ± 8.60 (b)
*KY Macroinvertebrate Bioassessment Index	57.97 (Good/Excellent)	59.90 (Excellent)	38.44 (Fair)

* Not conducive to be statistically analyzed.

When it became apparent that the streams flood differently (Blood River waters never left the channel and patchiness of flooding seemed to vary between the other two streams), we calculated the percent of tracking tubes containing silt after two rain events. At the unchannelized stream, an average of 27.18 ± 4.7% of the tracking tubes contained silt when flood waters receded. The restored stream had more than twice as many tubes flood compared with the unchannelized stream. No tubes were flooded at the channelized stream during the two rain events (Table 1).

Vegetation

Post-hoc tests revealed that the unchannelized stream had significantly more canopy cover than either the channelized or restored stream (Kruskal-Wallis, $P < 0.0001$; LSD $P < 0.05$; Table 1). Four sample points from the restored stream, which were cleared of trees during the restoration process, were removed from the analysis, but this did not change the observed pattern. Vertical cover profiles differed considerably among the three streams. There was a significant stream × vertical cover zone (i.e., 0–0.125, 0.125–0.25 m, etc.) interaction (ANOVA, $P = 0.002$). The interaction remained after removing four restored stream sample points from the analysis (ANOVA, $P < 0.0001$). The unchannelized stream tended to have the most vegetation 0–1 m above ground level. The restored stream tended to have taller vegetation with most occurring 1.75–2.25 m above ground (Figure 2). As with canopy cover, overall plant species richness (plant = herbaceous, vine, and shrub species) was significantly higher at the unchannelized stream than at either the channelized or restored streams (Kruskal-Wallis, $P < 0.0001$; Tukey $P < 0.05$; Table 1).

Macroinvertebrates

The Kentucky Macroinvertebrate Bioassessment Index (KMBI) score was highest for the unchannelized stream, followed by the channelized stream and then the restored stream (Table 1). Using nominal categories derived from the MBI, the unchannelized stream was classified into the highest category of stream "health". Thus, the unchannelized stream was most similar to unaltered, healthy streams that were used as references for the MBI. The channelized stream was classified at a level slightly below the unchannelized stream, but the restored stream was classified at a much lower level of stream health than either of the other two streams (Table 1).

Mammal Surveys

The presence of mammals ranging in size from deer mice to white-tailed deer (*Odocoileus virginiana* Zimmerman) was recorded by track plots on muddy banks, tracking transects in snow, and motion-sensitive cameras. Terrestrial mammal species richness, as determined by track plot surveys, differed significantly among streams (Kruskal-Wallis, $P = 0.0016$). The unchannelized and restored streams both had significantly higher species richness than the channelized stream (LSD, $P < 0.05$). Richness at unchannelized and restored streams did not differ significantly (LSD, $P > 0.05$; Figure 3).

Analysis for effects of seasons showed some variability in the pattern of mammal species

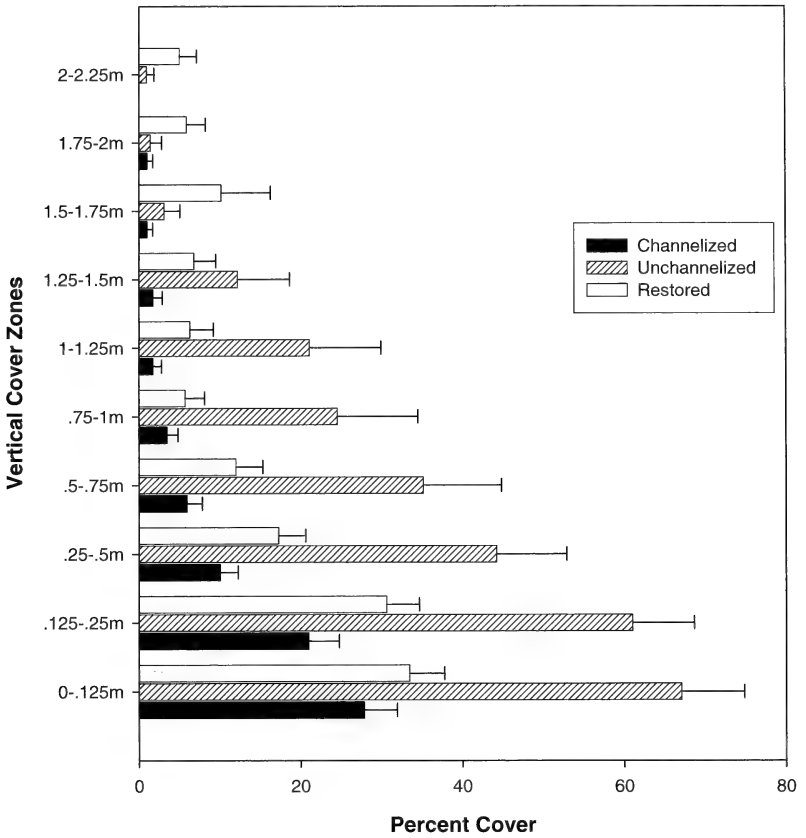

Figure 2. Vertical cover profiles, as measured at 10 height ranges, of vegetation at each stream ($\bar{X}$ +1 SE).

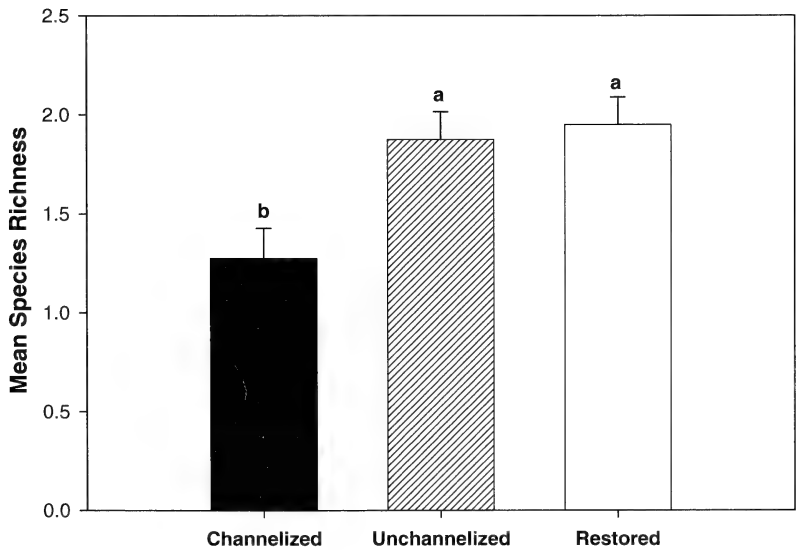

Figure 3. Terrestrial mammal species richness, as determined by track plot surveys. Means (+1 SE) that are significantly different have different letters (Kruskal-Wallis, $P = 0.0016$; LSD, $P < 0.05$).

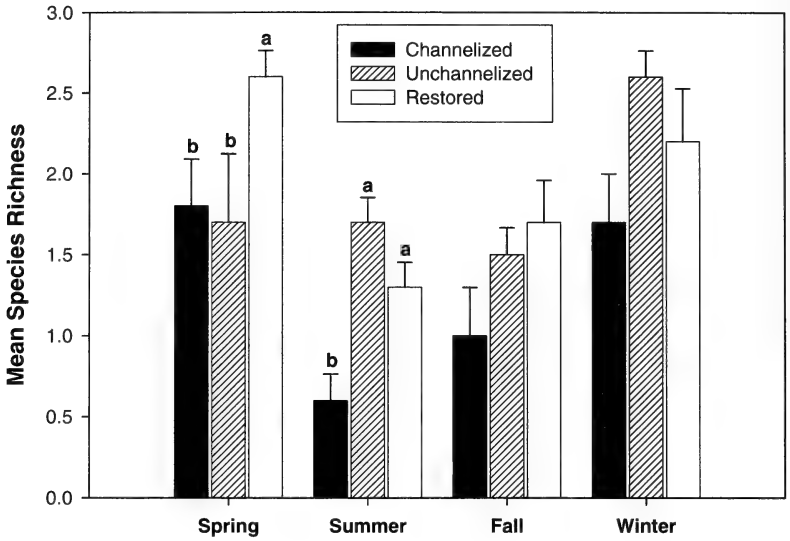

Figure 4. Terrestrial mammal species richness for each season as determined by track plot surveys. Means (+1 S.E.) that are significantly different have different letters (Kruskal-Wallis, $P < 0.05$; LSD, $P < 0.05$).

richness. The channelized stream tended to have low mammal species richness, but only significantly so during summer (Kruskal-Wallis, $P = 0.001$; LSD, $P < 0.05$; Figure 4). During the spring season, the restored stream was significantly higher in mammal species richness than the other two streams (Kruskal-Wallis, $P = 0.06$; LSD, $P < 0.05$; Figure 4).

Terrestrial mammal species richness, as determined by snow transect surveys, also differed significantly among streams (ANOVA, $P = 0.0006$). The unchannelized stream had significantly higher richness than the channelized stream (Tukey, $P < 0.05$), but the restored stream did not differ from the other two (Tukey, $P > 0.05$; Figure 5). There was no significant difference among years ($P = 0.78$) and no significant stream by season interaction ($P = 0.15$). Data for track plot surveys in winter (Figure 4) followed the same pattern as observed for snow tracking data.

Motion-sensitive cameras did not record significantly more mammal species at any one stream ($P = 0.90$), nor was there a significant stream by season interaction ($P = 0.43$). There was a difference among seasons (ANOVA, $P = 0.03$), however. Cameras recorded significantly more species during fall than during spring or summer sample periods (Tukey, $P < 0.05$).

We conducted 7400 tube nights at the channelized stream, 7360 at the unchannelized stream, and 7240 at the restored stream (tube night = number of tubes with tracking tube papers in them for all 10 nights × 10 nights × 4 seasons). No tracks from rat species (hispid cotton rat, *Sigmodon hispidus* Say and Ord; marsh oryzomys, *Oryzomys palustris* Harlan; roof rat *Rattus rattus* L.; or brown rat, *R. norvegicus* Berkenhout) or small shrews (least shrew, *Cryptotis parva* Say; or *Sorex* spp. L., Soricidae) were observed. Indices of mouse ($P = 0.63$), vole ($P = 0.39$), and shrew ($P = 0.21$) activity did not differ significantly among streams.

There also was no difference in bat activity among streams ($P = 0.24$) or seasons ($P = 0.17$). There was a stream by season interaction that was marginally significant (ANOVA, $P = 0.067$). The channelized stream had significantly lower bat activity than the restored stream during July (Tukey, $P < 0.05$; Figure 6). In addition, the pattern of mean bat activity among streams differed among months. During June and August mean bat activity tended to be lower at the restored stream, but during July it was highest at the restored stream.

DISCUSSION

The unchannelized and restored streams had the highest mammal species richness overall, while the channelized stream was lowest in species richness. Lower mammal

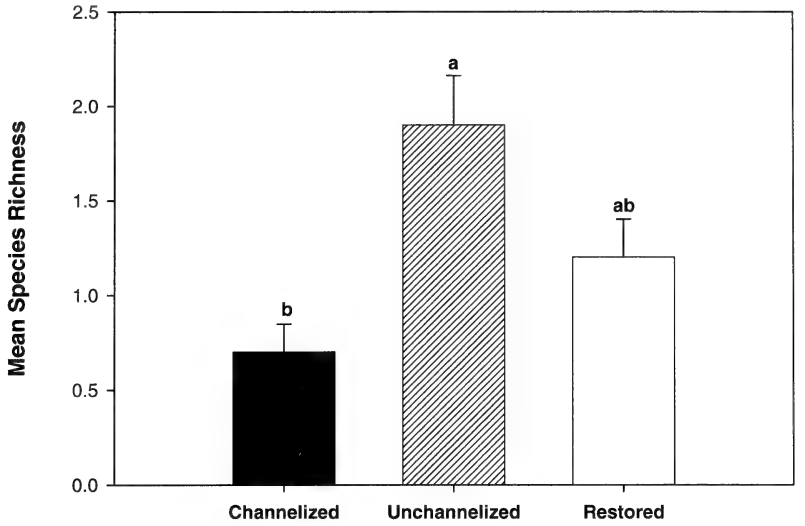

Figure 5. Terrestrial mammal species richness from transects conducted in snow. Means (+1 SE) with different letters are significantly different (ANOVA, $P = 0.0006$; Tukey, $P < 0.05$).

species richness was expected in the channelized stream riparian zone based on past research (Barclay 1980; Gray and Arner 1977; Possardt and Dodge 1978). The high mammal species richness found in the restored stream, however, was not necessarily expected because restoration created a recent habitat disturbance. Stream channelization is known to decrease riparian habitat heterogeneity (Simpson et al. 1982). We found differences in plant species richness and diversity, possibly linked to flood regime and microhabitat variability, which likely contributed to the differences in mammal species richness among streams.

Estimates of mammal species richness at the three streams differed depending upon the assessment method used and the season. According to track surveys on stream banks, the highest richness of mammals was at the

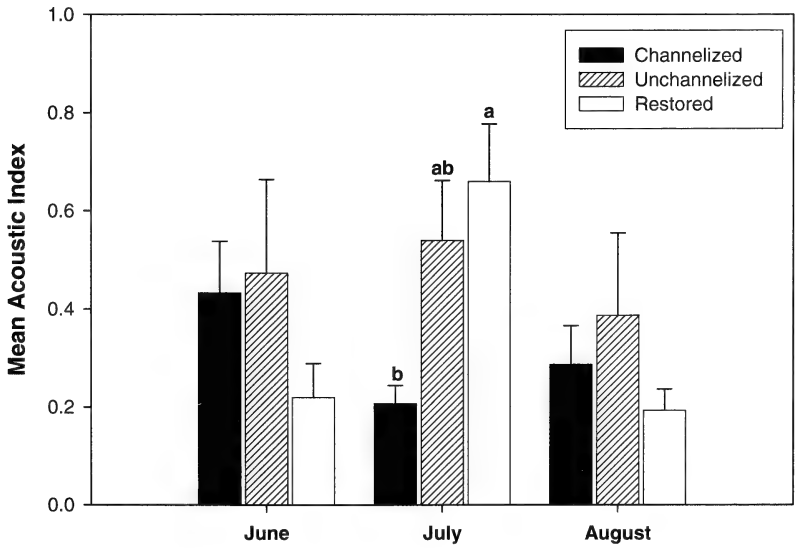

Figure 6. Mean (+1 SE) bat activity represented in terms of the acoustic index (# of minutes with bat activity/# minutes sampled). Streams with different letters are significantly different (Tukey, $P < 0.05$).

unchannelized and restored streams. In winter, however, both snow tracking and track plot surveys indicated that mammal species richness was higher at the unchannelized, but not the restored stream, compared with the channelized stream. No differences in species richness among streams resulted using motion-sensitive cameras. The lack of difference was in disagreement with results from tracking methods but was probably a product of high variability and low sample size (three cameras). Because all cameras were set at one stream before being moved to another stream, weather might have influenced mammal activity enough to mask any differences that were present among streams. It is also possible that there really were no differences among streams, but this was unlikely because we found significantly higher mammalian species richness at the unchannelized stream using both tracking methods and at the restored stream using bank track plots.

We found no differences in small mammal activity or species assemblage among stream types. Previous studies found differences in small mammal abundance (Possardt and Dodge 1978; Barclay 1980) and diversity (Possardt and Dodge 1978) between channelized and unchannelized streams. Conversely, Weeks (1982) found that water vole (*Arvicola terrestris* Linnaeus) abundance recovered quickly after channelization. Further splitting of the category of small mammals that we identified collectively as mice might reveal differences in small mammal communities among streams. For instance, cotton deermouse (*Peromyscus gossypinus* Le Conte), a species associated with bottomland hardwood forests, is uncommon in far western Kentucky (Derting and Somerlot 2002). Their limited distribution potentially could be a result of western Kentucky's history of stream channelization.

The channelized stream had significantly less bat activity than the restored stream during July. The difference was of limited reliability, however, because it was found after investigating a stream × month interaction that was only marginally significant ($P = 0.067$). Zielinski and Gellman (1999) highlighted the importance of having a large sample size (many nights of sampling) when using indices of bat activity. Future studies should incorporate more samples, and/or monitor streams from all treatments during the same night by using multiple observers, in order to remove the effect of weather on sample variability. Future studies also need to investigate species-specific bat activity because, although total bat activity did not differ among stream types, species with specific habitat or aquatic insect diet requirements might be affected more by channelization or restoration.

Higher plant species richness might contribute to the higher mammal richness found at the unchannelized stream. Animal species richness has been related to plant species richness (e.g., Siemann et al. 1998) or at least to the same environmental variables that influence plant richness (Hawkins & Pausas 2004). Plant species richness was highest at the unchannelized stream (Table 1), which could indicate the presence of more microhabitats and more vertebrate niches. There are several possible reasons for the higher plant species richness at the unchannelized stream. These include lack of disruption of the natural community of plants, more variation in bank angles and heights (Table 1), and an intermediate level of flood disturbance (Table 1).

Lack of disruption of the natural plant community and presence of physical microhabitats created by variability in bank height and angle probably have a synergistic effect on plant richness at the unchannelized stream. The restored stream also has high variability in physical bank characteristics, but a large proportion of the sample area was disturbed and homogenized recently by earthworks necessary for restoration. The intermediate disturbance hypothesis (Connell 1978) is a viable explanation for high plant species richness at the unchannelized stream. As compared with larger and smaller streams, Nilsson et al. (1989) found a peak in native plant species richness adjacent to mid-order streams and related their findings to the intermediate disturbance hypothesis. In mid-order streams there is enough flood disturbance present to constantly modify available niches, but not enough to decimate all species. All three of our streams were mid-order streams, but the channelized stream rarely left its banks in the area studied, and the

restored stream flooded extensively after rain events (according to tracking tube flood index and anecdotal observations of debris piles and sediment deposits). Higher plant species richness found at the unchannelized stream might be explained by its intermediate, potentially patchy pattern of flooding compared with the channelized and restored streams.

The major effect of restoration seems to have been on the invertebrate community, which needs to be considered when evaluating costs of restoration for aquatic ecosystems. Eventually, the restored stream could recover enough of its original flood regime and in-stream habitat to be more similar to the unchannelized stream than the channelized stream; however, increasing leaf retention and habitat heterogeneity in the channel could speed recovery of the invertebrate community (Laasonen et al. 1998; Muotka and Laasonen 2002).

Riparian habitats add diversity to the landscape (Sabo et al. 2005) and function as dispersal corridors (Beier and Noss 1998; Tewksbury et al. 2003); therefore, activities that alter streams have implications for much more than a localized reach. Continued study of stream alteration is needed for several reasons. First, channelized streams change over time (Shankman 1996), yet most studies of the terrestrial effects of channelization were conducted more than 20 years ago. Second, restoration projects offer many opportunities to investigate the efficiency and practicality of different restoration strategies in restoring flood regimes and natural communities to streams. Third, the results of restoration studies can be broadly applicable to other types of ecological restoration. For instance, in our study remnants of stream channel were connected with each other rather than construction of an entirely new channel. Leaving remnants reduced the amount of area disturbed by excavation and might have provided source populations of organisms that were able to disperse into the excavated riparian zone. In a similar manner, prairie restorations are often more successful when prairie remnants are located nearby (i.e., Kindscher and Tieszen 1998).

Future projects need to focus on the effects of channelization and restoration on a single guild of species (e.g., chiropterans, small mammals, medium-large mammals). Our study sacrificed a large sample size of streams of each type in order to look at a more whole picture of the three riparian ecosystems. We also were limited by the number of restored streams of similar order in our study area. In focusing on a single species guild and by using the relatively efficient methods used in our research (i.e., track surveys, tracking tubes, and echolocation surveys), future investigators would be able to gather data for several channelized, unchannelized, and restored streams.

Stream channelization negatively effects in-stream habitat and riparian corridors (Simpson et al. 1982; Brookes 1988). The stream restoration project addressed in this study did not cause excessive disturbance to the riparian zone flora or fauna. In fact, two years after restoration, many parameters at the restored stream already were more similar to an unchannelized stream than a channelized stream. However, in-stream habitat restoration should be included where projects are aimed at expedient rehabilitation of a functioning riparian system. Incorporation of habitat remnants (e.g., old stream channels) might be one manner in which this project minimized disturbance to organisms living in the riparian zone and also minimized the amount of time required for riparian organisms to colonize the restored riparian habitat.

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Extended Longitudinal Studies of Family Size and the Human Sex Ratio

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ABSTRACT

As the fourth over a span of about 40 years, this study further characterizes the human sex ratio of families of students enrolled at Western Kentucky University, Bowling Green, KY. Student respondents provided data on the parental, present, and projected generations. Survey results permitted calculations of average family sizes and sex ratios that were used in comparisons with results of the previous studies. Average numbers of children were 4.04, 2.58, and 2.45, and secondary sex ratios (males:100 females) were 102, 108, and 141; respectively, for the parental, present, and projected generations. Average family size decreased progressively during the period of study. Secondary sex ratios ranged from 97 to 108 males:100 females. Gender composition of the first two and three children influenced parities in two ways, namely preference for both genders and preference for males. Results of the present and previous studies indicated that the most desired families consisted of two children, both genders, and the male born first. Realization of this preference would contribute toward a balanced sex ratio. Other preferences included more male than female children. Overall, sex ratios for desired families in the four studies ranged from 133 to 141 males:100 females.

INTRODUCTION

Continued expansion of the human population coupled with shrinking availability of natural resources threatens to exceed the supportive capacity of the global environment (Gray et al. 1995). Limiting population growth and conserving natural resources are critical in sustaining both the environment and human population. The human population is a function of the number of family units and the number of children per unit.

Gender composition of children continues to be a force in determining family size and social stability (Call and Gray 1996). At the family level, gender composition of children within families has been found to influence the parents decision to have, or not to have, additional children (Thomas 1951; Loyd and Gray 1969). At the society level, enforcement of social policies that limit the number of children per family, without regard to gender preference, can seriously disrupt the sex ratio balance (Qi and Mason 2005).

Parents have identified two different gender preferences for their children, namely both sexes and more males. Presence of both sexes in the first two or three children resulted in fewer additional children in studies conducted in Britain (Thomas 1951), the United States (Loyd and Gray 1969; Gray 1972; Gray and Morrison 1974; Call and Gray 1996), and China (Gray et al. 1995). Male preference was evident in studies conducted in Korea (Park 1978), in the United States (Gray 1982; Call and Gray 1996) and in India (Mutharayappa et al. 1997). In male preference, more one-child and two-child completed families consisted of more males than females. In completed families of three children, more consisted of three males or two males and one female than three females or two females and one male (Call and Gray 1996). Male or son preference is especially pronounced in societies of Asia and Africa. Mutharayappa et al. (1997) concluded that elimination of son preference in India would lower the fertility level by about 8%. The reduction would have major impact on population growth rate and could produce important social benefits.

Gray and co-workers (Loyd and Gray 1969; Gray 1972; Gray 1982; Call and Gray 1996) conducted transgenerational studies by sampling college students at Western Kentucky University at approximately 10-year intervals. The results showed a decrease in family size, a rather consistent secondary sex ratio, and a pronounced preference for desired families of two children consisting of both sexes with male born first. Overall, the desired families included more male than female children and would distort the secondary sex ratio in favor of males.

Objectives of the present study were to authenticate and expand results of previously

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Table 1. Average number of children (a) and sex ratios (b) of families of three generations at four time periods.

	Time periods and studies							
	1968–72[1]		1979–82[2]		1992–96[3]		Present survey[4]	
Generation	a	b*	a	b	a	b	a	b
Parental	4.56	103	4.73	103	4.24	105	4.04	102
Present	3.32	101	3.7	101	2.7	97	2.58	108
Desired	2.46	138	2.6	133	2.35	136	2.45	141

* Males:100 Females.
[1] Loyd and Gray (1969) and Gray (1972).
[2] Gray (1982).
[3] Call and Gray (1996)
[4] Present survey.

reported studies, especially those findings identified through the present series. More specifically, the objectives were to determine family sizes and sex ratios as influenced by time and generation, to measure effects of gender composition of existing children on family size, and to ascertain association between sexes of children within families.

METHODS

This study is the fourth in a longitudinal series conducted at approximately decadal intervals (1968–72, 1979–82, 1992–96, 2007), thereby extending the time span to approximately 40 years. This and the three previous studies were based upon data obtained from students enrolled in large introductory courses in Ogden College of Science and Engineering and the College of Health and Human Services, Western Kentucky University. The student survey questions that had approval of the University's Human Subjects Committee requested only information on number of children by gender and order of birth.

In the present study, 884 (equal numbers of females and males) students completed data forms providing information on three generations: parental, present, and projected. For the parental generation, data were combined for the maternal and paternal parents' families for average size and sex ratio. For the present generation, data were obtained on gender of children by order of birth permitting calculations of average size, composition and relationships among sexes, and the effect of gender composition of children on family size. For the projected generation, student respondents were asked to indicate their desired number of children and gender by order of birth. These data were used in calculating average family size and sex ratios by order of birth for a projected generation. Procedures employed in this fourth study were patterned after those of the previous three studies (Loyd and Gray 1969; Gray 1982; Call and Gray 1996).

RESULTS AND DISCUSSION

Results of the present study substantiated those of the previous studies by supporting on-going trends. Beginning with the second study (1979–82), average numbers of children per family decreased progressively (Table 1). The decrease was approximately 0.69 and 1.12 children per family for the parental and present generations; respectively. Increases in parental and present family sizes between the first and second studies were accredited to enhanced financial aid enabling more students from larger families to attend college (Gray 1982). Within each study period the number of children per family decreased by approximately 1.0 to 1.50 from the parental to the present generation. Desired family size by respondents fluctuated slightly around an average of 2.46 throughout all studies. This average included those respondents wanting zero children, whereas, the parental and present generation averages included only families with children. In the current study, 79 of 884 respondents wanted no children (Table 3). Excluding those data and using only those respondents wanting children, the average desired number became 2.69. This average is more valid for comparison with average family sizes of parental and present generations because in those generations only families who had children were included in the survey.

In the earlier studies (Table 1), average numbers of children in the present generation were about one child more per family than in

Table 2. Effect of gender composition of existing children on subsequent parities in families of the present generation at four time periods.

Gender Combination	Time periods and studies			
	1968–72[a]	1979–82[b]	1992–96[c]	Present Survey[d]
	% families with no further parities			
First Child				
Female		3.2	13.6	6.6
Male		6.7	11.6	5.6
Total		5	12.6	6.1
First two children*				
Female-female		19.8	38.4	45.7
Male-male		26.8	42.8	54.3
Same gender	32.4	23.3	40.6	50
Different gender	38.4	30	50	55.2
Total	35.4	26.6	45.3	52.6
First three children*				
Female-female-female		36.6	50.8	38.4
Male-male-male		34.7	62.1	61.5
Same gender		35.6	56.4	50
Different gender		37.7	54.6	74.4
Total		36.6	55.5	62.2

[a] Loyd and Gray (1969) and Gray (1972).
[b] Gray (1982).
[c] Call and Gray (1996).
[d] Present survey.
* $P<0.05$ that increases in family size are independent of composition of sexes of existing children.

the projected generation. In the later studies, averages for the present (2.58) and desired (2.45) generations were closer, suggesting that parents were approaching satisfaction with the number of children per family. Both of these numbers are higher than the 2.1 children per family considered necessary for replacement level (Mutharayappa et al. 1997). Secondary sex ratios (males:100 females at birth) ranged from 97:100 to 108:100 for the parental and present generations (Table 1) and were within ranges reported for other populations (Stern 1960). However, calculated sex ratios for respondents' desired family sizes, combinations, and permutations of sexes of children resulted in consistently wider ratios ranging from 133 to 141 males:100 females.

The effect of gender composition of existing children on subsequent parities within families was relatively consistent for the four time periods (Table 2). Whether the first child was female or male had no consistent impact on further births. Gender composition of the first two and three children influenced subsequent parities in two ways. First, there was evidence of preference for both genders. When both genders were present in the first two children, approximately 6% fewer families had additional children. In the present study more families ceased having children when both genders were represented in the first three children. Second, there was evidence of preference for males. When same gender was separated into female-female and male-male, approximately 6% fewer families had additional children when the first two were male-male than female-female. In the 1992–96 period, approximately 11% (62.1 vs. 50.8) more families ceased having children when the existing three were all males than when all were females. In the 2007 survey, approximately 23% (61.5 vs. 38.4) more families ceased having children when the existing three were males than when all were females.

Survey respondents were asked to indicate for their own desired families the number of children and the combinations and permutation of sexes (Table 3). Results of the present study and the previous studies showed that the most desired family consisted of two children, both genders, with the male born first and the female second. Approximately one-half of all the desired families were of this format. About one-fourth of the desired families were for three children. The most preferred three-child family consisted of one

Table 3. Desired family size, combination, and permutation of sexes of children in the projected generation at four time periods.

Number of children desired	Combinations/Permutations of sexes	Time periods and studies			
		1976[a]	1979–82[b]	1992–96[c]	Present Survey[d]
		% Total survey respondents			
0		7.5	7	3.6	8.9
1	One female	0.8	0.9	3.2	2.5
	One male	3	2.2	3	1.1
	Total	3.8	3.1	6.2	3.6
2	Two females	1	0.4	1.4	0.5
	Two males	4	4	7.7	7.2
	One female one male	*39.9*	*39.2*	*46.6*	*34.3*
	Permutations				
	Female-male	5.4	6.4	7	6.2
	Male-female	34.6	32.8	39.5	28.1
	Total	44.9	43.6	55.7	42
3	Female-female-female	0.4	0.2	0.3	0
	Male-male-male	1.2	2.4	1.2	1.2
	Two females one male	*2.8*	*6.1*	*4.4*	*4.6*
	Permutations				
	Female-female-male	0.5	0.6	0.7	0.7
	Female-male-female	1	2.2	2	1.8
	Male-female-female	1.4	3.4	1.7	2.1
	One female two males	18.5	16.8	17.6	18.8
	Permutations				
	Female-male-male	1.5	0.7	0.8	1.1
	Male-female-male	9.1	8.9	10.2	8.5
	Male-male-female	8	7.3	6.6	9.2
	Total	22.9	25.5	23.5	24.6
4*	Two females two males	11.8	13	5.9	10.3
	Permutation				
	Male-female-male-female	6.2	7.2	2.9	5.7
	Total	16.2	16	9.1	14
>4**		4.7	5	1.6	6.9

* For desired families of 4 children, data are presented only for the most preferred combination (2females 2males) and permutation (male-female-male-female).

** For desired families of more than 4 children, only the overall percent is presented.

[a] Gray and Morgan (1976).

[b] Gray (1982).

[c] Call and Gray (1996).

[d] Present Survey.

female and two males in a male-female-male order. When both genders were preferred in combinations, the first child was more frequently male. For families of 2, 3, and 4 children, combinations of both genders were preferred over combinations of same gender. For example, in 2-child families, the combination of one female and one male was preferred by approximately 40% of respondents over the time periods as compared with less than 10% who preferred either female-female or male-male.

Results of these studies also provide evidence of male or son preferences. In desired families (Table 3), male-male and male-male-male families were more frequent than female-female and female-female-female families; respectively. Preference for two males and one female greatly exceeded one male and two females in all studies. In present generation families, additional parities were less frequent when existing children were male-male and male-male-male than when female-female and female-female-female; respectively.

The overall secondary sex ratio for desired families was 141 males:100 females (Table 1). Analysis of the sex ratio data by order of birth for the first four parities showed strong preferences for the first-born to be male and

Table 4. Sex ratios by order of birth resulting from desired family size, combination, and permutation of sexes of children in the projected generation at four time periods.

Time periods/Desired family size	Order of birth			
	First	Second	Third	Fourth
	——males:100 females ——			
1976[a]				
1	454			
2	610	26		
3	761	104	118	
4	494	92	208	51
1979–82[b]				
1	300			
2	536	32		
3	681	98	99	
4	589	82	160	41
1992–96[c]				
1	94			
2	555	36		
3	513	83	122	
4	700	72	138	88
Present Survey[d]				
1	220			
2	528	23		
3	581	118	88	
4	771	60	128	71

[a] Gray and Morgan (1976).
[b] Gray (1982).
[c] Call and Gray (1996).
[d] Present Survey.

the second-born to be female (Table 4). The alternating male:female pattern continued through the third and fourth children.

Independence of gender outcome was examined by calculating linear correlation coefficients between genders of consecutive and nonconsecutive parities within families of the present generation. Coefficients for results of the present and previous studies were low in magnitude, variable in sign, and generally non significant. For example, in the first study, Loyd and Gray (1969) found a low but significant correlation ($r = 0.068$) between genders of successive children. However, the association between genders was not confirmed in the subsequent studies, including the present one in which correlation ($r = 0.007$) between genders of the first and second child was not significant. Given the preference for alternating gender of children in the desired families, positive correlations existed between genders of child one and three, two and four. Conversely negative correlations occurred between genders of child one and two, one and four, two and three, and three and four. Independence of gender ratios and family size was analyzed through comparisons of observed and expected binomial distributions for family sizes of one to four for the present generations. The observed sex ratio for each generation was used in the binomial expansion. Combinations of sexes within families of sizes one, three, and four occurred as expected based upon the binomial analysis. However, for families of size two, the calculated chi-square was 5.59 ($P < 0.07$). Examination of the observed and expected frequencies for the combinations (female-female, female-male, and male-male) revealed an excess of observed female-male and deficits in the female-female and male-male combinations, suggesting the possibility that preference for both genders is having an effect on the population. Agreement between observed and expected distributions of combinations of genders within families of various sizes was reported from other studies, including those by Loyd and Gray (1969) and Gray and Morrison (1974).

Given the dynamics of the human population, it is not feasible to study the same population over intervals of time. In the present series of studies, using college students provided a degree of constancy resulting from similarities in demographic backgrounds and educational pursuits. Although college students and their families are not typical of total society, college students usually represent completed families and are at the age to give serious thought to their own family preferences.

In these studies, average family size and sex ratio data were based upon birth data whether the fetus was live- or still-born. In contrast, census population data are based upon living persons regardless of age. The difference in methodologies created a level of incongruity in the results. For example, ongoing mortality would reduce family size. In the present study, family sizes for the parental generation (Table 1) were greater than four children per family. Census data (U.S. Census Bureau) for average family sizes in the United States and in Kentucky during these periods were approximately three children per family, or nearly one child less. Since proportionally

more males than females die during all stages of life (Stern 1960), the sex ratio shifts from an excess of males at birth to an excess of females in later life. Sex ratios for the present and parental generations in the present study generally indicated an excess of males (more than 100 males:100 females). Calculated sex ratios from census data (U.S. Census Bureau) for these periods, indicated an excess of females, with ratios ranging from 94 to 96 males:100 females.

In the present study, comparison of results for the time intervals was made difficult because the decadal intervals between studies were shorter than the approximate 20 to 25-year generation time.

Parental preference for specific combinations of genders within families was found to limit family size. The natural occurrence of these preferred combinations, both sexes and more males, was shown to decrease the likelihood of further parities. The preference for families consisting of two children with both genders would favor equal numbers and would support the established ratio of approximately 100:100. However, the male preference would distort the ratio resulting in more males and fewer females. Under natural conditions preference would impact additional children and would limit family size but likely would not significantly distort the sex ratio. Given the knowledge and technology, permitting parents to effectively obtain their gender preferences could result in an imbalance of the sex ratio. Imbalanced sex ratios are beginning to create social discord in countries with legal limits on family size (Qui and Mason, 2005).

Average family size and number of families determine the human population. During the span of these studies, average size decreased while the number of families increased resulting in the continued population growth.

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Organization and Evolution of Fibrillarin Gene Introns

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ABSTRACT

Eukaryotic genes possess introns that must be spliced out following transcription. The origin of introns remains an unanswered question, one that is characterized by two different views: either they are ancient and generally undergoing loss or they are the result of more recent gain. We studied the intron organization of the fibrillarin gene from the sequenced genomes of twenty-five species representing all four eukaryotic kingdoms. Results showed that more complex, multicellular species had generally larger numbers and lengths of introns. Twenty-two different intron positions were used by the 127 introns found collectively in the fibrillarin genes from the twenty-five species. Several of these were shared in species across multiple kingdoms, a finding that was interpreted to indicate that these introns were present in early eukaryotic ancestors, as predicted by the introns-early model. In the ten deuterostome species examined, the fibrillarin introns showed a high level of evolutionary conservation, and the variations that did exist were best explained by four independent intron losses and no intron gain. The fungi as a group, however, showed much greater variation in the fibrillarin intron patterns, a result consistent with extensive intron loss, more recent intron gain, or a combination of both.

KEY WORDS: Fibrillarin, genome evolution, intron evolution, gene structure

INTRODUCTION

One of the biggest surprises in biology in the last century has been the discovery of introns in the coding regions of eukaryotic genes. Although considerable attention has been given to intron structure and organization, it is still not clear how or when introns first appeared or what function they might have (Lynch and Richardson 2002; Rodriguez-Trelles et al. 2006). Because prokaryotic organisms lack introns, the question arises as to whether introns are ancient entities that have been weeded out of prokaryotes, the introns-early model (Gilbert 1987), or are more recent additions to eukaryotic genomes only and thus were never present in prokaryotes, the introns-late model (Logsdon et al. 1995). Each model has evidence that supports it, and the consensus seems to be that the answer is some of both: some introns may indeed be ancient, but recent addition of introns also has occurred. For example, Rogozin et al. (2003) analyzed the introns of 684 orthologous genes from nine genomes and found that many introns were conserved in several kingdoms, representing an estimated evolutionary time of 1.5 billion years. Using maximum parsimony phylogenetic analysis, these authors reported evidence of lineage-specific intron loss and gain, with gains generally outnumbering losses. In contrast, Roy and Gilbert (2005) applied maximum likelihood phylogenetic analysis to the same data set of 684 genes and arrived at a somewhat different conclusion that early eukaryotic ancestors were very intron-rich and the subsequent rate of intron loss has been substantially higher than the rate of gain. Neilson et al. (2004) studied the introns in four fungal genomes and concluded that intron losses and intron gains had occurred roughly equally over the last 330 million years of evolution. A study of the introns in the subtilisin genes of 15 fungal species yielded evidence for a mixed evolutionary history of both intron loss and gain (Wang et al. 2005).

In order to address the issues of intron organization and evolution, we conducted an analysis of the introns of the gene for the protein fibrillarin. This protein is highly conserved throughout eukaryotes (Christensen et al. 1986), and homologs also are found in Archaea (Amiri 1994). However, fibrillarin homologs have not been found in Bacteria. Fibrillarin has been shown to carry out essential functions in ribosome biogenesis via

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Table 1. Accession numbers from NCBI (GenBank) for the fibrillarin gene data for the 25 species used in this study.

Species	Accession Number
Homo sapiens (human)	NP_001427.2
Pan troglodytes (rhesus monkey)	XP_512658.2
Canis familiaris (domestic dog)	XP_533671.2
Bos taurus (cattle)	XP_581057.3
Mus musculus (mouse)	NP_032017.2
Rattus norvegicus (rat)	NP_001020814.1
Monodelphis domestica (opossum)	XP_001369001.1
Danio rerio (zebrafish)	NP_998167.1
Ciona intestinalis (sea squirt)	288781*
Strongylocentrotus purpuratus (sea urchin)	XP_001175797.1
Drosophila melanogaster (fruit fly)	NP_523817.1
Anopheles gambiae (mosquito)	XP_309401.3
Apis mellifera (honeybee)	XP_624378.1
Caenorhabditis elegans (nematode)	NP_506691.1
Caenorhabditis briggsae (nematode)	gi:39583363
Neurospora crassa (ascomycete mold)	XP_961478.1
Cryptococcus neoformans (basidiomycete)	XP_567779.1
Coprinopsis cinerea (basidiomycete)	EAU80700.1
Aspergillus fumigatus (ascomycete mold)	XP_752792.1
Ustilago maydis (basidiomycete)	XP_759951.1
Arabidopsis thaliana (thale cress)	AF265547
Oryzae sativa (rice)	NP_001054787.1
Theileria parva (apicomplexan)	XP_764432.1
Plasmodium falciparum (apicomplexan)	XP_001348241.1
Dictyostelium discoideum (slime mold)	XP_646371.1

* DOE Joint Genome Institute *Ciona intestinalis* database protein ID.

interactions with many small nucleolar RNAs (Tollervey et al. 1993). On the assumption that this ribosomal processing machinery is a remnant of pre-biotic cellular chemistry, it is presumed that fibrillarin is an ancient protein that, together with the snoRNA machinery, has been lost in the Bacteria. If these assumptions were true, fibrillarin should be an excellent model gene to look at patterns of intron evolution in eukaryotes.

The goals of this project were to determine the number, size, and distribution of fibrillarin introns in species that have had their genomes sequenced, to determine the positions of the introns in relation to the fibrillarin amino acid sequence, and to examine these data in light of the introns-early and introns-late models. If the introns-early model were correct, evidence of conserved fibrillarin intron positions in widely divergent taxa should be found, and variation in intron numbers should be attributable to intron loss. In contrast, if the introns-late model were correct, evidence of conserved intron positions across divergent taxa should not be found, while evidence of recent intron gain should be present.

MATERIALS AND METHODS

Fibrillarin gene sequences were retrieved from the National Center for Biotechnology Information (NCBI) databases accessible at www.ncbi.nih.nlm.gov. Data were collected from 25 species whose sequenced and annotated genomes were available as of June, 2007 and whose fibrillarin genes contained one or more introns (Table 1). The 10 species of yeasts for which no fibrillarin introns were present were *Candida albicans*, *Candida glabrata*, *Debaryomyces hansenii*, *Encephalitozoon cuniculi*, *Kluyveromyces lactis*, *Pichia stipitis*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Yarrowia lipolytica*. The four species of protists for which no fibrillarin introns were present were *Cryptosporidium hominis*, *Giardia lamblia*, *Leishmania infantum*, and *Trypanosoma cruzi*. All species have a single ortholog for fibrillarin except for the plant *Arabidopsis thaliana* where there are two genes, products of an ancestral genome duplication (Vision et al. 2000). Both genes have the same intron/exon pattern, therefore only one (FIB1) was used in this analysis. Multiple sequence alignments

were conducted using CLUSTAL W (Thompson et al. 1994) with the default settings.

RESULTS

Numbers of Introns in Eukaryotic Fibrillarin Genes

The highest numbers of introns, 7–9, were found in the deuterostome animals, with the next highest, 6, found in the two plant species (Table 2). Simpler animals and fungi had an intermediate number, 2–5, while several protists only had one intron. An exception was the protistan parasite *Theileria parva* whose fibrillarin gene had four introns. The 10 species of yeasts and four additional species of protists were found to have fibrillarin genes with no introns. The general pattern found was that the more complex multicellular organisms had larger numbers of introns, while the simpler unicellular organisms had fewer or none at all.

Positions of Introns in the Fibrillarin Gene Sequence

Determination of the specific positions of introns, in relation to the fibrillarin amino acid sequence, revealed 22 different sites collectively among the 127 introns found in the 25 species examined (Figure 1). The intron positions were designated A through V, beginning with the amino terminal end (Figure 1). Because an intron position is precisely defined by its phase relative to amino acid codons in the DNA (phase 0 introns are inserted between adjacent amino acid codons, phase 1 introns are inserted after the first nucleotide of a given codon, and phase 2 introns are inserted after the first two nucleotides of a codon), introns from two or more species were considered to have the same position only if they occurred at the same amino acid(s) and had the same phase (Figure 1). A less precise definition was applied for the introns designated B or C, which occur within the amino terminal glycine/arginine-rich (GAR) domain of fibrillarin. This region is poorly conserved, has simple repetitive sequences, and generates an alignment with multiple gaps. As such, it was not possible to clearly define whether introns found in this region in two different species' fibrillarins were in fact at the same position or not. An intron in the N-terminal portion of the GAR domain was arbitrarily designated B, while one toward the C-terminal portion was designated C. Most fibrillarins contained only a single GAR domain intron; however, three species were found to have two GAR domain introns and, in such cases, the introns were designated B and C, respectively.

Within the fifteen animal kingdom species studied, nine different intron positions were seen (Table 2). The few intron positions found in the protostome species represented a subset of the larger set (all nine) found in the deuterostome group. Of the nine intron positions found in the deuterostome group, five (A, F, K, N, and Q) were present in all ten of the deuterostome species (Table 2). Furthermore, four (A, F, K, and Q) of the six intron positions found in plants were identical to four found throughout the deuterostomes. The same four positions were found in the honeybee, *Apis mellifera*.

The fungal kingdom was more divergent. Within the five species examined, twelve different intron positions were seen, and eight of these were only seen in fungi, six were present in only one of the fungal species, respectively. Two of the fungal intron positions were the same as those found in animals and plants (A and K). Several fungal introns lay within the GAR domain.

The plant kingdom only had two species in this analysis, one a eudicot and the other a monocot. Both had the same six intron positions conserved (A, F, I, K, O, Q). Protists generally had one or no introns in the fibrillarin genes. As noted above, the fibrillarin gene of *Theileria parva* contained four introns. Three of the positions were unique to this organism (J, L, and R), while one position (F) also was seen in animals and plants.

Several common intron positions were seen in species from different kingdoms. Intron Q was found in both animals and plants; intron K was found in animals, fungi, and plants; intron F was found in animals, plants, and protists; and intron A was found in all four of the kingdoms. Higher animals shared more common intron positions with plants (four) than with any other divergent groups, including the insects and nematodes to which they are more closely related.

Table 2. Intron data for the fibrillarin genes from 25 eukaryotic genomes. Individual intron positions within the amino acid sequence are named using an alphabetical system beginning with the N-terminus. Intron A of *Bos taurus* has an annotation error in GenBank such that its length could not be determined, however it is present (y).

Species (Introns Present)	No. of Introns	Intron Designation (Phase)																			
		A (1)	B(1)	C(1)	D (0)	E (1)	F (0)	G (0)	H (0)	I (0)	J (1)	K (0)	L (0)	M (1)	N (1)	O (0)	P (1,2)	Q (0)	R (1)	S (1)	T (1)	U (1)	V (2)
ANIMALS																							
Homo sapiens (A,C,E,F,K,N,Q,V)	8	5503		101		86	1027					1191			1042			1742					105
Pan troglodytes (A,C,E,F,K,N,Q,V)	8	5097		101		86	998					1199			1039			1744					105
Canis familiaris (A,C,E,F,K,N,Q,V)	8	4997		114		90	1243					896			1016			1255					109
Bos taurus (A,C,E,F,K,N,Q,V)	8	y		89		88	989					2907			1008			717					84
Mus musculus (A,C,E,F,K,N,Q,V)	8	4685		98		91	776					1117			929			580					87
Rattus norvegicus (A,C,F,K,N,Q,V)	7	4120		99			826					1254			949			573					101
Monodelphis domestica (A,C,E,F,K,N,Q,V)	8	989		129		161	474					433			517			98					124
Danio rerio (A,B,E,F,K,N,Q,V)	8	1711	1953			95	730					716			78			86					1778
Ciona intestinalis (A,B,C,F,K,N,Q)	7	242	67	177			268					105			162			57					
Strongylocentrotus purpuratus (A,B,C,E,F,K,N,Q,V)	9	2400	1045	576		1042	742					692			1688			1562					1625
Drosophila melanogaster (A,F)	2	118					381																
Anopheles gambiae (A,F)	2	288					292																
Apis mellifera (A,F,K,Q)	4	445					86					324						106					
Caenorhabditis elegans (A,B,N)	3	48	47												47								
Caenorhabditis briggsae (A,B,N)	3	50	44												42								
Animal Total	93																						
FUNGI																							
Neurospora crassa (B,C,G,P,T)	5		236	62				61									58				61		
Cryptococcus neoformans (C,H,M,S)	4			60					59					52						57			
Coprinopsis cinerea (K,M,U)	3											80		62								68	
Aspergillus fumigatus (B,D)	2		185		62																		
Ustilago maydis(A,P)	2	113															65						
yeast species (10)	0																						
Fungi Total	16																						
PLANTS																							
Arabidopsis thaliana (A,F,I,K,O,Q)	6	249					82			96		97				671		98					
Oryza sativa (A,F,I,K,O,Q)	6	80					318			144		36				1284		35					
Plant Total	12																						
PROTISTS																							
Theileria parva (F,J,L,R)	4						33				30		29						31				
Plasmodium falciparum (A)	1	286																					
Dictyostelium discoideum (A)	1	364																					
Other protist species (4)	0																						
Protist Total	6																						
TOTAL INTRONS	127																						

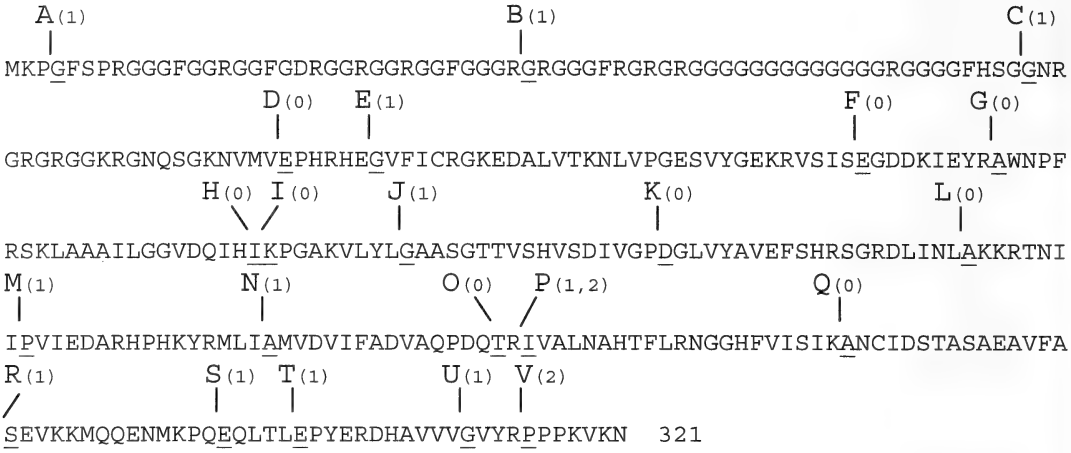

Figure 1. The collective fibrillarin intron positions found in 25 eukaryotic genomes. The human fibrillarin amino acid sequence was arbitrarily chosen as a guide sequence. Intron insertion positions lie in front of (for phase 0 introns) or within (for phase 1 or 2 introns) the codon of the underlined amino acids and are named alphabetically beginning at the N-terminus. Intron phases are given in the parentheses after each intron.

Lengths of Introns in the Fibrillarin Genes

Introns varied from a low of 29 base pairs in *T. parva* to a high of 5,503 base pairs in humans (Table 2). The introns of deuterostome animals were on average the longest and showed the greatest variation in length, while fungi and protists (albeit a small sampling) had very short introns with less length variation. Twelve of the sixteen fungal introns averaged 60 ± 4.0 base pairs each, while the four introns of *T. parva* averaged 30 ± 1.7 base pairs. Introns found at the same position in multiple species also generally exhibited great variation in length. For example, intron F, present in animals, plants and one protist, varied from a low of 33 base pairs in the protist to a high of about 1000 base pairs in mammals.

Evidence of Intron Loss but not Intron Gain in the Fibrillarin Genes of Deuterostomes

Because a fairly large number and diversity of deuterostomes (ten, including vertebrates, invertebrate chordates, and echinoderms) have had their genomes sequenced, this clade presented a good opportunity to examine whether the fibrillarin intron patterns revealed evidence of loss and/or gain over the course of about the last 670 million years, since the estimated protostome-deuterostome split (Ayala et al. 1998). Phylogenetic analysis using parsimony supported a pattern of fibrillarin intron evolution among the ten deuterostome species examined that involved four intron losses and no gains (Figure 2). The sea urchin *Strongylocentrotus* retained the same nine introns presumed to be in the

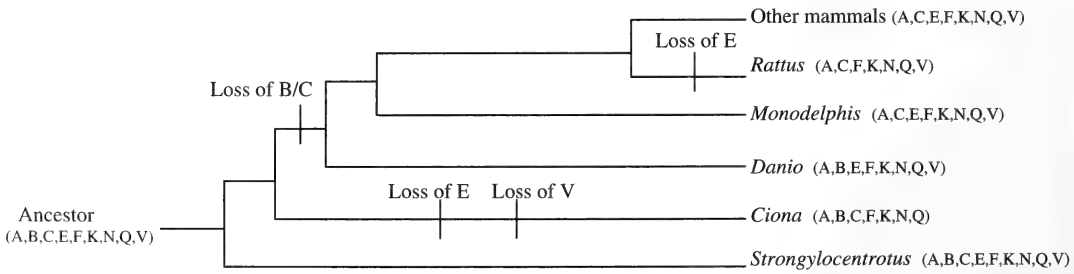

Figure 2. Cladogram showing the most parsimonious model for the evolution of the fibrillarin intron patterns in the deuterostome species. "Other mammals" includes human, chimpanzee, dog, cow, and mouse, all of which have identical intron patterns. Letters in parentheses refer to introns present.

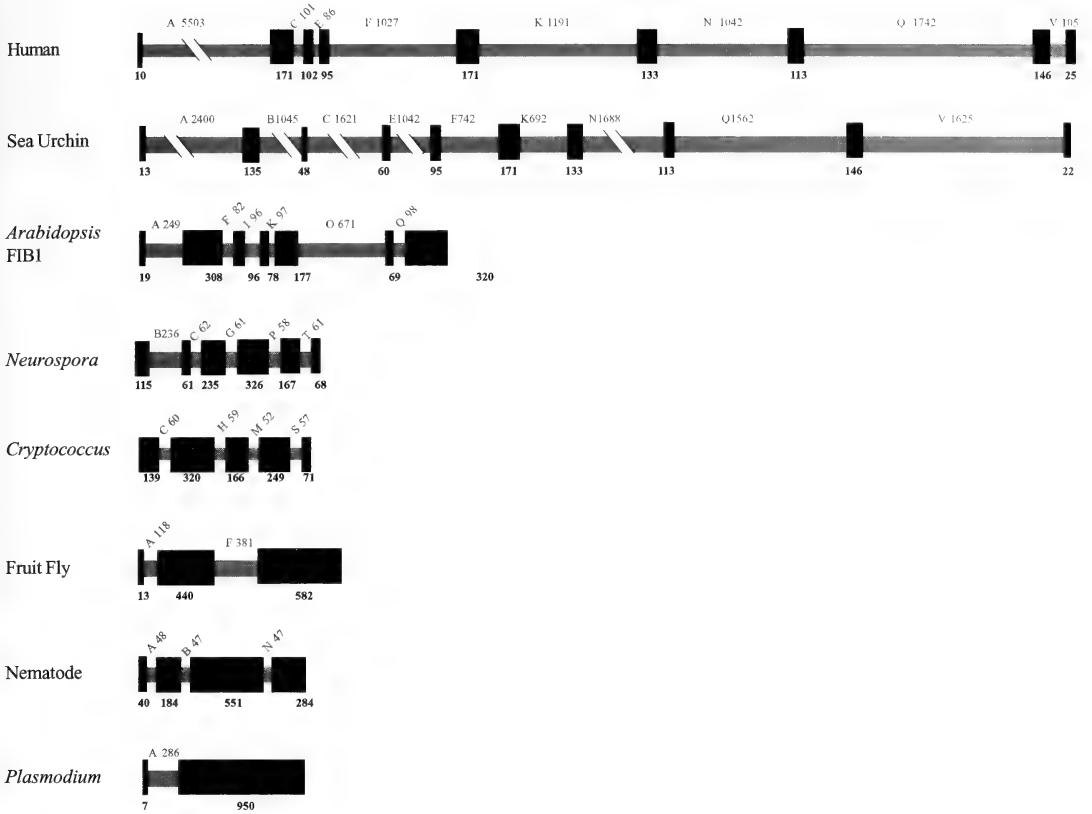

Figure 3. Comparative fibrillarin intron-exon organization from eight representative species. Exons are designated by wide bars, while introns are designated by narrow bars. Letters A–V refer to sequential intron positions beginning with the amino-terminal end of the coding region. Accompanying numbers refer to exon and intron lengths, respectively.

common ancestor. A loss of one of the two ancestral GAR domain introns (B or C) occurred early in the vertebrate lineage, losses of introns E and V occurred in the sea squirt *Ciona*, and an independent loss of intron E occurred in rat. No evidence of gains was found with this data set.

DISCUSSION

Our results permit us to draw several conclusions about the introns found in the fibrillarin genes and relate these to the larger question of the origins of introns. First, there are greater numbers of introns in the fibrillarin genes of more complex organisms than seen in simpler organisms. Multicellular species invariably have several introns in the fibrillarin gene, whereas many unicellular species have none or only one (Figure 3). This is particularly apparent in the fungi where the five multicellular species all had fibrillarin introns, whereas ten unicellular yeast species all lacked fibrillarin introns. This observation is consistent with what has been reported in general for eukaryotic introns, i.e., the genes of increasingly complex organisms exhibit more introns than those of less complex organisms (Roy and Gilbert 2007). The introns-early model accounts for this by the gradual loss of pre-existing introns (in the last universal common ancestor, LUCA) over evolutionary time such that simpler, rapidly-reproducing species lost most or all introns due to genome streamlining, whereas more complex, slowly-reproducing species retained more introns (Roy and Gilbert 2007). The introns-late model accounts for this by suggesting that introns entered early eukaryotic genomes via lateral gene transfer and have been gained disproportionately in the multicellular lineages (Palmer and Logsdon 1991; Koonin 2006).

Second, although intron positions appear to be relatively stable (for example, throughout

the ten deuterostome species or the two plant species), the lengths of the introns at a given position are highly variable but, in general, tend to be shorter in less complex species (Figure 3), a pattern observed generally throughout eukaryotes (Vinogradov 1999). Assuming that introns at the same position are homologs, there must be a mechanism whereby the lengths can change rather quickly (Parsch 2003). In several groups of species it was noted that the introns exhibit a characteristic, minimal length. For example, the nematode fibrillarin genes consistently have introns about 45–50 base pairs long, the fungal fibrillarin introns are around 60 base pairs long, and the protist, *Theileria*, fibrillarin introns are all about 30 base pairs long. We interpret these sizes to reflect the minimal lengths that an intron can be in order for the splicing machinery within a particular group of species to still be functional (Parsch 2003; Kupfer et al. 2004).

Third, several of the fibrillarin introns likely were present in an early ancestor of eukaryotes, consistent with the introns-early model (Gilbert 1987; Roy and Gilbert 2007). Of the 22 fibrillarin gene intron positions found throughout the 25 species examined, four of the positions (A, F, K, and Q) have introns in one or more species in at least three kingdoms, and intron A is found in all four kingdoms. In general, the number of different intron positions used in fibrillarin (22) is low compared with the total number of introns present (127) in the 25 species, a finding also consistent with an introns-early model characterized by inheritance of pre-existing introns combined with gradual loss (Roy and Gilbert 2007). The pattern of fibrillarin introns seen in the ten deuterostome species supports the idea in that it is most parsimoniously explained by a common ancestor with nine introns (as in the sea urchin *Strongylocentrotus*), and descendants having undergone four separate intron loss events with no gains. This interpretation is further supported by evidence of sequence homology seen in the deuterostome introns with the degree of sequence similarity correlating with the degree of evolutionary relatedness among the species (data not shown). A similar pattern of extensive loss with no gain has been reported for mammalian genes in general (Coulombe-Huntington and Majewski 2007). Analysis of the recently determined sequence of a sea anemone genome led to the conclusion that ancestral eumetazoan genes were intron-rich, revealing intron patterns often highly conserved during the evolution of animals (Putnam et al. 2007). Therefore, the pattern of intron loss, coupled with little or no gain, seen in the fibrillarin gene in the deuterostomes is not peculiar to this gene, but appears to be a pattern common to many genes.

Fourth, plant and animal fibrillarins have an unusually high number of intron positions in common (four: A, F, K, and Q), higher than the number expected on the basis of their phylogenetic distance alone. We are not the first to observe this; it appears to be a common feature of many genes of humans and *A. thaliana* (Rogozin et al. 2003). An explanation for this finding is not obvious, but assuming it is not due to parallel gain (Sverdlov et al. 2005), the simplest conclusion is that the common ancestor of the plant and animal lineages possessed the four introns the descendants have in common. Because animal-plant divergence is estimated to have occurred about 1.6 billion years ago (Wang et al. 1999), these four introns must have been present at least by that time. Furthermore, the fibrillarin gene from rice (*Oryza sativa*), a monocot, contains the same six introns as the *A. thaliana* fibrillarins. This suggests that the gene has not gained or loss introns in these lineages since the monocot-eudicot split, about 200 million years ago (Paterson et al. 2004).

Fifth, the fibrillarin genes of the five representative species from the fungal kingdom show a more variable pattern of introns as compared with animals and plants. Of the 16 introns present collectively in the fungal fibrillarins, 12 different intron positions are utilized, and eight of these are positions only seen in the fungi. If the introns-early model is correct, then fungi have diverged substantially from plants and animals, and even from each other. Such an interpretation would require significant, differential intron loss within the fungal lineages, relative to the animals and plants. A higher level of intron loss than intron gain has been reported for fungi (Carmel et al. 2007). A different interpretation is that substantial intron gain might have taken place preferentially in the fungi, subsequent to their split from the animals

and plants. In this way, intron gain could account for the large number of unique positions seen in the fungi (Nielsen et al. 2004).

Paralogous genes, resulting from ancestral gene duplications, are useful in establishing the ancestral state such that changes in one paralog but not the other in a descendant species most likely occurred after the time of the duplication. However, the study of intron patterns of paralogous genes in several different diverse lineages has generated conflicting results; Babenko et al. (2004) concluded that intron gains outnumber losses, while Roy and Penny (2007) concluded that losses outnumber gains. The differences are attributed to the specific analytical tests used. *Arabidopsis thaliana* has a large number of paralogs due to major genome duplication events (Vision et al. 2000), but the two *A. thaliana* paralogs of fibrillarin have undergone no change in their intron/exon structure. This is consistent with a recent study of over 2500 paralogs of *A. thaliana* that found that about 95% of the genes had not changed in intron structure since the time of the duplication (Knowles and McLysaght 2006).

A current limitation in this study is the availability of genomes equally representative of the diversity of life on earth. The animal group, particularly mammalian, has a disproportionately high number of genomes completed because of sequencing priorities. In contrast, the plant and fungal groups (and lower animal phyla) are less represented at the current time. As more data become available on biologically diverse and interesting genomes, it should be possible to fill in many of the gaps in our understanding of intron structure, organization and evolution.

In summary, the patterns of introns in the fibrillarin genes of eukaryotic species presents a complex picture that is not easily interpretable within the context of the introns-early or the introns-late concepts. However, the fibrillarin intron pattern reported here tends to favor the introns-early model in that some of the introns were apparently present in the earliest eukaryotic ancestors, many introns have been stably inherited over reasonably long evolutionary time, a small number of specific intron positions are used relative to the total number of introns present, and evidence for specific intron loss, but not intron gain, is documented in one group.

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LITERATURE CITED

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NOTES

Insect Prey Consumed by a Bachelor Colony of Virginia Big-Eared Bats, *Corynorhinus townsendii virginianus* Handley, in Lee County, Kentucky—The federally endangered Virginia big-eared bat occurs in Kentucky in a contiguous ten county region on the Cumberland Plateau (Adam et al. 1994). The objective of this study was to supplement natural history information on this species in Kentucky by documenting the food habits of a bachelor colony in Lee County. Sampling occurred in a cave with a known history of occupancy by *C. t. virginianus*. Because the cave in question serves as a hibernaculum for both female and male Virginia big-eared bats, it represents a critical piece of habitat for *C. t. virginianus* and shall be referred to only as the "study cave". Topography in the vicinity of the study cave is mountainous to hilly with steep mountainsides covered by mixed mesophytic forest (Burford and Lacki 1998).

To confirm the cave was being used by *C. t. virginianus* during the study period, the senior author conducted emergence counts prior to each sampling visit. Whitaker (1987) suggested fecal pellet analysis was a reasonable way to assess bat food choices, particularly if the animals were rare or endangered. Fecal pellets deposited by male Virginia big-eared bats were collected from cloth sheets placed on the floor of the study cave (Bauer 1992). Collection dates were 1 June; 17 and 27 July in 1990 and 18 May, 7 June, 5 July, and 12 August in 1991. All fecal pellets collected during a sampling visit were pooled. Thirty subsamples (3 fecal pellets per subsample) were taken from each pooled sample and analyzed following the procedure of Whitaker (1987). Prey items in the 210 fecal pellet subsamples were identified to Order. Dietary results were expressed as frequency of occurrence.

Identified food items attributable to the Order Lepidoptera (moths) and Coleoptera (beetles) occurred with the highest frequencies (Table 1). Other arthropod orders identified included Araneida (spiders) Orthoptera (crickets, roaches, katydids), Diptera (true flies), Hymenoptera (ants, wasps, bees), and Hemiptera (true bugs) (Table 1). The preponderance of moths in the diet of Kentucky *C. t. virginianus* supports similar trends noted in the vicinity of our sampling area (Burford and Lacki 1998) and other feeding studies within the range of this species (Dalton et al. 1986; Clark 1991; Sample and Whitmore 1993).

Table 1. Frequency of occurrence for food items identified in Virginia big-eared bat fecal pellets from Lee County, KY.

	Sampling Periods					
	1991		1991			
	June	July	May	June	July	August
CLASS INSECTA						
Lepidoptera	90	100	100	100	100	100
Coleoptera	55	77	33	67	60	47
Orthoptera	13	3				3
Diptera	3	3			3	3
Hymenoptea			3			
CLASS ARACHNIDA						
Araneida	10	10	3	3	3	

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NOTES

Six Noteworthy Vascular Plants for Kentucky: Two State Records and Four Range Extensions—Since the authoritative work of Jones (2005), *Plant Life of Kentucky: An Illustrated Guide to the Vascular Plants*, recent collections and a herbarium search have documented two new vascular plants for the Commonwealth of Kentucky and additional county record range extensions for four other taxa. Four of these plants were discovered in the Berea College Forest of Madison and Rockcastle Counties. All representative vouchers have been deposited at the Berea College Herbarium (BEREA) with available duplicates on file at Appalachian State University Herbarium (BOON) and Eastern Kentucky University Herbarium (EKY). The precise label information for each species from herbarium specimen(s) is presented with the significance of the report for Kentucky. Nomenclature and vernacular names follow Weakley (2008).

Aphanes microcarpa (Boiss. & Reut.) Rothm. (Rosaceae)—**Calloway County**: Hancock Biological Station, 561 Emma Drive, Murray, mowed yard in front of Main Laboratory Building in brown silty loams, latitude 36°44′2.9″N and longitude 88°06′58.0″W and 122 m. elev. in the Rushing Creek Quadrangle. Associates: *Cardamine hirsuta* L., *Krigia caespitosa* (Raf.) K.L. Chambers, *Kummerowia stipulacea* (Maxim.) Makino, *K. striata* (Thunb.) Schindler, *Plantago lanceolata* L., *P. rugelii* Descne, *Sherardia arvensis* L., *Taraxacum officinale* G.H. Weber ex Wiggers, *Trifolium dubium* Sibth., and *T. repens* L. Occasional, naturalized annual from southern Europe; 06 June 2006; *Derick B. Poindexter 06-152* (BEREA, BOON).

Significance. Thompson (2007) reported Slender Parsley Piert from the culturally disturbed habitat at the Hancock Biological Station of Murray State University in western Kentucky. Based on the Poindexter collection, it represents only the second documented county in Kentucky. Abbott et al. (2001) collected this exotic plant for the first time in Madison County in 1992. Browne and Athey (1992) and Medley (1993) did not list *Aphanes microcarpa* for Kentucky. It is included in Jones (2005) based on Abbott et al. (2005) and was mapped by Campbell and Medley (2006) and the USDA, NRCS (2008) from Abbott's Madison County state record. Habitats for this often overlooked inconspicuous annual include lawns, waste places, fields, pastures, and roadsides (Gleason and Cronquist 1991; Weakley 2008).

Erysimum capitatum (Douglas ex Hook.) Greene var. ***capitatum*** (Brassicaceae)—**Madison County**: Berea, Berea College Forest, near Brushy Fork Creek in an open grassy field with sandy shaley soils; latitude 37°33′46.0″N, longitude 84°17′26.0″W, and elev. 307 m., Berea Quadrangle. Associates: *Barbarea vulgaris* R. Br., *Cardamine hirsuta* L., *Erigeron philadelphicus* L., *Galium aparine* L., *Geranium carolinianum* L., *Lamium purpureum* L., *Poa pratensis* L., *Schedonorus arundinaceus* (Schreb.) Dumort, and *Solidago canadensis* L. Scarce (7–10 scattered plants) from unknown seed sources: 15 May 2008. *Ralph L. Thompson 08-296* (BEREA, BOON, EKY), *Glen E. Dandeneau 08-02* (BEREA).

Significance. This is the first report of Western Wallflower for the Commonwealth of Kentucky. It was not listed in Browne and Athey (1992), Jones (2005), Medley (1993), or Campbell and Medley (2006). *Erysimum capitatum* var. *capitatum* is a widespread native biennial or short-lived perennial of the western United States. It is mapped eastward to KS, OK, TX, AR, IA, WI, MI, all the contiguous states to Kentucky, and Maryland by the USDA NRCS (2008). The Great Plains Flora Association (1986) reported it from MN, ND, SD, and NE, but it was not mapped in these states by the USDA, NRCS (2008). Habitats include open slopes, calcareous and shale rocky outcrops, road cuts, and open wooded hillsides (Great Plains Flora Association 1986; Weakley 2008).

Hosta ventricosa (Salisb.) Stearn (Hemerocallidaceae)—**Rockcastle County**: John B. Stephenson Memorial Forest State Nature Preserve within the Berea College Forest, 1.8 mi N of Disputana and 0.9 mi ENE on Anglin Falls Road; latitude 37°29′33.2″N, longitude 84°13′31.0″W, and 247 m elev., Johnetta Quadrangle. Infrequent, three large colonies growing in sandy loams of the Anglin Creek floodplain. Associates: *Agrimonia parviflora* Aiton, *Carex lurida* Wahlenb., *Conoclinium coelestinum* (L.) DC., *Dichanthelium clandestinum* (L.) Gould, *Impatiens capensis* Meerb., *Lobelia cardinalis* L., *L. siphilitica* L., *Microstegium vimineum* (Trin.) A. Camus, *Sanicula odorata* (Raf.) Pryer & Phillippe, and *Solidago canadensis* L. Scarce, East Asian perennial; 17 July 2004. *Ralph L. Thompson 04-920* (flower) (BEREA, EKY); 8 September 2007, *Ralph L. Thompson and Edward W. FitzGerald 07-365* (fruit) (BEREA). **Lee County**: Riparian habitats along Little Sinking Creek; Little Sinking Creek Road, ca. 0.1 mi NE of jct. with Ky 1036; N of Greely, KY. Associates: *Alnus serrulata* (Aiton) Willd., *Asimina triloba* (L.) Dunal, *Cornus alternifolia* L. f., *Pyrularia pubera* Michx., and *Staphylea trifolia* L. Colony of 12–14 crowns on low sloping creek bank; 30 June 1996. *T. J. Weckman, J. C. Weckman, J. E. Rozeman 2920* (EKY).

Significance. Blue Plantain Lily was not reported for Kentucky by Jones (2005) or Browne and Athey (1992). Medley (1993) listed it at edges of mesic woods in Lee and Wolfe Counties of the Cumberland Plateau. Campbell and Medley (2006) mapped these two counties based on two collections from old home sites and gardens. Because it is readily grown as an ornamental, they stated, "it does not seem generally able to become naturalized." These were the same two counties mapped by the USDA, NRCS (2008) and was the basis for state recognition in FNA (2002).

The new population on the Northern Cumberland Plateau from Rockcastle County was composed of three

colonies over 1.0 m × 1.5 m, each on a wet meadow floodplain of Anglin Creek. We believe the Blue Plantain Lily had reproduced from seed and consider these populations to have been locally naturalized. The 1996 collection from Little Sinking Creek being the second report from Lee County, was from a habitat similar Anglin Creek, and these plants were viewed "as naturalized" (T.J. Weckman, pers. comm., 26 May 2008.). The available evidence suggests that *Hosta ventricosa* has become established at several sites in the state and should now be considered as a member of the naturalized flora of Kentucky. Thompson and Fleming (2004) did not list it in the vascular flora of Anglin Falls Ravine because it was not present at the time collections were made in 1997–1998. Habitats include disturbed open areas, although it is frequently grown in anthropogenic settings as a shade ornamental (FNA 2002; Weakley 2008).

Nuttallanthus canadensis (L.) D.A. Sutton (Plantaginaceae)—**Madison County**: Berea, Berea College Forest, Cross-Country Trail, 0.2 mi past foot bridge crossing to the east side of Brushy Fork Creek between open area of blackberries and Virginia pine copse adjacent to an old field, latitude 37°33′50.6″N, longitude 84°17′29.4″W, and elev. 305 m., in the Berea Quadrangle. Associates: *Acer rubrum* L., *Dichanthelium acuminatum* (Sw.) Gould & C.A. Clark, *Packera anonyma* (Alph. Wood) W.A. Weber & A. Löve, *Pinus virginiana* Mill., *Potentilla simplex* Michx., and *Rubus argutus* Link. Rare; 24 May 1997. *Rudy A. Gelis 310* (BEREA).

Significance. Canada Toadflax is documented for the first time in Kentucky based on Jones (2005), Medley (1993), Campbell and Medley (2006), and USDA, NRCS (2008). Although reported for Kentucky by Browne and Athey (1992), their inclusion is based on a misidentified specimen. Jones (2005) stated that this plant, "is to be expected." This widespread annual/biennial has been mapped throughout the eastern United States, except for Kentucky, by the USDA, NRCS (2008). Habitats are quite varied and include relatively dry or sterile sandy, loamy soils, ruderal roadsides and meadows, as well as more natural areas such as thin soils over rock outcrops (Gleason and Cronquist 1991; Weakley 2008).

Sagina procumbens L. (Caryophyllaceae)—**Boone County**: First rest stop south of Florence, KY, on I-75 at mile marker 176; latitude 38°56′19.95″N, longitude 84°38′1.30″W; and elev. 296 m, Union Quadrangle. Individuals were found growing in brick sidewalk crevices surrounding the main rest stop building and entranceway. Associates: *Bryum argenteum* Hedw., *Chamaesyce maculatum* (L.) Small, *Cyperus squarrosus* L., *Plantago major* L., and *Poa annua*. Frequent (100+ plants), native perennial; 7 April 2007. *Derick B. Poindexter 07-155* (BOON), *Ralph L. Thompson 07-299* (BEREA).

Significance. This is the first published record of Northern Pearlwort from Boone County and only the second report for Kentucky. Prior indications of this plant in Kentucky were based on Medley (1993) and Campbell and Medley (2006) who listed a record from Bracken County and an ambiguous listing for Kentucky by the PLANTS Database (USDA, NRCS 2008). *Sagina procumbens* was not accredited to Kentucky in Browne and Athey (1992) and FNA (2005) although it was listed as "to be expected" by Jones (2005).

The perennial habit, persistent basal leaves, and 4-merous perianth of this taxon distinguish it from its more widespread and sympatric congener, *S. decumbens* (Elliott) Torr. & A. Gray. The absence of *S. procumbens* in adjacent counties in Ohio (USDA, NRCS 2008) is enigmatic but a testament to the sporadic occurrence of this taxon to occupy weedy, moist habitats such as trail margins, pathways, parking areas, and crevices (FNA 2005; Weakley 2008).

Thermopsis mollis (Michx.) M.A. Curtis ex A. Gray (Fabaceae)—**Madison County:** Berea College Forest, Lower Silver Creek, an old-field adjacent to a *Pinus-Querons-Carya-Acer* stand, ca. 0.2 mi south of KY 21 and 1.9 mi east of Berea city limits: latitude 37°32′54.0″N, longitude 84°14′31.0″W, and elev. 305 m, Berea Quadrangle. Associates: *Andropogon virginicus* L., *Carex hirsutella* Mack., *Danthonia spicata* (L.) P. Beauv., *Houstonia caerulea* L., *Krigia biflora* (Walt.) S.F. Blake, *Packera anonyma* (Alph. Wood) W.A. Weber & A. Löve, *Potentilla simplex* Michx., *Rosa carolina* L., *Rubus flagellaris* Wild., *Tridens flavus* (L.) Hitchc Rare: a population of 25–35 flowering stems: 04 May 2007. *Ralph L. Thompson and John T. Perry 07-390* (BEREA).

Significance. Appalachian Golden-Banner is state-listed as "Endangered" by the KSNPC (2000). Our Madison County voucher is the second county recorded for the Commonwealth of Kentucky. This population is from one of two sites in the Berea College Forest, and "it represents a natural population that has not been planted" (John T. Perry, pers. comm., 04 May 2008). Browne and Athey (1992) and Jones (2005) list it for Kentucky. The first occurrence in Kentucky was from a 1982 specimen cited by Medley (1993) from Clay County. The Clay County specimen was the basis for this taxon being mapped in Kentucky by Campbell and Medley (2006) and USDA, NRCS (2008). Isley (1998) did not map it for Kentucky. This native perennial is found on a few rich sandy slopes to dry woods and ridges in the southern Appalachian region (Isley 1998; Weakley 2008).

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Abstracts of Some Papers Presented at the 2007 Annual Meeting of the Kentucky Academy of Science

Edited by Robert J. Barney

AGRICULTURAL SCIENCES

Performance of Pacific White Shrimp *Litopenaeus vannamei* Raised in Static Heterotrophic Tank Systems Exposed to Different Light Sources and Intensities. DAVID R. WOOD*, RUSSEL S. NEAL, BRIAN M. BOUDREAU, SHAWN D. COYLE and JAMES H. TIDWELL, Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601.

The market for shrimp has consistently shown high consumer demand. To support the development of inland shrimp culture in the United States, a trial was conducted to evaluate the effect of different light regimes on shrimp cultured in intensive heterotrophic systems. The trial used 3.8-m^3 polyethylene tanks with five light treatments: 1) Sunlight with natural diurnal cycle acting as control (midday - 718 lux); 2) one metal halide light (1074 lux); 3) one fluorescent light (214 lux); 4) two fluorescent lights (428 lux); and 5) three fluorescent lights (642 lux). There were three replicate tanks per treatment and each was separated by black plastic to prevent light contamination. Each tank was stocked with a density of 465 shrimp/m^2 at an initial mean weight of 0.4 grams. Tanks were harvested after 92 days and final average individual weight, percent survival, harvest yield, and FCR were determined. Light treatment had a significant impact ($P < 0.01$) on average individual weight, survival, harvest yield (kg/m^2), and feed conversion ratio. Differences in production parameters among shrimp in the Natural Light (control), Metal Halide, and 1-Fluorescent treatments were not significantly different ($P > 0.05$). However, there was a linear negative relationship ($P < 0.01$; $R^2 = 0.758$) between the number of fluorescent fixtures and harvest yield, survival, and feed conversion efficiency. Decreased survival in 2 and 3-Fluorescent fixture treatments appeared to be related to greater concentrations of filamentous bacteria in those tanks. Natural Light, Metal Halide, and low levels of fluorescent light appeared to inhibit, or did not promote, high concentrations of those bacteria.

A Spacing Calculator for Mixed Plantings. MICHAEL K. BOMFORD, Community Research Service, Kentucky State University, Frankfort, KY 40601.

The most appropriate way to compare mixed plantings to pure stands is a matter of debate among ecologists and agricultural scientists but a consensus has emerged that mixed plantings tend to be more productive. Few horticulture extension materials offer practical advice for designing mixed plantings. One exception, a popular series of gardening manuals, recommends that plant spacing in mixtures be set to the mean of monoculture spacing recommendations for component crops. In most cases this results in a lower plant density in mixtures than in segregated pure stands, reducing the likelihood of realizing a mixture yield advantage. An improved method for calculating mixture spacing is proposed that sets total plant density in mixed plantings equal to the combined density of segregated pure stands. This method is intended to allow meaningful comparisons of mixtures to pure stands, and increase the likelihood of realizing a yield advantage from mixed planting. The method derives mixture spacing recommendations from monoculture spacing and the proportion of the mixture accounted for by each crop. A mixture spacing calculator that uses the method is available online (http://organic.kysu.edu/Companion_spacing.html).

Evaluation of Alternative Bioethanol Feedstock Crops. JOHN A. RODGERS*, MICHAEL K. BOMFORD, CHANGZHENG WANG, BRIAN GEIER and ANTHONY SILVERNAIL, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Growing concern about foreign energy dependence has led to increased interest in biofuels as domestically-produced, alternative energy sources. The United States is now the world's largest producer of ethanol fermented from plant carbohydrates. More than 95% of this ethanol is currently made from the grain of corn (*Zea mays* L.) through a production process that consumes 75–90% as much energy as is available from the fuel. Other crops may offer superior energy returns, or be more compatible with sustainable production systems suitable for adoption by limited-resource farmers. We compared carbohydrate yield of corn, Jerusalem artichoke (*Helianthus tuberosus* L.), sweet potato (*Ipomoea batatas* L.), and sweet sorghum (*Sorghum bicolor* L.) grown organically in irrigated 8 m^2 plots in a completely randomized design with four replicates. Sweet potato and sweet sorghum, respectively, produced approximately 580 and 280% more carbohydrate per unit cultivated area than sweet corn. Sweet sorghum and sweet potato show potential as alternative biofuel feedstock crops, compatible with low input production systems.

Grain Yield Loss Due to Variation of In-row Spacing of Corn. TODD BALLARD*, MARVIN RUSSELL, ELMER GRAY, TODD WILLIAN and MARTIN STONE, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Uneven plant spacing within rows of corn (*Zea mays* L.) usually affects grain yield negatively. Average plant spacing is an incomplete index of density; plant distribution must also be considered. In 2007, relationships between corn yields and in-row spacings were examined using different indices of spacing (distance between

individual plants, average plant spacings, and standard deviations) on four producer managed plots and 27 researcher managed plots. Linear correlations between these indices and grain yield were calculated. Researchers at Purdue University (AGRY-94-02) used the statistical standard deviation, σ, to relate in-row spacing variability and grain yield. A linear model of loss for $\sigma > 2$ inches was proposed as $Y = Y0\text{-}a\sigma$ with slope varying from 2.5 to 5.0 bushels per acre depending on genotype and environment. For most plots, σ did not vary enough between rows to demonstrate a significant linear relationship. However, for one plot the correlation was -0.84 and significant; σ was 4.4 resulting in predicted yield loss of 26 Bu/A. A more comprehensive model (Ducan's Grain Yield Model) is being tested to define the relationship between spacing and yield.

Early Fall Aerification and Topdressing Effects on Bermudagrass (*Cynodon dactylon*) P. B. WOOSLEY, T. SEYMOUR and B. WEST*, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

Spring dead spot is a common disease of bermudagrass in Kentucky and throughout the South. The pathogen attacks the root system of bermudagrass in the fall resulting in dead circular patches of turf once green-up occurs in the following spring. Different aerification types and topdressing with sand in the fall were evaluated on 'Quickstand' bermudagrass. Topdressing treatments resulted in faster spring green-up and better turf quality in the late fall and spring. Solid tine aerification was similar to the control in regards to bermudagrass quality and green-up while linear aerification significantly reduced bermudagrass green-up and quality. Improving drainage in the fall by aerification and increasing spring green-up could help reduce the incidence and effects of spring dead spot.

Landscape Construction at the Felts Log Cabin. ELIZABETH ALEWINE* and MARTIN STONE, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The felts Log Cabin is located on the campus of Western Kentucky University. Built in the early 1800's by Archibald Felts, the cabin was occupied by his descendants until 1968. The dogtrot floor plan, V- notched logs, and stone chimneys are some of the historical architectural features of the cabin. It was donated to the Kentucky Library and Museum at WKU in 1980, and now serves as on- site exhibit of early frontier life in Kentucky. The new landscape design for the cabin includes a kitchen garden with period-appropriate plants and out door demonstration areas. The inventories and journals of the Shaker community in South Union provided the basis for the vegetables used in the kitchen garden, including 'Late Flat Dutch' cabbage and 'Long Scarlet' radish. Dye plants, such as Bloodroot and Virginia creeper, are included in the kitchen garden, and the front of the cabin will be used to display examples of field crops, including 'stowell's Evergreen' corn. An area close to the cabin has been designed for a native plant display. Construction of these gardens in the spring of 2008 will involve the removal of grass around the cabin in keeping with historical accuracy. Combined with the cabin's location on campus, this will increase the potential for soil erosion. A fence and plants that are intended to act as vegetative filters are included in the design to help slow water runoff, and the use of raised planting beds and mulch to cover the bare soil will minimize soil loss. The native plant garden is intended to act as an introduction to the cabin, and will provide a selection of plants native to forests of Kentucky. Many plants are not typically seen outside of wild woodland settings, such as Strawberry bush (*Euonymus americanus*), several Trillium, Bird's-Foot Violet (*Voilapedata*), and Rattlesnake Orchid(*Goodyera pubescens*), and should increase visitors' enjoyment of the entire display. A path will connect the native garden to the cabin exhibit, and an informational pamphlet about the cabin and native gardens will be provided to the visitors.

Evaluation of Stocking Density and Light Intensity on the Growth and Survival of the Pacific White Shrimp (*Litopenaeus vannamei*) Reared in Zero Exchange Systems. RUSSELL S. NEAL*, BRIAN M. BOUDREAU, SHAWN D. COYLE and JAMES H. TIDWELL, Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601.

The majority of research on heterotrophic systems has been performed in greenhouses in tropical or sub-tropical environments. In temperate regions, insulated agricultural buildings, such as those used for growing poultry or swine, could provide more economical temperature control. However, natural sunlight may be important to these complex microbial populations. The objective of this study was to evaluate the effects and interactions of stocking density and light level on the growth and survival of *Litopenaeus vannamei* in zero exchange heterotrophic systems. This could provide essential information for efficient system design in temperate regions. In a greenhouse twelve, 3,800-L conical-bottom tanks were filled with de-chlorinated city water, salted to 25 ppt salinity, inoculated with heterotrophic bacteria, and randomly assigned one of four different treatments combinations. The study was a 2×2 factorial with main effects being stocking density (139 shrimp/m^2 vs. 278 shrimp/m^2) and light (natural light vs. low level artificial). Natural light tanks received ambient greenhouse light. Black plastic covered one half of the greenhouse and a single, 60-watt incandescent light bulb was used as a light source for each low-light tank. Juvenile *Litopenaeus vannamei* (0.40 ± 0.28 g) were stocked at random into each tank. After 12 weeks, there was a statistically significant ($P < 0.05$) interaction between density and light level for survival, average weight, and harvest weight. However, natural light significantly increased production ($P < 0.05$) at both stocking densities. Future research

should investigate how light spectrum and intensity affects the microbial population of these systems.

Total Replacement of Fish Meal with Commercial Yeast Extract (NuPro©) and Soybean Meal in Practical Organic Diets for Nile tilapia, *Oreochromis niloticus*. KIMBERLY A. TROSVIK*, LINDA S. METTS, KENNETH R. THOMPSON and CARL D. WEBSTER, Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601.

Fish meal is the main protein source in most fish diets, and it also provides essential nutrients necessary for growth. These attributes, added to the competition for its use in livestock and poultry feeds, increase its demand, making it the most expensive macro-ingredient of a fish diet. There are many potential alternative protein sources, with both soybean meal and yeast being two favorable ingredients. The objective of this study was to assess organically-certified yeast and soybean meal as a complete protein replacement for fish meal in organic tilapia diets. A 6-week feeding trial was performed in a recirculating system comprised of 36, 10.0-L tanks stocked with 20, 200 mg fish per tank. Fish were fed three times daily one of five formulated diets with 7 replicates per diet. Diet 1 was formulated similar to a commercially-available diet. Diets 2, 3, 4, and 5 contained no fish meal, but instead contained 10%, 20%, 30%, and 40% NuPro©, respectively. Organic soybean meal was the second major protein ingredient in all diets. At the end of the feeding trial, tilapia fed Diet 1 had a significantly ($P < 0.05$) higher final weight and specific growth rate than fish fed other diets. Fish fed Diet 1 also had a significantly lower feed conversion ratio than fish fed other diets. Results indicate that diets which contained NuPro© were not adequate for optimal growth in small tilapia; however, further studies must be done as very little is known about the effects of NuPro© on fish.

Conifer Decline at Baker Arboretum. PATRICK HARRIS*, MARTIN STONE, DANA KING, KRISTIN GOODIN and JENNIE DANKS, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

The Baker Arboretum is a 15 acre private garden, established over 15 years ago near Bowling Green, which serves as a horticultural teaching and research facility for students and faculty at Western Kentucky University. The horticultural collections specialize in dwarf conifers, Asian maples, and American and Asian dogwoods and their hybrids. Over the past few years, an apparently non-pathogenic decline has been seen in the growth of some conifers. By noting the position of the terminal bud scar, textural, and morphological changes in the stem, the growth of 33 specimens from four genera were noted for the past four years. Growth of 13 taxa were significantly reduced for the most recent years, 2006 and 2007, compared to the previous year's growth, 2004 and 2005. Tests revealed soil pH between 7.0 and 7.6 which was elevated for these acid-loving plants. Base saturation for soil calcium was excessive (up to 92%) compared to magnesium. Irrigation water did not contain excessive calcium but was low in magnesium and may be a contributing factor. Further research will continue and a complementary in-ground study is underway on site using *Chamaecyparis* and *Picea*.

Baby Corn as a Potential Crop in Kentucky. MARTIN STONE*, ELMER GRAY and TODD WILLIAN, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

Baby corn is the young, unfertilized ears of corn and is usually consumed fresh or canned. There is little difference between sugar enhanced and field corn cultivars for taste or sugar content in baby corn. There is a narrow range of size tolerance and size specifications that must be followed for both length and width of the ears. Husked ears must be between 5–10 cm long, yellow, with uniform and straight rows with the bottom-end width between 1.0–1.5 cm. Currently found primarily in Asian cuisine, it is in demand by high-end American restaurants. The United States is the world's largest consumer of baby corn but very little acreage is produced domestically. Therefore, the U.S. is also the world's leading importer. Baby corn requires similar agronomic and environmental conditions as field corn. It may suffer fewer late season pests because it is harvested around mid-season thereby avoiding many late season pests. For this reason, it may be a crop suitable for high value organic production. Plants may be picked several times during the growing season and any given plot of corn may be picked up to ten occasions each season. A relatively young crop with much potential for domestic and world growth, it is a potentially successful high value crop for Kentucky producers. Restaurants and farmer's markets are likely sales outlets.

Preliminary Evaluation of Baby Corn at Bowling Green, Kentucky in 2007. LARAMIE STONE*, Warren East High School; ELMER GRAY, TODD WILLIAN and MARTIN STONE, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

Baby corn is the edible, young, unfertilized ears of corn and is usually consumed fresh or canned. A field trial was conducted during the 2007 growing season at the Western Kentucky University research farm. Three cultivars of field corn with varying degrees of genetic flex, DeKalb 65–47 (low), DeKalb 63–46 (medium), and DeKalb 64–78 (high) were planted on 30 inch rows. Plots were four rows wide, 30 feet long, and data was taken on the center two rows only. Three plant population densities of each flex cultivar were planted at 36,000, 46,000, and 57,000 plants per acre. Baby corn was hand harvested on 3 to 4 day intervals beginning at 60 DAP (days after planting) and continued for ten harvests through 92 DAP. The highest population densities produced the greatest cumulative production. The highest flex cultivars produced the greatest cumulative production followed by medium and then low flex cultivars. However, for much of the season,

the average ear size was too great to meet market specifications. Later in the season when the average ear size was acceptable, the ears were cosmetically unmarketable. Field corn appears to be a viable specialty crop for Kentucky producers but more refinement is required in cultivar and plant density selection.

Lifespan of Rumen Microbes in Rumen Transfaunate. FELICIA POOL* and JENKS BRITT, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

The rumen is a fermentation vat where bacteria and protozoa convert feedstuffs to volatile fatty acids, methane, carbon dioxide, ammonia, and microbial cells. The purpose of the present study was to determine how long the microbes present in a cow's rumen can survive outside of the rumen. When the natural fauna of the rumen is killed via antibiotics, lack of sodium bicarbonate, or other cause, contents of a healthy cow's rumen can be used to re-inoculate the affected cow's rumen; this process is called transfaunation. Three samples were taken from the rumen of a cow via a rumen cannula. Samples 1, 2, and 3 were stored at 21, 38, and 2°C, respectively. Before the samples were subjected to different temperatures and at 4-hour intervals after that, the samples were observed for motility and gas production. Motility is indicative of protozoan survival; gas production indicates bacterial survival. After all motility had ceased, the samples were stored at 38°C to determine how long they would produce gas. At first observation, the order of diminishing activity was sample 2, 3, 1. Sample 3 remained motile the longest; sample 1 produced gas for the longest period of time. The results demonstrate that protozoa survive equally well at 21 and 2°C; they did not survive as well at 38°C. Bacteria survived the longest at 38°C, but did not survive well at 2°C. These results demonstrate that rumen transfaunate should be stored anaerobically in a thermos and used within a few hours of collection.

Teat Order Establishment of Neonatal Pigs and Its Relationship to Weight Gain. AMANDA GRAY, SARAH THOMAS and GORDON JONES, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The nursing patterns of 348 neonatal pigs from 39 crossbred litters at the Western Kentucky University farm were observed. The time required to establish a teat position and observations on the retention of that position were recorded daily in 1-hour intervals from birth to weaning. Pigs were cross fostered and observed to determine if the same or a different teat position was established on the foster sow. The effect of teat position on weight gain was also determined by weighing the pigs at birth and at weaning. Analysis of data indicated that teat order was established between 24 and 48 hr after birth. Anterior teats (teats 1–2) were preferred over posterior teats (teats 6–7) with 100% of sows having pigs established on anterior teats compared to only 74% on posterior. After fostering, only 12.5% of pigs remained on the same teat as on their birth sow. Seventy-five percent of fostered pigs chose a teat that was not previously selected for nursing by any of the pigs in the foster sow's litter. Pigs with greater birth weights tended to nurse from the middle teats (teats 3–5) while the smaller pigs were located on the most anterior and posterior teats. Birth weight accounted for 18% of the variation in weaning weight. No significant relationship was found between teat order and weight gain. This research suggests the need for additional data to determine the effects of cross-fostering on weight gain, morbidity, and mortality of pigs.

A Classroom Project to Engage Turfgrass Management Students. P. B. WOOSLEY* and J. E. KESSELL, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

Students were given the assignment in a turfgrass management class to grow and care for a turf baby. Students were given an empty pot and some turfgrass seed. The students were responsible for choosing the soil, mowing height, fertility program, and growing environment for their turf babies. Students were also asked to document certain stages of development in a turf baby book. Eleven different turfgrass species were used. The project helped students learn about turfgrass establishment, growth and development, fertility, management practices, and specie identification.

The Kentucky State University Ornamental Pawpaw Project. KIRK W. POMPER*, SHERI B. CRABTREE, JEREMIAH D. LOWE and R. NEAL PETERSON, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

There is a strong demand for new plant materials with ornamental potential for the Kentucky nursery industry. *Asimina triloba* (Pawpaw) is a tree native to the eastern U.S., producing large red flowers and large, edible fruit. *Asimina reticulata* (Netted Pawpaw) is native to Florida and southern Georgia and is a large shrub that produces elongated white flowers and inedible fruit. The objective of this project was to examine the potential of *A. triloba* and *A. reticulata* crosses as ornamental trees or bushes for the Kentucky nursery industry. Crosses (F1) between *A. triloba* and *A. reticulata* were performed in 1993. Greenhouse grown seedlings were field planted in 1995 and resulted in seedling shrubs. In 2001, shrubs produced red flowers that displayed a range of flower shapes; fruit was infrequent and inedible. In 2005, fruit was collected and seeds extracted from these plants. In 2006, F2 seedlings were then propagated in containers in the greenhouse from the open pollinated seeds collected from these F1 selections. About 40 seedlings germinated, grew vigorously, and became dormant in fall of 2006. Seedlings were over-wintered at 4°C. In 2007, seedlings began to flower in the greenhouse. Selections of note were: KSUOR001P (pink flowers), KSUOR002Y (yellow), KSUOR003Y (yellow-green), and KSUOR004R (red).

These seedlings showed great variation in flower form and color, and would be desirable to the ornamental industry. Clonal propagation methods and winter hardiness will be evaluated over the next 5 years.

Taste Test Evaluations of Primocane Fruiting Blackberries from the University of Arkansas Breeding Program Grown in Kentucky. JEREMIAH D. LOWE[1]*, KIRK W. POMPER[1], SHERI B. CRABTREE[1], JOHN R. CLARK[2] and JOHN G. STRANG[3]. [1]Atwood Research Facility, Land Grant Program, Kentucky State University, Frankfort, KY 40601; [2]Fruit Culture & Breeding, 316 Plant Science Bldg., University of Arkansas Fayetteville, AR 72701; and [3]Department of Horticulture, N-318 Agricultural Sciences North, University of Kentucky, Lexington, KY 40546. (jeremy.lowe@kysu.edu).

Primocane fruiting blackberries have the potential to produce a niche-market crop for Kentucky growers from late summer until frost. In June 2006, six selections of primocane fruiting blackberries from the University of Arkansas breeding program (APF-27, APF-40, APF-41, APF-42, APF-46, and APF-77) and the commercially available primocane fruiting cultivars Prime-Jim® and Prime-Jan®, were established at the Kentucky State University (KSU) Research Farm. In August of 2007, taste test evaluations were performed on freshly harvested fruit from all selections. In taste tests held at KSU and University of Kentucky, 21 participants sampled three fruit from each selection and were asked to evaluate the following characteristics on a scale of 1 to 5: flavor, sweetness, bitterness, tartness, color (all, 5 = intense, 1 = mild), texture (5 = hard, 1 = soft), appearance (5 = like, 1 = dislike), and seediness (5 = seedy, 1 = not seedy). Participants were also asked to award points to their favorite three selections (3 points = 1st place, 2 points = 2nd place, 1 point = 3rd place). Statistically significant differences were detected among the selections in all categories with the exception of tartness and seediness. The selection APF-40 ranked highest in flavor, sweetness, color, and appearance and ranked lowest in bitterness, tartness, and seediness. APF-40 received the highest score of 29 points and Prime-Jim® scored the lowest with one point. Initial taste test trials indicate consumers prefer the advanced primocane fruiting selections over currently available primocane fruiting blackberry cultivars; however, additional evaluation will be needed over the next several years.

Phenolic Content and Antioxidant Capacity of Fractioned Extract of the Pawpaw Cultivar 'PA Golden'. HIDEKA KOBAYASHI*, CHANGZHENG WANG and KIRK W. POMPER, Human Nutrition Program, Kentucky State University, Frankfort, 40601.

Pawpaw (*Asimina triloba* (L.) Dunal), a deciduous species of the eastern U.S., bears the largest edible fruit of all native trees. Investigations have previously examined phenolic content (PC) and antioxidant capacity (AC) of 14 pawpaw genotypes at full maturity. However, the high sugar content of pawpaw pulp has hindered a further elucidation of phytochemical constituents by chromatographic analysis. The objective of this study was to identify high PC and AC fractions with low sugar content. In an effort to remove sugar, pawpaw pulp was fractioned by using vacuum chromatography. Acetonic (AcOH) extract was first made with fruit pulp of the popular pawpaw cultivar 'PA Golden', which was lyophilized and then reconstituted with double distilled water (DDH_2O), followed by fractionation with different solvents in the order of DDH_2O (F1), 50% methanol (MeOH) (F2), 100% MeOH (F3), 100% AcOH (F4), and 50% AcOH (F5). Fractioned extracts were lyophilized and PC and AC were estimated with Folin-Ciocalteu assay and ferric reducing/antioxidant power (FRAP) assay. Absorbance readings for both Folin-Ciocalteau (760 nm) and FRAP (595 nm) assays were recorded up to 2 hr. F5 had the greatest PC and AC of pawpaw fractions examined. Interestingly, PC and AC of F2 were much lower than those of F5. These findings are a direct contrast to previously published reports on PC of fractioned lowbush blueberry extract, revealing considerable differences between blueberry and pawpaw phenolic constituents and antioxidant components in acquired fractions.

The Effect of Kaolin Clay (Surround WP) on Black Rot Disease in Grapes. SANJUN GU*, ANGIE WHITEHOUSE and KIRK W. POMPER, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Grape black rot (*Guignardia bidwellii*) is a devastating fungal disease in Kentucky. Production of grapes is severely limited if this disease is not controlled. The objective of this study was to investigate the effect of Surround WP (kaolin clay) on the control of black rot disease in grapes. 'Norton', 'Chambourcin' and 'Chancellor' grapes, which were planted in randomized complete blocks in 2003 at the Kentucky State University Research Farm, were sprayed every 7–10 days with Surround WP, Manzate fungicide, or water (control) from the time of budbreak (late April) to veraison (late August). Disease incidence on leaves and berries was recorded at veraison. The disease incidence and severity on leaves, based on the percent of leaves with lesions and the number of lesions per leaf, was most severe in vines treated with Surround (26%, 2.8), followed by control (18%, 1.4) and Manzate (9%, 0.3). The disease incidence on fruit showed no difference ($P = 0.55$) between treatments; vines treated with Surround, Manzate, or water had 61%, 51%, and 63% of berries infected, respectively. Surround and Manzate did not affect vine phenology, harvest time, number of fruit clusters per vine, or fruit composition (berry weight, sugar content, and pH). Surround WP did not prevent black rot infection of grape leaves or berries.

Effect of Soybean Oil and Stylet-Oil on Delaying Budbreak in Grapes. SANJUN GU*, HEATHER PENN and KIRK W. POMPER, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Grape production in Kentucky is challenged by late spring frosts, which can damage expanding buds, shoots,

flowers and florets, and cause subsequent loss of yield and fruit quality. However, cultivars with late budbreak, either natural or induced, may avoid late frost damage. The objective of this study was to determine if dormant oil sprays delayed grape vine budbreak. Dormant 'Cabernet Franc', 'Lemberger' and 'Traminette' grapevines were sprayed with soybean oil or Stylet-Oil, at 0%, 5% or 10%, on March 5th and 27th, 2007. One-year-old dormant canes from treated and untreated vines were collected one day after oil application, cut to one-bud segments, and forced at 25°C under constant fluorescent lighting to induce budbreak. After oil application, phytotoxicity of soybean oil and Stylet-Oil to grape buds and shoots was evaluated; higher oil concentrations resulted in increased bud mortality. There was no significant difference in phytotoxicity among cultivars. Oil treatments applied on either date did not delay budbreak for any cultivars, except for 'Traminette', which showed delayed budbreak of 6 or 8 days when 5% or 10% Stylet-Oil was applied on March 5th, respectively. The dormant status of vines influenced the effect of oil application, based on application date. Days to 50% budbreak were constant in 'Cabernet Franc' and 'Lemberger', but were reduced sharply in 'Traminette' from February to April.

Assessment of Annonaceous Acetogenin Activity in Ripe Pawpaw (*Asimina triloba*) Fruit and Twig Tissue. LATRICE FAULKNER*, JEREMIAH D. LOWE and KIRK W. POMPER, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

Pawpaw [*Asimina triloba* (L.) Dunal] contains Annonaceous acetogenins, which are promising new anti-tumor and pesticidal agents, present in extracts of twigs, fruit, seeds, roots, and bark. Ripe fruit potentially represent a large source of biomass for the extraction of acetogenin compounds. Identification of high acetogenin fruit concentration is difficult due to fruit availability and perishability. Identification of high acetogenin genotypes using twigs could provide a rapid screening method for high acetogenin selections. The objective of this study was to determine if there is a correlation between acetogenin content in ripe fruit and twigs. Five ripe fruit and actively growing twig tissues were harvested from each of the pawpaw cultivars NC-1, Sunflower, Taytwo, Wabash, and Wells. Fruit pulp was homogenized, placed in ziplock bags, and stored at -15°C until extraction. Pulp and dried twig tissue was extracted with 95% ethanol. The Brine Shrimp Test (BST) bioassay was employed to assess acetogenin activity. The BST identified acetogenin activity in the pulp and twig tissue of all cultivars examined. There was a high positive correlation between twig and fruit activity. There was low fruit and twig activity in the cultivars Sunflower and Wells, and high activity in both tissues in the cultivar NC-1. Other cultivars showed intermediate activity in both fruit and twig tissues. Twig tissue can be used as a rapid screening method to identify high acetogenin genotypes.

Leaf Size and Stomatal Density Varies by Pawpaw (*Asimina triloba*) Variety. SHERI B. CRABTREE*, KAMERO NEBLETT and KIRK W. POMPER, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

The pawpaw [*Asimina triloba* (L). Dunal] is a native tree fruit common to the eastern U.S. As the satellite site for the USDA National Clonal Germplasm Repository for *Asimina* species, goals of the KSU pawpaw research program include description and classification of unique germplasm. Leaf stomatal density has not been previously calculated for pawpaw. Plants with fewer stomata on their leaf surfaces are generally better-adapted to dry conditions. The objective of this study was to examine leaf morphology and frequency of stomata in 10 pawpaw cultivars. Five leaves were collected from 10 different pawpaw cultivars (Shenandoah, Rappahannock, Middletown, Mitchell, NC-1, Overleese, PA-Golden, Sunflower, Taytwo, and Wells) at the KSU research farm. The length, width, and thickness of each leaf were measured, and leaf stomata were counted using fingernail polish leaf impressions and a compound light microscope at 400× magnification. Differences in leaf size among cultivars were observed. The cultivars Wells, Taytwo, and PA-Golden had the longest leaves (over 18 cm), while Taytwo, Wells, and Overleese had the widest leaves (over 8 cm). The cultivars with the largest leaf area were Taytwo (166 cm^2) and Wells (156 cm^2). Leaf thickness did not significantly vary among cultivars. Stomatal density varied significantly by cultivar, with Taytwo having the most stomata per mm^2 (415) and Shenandoah and Middletown the fewest (144 and 152/mm^2). These cultivars could potentially be more drought-tolerant than others due to their low stomatal density.

Assessment of Clonality in Native Kentucky Pawpaw Patches Using Intersimple Sequence Repeat Markers. LAUREN A. COLLINS*, LI LU, JEREMIAH D. LOWE, SHERI B. CRABTREE and KIRK W. POMPER, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601.

Pawpaw [*Asimina triloba* (L.) Dunal] is a fruit tree native to the Southeastern area of the United States. Pawpaw is high in nutritional value, and could possibly be a new potential crop for farmers in Kentucky. Kentucky State University serves as the USDA National Clonal Germplasm Repository, or gene bank, for pawpaw. Assessing genetic diversity and evaluating pawpaw germplasm for the repository collection is a top priority. Pawpaw is usually found in the understory of hardwood forests, often in large patches. Pawpaw forms rootsuckers and therefore it has been suggested that most pawpaw patches are clonal in nature. Clonality of patches would affect sampling strategies in assessing genetic diversity. The objective of this study was to utilize intersimple sequence repeat (ISSR) markers to determine if DNA fingerprint patterns indicate that all trees in a patch are clones. Leaf samples were collected from trees in three

native patches in central Kentucky. DNA was extracted from leaves using a Dnamite plant kit. The DNA concentration was quantified, and diluted to 1 ng/ul. The diluted DNA was amplified by the polymerase chain reaction (PCR) using the ISSR primers UBC841T or UBC841C and then run on a 2% agarose gel overnight to separate PCR products. The gel was stained with ethidium bromide, photographed and scored. DNA fingerprints identified at least two genotypes in each patch, indicating the patches were not entirely clonal. This information will impact future sampling strategies in assessing genetic diversity in pawpaw.

Constructed Wetland Microcosms for Remediation of Water Contaminants. ERIC T. TURLEY* and GEORGE F. ANTONIOUS, Kentucky State University, Land Grant Program, Department of Plant and Soil Science, Frankfort, KY 40601.

The increased use of agrochemicals such as synthetic pesticides and fertilizers demands practices for remediation of these environmental contaminants. Constructed wetlands can be designed and utilized to reduce or eliminate some of the negative effects of agrochemicals on runoff water quality. At Kentucky State University Research Farm, twelve constructed wetland microcosms were established. Six microcosms were filled with river gravel and six filled with limestone (size #2). Eighteen sweet flag (*Acorus calamus*) were planted in 6 microcosms (nine in 3 river gravel and nine in 3 limestone microcosms). Microcosms were used to study their performance for removal of orthophosphate ions, NO_3-N, NH_4-N, turbidity, and total suspended solids (TSS) from surface waste water discharge. Biological oxygen demand (BOD_5), pH, dissolved oxygen (DO), and temperature were also measured. Plants were fertilized with Peters and appropriate water level in microcosms was maintained. Plants were also monitored for growth and performance in the two types of rocks. Water samples were collected at regular time intervals to monitor performance of microcosms. The results revealed that microcosms containing the two types of rocks, limestone or river gravel, and planted with sweet flag had 16% increase in dissolved oxygen compared to microcosms without sweet flag plants. The NO_3-N content in microcosms containing crushed limestone was reduced by 40.1% compared to microcosms containing river gravel.

Natural Capsaicin in *Capsicum chinense*: Concentration vs. Origin. HU YOON-HYEON* and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601; TERRY BERKE, Seminis Seed Co., Woodland, CA; and ROBERT L. JARRET, USDA/ARS Plant Genetic Resources Conservation Unit, Griffin, GA 30223.

Capsaicin [N-vanillyl-8-methyl-6-(E) noneamide] is the most pungent of the group of compounds known as capsaicinoids in chili peppers. A survey was conducted to screen fruits of 307 hot pepper accessions of *Capsicum chinense* selected from the USDA germplasm collection for their major capsaicinoids content (capsaicin and dihydrocapsaicin). Seeds of *C. chinense* from fruits originated from Belize, Brazil, Colombia, Costa Rica, Ecuador, Mexico, Peru, Puerto Rico, Suriname, United States, and Venezuela were planted in the field in Woodland, California.

Mature fruits were harvested and their capsaicinoids were quantified. Concentrations of capsaicin and dihydrocapsaicin varied between origins and between accessions (genotypes) of the same origin. Statistical analysis revealed that fruits obtained from seeds originated in Mexico, Brazil, and United States produced the greatest concentration of total capsaicinoids. Among all accessions analyzed, accession PI-438644 (Mexico) had the greatest total capsaicinnoids content (2.38 mg g^{-1} fresh fruit) and accession PI-441619 (Brazil) had the greatest capsaicin content (2.2 mg g^{-1} fresh fruit), while PI-441623 (Brazil) had the greatest dihydrocapsacin content (1.8 mg g^{-1} fresh fruit). PI-640900 contained the greatest capsaicin content (1.6 mg g^{-1} fresh fruit) among all accessions selected from United States. Quantification of capsaicinoids in the selected accessions allowed the identification of accessions with greatest levels of capsaicinoids. Accessions PI-438644 (Mexico), PI-441619 (Brazil), and PI-640900 (United States) were identified as potential candidates for mass production of capsaicinoids, or for breeding of varieties having greatest capsaicinoids content.

Screening *Brassica* Species for Glucosinolate Content. GEORGE ANTONIOUS* and MICHAEL BOMFORD, Community Research Service, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601 and PAUL VINCELLI, Department of Plant Pathology, University of Kentucky, Lexington KY 40546.

Glucosinolates (GSL), a group of naturally occurring thioglucosides, are compounds in Cruciferous crops that can be used as biofumigants because their hydrolysis products, isothiocyanates, are toxic to some soil-borne plant pathogens. Our goal was to survey *Brassica* accessions from the National Germplasm Repository to identify potential cover crops that could be soil-incorporated to eradicate sclerotia (survival bodies) of *Sclerotinia sclerotiorum*, an important pathogen of winter vegetables grown in unheated high tunnels and oospores of *Phytophthora* blight in summer-grown bell peppers. Ten accessions that demonstrated relative cold tolerance, rapid maturity, and superior biomass production were selected from 48 accessions. The selected accessions were grown under fall greenhouse (low stress), winter high tunnel (moderate stress), and spring field (high stress) conditions. These included seven accessions of *Brassica juncea* (Indian mustard), one of *Brassica napus* (oil seed rape), one of *Brassica campestris* (field mustard), and one of *Eruca sativa* (arugula). Quantification of total GSL was

based on inactivation of the endogenous thioglucosidase and liberation of the glucose moiety from the GSL molecule by addition of standardized thioglucosidase and colorimetry. GSL concentration of greenhouse, high tunnel, and field-grown plants averaged 24, 36 and 80 μ moles g^{-1} fresh weight (leaves and stems), respectively. Accessions with the highest GSL content tended to be *B. juncea*. We conclude that environmental stress on growing plants can increase the level of GSL in *Brassica* plants.

Yield and Quality of Hot Pepper Grown with Various Soil Amendments at Kentucky State University Farm. JAMI A. ROGERS* and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Environmental Toxicology Program, Kentucky State University, Frankfort, KY 40601.

Optimizing crop yield without sacrificing quality is the goal of all farmers. Amending the soil can be an inexpensive way of increasing crop yield. The objective of this study was to determine which of three soil amendments (yard waste compost, sewage sludge, or wood chips) would most positively affect the yield and quality of peppers grown at KSU Research Farm. Five Capsicum accessions were selected from the USDA Capsicum germplasm collection based on our previous research. Two accessions from *Capsicum chinense*, two from *C. baccatum*, and one from *C. annuum* were selected. After each harvest mature fruits were weighed and counted. Length, width, wall thickness and color of fruits as well as plant length and width were also recorded. There was no significant difference between the treatments within the individual fruit parameters such as fruit weight, length, width, and wall thickness. There were significant differences between plant length and width with sewage sludge and yard waste compost producing significantly larger plants than either wood chips or no mulch treatments. Yard waste compost and sewage sludge also produced significantly greater yields than wood chips and no mulch.

Heavy Metals in Soil, Runoff Water, and Eggplant Fruits from Soil Amended with Sewage Sludge and Yard Waste Compost. MICHAEL SOMUAH*, ZACHARY RAY, TEJINDER KOCHHAR and GEORGE ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601 and JOHN SNYDER, Department of Horticulture, University of Kentucky, Lexington, KY 40546.

Contaminated surface water has become a critical environmental problem. Land application of soil amendments such as sewage sludge can increase concentration of nutrients and heavy metals in surface runoff and their accumulation in edible portions of plants at harvest. A field study was conducted on a silty-loam soil of 10% slope at Kentucky State University Research Farm. Eighteen plots of 22 × 3.7 m each were separated using metal borders and the soil in six plots was mixed with sewage sludge and yard waste compost (SS-YW) each at 15 t $acre^{-1}$, six plots were mixed with sewage sludge at 30 t $acre^{-1}$, and six unamended plots were used for comparison purposes. Plots were planted with eggplant, *Solanum melongena*, as the test plant. The objectives of this investigation were to: 1) assess chemical and physical properties of soil following addition of soil amendments and their impact on the yield and quality of eggplant fruit; 2) determine the effect of soil amendments on the transport of nutrients (NO_3-N and NH_4-N) and heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, and Mo) into surface water; and 3) investigate the effect of this practice on heavy metal bioavailability in eggplant fruits at harvest. Concentrations of heavy metals in soil were below the USEPA limits. Total marketable yield and quality of fruits were greatest in SS-YW treatment.

Agricultural and Environmental Laws in Kentucky: An Overview. CAROLINE R. WILSON* and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Water Quality and Environmental Toxicology, Kentucky State University, Frankfort, KY 40601.

Investigation of air and water quality in Kentucky, along with an overview of mandated laws established to protect the environment from ecological harm is growing because of increasingly harmful toxins being inadvertently consumed by ecological species. Global warming and depletion of the rate of extinction in certain plant and animal species due to environmental hazards are rising at alarming rates. Plants, animals and humans alike are being affected from poor soil and water quality, deterioration in air quality and increased consumption of toxic airborne substances. Previous laws were implemented to ensure that farmers use safe pesticides and producers manufacture products that minimize toxins such as pesticides, lead, photochemical oxidants and particulates. The recent discovery of lead found in imported products has caused a massive recall and influx of questions about environmental safety in the U.S. Laws were enacted and are being regulated across the nation to help control an overflow of toxic chemicals in our drinking water, air quality and agricultural crops. This overview investigates current legislation and recent changes to environmental laws and the importance of managing sustainability in our ecosystem.

Pollen from Genetically Modified Corn Does Not Affect Honey Bee Queen Rearing Behavior. THOMAS C. WEBSTER*, JOHN D. SEDLACEK and MARK MORIATY, Community Research Service, Kentucky State University, Frankfort, KY 40601.

The effects of genetically engineered Bt corn pollen, conventional corn pollen and sunflower pollen on honey bee queen rearing activity were compared. Cages containing approximately 200 worker bees were each fed a diet containing one of these pollen types. Female honey bee larvae were then placed into small plastic cups and introduced to the cages to stimulate the worker bees to deposit royal jelly as part of queen rearing behavior. Royal jelly provisioning was similar for cages fed Bt corn pollen (34.6 ± 4.5 mg), conventional corn (32.0 ± 6.1 mg) or sunflower (34.9 ± 5.7 mg) pollen. However, the growth of larvae over two days was greatest in cages of bees fed

sunflower pollen (18.3 ± 0.3 mg) and similar for larvae in cages fed Bt corn pollen (15.8 ± 0.5 mg) and conventional corn pollen (15.6 ± 0.3 mg). The proportion of introduced larvae accepted by the bees was equivalent for cages of bees fed each of the three diets. These results support other studies that indicate no harmful effects of Bt corn pollen on honey bees, and that corn pollen is less nutritious than sunflower pollen.

Ground Beetle Species Richness and Abundance in Organic, Conventional, and Genetically Engineered Bt Sweet Corn. KAREN L. FRILEY* and JOHN D. SEDLACEK, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Ground beetles (Coleoptera: Carabidae) are predators of many insect pests in agroecosystems. Economically important insect pests that they prey upon in sweet corn fields include corn earworm, *Helicoverpa zea* (Boddie); European corn borer, *Ostrinia nubilalis* (Hűbner); southwestern corn borer, *Diatreae grandiosella* Dyar; fall armyworm, *Spodoptera frugiperda* (J.E. Smith); and Japanese beetle, *Popillia japonica* Newman. Sweet corn was grown using organic, conventional, and genetically engineered production practices. Concerns regarding reduction in biodiversity and non target impacts in genetically engineered crops have been expressed. Thus, the objective of this research is to determine ground beetle species richness and abundance in the three cropping methods of sweet corn. Pitfall traps were used to capture ground dwelling insects. Four pitfall traps were placed equidistant from edges and from each other within the middle row of corn in two of three subplots in each plot. Pitfall traps were made from 473 cc plastic cups. Two cups were placed in each hole so the edge was flush with the soil surface. A rain cover, supported by 3 wooden blocks, was made from a 23 cm diameter plastic plate. A 118 cc 1:1 ratio of ethylene glycol and distilled H_2O was placed in each trap to preserve insects until collected. Pitfall traps were serviced at weekly intervals throughout the growing season. Total numbers of ground beetles increased from June 24 to July 25 in each cropping type. Initial analyses for three sampling periods revealed greater numbers of carabids in Bt plots than organic and conventional plots. Bt plots also had the greatest number of different species. *Harpalus pensylvanicus*, *Cyclotracheus soldalis*, and *Stenolophus* sp. were the most abundant ground beetle species caught.

Taste Preferences For Sweet Corn Grown Organically, Conventionally or Genetically Engineered Among Patrons of Two Farmers' Markets in Central Kentucky. JOHN D. SEDLACEK*, SUSAN B. TEMPLETON and KAREN L. FRILEY, Community, Research Service, Kentucky State University, Frankfort, KY 40601.

We examined consumer knowledge and taste preferences concerning sweet corn grown organically, conventionally and that which is genetically engineered. Consumer knowledge was quantified using a 10 question survey administered at the Lexington and Frankfort Farmers' Markets. The questionnaire quantified attitudes and perceptions concerning sweet corn grown using each of the three cropping types. The survey culminated with taste paneling of corn harvested from each of the three cropping methods. An informal acceptance and sensory evaluation using informed/blind testing procedures was employed. Color, juiciness, sweetness, crispness, overall appearance, overall flavor, and overall texture were evaluated. The majority of respondents were female, Caucasian, non-farmers ranging in age from 45–64 years. Before completing the taste perception component of our survey, 49% of the respondents believed that the organically grown sweet corn would taste better. Surprisingly, for each market and year of the study, respondents preferred the genetically engineered sweet corn over that which was conventionally and organically grown for color, juiciness, sweetness, and crispness characteristics. Overall, taste test respondents preferred the genetically engineered sweet corn to the organically grown sweet corn by at least a 2.4:1 ratio for appearance, 2.6:1 for flavor, and 2.4:1 for texture. Differences in fertilizer amendments between the genetically engineered and conventional plots vs. the organic plots may be responsible for these differences. Another possible explanation could be weed pressure in the organic plots that was much more severe than in the conventional and genetically engineered plots.

Populations of Lady Beetles and Green Lacewings in Sweet Corn Grown With Organic, Conventional or Biotechnology Enhanced Cropping Methods. LESLYE S. BRENT*, KAREN L. FRILEY and JOHN D. SEDLACEK, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Sweet corn is among the most important and valuable vegetables grown in Kentucky during summer months. Important insect pests in sweet corn fields include corn earworm, *Helicoverpa zea*; European corn borer, *Ostrinia nubilalis*; southwestern corn borer, *Diatreae grandiosella*; and fall armyworm, *Spodoptera frugiperda*. Sweet corn was grown using organic, conventional, and genetically engineered production practices. Concerns regarding negative impacts on biodiversity and non-target beneficial insects in genetically engineered crops have been voiced. Therefore, the objective of this research is to determine lady beetle species composition and abundance and green lacewing abundance in the three cropping methods of sweet corn. Yellow sticky traps 232 cm^2 in area were used to capture flying insects at tassel and silk height during anthesis. Four sticky traps were placed equidistant from edges and from each other within the middle row of the center corn subplot in each plot. Pitfall traps were serviced weekly for 3 weeks. Pink Lady Beetle, *Coleomegilla maculata*, was the most abundant lady beetle caught followed by the Asian multicolored lady beetle, *Harmonia axyridis*. Few spotless lady beetle, *Cycloneda munda*; mildew-eating lady beetle, Psyllobra viginumalata; seven spotted lady beetle, *Coccinella septempunctata*; parenthesis lady beetle, *Hippodamia parenthesis*; and variegated lady beetle, *Hippodamia*

variegata; were captured. Green Lacewings, *Crysoperla carnea*, were captured in small numbers.

BOTANY

Floristic Survey of the Berea College Forest, Jackson, Madison, and Rockcastle Counties, Kentucky. RALPH L. THOMPSON, Berea College Herbarium, Department of Biology, Berea College, Berea, KY 40404.

A survey of the vascular flora of the Berea College Forest (BCF) in east-central Kentucky was made during 2002–2007. All BCF specimens in the Berea College Herbarium were also examined. Founded in 1897, BCF is the oldest established forest in Kentucky and one of the oldest managed forests in the United States. It consists of 3380 ha in parts of three counties: Jackson (656 ha), Madison (2343 ha), and Rockcastle (381 ha). A tract of 2703 ha of the BCF in Madison County has been listed as a Historic District by the National Register of Historic Places of the National Park Service, and a 51 ha tract of Mixed Mesophytic Forest in Rockcastle County has been set aside in perpetuity as the John B. Stephenson Memorial Forest State Nature Preserve. Vegetation is comprised of Western Mesophytic Forest, Mixed Mesophytic Forest, secondary succession areas, wetland habitats including five reservoirs, planted prairie species sites, and other non-forested anthropogenic-influenced areas. The known vascular flora is composed of 1015 specific and infraspecific taxa in 513 genera from 139 families. A total of 2107 herbarium specimens are cited in the annotated list: 547 from Jackson County, 950 from Madison County, and 610 from Rockcastle County. Taxonomic classification of species is Equisetophyta (2), Lycopodiophyta (4), Polypodiophyta (33), Pinophyta (13), and Magnoliophyta (963). One hundred ninety-five taxa (19.21%) are naturalized, introduced, adventive, or cultivated exotics. The largest families in species richness are the Asteraceae (141), Poaceae (100), Cyperaceae (64), Fabaceae (56), and Rosaceae (36). Four state-listed occurring naturally in/or bordering BCF are *Castanea dentata* (endangered), *Juglans cinerea* (special concern), *Paxistima canbyi* (threatened), and *Thermopsis mollis* (endangered). Four state records documented in the BCF are *Nuttallanthus canadensis*, *Polygonum densiflorum*, *Viburnum sieboldii*, and *Vicia tetrasperma*.

CHEMISTRY

Atom Efficient Syntheses of Short Push-pull Chromophores. KRYSTAL D. HOLLEY, TIFFANY R. SHORTER and JEANNE L. KUHLER*, Department of Physical Sciences, Auburn University, Montgomery, AL 36124.

Push-pull chromophores are molecules which allow for efficient intramolecular delocalization of π-electrons which makes them useful for many biological processes and for making logic devices that can essentially be turned "on" or "off." This fluorescent "on-off" feature is the result of the intramolecular movement of the π-electrons. This movement of the π-electrons is made possible in a molecule which has one portion of its structure that can donate electrons temporarily at one side of the molecule (donor) while another portion of its structure can accept this electron density (acceptor). A series of substituted phenylenediamines have been efficiently transformed to their carbamate derivatives in high yields under mild conditions. These reaction conditions are sensitive to the electron-donating and electron-withdrawing properties of the attached subsitutents. Electron-donating substituents are shown to facilitate carbamate formation under neutral reaction conditions without the need of a catalyst, while electron-withdrawing substituents on the aromatic amine require the assistance of a Lewis acid catalyst to produce the carbamate. Reaction progress has been followed using TLC, and all products have been characterized using ^{1}H NMR, ^{13}C NMR, FTIR, and GC-MS to further enhance the learning experience for undergraduates. These optimized reaction conditions should prove to be useful for a variety of applications including the synthesis of push-pull chromophores, organic dyes, and the tetraalkyl-phenylenediamine donor-acceptor systems, which are useful as molecular devices.

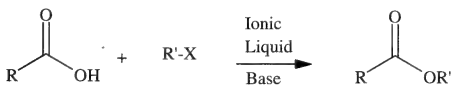
Efficient Mitsunobu Reactions of Protected Amino Acid Derivatives for Undergraduates. WHITNEY K. HELMS, OLUWAGBEMIGA I. KORIKO and JEANNE L. KUHLER*, Department of Physical Sciences, Auburn University, Montgomery, AL 36124.

The Mitsunobu reaction is a highly useful stereoselective reaction for the synthesis of pharmaceutical products due to its high degree of inversion of configuration involving alcohols. However, hindered alcohols often produce lower yields of the synthetic target. Another limitation of the Mitsunobu reaction is the requirement for an acidic reaction partner with a pK_a value of less than 10. Herein we report the use of the catalyst *N,N,N′,N′*-tetramethyl azodicarboxamide (TMAD) instead of the diethyl azodicarboxylate (DEAD) with triphenylphosphine (TPP) catalyst system to apply the Mitsunobu reaction to several tertiary alcohols, and also to both enantiomers of the amino acid derivatives of 1-Amino-2-propanol, Valinol, 2-Phenylglycinol, Phenylalaninol, and Methioninol. Reaction progress has been followed using TLC, and all products have been characterized using ^{1}H NMR, ^{13}C NMR, and FTIR to further enhance the learning experience for undergraduates. This TMAD catalyst provides the additional advantage of producing fewer of the problematic by-products often associated with the traditional DEAD/TPP conditions, such as formation of phosphine oxide and hydrazinecarboxylate.

An Esterification Reaction Without an Acid Catalyst for Undergraduates. ROBEY T. BROOKS, WHITNEY K. HELMS, KRYSTAL D. HOLLEY, PRATIK P. PATEL and JEANNE L. KUHLER*, Department of Physical Sciences, Auburn University, Montgomery, AL 36124.

The ester functional group is ubiquitous and occurs in a wide variety of products ranging from fragrances, monomers, and plasticizers to common local anesthetics used to relieve the pain of burns, toothaches, earaches, cold sores, cuts, and abrasions. Although there are several

methods for the esterification reaction, most involve acidic conditions and the use of great quantities of organic solvents, thus generating waste which is expensive for industry to dispose of safely. The most common method to prepare esters has been the acid-catalyzed Fischer esterification reaction between an alcohol and a carboxylic acid. However, due to its dependence on an acid catalyst, it is limited to molecules which are not sensitive to acid. Also this Fischer esterification reaction does not always produce useful yields of ester product when the alcohol is tertiary because elimination byproducts can form. Therefore, a complimentary method has been developed which uses alkyl halides instead of alcohols and basic conditions instead of acid. These conditions produce higher yields due to a change to an alternative mechanism which does not involve an equilibrium process. An additional advantage of this method is the stereospecific inversion of configuration. Reaction progress was followed using TLC, and all products have been characterized using ^{1}H NMR, ^{13}C NMR, and FTIR to further enhance the learning experience for undergraduates.



Design and Development of Palladium-iron Bimetallic Electrocatalysts for Polymer Electrolyte Fuel Cells. JESSICA R. PRICE[1]*, RICHARD D. COOKS[1], MARK A. CUNNINGHAM[2], XIAOPING WANG[3] and DEBORAH J. MYERS[3], [1]Department of Biology, Berea College, Berea, KY 40404; [2]Department of Chemistry, Berea, College, KY 40404; [3]Argonne National Laboratory, Argonne, IL 60439.

The path to more efficient energy sources for modes of transportation to replace the CO_2-emitting, low efficiency internal combustion engine, has led the Argonne National Laboratory of the U.S. Department of Energy to develop commercially competitive polymer electrolyte fuel cells (PEFCs). The purpose of this project is to design and develop bimetallic cathodic electrocatalysts for PEFCs with high electrochemical activity and high stability to replace more expensive platinum-based electrocatalysts. The bimetallic electrocatalysts reported in this study are composed of the precious metal palladium (Pd) and the base metal iron (Fe) fixed onto carbon support. The less expensive base metal, iron, is designed to comprise the core of the bimetallic alloy with a monolayer outershell consisting of palladium. The Pd-Fe electrocatalysts were synthesized by the impregnation method, utilizing $Fe(NO_3)_2$ and $Pd(NO_3)_2$ as metal precursors, producing bimetallic catalysts with a range of metal compositions. The precursor salts were reduced to the Pd-Fe bimetallic electrocatalyst in a dilute hydrogen atmosphere. Transmission electron microscopy, temperature programmed-reduction, and cyclic voltammetry using the rotating disk electrode were used to characterize the electrocatalysts' composition and particle size, reduction conditions for heat treatment, and catalyst stability and performance, respectively. The bimetallic catalyst with a molar ration of 30:70 (Pd:Fe), heat treated in regen gas at 620°C for 10 h, showed the highest activity of 65,31 mA/mg_{Pd} at 0.85 V. Further research will focus on maximizing catalyst performance by optimizing heat treatment conditions to minimize particle size with a core-shell morphology. The desired end result is a cost efficient bimetallic alloyed electrocatalyst that is cost efficient, has a high rate of oxygen reduction, a small particle size, and an activity of 440 mA/mg metal at 0.9 V (2010 DOE target).

COMPUTER & INFORMATION SCIENCE

Grid: The New Computational Infrastructure for 21st Century Data-Intensive Science. AKHTAR H. MAHMOOD, Department of Chemistry & Physics, Bellarmine University, Louisville, KY 40205.

Grid is the new IT infrastructure for 21st century science – a new computing paradigm that is poised to transform the practice of large-scale, data-intensive research in science. The Grid concept is *"as available as electrical power"*. The Grid will allow scientists worldwide to view and analyze huge amounts of data coming from the large-scale experiments in science. The Grid infrastructure can be perceived as a collection of "Virtual Organizations" (VOs), bringing together geographically and organizationally dispersed computational resources, such as CPUs, storage systems, communication systems, and data sources. The Grid is envisioned to be the next revolution to transform the internet and scientific computing. At Bellarmine University, we are planning to set up a Tier-3 grid site using the OSG (Open Science Grid) resources. OSG will allow physicists worldwide to view and analyze huge amounts of data flowing from the next generation of large-scale scientific experiments. Current status and developments in Grid Computing will be presented.

ENGINEERING

Estimation of Energy in the Winds at Madisonville, Kentucky. JANARDAN ROHATGI* and WILLIAM HOWARD MCGREGOR, Madisonville Community College, Madisonville, KY 42431.

At the Madisonville Community College a wind measurement and recording system was installed in November 2005. The system consists of wind velocity, direction, and temperature sensors mounted on a tower at an elevation of 30 ft above ground. This is stand alone equipment powered by batteries. The velocity and direction are measured at an interval of every two seconds, then the average of every 10-minute intervals is stored in a chip. The stored data in the chip are analyzed using custom built software. This work presents the analysis of these data for a period of about 454 days. Some features that characterize this location are: mean speed at 30 ft elevation = 4.9 miles per hour; max wind speed gust (2-second) = 53 mph; speed ≥ 7 mph = 24%. Because 3-bladed medium- or large-sized wind turbines are at an elevation 120 ft or more, mean wind speed of 7 mph corrected (employing well known 1/7 power-law) to 120 ft

elevation would be about 8.54 mph (~4 m/s). That means about 24% of the time, one can expect at least ~ 4 m/s. This will be equivalent to a power density of about 40/m^2. Assuming a rotor diameter of 17-m and employing Betz's coefficient we will have power of approximately 5350 W. Using capacity factor of about 30% we may expect approximately 14,000 kWh per year. This work analyzes wind data for this site and gives an estimate of energy generation for different scenarios.

GEOGRAPHY

Accuracy Analysis of the 2005 Kentucky Land Cover Data Set Update. DEMETRIO P. ZOURARAKIS[1]* and MICHAEL PALMER[2], [1]Kentucky Division of Geographic Information, Frankfort, KY 40601 and [2]Sanborn, Inc., Ann Arbor, MI 48108.

The final accuracy statistical analysis for the Kentucky Landscape Census (KLC), National Land Cover Dataset (NLCD) update for 2005 is presented. The goal for the change map accuracy assessment was to both determine how well change was captured and to assess the accuracy of the change classification. Accuracy techniques have been developed and applied successfully when validating a single time period. Scaling the procedure to incorporate two time periods becomes a daunting task, where the number of samples grows to 450 (9×50). Given the KLC change detection product has 13 classes, the required sample size would be 8450 (13 × 13 × 50) samples. Since not all changes are logical within the NLCD land cover classification system, that number would likely be smaller, but the number of required samples is still much greater than for a single date accuracy assessment and probably not feasible under most time and budget conditions. To accomplish this task, validation was completed in two steps. First the 2005 change areas were assessed as a single date land cover map. Second, a change mask was assessed as a binary change/no change map. Change was captured with a success rate of 96%. While a deterministic result shows 58.8% classification accuracy, a representative fuzzy assessment of the classification shows a favorable overall classification accuracy of 79.9%. Critical issues with respect to reference data, sampling and class rarity have been addressed to provide a statistically valid accuracy statement of the KLC 2005 change map.

GEOLOGY

Fecal Microbe Distribution and Abundance Used as a Possible Proxy for Nutrient Source Identification in Eutrophic Wilgreen Lake, Madison County, Kentucky. THERESA A. AGUIAR* and WALTER S. BOROWSKI, Department of Geography and Geology, Eastern Kentucky University, 521 Lancaster Avenue, Richmond, KY 40475-3102.

Wilgreen Lake is a eutrophic lake that has been listed on the EPA's 303d list as nutrient impaired. Potential sources of this impairment are from humans, cattle manure and fertilizers. We suspect that the majority of nutrients originate from human sources. As a possible proxy for nutrient input, we test our hypothesis by examining fecal microbe distribution and abundance in the lake. We took water samples at 19 sampling locations on 4 occasions. Sampling spanned 26 June to 15 August with the last 3 sampling events occurring at roughly two-week intervals. These samples were then processed using IDEXX methods, which count total coliform and *Escherichia coli* colonies. For sampling localities with microbial abundance that exceeded maximum sensitivity (>2419 cfu/100 ml), we performed dilutions of 1:4 or 1:10. Both total coliform and *E. coli* levels are elevated at sites adjacent to septic tank clusters. There is a gradual decline in microbial abundance distal to these populated areas and microbe levels are low in the third lake tributary that drains only cattle pasture. This strongly suggests that the source of microbial input is from septic systems; however, we cannot eliminate the possibility that fecal microbes are introduced into the lake via inflows, because of high microbial abundance in their waters. We plan on using Reverse-Transcription Polymer Chain Reaction (RT-PCR) techniques to distinguish between human and cattle sources of *Bacteroides* to potentially eliminate the inflows as a microbial source.

The "Anti-Museum": A Review of the Answers in Genesis Creation Museum. DANIEL PHELPS, Kentucky Paleontological Society, Lexington KY 40514.

The new Creation Museum near Petersburg, Kentucky was visited soon after it opened in May 2007. During its formative stages the Creation Museum christened itself "The Anti-Museum." Various displays and videos at the Anti-Museum were reviewed. These media show that the Anti-Museum is one of the weirdest accomplishments of the creationist movement. The Anti-Museum is unique because of its size and cost ($27 million). The Anti-Museum's success or failure will gauge the market for old-fashioned "scientific" and Biblical creationism, especially at a time when Intelligent Design Creationism (IDC) is perceived as a more dangerous threat to science education. The Anti-Museum is projected to have had 250,000 visitors by November 2007. These attendance numbers are well beyond the most optimistic projections of Answers in Genesis. The Anti-Museum is no threat to science, but may hinder science education and the public's science literacy, both regionally and beyond.

Conodonts from Target Bedrock and Impact Breccias of the Haughton Impact Structure, Devon Island, Nunavut, Canada. WESLEY C. SMITH* and CHARLES E. MASON, Department of Physical Sciences, Morehead State University, Morehead, KY 40351; JOHN E. REPESKI, U.S. Geological Survey, MS 926A National Center, Reston, VA 20192; PAULA LINDGREN, Department of Geology and Petroleum Geology, University of Aberdeen, Aberdeen AB24 3UE, UK; JOHN PARNELL, Department of Geology and Petroleum Geology, University of Aberdeen, Aberdeen AB24 3UE, UK; and PASCAL LEE, Mars Institute, SETI Institute, and NASA Ames Research Center, MS 245-3, Moffett Field, CA 94035-1000.

Samples of carbonate bedrock (n = 5) near, and melt breccia clasts (n = 31) within the Haughton impact

structure (Tertiary), Devon Island, Canada (75.2°N Lat.; 89.4°W Long.) yielded 95% recovery of conodonts. This study is the first to examine conodonts from Haughton Crater, which provide new data on the impact and its thermal history. Most of the target bedrock samples produced conodonts that are indicative only of Late Ordovician age (for some, a Silurian age is possible), which is consistent with the Allen Bay Formation (Upper Ordovician-Lower Silurian) as mapped in the area. Most of the samples of clasts from breccias also fall within this age range; however, some contain faunas of latest Early to earliest Middle Ordovician age, consistent with the ages of some of the underlying units, e.g., the Eleanor River Fm., displaced as ejecta and as parts of the central uplift. Conodont color alteration index (CAI) values in the regional bedrock are about 1.5, indicating minimum post-depositional, long-term heating in the approximate range of 50–90°C. Some samples contain conodont elements having higher CAI values - up to 4. Some of these samples also contain co-occurring conodonts having several different CAI values, and some of the elements in these samples display surface features characteristic of the effects of contact with hydrothermal conditions. These surface features, and the co-occurrence of multiple within-sample CAI values, are another of the many indicators of hydrothermal activity associated with the impact. Conodonts may provide some additional constraints on some of the thermal history parameters of this event. For example, one larger carbonate clast (~25 cm × 20 cm × 10 cm) was split into two samples, the outer edges and the center of the clast, which were processed separately. CAI of conodonts in the outer sample range from 3.5 to 4, whereas most elements from the inner part of the clast range from 2 to 3, demonstrating the insulation effect of the poor heat conductivity of rock. Splits of these two samples processed by one of us (PL) using biological marker maturity parameters (pregnane/sterane and tricyclic terpane/hopane) also showed this insulation effect. Additional results of this study include: 1) confirms the use of chemical biomarkers for thermal maturation, 2) correlation of Devon rocks with Greenland, and 3) discovery of a new species of the rare conodont genus *Wandellia*.

Establishment of an Undergraduate Research Program for the NASA Haughton-Mars Project. MEGAN E. ENNIS[1,2]*, CHARLES E. MASON[1], JAMES W. ATWOOD[2] and WESLEY SMITH[1], [1]Department of Physical Sciences, Morehead State University, Morehead, KY 40351; [2]Department of Space Science, Morehead State University, Morehead, KY 40351.

The NASA Haughton-Mars Project (HMP) base camp is located on the rim of the Haughton impact crater on Devon Island in the Canadian High Arctic. Dr. Pascal Lee established HMP as a Mars analog site in 1997. The focus of this project is to develop a funded, nationally competitive HMP undergraduate research program, which will allow undergraduates to participate in this exciting, fieldwork-based geology, and planetary science research program. During the 2006 HMP field season the PI and one MSU undergraduate (Wesley Smith) worked on a collaborative project on biomarkers with Dr. John Parnell and his research group from Aberdeen University in Scotland. Here we utilized the conodont color alteration index or CIA to check results of chemical biomarkers taken from melt breccia clasts by Dr. Parnell's research group. Our study, using CIA of conodonts, gave the same temperatures reached by the melt breccia clasts as the chemical biomarkers thus corroborating the results of their study. Also, in this study besides such things as the discovery of new conodont species, an insulation effect was noted between the inner and outer parts of the large melt breccia clasts. During the 2007 HMP field season two MSU undergraduate students, Megan Ennis and James Atwood, accompanied the PI. Megan's primary project was to collect large melt breccia clasts from around the crater to examine the insulation effect noted in the 2006 study. James' primary project was to work with Dr. Lee in the development of a plan for a SETI antenna site at HMP.

HEALTH SCIENCES

Extended Longitudinal Studies of Family Size and the Human Sex Ratio. ARCHANA LAKKARAJU* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

As the fourth study over a span of about 40 years, this study further characterizes the human sex ratio of families of students enrolled at Western Kentucky University. Student respondents provided data on the parental, present, and projected generation. Survey results permitted calculations of average family sizes and sex ratios which were used in comparisons with results of the previous studies. Average numbers of children were 4.04, 2.58, and 2.45, and secondary sex ratios (males: 100 females) were 102, 108, and 141; respectively, for the parental, present, and projected generations. Average family size decreased progressively during the period of study. Secondary sex ratios ranged from 97 to 108 males: 100 females. Gender composition of the first two and three children influenced parities in two ways, namely preference for both genders and preference for males. Results of the present and previous studies indicated that the most desired families consisted of two children, both genders, and the male born first. Realization of this preference would contribute toward a balanced sex ratio. Other preferences included more male than female children. Overall, sex ratios for desired families in the four studies ranged from 133 to 141 males: 100 females. Attainment of these results would imbalance the human sex ratio creating social discord.

Plate Waste – What Head Start Students Won't Eat. SUSAN TEMPLETON* and MARTHA MARLETTE, Human Nutrition Research, Kentucky State University, Frankfort, KY 40601.

The Head Start Program provides breakfast and lunch (morning) or lunch and snack (afternoon) for students in

their half-day program. To determine the actual benefit students receive from these meals, uneaten food was collected from 644 plates after 169 meals (58 breakfasts, 87 lunches, and 24 snacks) served to 114 four-year-olds at a local Head Start Center. Individual leftover food items were separated and weighed. A sample plate was also collected at each meal. Before and after photos were taken of each plate, and compared to sample plate photos to improve initial serving size estimates; initial servings were also adjusted to reflect additional helpings taken. Plate waste was calculated by comparing leftovers to total amount given for each food item. Percent of waste varied by food group and meal, and by which program (morning or afternoon) the student attended. Overall waste was high: meat substitutes (eggs and beans) 65%, vegetables 64%, mixed dishes 51%, fruit 49%, grain items 43%, dairy items 41%, and meats 40%. All food group pairings except dairy-grains, fruit-mixed dish, grains-meat, meat substitutes-vegetables, and dairy-meat were significantly different ($P < 0.05$). Breakfast had higher waste for dairy products (49%), lunch had higher waste for fruit (57%), and snack lower waste for grains (49%), all significant at $P < 0.001$. Overall lunch waste was significantly higher for afternoon students, 60% vs. 47% for morning students ($P < 0.001$). Many children are not consuming enough to receive the full benefit because they do not find the meals appetizing or have eaten before coming to school.

Weight Status, Eating Habits, and Activity Patterns of Kentucky State Fair Attendees. MARTHA MARLETTE* and SUSAN TEMPLETON, Human Nutrition Research, Kentucky State University, Frankfort, KY 40601.

Kentucky ranks seventh highest in the nation in obese adults, and third in overweight children. KSU Nutrition and Health Research Staff measured body fat percentage by bioelectrical impedance analysis (BIA) for 941 adults and measured height and weight to calculate Body Mass Index (BMI) for 115 boys and girls under age 21 during the eleven (11) days of the 2007 Kentucky State Fair. Each participant also completed a survey on diet and physical activity habits and received counseling on how to manage their diet and exercise in order to achieve/maintain a healthy weight. Both BMI and BIA were classified by age and gender standards. BMI results indicated 65.8% of girls and 55% of boys were Normal, 20.5% of girls and 20% of boys were At Risk of Overweight, and 11% of girls and 25% of boys were Overweight. BIA values indicated 46.9% of females and 49.7% of males were Healthy, 25.6% of females and 27.4% of males were Overfat, and 16.3% of females and 21% of males were Obese. These data indicate that more males than females were obese. When compared with At Risk and Overweight children and Overfat and Obese adults, Normal children and Healthy adults reported more frequent positive behaviors including exercise and fruit consumption ($P < 0.001$), and less frequent negative behaviors such as TV viewing/video game usage, eating at fast food restaurants ($P < 0.001$), breakfast skipping ($P < 0.05$), and skipping of other meals ($P < 0.01$). More encouragement of proper diet and exercise habits is needed for all ages.

The Effect of Flavonoids on the Changes of Zinc and Copper Concentrations in Tissues of Rats. FREDERICK N. BEBE and MYNA PANEMANGALORE, Nutrition and Health Program, Kentucky State University, Frankfort, KY 40601.

The consumption of purified flavonoids as dietary supplements increases the possibility of toxicity at high levels. It is, therefore, important to assess safe levels of flavonoids for humans, and also determine if they interact with essential trace metals. The objective of this study was to determine the effect of administering different levels of a flavonoid mixture and determine changes in the concentration of zinc and copper in tissues of rats. Male SD rats (150–175 g, 6/group) were fed the AIN-93 diet. A mixture of equal amounts of flavonoids, quercetin, rutin and catechin, were fed at 5, 25, 50, 100 and 200 mg/kg bw (Groups: CON, FM5, FM25, FM50, FM100, FM200) in 0.1% PEG orally for 30 days. There was a significant reduction in copper retention in all tissues especially at high levels (100 and 200 mg/kg bw) of flavonoid exposure. In the liver, Cu declined by an average of 15% and 23% in the higher FM groups compared to the CON; in the kidney, the decline in Cu retention ranged from 6% to 44%. In the small intestines and the small intestinal mucosa, Cu retention declined significantly at higher levels of flavonoid exposure: FM100 (35 and 17%) and FM200 (58 and 26%), respectively, compared to CON. The changes in Zn concentrations in the liver and kidney of the FM groups were less significant than that of Cu. These data together with previous data suggest that high level consumption of flavonoids could compromise Cu homeostasis in tissues. (USDA, Evans Allen Grant # KYX-50-03-17H)

Tolerance of Cadaver Spines: Can Traction Force Cause a Ruptured Disc? CHARLES BARRETT JR.*, BENJAMIN RAY and DAVID PORTA, Department of Biology, Bellarmine University, Louisville, KY 40205.

Vertebral Axial Decompression (VAX-D®) is a treatment for low back pain from herniated discs. It consists of a traction machine that stretches the back to decompress the spine. A patient typically undergoes 15 cycles of tension (up to 75 lbf) and relaxation (20 lbf) in one 45-minute treatment. A 2003 case study published in the Mayo Clinic Proceedings described a man who supposedly suffered a ruptured L5-S1 disc from VAX-D treatment. There were no force plots provided and no concomitant injuries described. This series of experiments attempted to replicate the noted injury by applying traction force to 8 dissected embalmed cadaver spines (6 females age 55–83 and 2 males age 55 and 84). The L-1 vertebra was potted using Easy-Flo 60 polyurethane casting resin and fixed in place. A winch applied traction force to the pelvis through an in-line force transducer

(Omega DLC 101-5K). The transducer signal was fed to a PC equipped with InstruNet World Win32 software which plotted the force over time. Traction force was increased over 5–10 seconds until gross failure was detected. The potting failed in two specimens (at 128 and 218 lbf.). In the remaining six, the failure force ranged from 195 to 270 lbf and no isolated ruptured discs were produced. Three tests resulted in sacral fractures (avg. 237 lbf) and three resulted in vertebral fractures (avg. 226 lbf.) with associated ligamentous injuries. These results do not support the notion that a properly used traction machine would cause an isolated ruptured disc.

PHYSICS & ASTRONOMY

Development of a G/T Elevation Profile of the Morehead State University 21 Meter Space Tracking System and Radio Telescope. MEGAN E. ENNIS*, BENJAMIN K. MALPHRUS, JEFFERY KRUTH and JAMES W. ATWOOD, Department of Space Science, Morehead State University, Morehead, KY 40351.

The Morehead State University 21 Meter Space Tracking Antenna and Radio Telescope is a medium aperture centimeter-wave length radio telescope designed to permit the investigation of a wide variety of radio sources at different frequencies, using L-Band, S-Band, and Ku-Band, and also to provide space tracking services for satellite telecommunication applications. The performance of this instrument as a radio telescope is highly dependent upon the antenna's radio frequency (RF) performance characteristics. Primary among these are antenna gain and system temperature. This study of determining the antenna gain and system temperature will be accomplished primarily using only the L-Band, which operates on a frequency range from 1.4–1.7 GHz and Ku-Band (11.2–12.7 GHz) and may eventually expand to cover S-Band. The antenna gain is a measure of the directivity and the telescope's ability to convert radiation energy to output energy (and vice versa via the theory of antenna reciprocity). While it is difficult to directly measure antenna gain or system temperature, a characteristic known as G/T (often referred to as the antenna figure of merit) can be empirically measured. Antenna gain can be determined by integrating under the antenna radiation pattern. System temperature can be numerically derived from the antenna gain and G/T measurements. The value of G/T serves as a predictor of the instrument's ability to detect and measure characteristics of distant astronomical sources. The initial results of G/T measurements at L- and Ku-Band are presented, as well as initial measurements of G/T verses elevation angle (elevation profile) made using radio sources.

Evidence of New Subatomic Particles With Heavy Quarks. AKHTAR H. MAHMOUD, Department of Chemistry & Physics, Bellarmine University, Louisville, KY 40205.

Particle accelerators collide matter and antimatter head-on, and the energy released from this collision recreates the primordial universe, on a small scale for very short times. At a few microseconds after the Big Bang, a host of subatomic particles known as charmed baryons populated the universe, which soon decayed into protons and neutrons, the primary constituents of the nucleus of the ordinary atom. The mass spectroscopy of subatomic particle provides crucial clues about the structure of Baryons in the early universe. In recent years, both the CLEO experiment at CESR (Cornell Electron Storage Ring) and the BABAR experiment at SLAC (Stanford Linear Accelerator Center) have reported the evidence of several new charmed baryons. These intense high energy collisions convert energy into matter inside the detector at the femtoscale level, creating new types of matter with the heavy charm quark. Evidence of new subatomic particles from the CLEO and the BABAR experiments will be presented.

PHYSIOLOGY & BIOCHEMISTRY

The Role of Transforming Growth Factor Beta Receptor I (TGFβRI) Variants in Cell Proliferation During Development of Uterine Cervical Neoplasia. DIANA LAMPE*, JAMES SCHAEPER, ALLISON FOERTSCH, STEVEN PFEIFFER, LARRY DOUGLASS and JULIA CARTER, Wood Hudson Cancer Research Laboratory, Newport, KY 41071.

Cervical cancer (CxCa) is the second most frequently diagnosed cancer in women worldwide. Human papilloma virus (HPV) is necessary but not sufficient for CxCa development. Transforming growth factor-beta (TGF-β) inhibits normal epithelial cell growth but promotes mesenchymal cell growth. Decreased TGF-β is associated with cancer initiation. Increased TGF-β is associated with cancer invasion and metastasis following epithelial to mesenchymal transition (EMT). TGFβRI is a key element in TGF-β signaling. Here, we studied two germline variants in TGFβRI, Int7G24A and TGFβRI*6A. We hypothesized that TGFβRI variants cause decreased TGF-β signaling resulting in increased proliferation in normal cervix and increased susceptibility to cervical cancer. To test this hypothesis, formalin-fixed, paraffin-embedded tissues from four stages of CxCa progression were stained immunohistochemically using a monoclonal mouse antibody to Ki 67 (MIB-1) to detect proliferating cells. Proliferation was determined semiquantitatively (1+, 2+, 3+). Variant status in 96 patients was determined using DNA extraction, polymerase chain reaction, gel electrophoresis, and capillary electrophoresis. Cell proliferation increases significantly during CxCa progression ($P = 0.0001$). Nearly 62% of patients without CxCa did not carry either of the variants. In contrast, 59% of the women with invasive CxCa were TGFβRI variant carriers. Cell proliferation was higher in normal tissue from variant carriers compared to normal tissue from wild type patients. Cell proliferation was significantly increased in tumors from wild type CxCa patients compared to the variant carriers. These preliminary data suggest that TGFβRI variant carriers are more susceptible to CxCa initiation but that EMT may occur more frequently in tumors of non-carriers.

The Importance of Different Regions of the Face and Temperature in Eliciting the Human Diving Reflex. SAM WEIGEL* and THOMAS E. BENNETT, Department of Biology, Bellarmine University, Louisville, KY 40205.

The diving response is characterized by bradycardia, decreased cardiac output, increased arterial blood pressure, redistribution of regional blood flow, and perhaps an active contraction of the spleen. Holding one's breath alone is enough to trigger the diving response, but several other factors, when stimulated simultaneously with apnea, cause the response to be much more pronounced. A previous study (Shuitema and Holm, 1988) showed that the forehead and eyes are key in eliciting the diving reflex; however they failed to take into account varying temperatures and the role of gender. It is the goal of this study to take these factors into account as well as to monitor O_2 saturation during the diving reflex. Ten males (age 20–21) and ten females (29–21) will be participating in this study. Six areas of the face were isolated (forehead, eyes, ears, cheek, nose, and mouth) using wet towels and tested with and without apnea at three temperatures (0–3, 20–23, and 35–38°C). Resting heart rate with and without apnea was recorded as controls. A digital physiograph system (BIOPAC Systems, Inc.) was used to record heart and respiration rates and a pulse oximeter (NELLCOR, Inc.) was used for 0_2 saturation. A disk thermometer (Yellow Springs Instruments) was placed in the wet cloth to monitor stimulus temperature. Early results show that low temperatures on the eyes with apnea produce the strongest reflex and males are more pronounced than females. O_2 saturation change was minimal. (This study was approved by the IRB of Bellarmine University.)

Salivary Testosterone and Dehydroepiandrosterone (DHEA) as Predictors of Vocal Register in Adolescent Male Singers. WESLEY THOMAS*, THOMAS E. BENNETT, ALEXANDER T. SIMPSON and WILLIAM J. TIETJEN, Departments of Biology and Music, Bellarmine University, Louisville, KY 40205.

During puberty, hormones direct the growth and development of the larynx causing the characteristic 'voice drop' in males. The deepness of the voice and lower pitch frequencies directly correlate to the increased size of the larynx. As the vocal chords elongate, range descends and registers redevelop. The final size of the larynx after maturation reflects the hormonal environment and target organ sensitivity during puberty. A previous study (Harries et al. 1997) failed to show a correlation between salivary testosterone level and fundamental frequency in vocally untrained 13–15 year old boys. The goal of this study is to assess the relationships among androgenic hormones, morphologic parameters, and the vocal register in late adolescent males with formal vocal training. Sixteen males (age 16–19) attending a summer vocal program participated in this study. To control for diurnal fluctuations subjects were tested in one hour sessions in the afternoon. Salivary samples, collected at the beginning, middle and end of the session were polled and analyzed for testosterone and DHEA with commercial enzyme immunoassay kits (Salimetrcs, LLC). Height, weight, neck length, and neck circumference (2 locations) were taken and a health assessment questionnaire completed. Each subject's singing voice was digitally recorded and vocal register was determined following a series of vocal warm-up exercises. Vocal range and fundamental frequency were determined using computer software (Sound Forge). Preliminary analysis of the data suggests several relationships among the measured variables. (This study was approved by the IRB of Bellarmine University.)

PSYCHOLOGY

Pilot Comparison of the Aptitude Subscales of the Scholastic Abilities Test for Adults and the Wechsler Adult Intelligence Scale-III. ASHLEY KIRK* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Recent empirical data from large scale surveys of college students indicate marked growth among the rates of students entering colleges with learning disabilities relative to nearly twenty years ago. Psychoeducational tests are commonly used during the screening and diagnostic process for evaluating students for learning disorders when they present for evaluation. One of these measures, the Scholastic Abilities Test for Adults (SATA), is commonly used as an aptitude-ability discrepancy screener and is approved by the Educational Testing Board as a qualified achievement instrument. During the development stages of the SATA, preliminary correlations were reported between like aptitude scales of the SATA and the Wechsler Adult Intelligence Scale-Revised in a small sample of students (n = 35). No published studies have evaluated the current status of the psychometrics of the SATA, despite its usage. The present study reports pilot data concerning the validity of the aptitude scales of the SATA using the current version of the Wechsler Scales, the Wechsler Adult Intelligence Scale-III. Correlations of a smaller magnitude emerged for all three SATA aptitude scales relative to those reported using the WAIS-R. Ability-achievement discrepancies were calculated to evaluate the ability of the SATA to detect potential needs of students for follow-up testing for learning disorders. The sensitivity of the SATA to detect a learning problem was found to be low (50%). The results are discussed in relation to future needs for research evaluating the psychometrics of the SATA and its utility as a screening measure. Research supported by a grant from KY EPSCoR.

The Ability of the Wender Utah Rating Scale to Differentiate Among Mixed Symptoms of Major Depression, Anxiety Disorders, and AD/HD. CASSIE M. WATKINS* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a prevalent disorder with high rates of co-morbidity with psychiatric disorders such as mood and anxiety disorders.

Symptoms of AD/HD overlap with aspects of depression and anxiety, and the latter have been shown to produce false positives on AD/HD screening measures. Thus, accurate diagnosis of AD/HD can be complicated especially when symptoms of other psychiatric disorders are present. The current study examined the ability of the short form of the Wender Utah Rating Scale (WURS) to differentiate between mixed symptoms of Major Depressive Disorder, Anxiety Disorders, and AD/HD at a sublinical level. The WURS is a unique empirical instrument for evaluating adults' retrospective reports of childhood AD/HD. In particular, WURS scores can be used to accurately differentiate adult groups without AD/HD or psychiatric disorders, and those with either AD/HD, or Major Depression. Because comparative psychiatric data are lacking for anxiety symptoms for most AD/HD instruments, and none have addressed co-morbidity issues, the present study was conducted. Extensive psychosocial, AD/HD and psychiatric rating scale data were inspected and four subclinical groups were identified (AD/HD with Anxiety and/or Depression, Anxiety without AD/HD, Anxiety + Major Depression without AD/HD, and Control). It was predicted and found that the AD/HD group and the mixed Anxiety and Depression group scored significantly higher on the WURS relative to the control group. In addition, the AD/HD group had significantly higher WURS scores relative to all other groups. Research supported by a Morehead State University Undergraduate Research Fellowship and KY EPSCoR.

The Relationship of Substance Abuse Symptoms on the Psychiatric Diagnostic Screening Questionnaire and Substance Abuse Subtle Screening Inventory-3. JARED DILLOW* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Substance abuse is a serious, highly prevalent problem, especially among college-age adults. Current empirical data indicates nearly half of students nationwide engage in binge drinking which is associated with a variety of academic, interpersonal, legal, and medical and psychiatric problems. In addition, an estimated 1,700 students die in alcohol related deaths annually. Self-report scales are commonly used as part of national screening outreach to identify high risk students, and as part of psychotherapy to gage the severity and breadth of substance abuse symptoms. One particularly promising measure for a college population is the Psychiatric Diagnostic Screening Questionnaire (PDSQ). The PDSQ screens for symptoms of the most common DSM-IV-TR based disorders found in clients who present to outpatient psychotherapy. Data for this measure, however, are lacking for its substance abuse scales. In this study, the relationship between the alcohol and substance abuse subscales of the PDSQ and SASSI-3 were investigated. In a sample of three hundred undergraduates, it was predicted and found that moderate to strong correlations emerged between the SASSI-3 and both the PDSQ Alcohol abuse subscale ($r = 0.60$) and the PDSQ Substance abuse subscale ($r = 0.58$). These results are discussed in relation to use of the PDSQ for identifying students with potential substance abuse problems. Research supported by a Morehead State University Undergraduate Research Fellowship and a prior grant from KY Statewide EPSCoR.

Discriminant Validity of the Adult AD/HD Self Report Scale-v1.1 (ASRS-v.1.1) in a College Population. JESSICA E. HILL* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351 and WARREN LAMBERT, Somerset Community College, Somerset, KY 42501.

Attention Deficit/Hyperactivity Disorder (AD/HD) affects an estimated 8-million adults, including 2% to 8% of the college population. AD/HD rating scales are commonly used for screening and diagnosis of AD/HD in adults. However, many AD/HD rating scales are expensive, time consuming to administer and score, and contain questions which are more child-focused in their symptom context. To address these problems, the World Health Organization (WHO) published the Adult AD/HD Self Report Scale-v1.1. The ASRS-v1.1 is a free, publicly available AD/HD rating scale which assesses the DSM-IV-TR symptoms of AD/HD using questions with adult symptom contexts. To date, there are limited data concerning the validity of the ASRS-v.1.1 for use in screening and evaluation in a college population. The purpose of this study was to evaluate the ability of the ASRS-v1.1 to differentiate between college students with and without self-acknowledged AD/HD. Large scale survey data ($n > 1000$) was collected from students enrolled at a 4-year university and at a regional community college. Students reporting an existing diagnosis of AD/HD ($n = 44$) were identified and demographically matched with those without any positive history of significant AD/HD symptoms. Multiple diagnostic indicators in the survey data external to the ASRS-v1.1 were used to confirm group status. Using independent samples *t*-tests, it was predicted and found that ASRS-v1.1 scores from AD/HD students were significantly higher than for students without AD/HD. Research supported by prior grants from KY EPSCoR and the Institute for Regional Analysis and Public Policy at Morehead State University.

SCIENCE EDUCATION

Development of Biotechnology Courses to Enhance Aquaculture and Biology Programs and Recruit Students to Kentucky State University. LI LU[1]*, KIRK W. POMPER[1], KARAN KAUL[2], NARAYANAN RAJENDRAN[2] and JAMES TIDWELL[3], [1]Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601; [2]Carver Hall, Kentucky State University, Frankfort, KY 40601; [3]Division of Aquaculture, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

Biotechnology impacts human health, medical diagnosis, forensics, pharmaceuticals, nutrition, the environ-

ment, and agriculture. Training in biotechnology and molecular biology techniques is critical for students who wish to pursue careers in the life sciences and agriculture. The biotechnology industry in the U.S. and Kentucky has also almost doubled in the past few years creating new jobs for Kentucky's college graduates. In 2005, a USDA 1890 Institution Teaching and Research Capacity Building Grant was funded with the objectives to 1) support the instruction and development of two courses, "Understanding Biotechnology" and "Advanced Techniques in Biotechnology," 2) enhance laboratory experiences of the course "Cell Biology" with molecular techniques, 3) support individual undergraduate student research projects in biotechnology, and 4) support recruitment of undergraduate Biology and Aquaculture Master's students at KSU through high school recruiting visits, a biotechnology website, and increased KSU biotechnology library holdings. Over 30 students have already participated in classes supported by this grant. In the course "Understanding biotechnology," students extract DNA from plant and aquaculture species, and conduct techniques such as Southern blotting, Western blotting, PCR, bacterial transformations, and reporter gene screening. Molecular marker techniques to examine genetic relationships in the Annonaceae family have been conducted by students in the class and will be discussed. A web site was created to promote the KSU biotechnology program and can be found at http://biotech.kysu.edu/.

Online Courses: Are Students Learning? JOHN G. SHIBER, Division of Health Sciences, Kentucky Community & Technical College System – Big Sandy District, Prestonsburg, KY, 41653.

One hundred fifty-nine community college students enrolled in 106 courses from 12 general study areas from Fall-2006 to Fall-2007 were surveyed about their online credit course experience. Most found the course presentations easy to understand and were able to successfully complete all course requirements, but only 41% said they felt they had learned as much in this format as they would have in a regular classroom, i.e., 59% believed they would have learned better with traditional instruction. Math had the highest dropout rate and only 31% said they had learned better online. Communication, philosophy, information technology and business had even lower percentages on this point. Nearly half the respondents judged online tests as difficult-very difficult - 74% of math and 64% of science students judged them as such. Math students repeatedly commented that math belongs in the classroom. An average of 33% of all participants experienced technical difficulties with online classes, but most course grades were A, B and C. The majority said their courses were well-suited for online delivery and that they would take more online courses. The overall responses and comments suggest, however, that students may be trading off actual learning for convenience, especially in math but also in philosophy, communications, business, science, and information technology courses and that some courses are not appropriate for online learning and should not be offered in that format to all students. Further research along these lines, along with establishing certain criteria for accepting students into online programs and reviewing testing protocol, is encouraged.

ZOOLOGY

A Survey of Amphibians and Reptiles within a Constructed Wetland and Restored Stream Corridor in Campbell County, Kentucky. JEFFREY GOESSLING* and JOHN W. FERNER, Department of Biology, Thomas More College, Crestview Hills, KY 41017; PAUL KRUSLING, Scott High School, 5400 Old Taylor Mill Road, Taylor Mill, KY and SCOTT FENNELL, Center for Applied Ecology, Northern Kentucky University, Highland Heights, KY 41076.

A survey of amphibians and reptiles was conducted in a stream corridor restoration project from May to September 2007. The site included a restoration along about 2000 feet of the Moock Road Tributary of Three Mile Creek at the Bentwood Condominiums in Wilder, Campbell County, Kentucky. The restoration project was done between June and November 2003 and included stabilization of stream banks, construction of stream riffle complexes, restoration of stream sinuosity, creation of two stormwater detention wetlands, enhancement of an existing pond and enhancement of 25 acres of riparian woodland. A total of seven visits were made to the site between 2 May and 20 September 2007 for from one to two hours per visit. A total of six amphibian and seven reptile species were observed in the habitats within this conservation area, including the wetlands, pond, stream, fields and slopes. Two species of reptiles represent new geographic records for Campbell County: the common musk turtle (*Sternotherus odoratus*) and the red-eared slider (*Trachemys scripta elegans*). The design of the physical features in this restoration project was found to provide a successful corridor for colonization of the newly created wetlands by amphibians and reptiles from surrounding habitats.

Initial Studies of Ventral Eversible Gland Function in Velvetbean Caterpillars, *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). ANTHONY J. LENTZ, Department of Biology, Bellarmine University, Louisville, KY 40205.

A ventral eversible gland was discovered in the prothorax of velvetbean caterpillars (*Anticarsia gemmatalis*), a pest of soybeans and other plants in the southeastern United States. While such glands have been reported in a few other Noctuidae, only the fall armyworm (*Spodoptera frugiperda*) has been studied in depth (by Marti and others at USDA) and no function was determined. Glands were removed from 7 to 10-day old, cold-anesthetized velvetbean caterpillars to examine the impact on larval life cycle, onset of pupation and larval defensive response. Pilot studies suggest that larvae

without glands (1) pupate zero to two days sooner than intact larvae, (2) have comparable weight gain and survival, and (3) exhibit behavioral defenses similar to larvae with glands when provoked in simulated attacks. There is no evidence suggesting that these glands function in defense. Exploratory studies comparing the effect of gland removal on pupation of velvetbean caterpillars and the fall armyworm are underway.

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Kentucky Academy of Science Business Meeting
University of Louisville, November 10, 2007

President—Nigel Cooper

1. President Cooper called the meeting to order and thanked retiring Board members Miriam Kannan, Phil Lienesch, and Chris Lorentz, presenting each with a plaque to commemorate their service.
2. Nigel thanked all KAS members for helping him lead the Academy through a successful year, with special thanks to
   Ruth Beattie for her work with the Junior Academy,
   David White for the improvements he has made in the Journal,
   John Mateja for recruiting good speakers this year,
   Robin Cooper for his work on the superlative awards,
   Bob Creek for the countless details he takes care of every year,
   Rob Kingsolver for co-chairing the local arrangements committee, and
   Jeanne Harris for organizational support.

Vice President — Robin Cooper

Next year, the KAS meeting will be hosted by the University of Kentucky in Lexington.

Treasurer – Ken Crawford

1. KAS can expect an additional $80,000 from the reversion of the Athey grandchildren's education fund in December of this year.
2. The Board has decided that KAS should cover educational expenses remaining for the youngest Athey heir to complete her Masters degree.
3. The Wachovia account has grown from $400,000 to $500,000, not counting withdrawals made to fund KAS operations.
4. The 2007 budget is roughly balanced, with a small shortfall to be covered by our investment income.
5. The 2008 budget totals $104,000. The only significant change from the previous year is a higher amount allocated for the Junior Academy.

Executive Director – Jeanne Harris

1. We have registered c. 700 people for this annual meeting.
2. The Academy membership is 580, composed of 105 students, 396 regular members, 68 lifetime members, and 11 family members.
3. We have recruited two new institutional members: Lindsey Wilson College and Brescia University.

JKAS – David White

The December issue has been sent to Allen Press. Members should be receiving copies soon.

KJAS – Ruth Beattie

Please help recruit more high schools to the Kentucky Junior Academy of Science. We currently have only 7 active schools.

Nominations – David Olson

David announced the following new Board members and officers for 2008:

1. Nancy Martin—Vice President
2. Rob Kingsolver—Secretary
3. Ken Crawford—Treasurer
4. Cheryl Davis—At-large Representative
5. Richard Durtsche—Biological Sciences Representative

President Elect – John Mateja

John thanked Nigel Cooper for his service over the past year and presented a plaque from the Academy. Nigel handed over the KAS gavel and presented the traditional $100 gift to the President's Fund as his final act of service in a successful year for KAS.

New President's Remarks

John Mateja said he was happy to serve as KAS president and looked forward to the January meeting.

Meeting Adjourned

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