JOURNAL OF THE KENTUCKY ACADEMY OF SCIENCE

Official Publication of the Academy

Volume 70
Number 1
Spring 2009

All manuscripts and correspondence concerning manuscripts should be addressed to the Editor (david.white@murraystate.edu).

The JOURNAL is indexed in BioOne, Cambridge Scientific Abstracts, Selected Water Resource Abstracts, State Academies of Science Abstracts, and Zoological Record.

Membership in the Academy is open to anyone with an interest in science. Interested parties can join online at www.kyscience.org where membership options and benefits are listed. Please contact the executive director at executivedirector@kyscience.org for additional information.

The JOURNAL is made available as a PDF to all active members of the Academy (go to www.kentuckyscience.org). Hard copy subscriptions are available. Subscription rates for nonmembers are $50.00/year domestic, $60.00/year foreign. Back issues are $30.00 per volume.

The JOURNAL is issued in spring and fall. Two issues comprise a volume.

All other correspondence concerning memberships or subscriptions may be addressed to the Executive Director, Kentucky Academy of Science, P.O. Box 22579, Lexington, KY 40522-2579 or executivedirector@kyscience.org.

♾ This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

INSTITUTIONAL AFFILIATES

Enhanced Members

Berea College
Bellarmine University
Brescia University
Centre College
Eastern Kentucky University
Kentucky State University
Morehead State University
Murray State University
Northern Kentucky University
Spalding University
Transylvania University
University of Kentucky
University of Louisville
Western Kentucky University
Kentucky Community and Technical College System

Fellow

Kentucky Biomedical Research Infrastructure Network

Sustaining Member

Campbellsville University

Members

Asbury College
Kentucky Wesleyan College–Lindsey
Wilson College
Pikeville College
Thomas More College
University of the Cumberlands

INDUSTRIAL AFFILIATES

Enhanced Member

Kentucky Science and Technology Corporation

Patron

Lumins Associates

Sustaining Member

Third Rock Consultants

Members

Kentucky American Water
Wood Hudson Cancer Research Laboratory

Associate Members

Hoffman Environmental Research Institute

Pawpaw (*Asimina triloba*) in Kentucky. See article by Kirk W. Pomper, Jeremiah D. Lowe, Li Lu, Sheri B. Crabtree, and Lauren A. Collins, page 3, this issue.

JOURNAL OF THE KENTUCKY ACADEMY OF SCIENCE
ISSN 1098-7096
Continuation of
Transactions of the Kentucky Academy of Science
Volume 70 Spring 2009 Number 1

J. Ky. Acad. Sci. 70(1):3–11. 2009

Clonality of Pawpaw (*Asimina triloba*) Patches in Kentucky

Kirk W. Pomper,[1] Jeremiah D. Lowe, Li Lu, Sheri B. Crabtree, and Lauren A. Collins
Kentucky State University, Land Grant Program, 129 Atwood Research Facility, Frankfort, Kentucky 40601-2355

ABSTRACT

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree-fruit (see overleaf, page 2) native to the southeastern region of the United States. Kentucky State University serves as the USDA-National Clonal Germplasm Repository for pawpaw, therefore assessing genetic diversity across the pawpaw's native range is a high priority. Pawpaw is usually found in large patches as an understory tree and root suckering likely occurs. To determine if native pawpaw patches are clonal, DNA was extracted from leaf samples collected from trees in six native patches in three counties in central Kentucky. Two ISSR-PCR primers yielded three polymorphic and six monomorphic markers in the six patches. Three patches did not display any polymorphic markers in each patch, suggesting they were clonal. However, three other patches did show polymorphic markers within each patch, indicating these patches were not clonal and contained trees of at least two genotypes within each patch. This study suggests that to assess the genetic diversity of a pawpaw patch or local population, more intensive sampling strategies will be required.

KEY WORDS: *Asimina triloba* (L.) Dunal, Kentucky banana, understory tree, inter simple sequence repeat, ISSR DNA markers

INTRODUCTION

The North American pawpaw [*Asimina triloba* (L.) Dunal] is in the early stages of commercial production (Pomper and Layne 2005). This tree produces the largest edible fruit native to the United States that may reach up to 1 kg in size (Darrow 1975). The pawpaw fruit has both fresh market and processing potential. The fruit is very nutritious (Peterson et al. 1982); it has an almost tropical aroma, smooth custard-like texture, and flavors reminiscent of a combination of mango, banana, and pineapple (Layne 1996; Shiota 1991; Duffrin and Pomper 2006). Natural compounds in the leaf, bark, and twig tissues of pawpaw possess insecticidal and anti-cancer properties (McLaughlin 2008). The unique qualities of the fruit, ornamental value of the tree, and the potential for useful bioactive compounds suggest that pawpaw has great potential as a new high-value crop.

Pawpaws are native to mesic hardwood forests of 26 states in the eastern United States, including Kentucky (Chester et al. 1995) and surrounding states (Rheinhardt and Rheinhardt 2000; Larimore 2003; Lagrange and Tramer 1985), ranging from northern Florida to southern Ontario (Canada) and as far west as eastern Nebraska (Kral 1960). This small, deciduous tree may attain a height of 5 to 10 m and tends to be found in patches (Layne 1996). Pawpaws are often found growing as understory trees in the deep, rich fertile soils of river-bottom lands (Kral 1960; Callaway 1990; Callaway 1993; Young and Yavitt 1987). The pawpaw is diploid [n = 2x = 18, (Bowden 1948; Kral 1960)] and is pollinated by flies and beetles (Faegri and van der Piji 1971). Pawpaw flowers are strongly protogynous and are likely self-incompatible (Willson and Schemske 1980),

[1] Corresponding author e-mail: kirk.pomper@kysu.edu

Table 1. Location and description of pawpaw patches sampled for study.

Patch	Kentucky county	GPS coordinates	Approximate number of stems	Topography
A	Franklin	N38 07 44.57 W84 53 13.71	100	Top of hill
B	Franklin	N38 07 47.98 W84 53 10.23	500+	Top of hill
C	Woodford	N38 08 31.44 W084 51 32.58	100	Gradual slope
D	Woodford	N38 08 00.06 W084 51 07.62	30	Flat area
E	Menifee	N37 49 36.36 W083 38 28.74	50	Steep slope
F	Menifee	N37 49 22.92 W083 37 41.70	100	Steep slope

although some cultivars, such as 'Sunflower', may be self-fruitful. Pollinator limitation has often been suggested as an explanation for low fruit set (<0.5%) in wild patches (Willson and Schemske 1980). Low light levels in the understory may also limit flower bud formation during the previous summer. If flowers are formed and successfully pollinated, low light levels may also reduce photosynthate partitioning to fruit and reduce fruit set. If fruit is produced, the relatively large pawpaw seeds are well-adapted for dispersal by mammals such as coyotes and raccoons (Cypher and Cypher 1999).

The potential clonality of pawpaw patches and the interaction of this plant with other plant species in the forest is of interest to researchers (Hosaka et al. 2005, 2008; Cole and Weltzin 2005). Pawpaws often produce many root suckers, presumably forming large clonal patches, thus contributing to poor fruit set within a patch due to flower self-incompatibility. Clonality has been suggested as an adaptation by pawpaw to persist and spread on the forest floor. Hosaka et al. (2005) evaluated three possible functions of clonal growth related to pawpaw: (1) risk spreading through multiplication of stems, (2) enhanced establishment and survival of new stems, and (3) horizontal expansion growth of patches. These authors found no difference in stem turnover rate for patches of different size, indicating that stem production is more than sufficient to avoid patch extinction. They found no evidence that clonal growth contributes to extensive horizontal expansion of patches, suggesting that ensuring survivorship of new stems is the main ecological role of clonal growth in pawpaw. The origin of new stems (ramet or seedling) in patches was difficult to ascertain, but inspection of root connections by excavating pawpaw patches in an area adjacent to the study plot indicated that more than 90% of the stems appeared to be of clonal origin (Hosaka et al. 2005). Neither ramet occurrence over time nor mortality was correlated with light conditions; however, the structure of pawpaw patches and ramet size was influenced by canopy condition (Hosaka et al. 2008). Hosaka et al. (2005, 2008) did not determine if the patches were indeed clonal, and they did not determine if fruit were produced in the patches. Although pawpaw trees in patches reproduce clonally, whether patches consist of a single genotype, potentially from a single seed from the original fruit (which usually contains about 15 seeds), has not been examined.

Determining the clonality of pawpaw patches is important in developing strategies for the conservation of pawpaw genetic resources. In 1994, Kentucky State University (KSU) was designated as a satellite repository for *Asimina* preservation in the U.S. Department of Agriculture (USDA), National Plant Germplasm System (NPGS). As a result, germplasm evaluation, preservation, and dissemination are high priorities for KSU. The repository orchards currently contain over 2000 accessions collected from the wild in 17 states and more than 40 cultivars. One of the goals of the repository is to assess levels of genetic diversity in native populations, in the repository collection, and in commercially available cultivars. Another goal is to acquire unique germplasm to add to the collection that could be useful in future pawpaw breeding efforts. When sampling pawpaw patches for genetic diversity studies and for preservation of samples, the clonality of patches is an important consideration. If pawpaw patches are usually clonal, sampling methods can be less intensive. Examining clonality of pawpaw patches using DNA based markers, such as inter-simple sequence repeat (ISSR) marker systems (Pomper et al. 2003), would detect

Figure 1. Maps of the six patches in three Kentucky counties that were sampled in the genetic study.

genetic differences among trees in a patch, which is not possible with observational or morphological studies.

To test if native pawpaw patches are clonal or contain more than one genetically different tree, we utilized inter-simple sequence repeat (ISSR) DNA fingerprinting techniques to determine if DNA fingerprint patterns indicate pawpaw patches contained genetically different trees (seedlings) in a patch.

MATERIALS AND METHODS

Plant Material

Leaf samples were collected from 20 trees each from six different patches located in central

Table 2. Inter simple sequence repeat (ISSR) markers scored for DNA samples collected from 20 individual trees in six Kentucky pawpaw (*Asimina triloba*) patches: Patch A (Franklin Co.), Patch B (Franklin Co.), Patch C (Woodford Co.), Patch D (Woodford Co.), Patch E (Menifee Co.), and Patch F (Menifee Co.), with 1 indicating the presence of the marker and 0 indicating the absence of a marker.

	Patch and tree number																			
Marker	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20
841T-1470	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0**	**0**
841T-1380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-670	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-2800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1945	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1830	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1480	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-750	**0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0**	**0**	**0**
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20
841T-1470	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-1380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-670	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-2800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1945	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1830	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1480	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-750	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
841T-1470	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
841T-1380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-670	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-2800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1945	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1830	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1480	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-750	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20
841T-1470	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-1380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-670	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-2800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1945	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1830	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1480	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-750	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20
841T-1470	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-1380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-670	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-2800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1945	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1830	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1480	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-750	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	1	1	1

Table 2. Continued.

Marker	Patch and tree number																			
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
841T-1470	1	1	1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
841T-1380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841T-670	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-2800	1	1	1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
841C-1945	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1830	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-1480	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
841C-750	**0**	**0**	**0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Kentucky (Table 1 and Figure 1). The leaf samples were collected on a transect line across the largest dimension of each patch. Patches varied greatly in size; ranging from 30 to 500 stems. Leaf samples were stored in Ziploc plastic bags at −80°C until needed for extraction.

DNA Extraction

DNA was extracted from the pawpaw leaves using the DNAMITE Plant Kit (The Gel Company, San Francisco, CA). About 1~2 cm^2 of young leaf tissue was used. The DNA concentration and a 260/280 nm absorbance ratio were determined with GeneQuant™ *pro* RNA/DNA calculator (GE Healthcare, Piscataway, NJ). All samples were stored at −80°C until needed.

ISSR-PCR Amplification

The ISSR-PCR amplification was performed with GoTaq Flexi DNA polymerase (Promega Co., Madison, WI). The reactions were set up follows: 4 µl of 5× colorless GoTaq Flexi buffer, 0.4 µl of 10 mM dNTPs solution, 1.6 µl of 25 mM $MgCl_2$, 1.33 µl of 3 µM primer solution, 0.3 µl of 5 units/µl GoTaq DNA polymerase, 2 µl of diluted 1 ng/µl pawpaw DNA, and 10.37 µl of ddH_2O to bring the total volume to 20 µl. Based on prior screenings, two primers were synthesized for use in this study based on the primer UBC841 (University of British Columbia, Canada, microsatellite set #9) with the following sequences: UBC 841C (GAG AGA GAG AGA GAG ACC) and UBC841T (GAG AGA GAG AGA GAG ATC). The PCR amplifications were performed using GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). The PCR program consisted of an initial period of 94°C for 5 min, followed with 45 cycles of 45 sec denaturation at 94°C, 1 min annealing at 50°C, and 2 min extension at 72°C, and a final extension period of 10 min at 72°C. The PCR results were then stored at 4°C until electrophoresis. Products were separated by electrophoresis at 60 volts for 18 hrs using a 300 ml, 18 cm long, 2% agarose gel. Hyperladder I or II DNA markers (Bioline Inc., Boston, MA) were used as weight ladder standards. Gels were imaged using a Kodak Gel Logic 100 photo documentation system, and the PCR-amplified products analyzed using Kodak Molecular Imaging Software (Version 4.0.5, Kodak, Rochester, NY). At least three replicate gels were scored for each primer/patch.

Data Analysis

Scores were entered into a matrix, analyzed, and dendrograms constructed using NTSYSpc software, version 2.11T (Exeter software, Setauket, NY) The level of genetic similarity among trees in a patch was determined by Nei's genetic distance (Nei 1978). Dendrograms were constructed based on the matrix of the distances using unweighted pair-group mean analysis (UPGMA). A similarity matrix was generated using the Dice coefficient, S = 2Nxy/(Nx + Ny), where Nx and Ny are the numbers of bands observed in trees X and Y, respectively, and Nxy is the number of bands common to both clones (Dice 1945). The Dice values were then used to perform UPGMA cluster analysis and generate a dendrogram.

RESULTS AND DISCUSSION

For the two ISSR primers utilized, nine reproducible markers were amplified in the

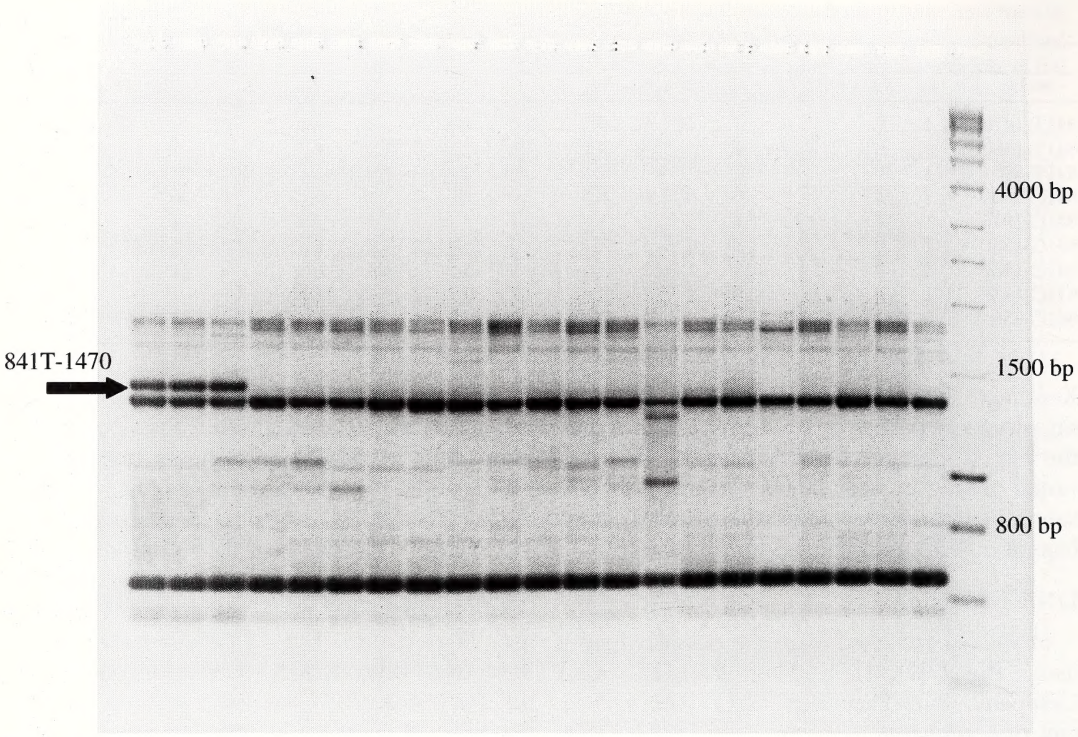


Figure 2. Photograph of a representative agarose gel of inter simple sequence repeat markers for 20 pawpaw trees in Patch F. Marker 841T-1470 is indicated by the arrow and occurs in three trees in the patch. A ladder of known band sizes can be found in the far right lane of the gel.

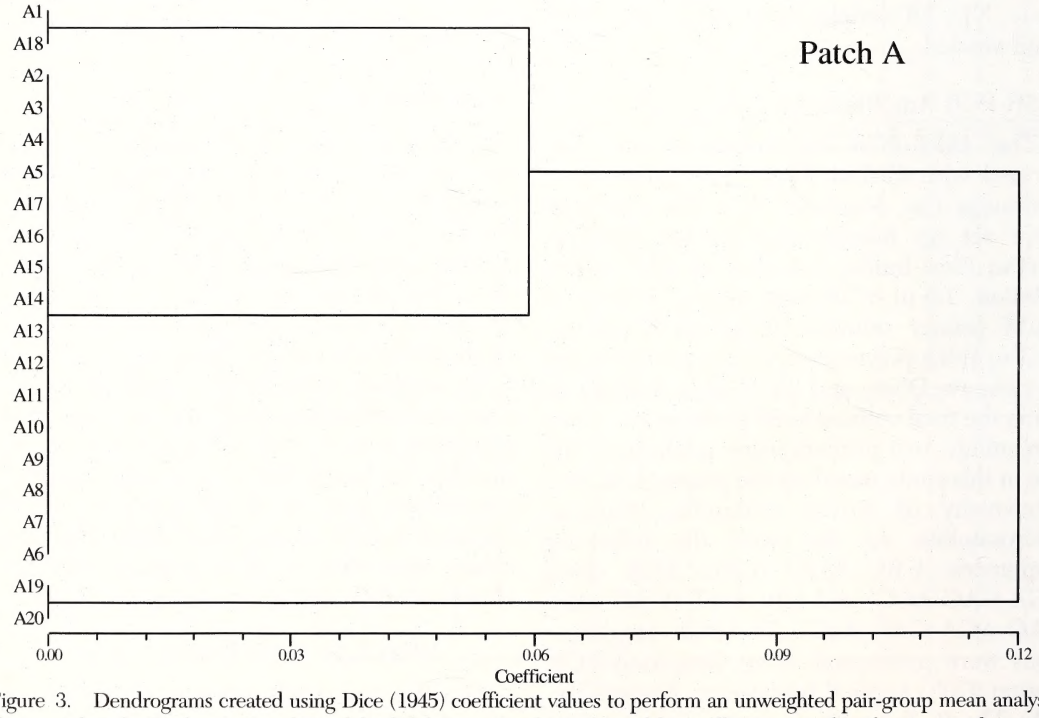
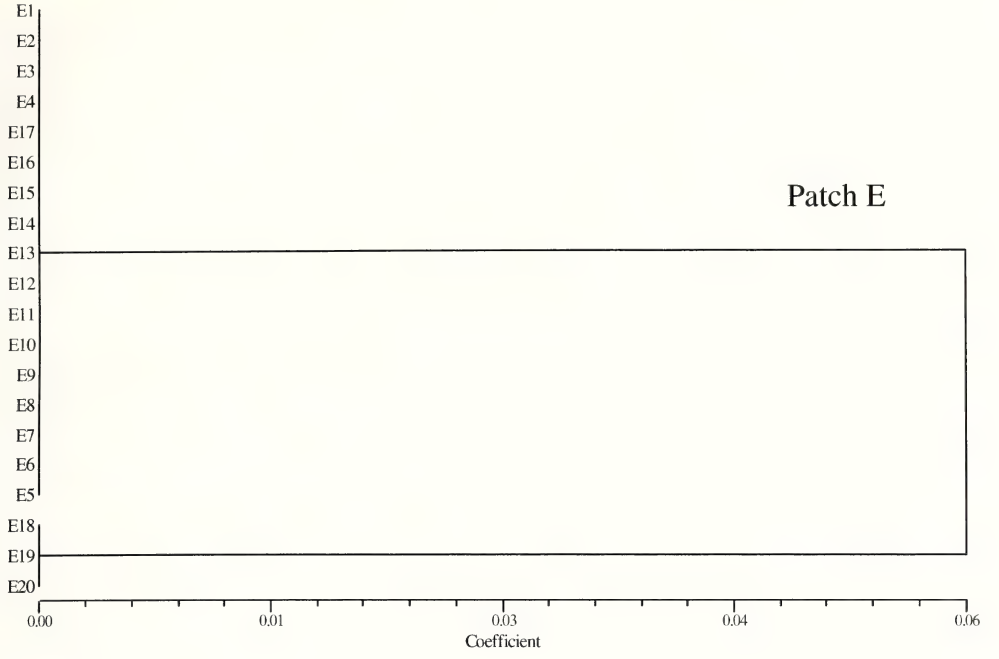


Figure 3. Dendrograms created using Dice (1945) coefficient values to perform an unweighted pair-group mean analysis cluster analysis for Patches A, E, and F and display the genetic relationships of the seedling trees to the other trees in the patch.



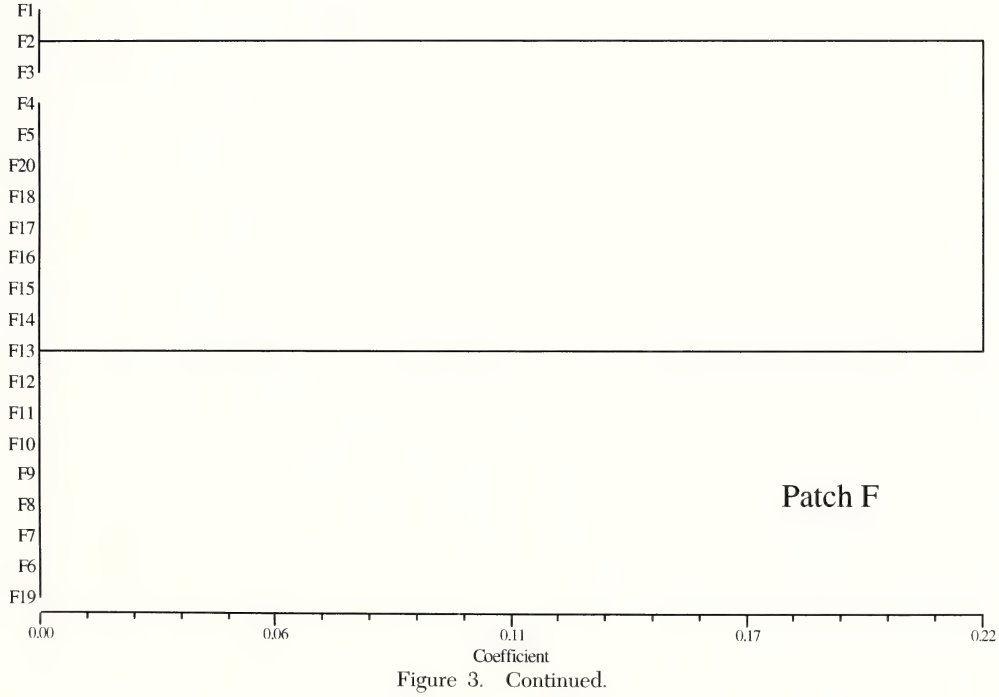


Figure 3. Continued.

six patches examined. Primer 841C produced six reproducible markers for the patches at 2800, 1945, 1830, 1550, 1480, and 750 bp in size. Primer 841T produced three reproducible markers for the patches at 1470, 1380, and 670 bp in size. There were three polymorphic markers, 841T-1470, 841C-2800, and 841C-750, and six monomorphic markers, 841T-1380, 841T-670, 841C-1945, 841C-1830, 841C-1550, and 841C-1480 scored across the patches (Table 2).

The marker data indicated that there were 3 genotypes represented in the 20 trees sampled across Patch A, indicating the patch was not completely clonal (Table 2). Each tree sampled in Patch B displayed all the same marker and all trees contained all the markers scored, suggesting the patch was clonal. Trees in Patch C all displayed an identical marker pattern, all trees were missing the markers 841T-1470 (bp) and 841C-750, suggesting the patch was clonal. Trees in Patch D displayed an identical marker pattern, all trees were missing the marker 841C-750, suggesting the patch was clonal. The marker data indicated that there were 2 genotypes represented in the 20 trees sampled across Patch E that did not contain the marker 841C-750, indicating the patch was not clonal. The marker data indicate there were 2 genotypes represented in the 20 trees sampled across Patch F that did not contain the marker 841C-750, but three trees did contain markers 841T-1470 and 841C-2800, indicating the patch was not clonal (Figure 2). Dendrograms of Patches A, E, and F display the genetic relationships of seed derived and clonally produced trees in the patch (Figure 3).

Patches B, C, and D did not display any polymorphic markers in each patch, suggesting these patches were clonal. However, Patches A, E, and F did show polymorphic markers within each patch, indicating these patches were not clonal and contained trees of at least two genotypes within each patch. It is possible that Patches B, C, and D do contain seedling trees that were not sampled when we collected on a transect line across the widest part of a patch. Additionally, it is possible that screening the trees sampled from Patches B, C, and D with additional ISSR primers could identify the presence of seedling trees.

Determining the clonality of pawpaw patches is important in developing strategies for the conservation of pawpaw genetic resources. In 1994, KSU was designated as a satellite repository for *Asimina* preservation in the USDA-NPGS and germplasm evaluation, preservation, and dissemination are high priorities (Huang et al. 1997, 1998, 2000; Pomper et al. 2003). To determine the level of genetic diversity in native populations and to acquire unique germplasm to add to the repository collection, the potential clonality of pawpaw patches is an important consideration in developing sampling strategies for wild pawpaw patches (Rogstad et al. 1991). If pawpaw patches are usually clonal, sampling methods can be less intensive. It is difficult to demonstrate that a patch is clonal or not. This would require that all trees in a patch be sampled. We have observed pawpaw patches of well over 500 stems and sampling all stems in a patch this size would be extremely labor intensive. In this study, 50% of the pawpaw patches that we examined were not clonal. A pawpaw fruit often contains more than 15 seeds. Therefore, non-clonal patches may be the result of multiple seeds germinating from an original fruit or from multiple seeds in the feces of an animal that had consumed one or more pawpaw fruit. Non-clonal patches could also be the result of sexual reproduction and fruit dropping into the patch allowing the expansion of the genetic base of the patch (Cypher and Cypher 1999; Peterson 1991). This study suggests that to assess the genetic diversity of a patch or local population, more intensive sampling strategies will be required. The examination of additional patches with additional primers will aid in the development of a patch sampling strategy for pawpaw.

ACKNOWLEDGMENT

This research was supported by USDA-SERD Grant project KYX-2005-03514 by K Pomper, K. Kaul, N. Rajendran, and J. Tidwell. We wish to thank K. Bates for his help in constructing maps of the patches.

LITERATURE CITED

Bowden, W. M. 1948. Chromosome numbers in the *Annonaceae*. American Journal of Botany 35: 377–381.

Callaway, M. B. 1990. The pawpaw (*Asimina triloba*). Kentucky State University. Publications. CRS-HORT1-901T.

Callaway, M. B. 1993. Pawpaw (*Asimina triloba*): A "tropical" fruit for temperate climates. Pages 505–515 in J. Janick and J. E. Simon (eds). New crops. Wiley, New York.

Chester, E. W., S. M. Noel, J. M. Baskin, C. C. Baskin, and M. L. McReynolds. 1995. A phytosociological analysis of an old-growth upland wet woods on the Pennyroyal Plain, southcentral Kentucky, USA. Natural Areas Journal 15:297–307.

Cole, P. G., and J. F. Weltzin. 2005. Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. Biological Invasions 7:477–488.

Cypher, B. L., and E. A. Cypher. 1999. Germination rates of tree seeds ingested by coyotes and raccoons. American Midland Naturalist 142:71–76.

Darrow, G. M. 1975. Minor temperate fruits. Pages 276–277 in J. Janick and J. N. Moore (eds). Advances in fruit breeding. Purdue University Press, West Lafayette, IN.

Dice, L. R. 1945. Measures of the amount of ecological association between species. Ecology 26:297–302.

Duffrin, M. W., and K. W. Pomper. 2006. Development of flavor descriptors for pawpaw fruit puree: A step toward the establishment of a native tree fruit industry. Family and Consumer Sciences Research Journal 35:118–130.

Faegri, K., and L. van der Piji. 1971. The principles of pollination ecology. Pergammon, New York.

Hosaka, N., S. Gómez, N. Kachi, J. F. Stuefer, and D. F. Whigham. 2005. The ecological significance of clonal growth in the understory tree, pawpaw (*Asimina triloba*). Northeastern Naturalist 12:11–22.

Hosaka, N., N. Kachi, H. Kudoh, J. F. Stuefer, and D. F. Whigham. 2008. Patch structure and ramet demography of the clonal tree, *Asimina triloba*, under gap and closed-canopy. Plant Ecology 197:219–228.

Huang, H., D. R. Layne, and R. N. Peterson. 1997. Using isozyme polymorphisms for identifying and assessing genetic variation in cultivated pawpaw [*Asimina triloba* (L.) Dunal]. Journal of American Society for Horticultural Science 122:504–511.

Huang, H., D. R. Layne, and D. E. Riemenschneider. 1998. Genetic diversity and geographic differentiation in pawpaw [*Asimina triloba* (L.) Dunal] populations from nine states as revealed by allozyme analysis. Journal of American Society for Horticultural Science 123:635–641.

Huang, H., D. R. Layne, and T. L. Kubisiak. 2000. RAPD inheritance and diversity in pawpaw [*Asimina triloba* (L.) Dunal]. Journal of American Society for Horticultural Science 125:454–459.

Kral, R. 1960. A revision of *Asimina* and *Deeringothamus* (Annonaceae). Brittonia 12:233–277.

Lagrange, R. L., and E. J. Tramer. 1985. Geographic variation in size and reproductive success in the paw paw (*Asimina triloba*). Ohio Journal of Science 85:40–45.

Larimore, R. L., D. T. Busemeyer, and J. E. Ebinger. 2003. Pawpaw, *Asimina triloba* (L.) Dunal (Annonaceae), in the prairie peninsula of Illinois, USA. Natural Areas Journal 23:356–361.

Layne, D. R. 1996. The pawpaw [*Asimina triloba* (L.) Dunal]: A new fruit crop for Kentucky and the United States. HortScience 31:777–784.

McLaughlin, J. L. 2008. Paw paw and cancer: Annonaceous acetogenins from discovery to commercial products. Journal of Natural Products 71:1311–1321.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Peterson, R. N., J. P. Cherry, and J. G. Simmons. 1982. Composition of pawpaw (*Asimina triloba*) fruit. Northern Nut Growers Association Annual Report 73:97–107.

Peterson, R. N. 1991. Pawpaw (*Asimina*). Acta Horticulturae 290:567–600.

Pomper, K. W., S. B. Crabtree, S. P. Brown, S. C. Jones, T. M. Bonney, and D. R. Layne. 2003. Assessment of genetic diversity of pawpaw varieties with inter-simple sequence repeat markers. Journal of American Society for Horticultural Science 128:521–525.

Pomper, K. W., and D. R. Layne. 2005. The North American pawpaw: Botany and horticulture. Horticultural Reviews 31:351–384.

Rogstad, S. H., K. Wolff, and B. A. Schaal. 1991. Geographical variation in *Asimina triloba* Dunal (Annonaceae) revealed by the M13 DNA fingerprinting probe. American Journal of Botany 78:1391–1396.

Rheinhardt, M. C., and R. D. Rheinhardt. 2000. Canopy and woody subcanopy composition of wet hardwood flats in eastern North Carolina and southeastern Virginia. Journal Torrey Botanical Society 127:33–43.

Shiota, H. 1991. Volatile components of pawpaw fruit (*Asimina triloba* Dunal). Journal of Agricultural and Food Chemistry 39:1631–1635.

Willson, M. F., and D. W. Schemske. 1980. Pollinator limitation, fruit production, and floral display in pawpaw (*Asimina triloba*). Bulletin of the Torrey Botanical Club 107:401–408.

Young, D. R., and J. B. Yavitt. 1987. Differences in leaf structure, chlorophyll, and nutrients for the understory tree *Asimina triloba*. American Journal of Botany 74:1487–1491.

J. Ky. Acad. Sci. 70(1):12–16. 2009.

Incidence of *Phoradendron leucarpum* (Viscaceae) at General Burnside State Park, Pulaski County, Kentucky

Ralph L. Thompson[1]

Berea College Herbarium, Biology Department, Berea College, Berea, Kentucky 40404

and

Katrina Rivers Thompson

Child and Family Studies Department, Berea College, Berea, Kentucky 40404

ABSTRACT

A survey of host trees infested with eastern mistletoe (*Phoradendron leucarpum*, Viscaceae) at General Burnside State Park in Pulaski County, Kentucky, was made in late 2008 and early 2009. It is the only island state park in Kentucky, and consists of 174.0 ha in the middle of Lake Cumberland, adjacent to the city of Burnside. A total of 244 mistletoe-infested trees from nine tree species in eight families were found. *Prunus serotina*, *Juglans nigra*, and *Ulmus americana* were the most common host trees. Mistletoe infestation had a greater occurrence in older, full-crowned canopy trees of open, exposed sunny habitats.

KEY WORDS: General Burnside (Island) State Park, eastern mistletoe, host tree specificity, *Phoradendron leucarpum*

INTRODUCTION

General Burnside State Park (GBSP), the only island park in Kentucky, encompasses 174.0 ha in the middle of Lake Cumberland, across from the city of Burnside (Figure 1), and lies 1.8 km south of Somerset off U.S. 27 in Pulaski County (Kentucky State Parks 2009). The island park is located at latitude 36°58′33″N and longitude 84°36′08″W. Elevation ranges from 225 m at the normal pool shoreline at Lake Cumberland to 276 m at the highest point, Bunker Hill (Figure 1). GBSP has been known as Bunker Hill, Chandler Island State Park, and General Burnside Island State Park. A botanical reconnaissance in April 2001 at GBSP revealed a high incidence of eastern mistletoe (*Phoradendron leucarpum* (Raf.) Reveal & M.C. Johnston), an epiphytic hemiparasite in the Viscaceae that infests various deciduous trees. This initial observation inspired the present field survey of eastern mistletoe at GBSP.

HISTORY

The island park is named for Major General Ambrose E. Burnside, the sideburn-whiskered Union general with the Ninth Army Corps, who established a camp and supply depot at the community of Point Isabel during the Civil War. The purpose was to fortify the site along a major lookout point, Bunker Hill, and control a portion of the Cumberland River from the Confederates. Afterwards, General Burnside accepted the surrender of Confederate forces at Cumberland Gap to secure eastern Kentucky and Tennessee from a strong Confederate presence. His camp at Point Isabel soon became known as Camp Burnside and the community was called Burnside by the end of the Civil War (Kentucky State Parks 2009).

In the 1940s, the Nashville District Corps of Engineers began a project to impound the waters of the Cumberland River by building the colossal Wolf Creek Dam, the 22nd largest dam in the United States. It was completed in 1950 and the Cumberland River was impounded for 163.0 km upstream creating Lake Cumberland with 2008 km of shoreline and a seasonal pool over 25,500 ha.

The rising waters of Lake Cumberland eventually covered the lower portions of Burnside forming a tear-shaped island (Figure 1). The island was suggested as an excellent site for a camping park by the Corp of Engineers, and it subsequently was transferred from the Corps to the Commonwealth

[1] Corresponding author e-mail: ralph_thompson@berea.edu

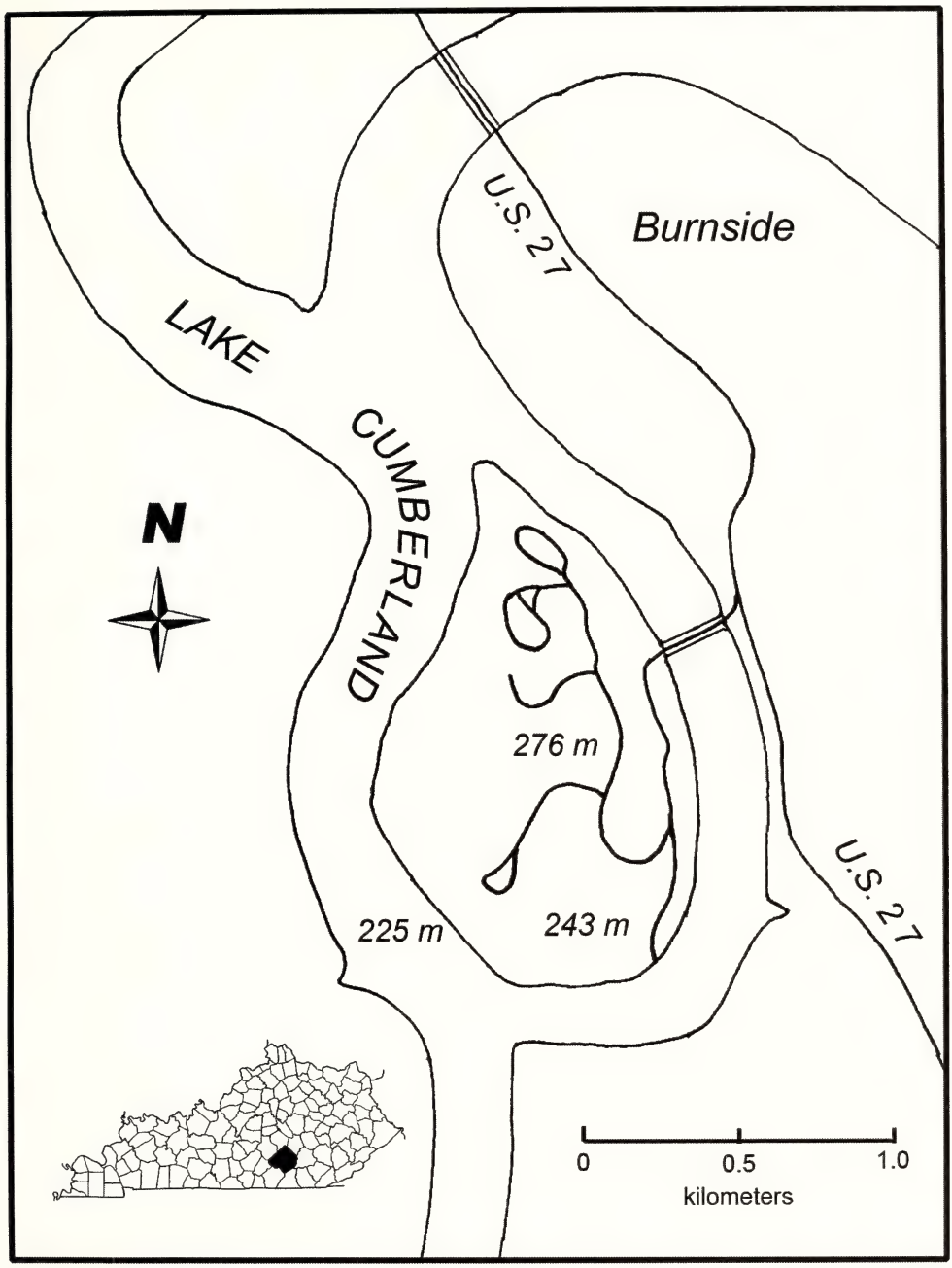


Figure 1. Map of General Burnside State Park, a 174 ha tear-shaped island situated in Lake Cumberland, Pulaski County, Kentucky. The highest elevation is Bunker Hill at 276 m above mean sea level. The park perimeter above cliff line is 243 m above sea level, and the mean lake pool is 225 m above sea level.

of Kentucky for fee simple on 3 February 1958. In 1959, a causeway bridge from U.S. 27 to the island was built by the Kentucky Department of Transportation (Kentucky State Parks 2009). A beach, boat ramp, camping area with 94 utility hookups, and nine-hole golf course were constructed in late 1959–1960. An 18-hole golf course, built in the mid-1970s, was completely renovated in 2007 to approximately 24.0 ha of green and fairway surface area and 37.0 ha of rough bordering land.

The island park was initially called Chandler Island State Park to honor Governor A. B. "Happy" Chandler after transfer of the land to the Commonwealth of Kentucky. However, the Burnside and Somerset Chambers of Commerce recommended the park be renamed as General Burnside Island State Park, and the Kentucky Parks Board approved the name change on 28 May 1960. The name has changed through the years, and it is now known as General Burnside State Park (Kentucky State Parks 2009).

THE STUDY AREA

GBSP lies entirely within the Eastern Highland Rim of the Interior Plateaus Ecoregion (Keys et al. 1995; Woods et al. 2002). Küchler (1964) and Woods et al. (2002) classified the vegetation for this region of Kentucky as Oak-Hickory (*Quercus-Carya*) Forest. The forest stands of GBST include mosaics of Oak-Hickory (*Quercus-Carya*) and Oak-Ash-Elm (*Quercus-Fraxinus-Ulmus*) types on the upper dry sites and an eastern red cedar (*Juniperus virginiana* L.) type on the uppermost drier limestone-exposed sites. These forest types are intermixed with several calcicolous trees including black cherry (*Prunus serotina* Ehrh.), common hackberry (*Celtis occidentalis* L.), honey locust (*Gleditsia tricanthos* L.), and black walnut (*Juglans nigra* L.).

The geology of GBSP consists primarily of Mississippian limestones (Taylor et al. 1975). The Ste. Genevieve limestone member of the Monteagle Limestone are present from 256–276 m elevation and often forms rock outcrops. St. Louis limestone lie from 225–256 m elevation. Concordantly, below 225 m elevation lie the Mississippian limestones of the Salem and Warsaw Formations (Taylor et al. 1975). The underlying and exposed bedrock is comprised mainly of medium to light gray, medium-grained limestone interbedded with some chert, claystone, and siltstone (Taylor et al. 1975).

The soils of GBSP are classified as the Talbott, Waynesboro, and Brookside series (Ross 1974). The Talbott rock silt loams occupy the higher elevations from 260 to 276 m on the north-central part of GBSP. Talbott series are 80.0 cm deep, well-drained, residual limestone soils of gently 6 to 12% side slopes, ridgetops, and rock outcrops (Ross 1974). Waynesboro loams are found on a large portion of the island including the golf course from 250 to 260 m. These loam soils are 144 to 229 cm deep, well-drained, old alluvium of limestone 6 to 12% side slopes and ridges (Ross 1974). Waynesboro clay loam series are found on severely eroded ridge slopes around the eastern part near boat ramp from 240 to 250 m to the island causeway with 12 to 30% slopes. The Brookside outcrop complex of steep hillsides and rock outcrops on 30 to 75% slopes encloses GBST in a narrow band from 225 to 240 m near the normal pool shoreline of Lake Cumberland. Brookside soils are 74.0 to 99.0 cm deep, well-drained, colluvial limestone soils (Ross 1974).

METHODS

We investigated the occurrence of eastern mistletoe in host trees by walking the complete upper island terrain of GBSP. Nikon Monarch 8 × 42 power binoculars were used to spot visible signs of mistletoe infestation, i.e., broom die-back, limb and trunk swellings, cankers, and clusters. Representative vouchers of mistletoe with accompanying host twigs were collected with the use of a 12 m extendable fiberglass linesman pole, processed, and deposited in the Berea College Herbarium. Seven field trips were made to inventory eastern mistletoe and gather descriptive data from late December 2008 through middle March 2009. Plant nomenclature followed Jones (2005).

RESULTS AND DISCUSSION

General Burnside State Park was a unique and ideal site for a survey of *Phoradendron leucarpum*. As a small island isolated by Lake Cumberland, GBSP has the higher elevations of the contiguous mainland terrain with many anthropogenically-created open habitats and an abundance of deciduous calcicolous trees to serve as hosts for eastern mistletoe.

The GBSP topographic open terrain is a complex consisting of wooded groves and scattered canopy trees among and adjacent to the golf course, forested edges along paved roads, a large camping and maintenance building area, and the forested border of the park contiguous to the steep wooded hillsides

Table 1. Host specificity of *Phoradendron leucarpum* in General Burnside State Park, Kentucky.

Tree species	Total	Percentage
Prunus serotina Ehrh.	88	36.06
Juglans nigra L.	69	28.28
Ulmus americana L.	38	15.57
Fraxinus americana L.	19	7.79
Gleditsia triacanthos L.	14	5.74
Celtis occidentalis L.	7	2.87
Acer saccharinum L.	6	2.46
Diospyros virginiana L.	2	0.82
Carya ovata (Mill.) K. Koch	1	0.41
Total: 9	244	100.00

down to Lake Cumberland. This high degree of variability in open habitat proved most favorable for host tree infestation. In previous eastern mistletoe studies, avian vectors of mistletoe berries have been shown to prefer tall, mature canopy trees in open habitats of higher topographic elevations (Thompson and Noe, Jr. 2003; Thompson and Poindexter 2005; Thompson et al. 2008).

A total of 244 mistletoe-infested trees from nine tree species in eight families were found at General Burnside State Park (Table 1). Occurrence of mistletoe was greater in the older, tall, full-crowned canopy trees of upper elevations in open sunny habitats. Trees with mistletoe often were solitary, scattered, or in small wooded groves. Black cherry, black walnut, and American elm were the most prevalent host trees (Table 1), and they also displayed heavy infestations (31 to 100 clusters). A few black cherry and black walnut trees showed such extensive infestations (100 to 150+ clusters) that mortality of the host trees appeared imminent. Four lesser host trees were white ash, honey locust, common hackberry, and silver maple (*Acer saccharinum* L.). These host trees tended to have light (1 to 10 clusters) to moderate (11 to 30 clusters) infestations. Two persimmon trees (*Diospyros virginiana* L.) and one shagbark hickory (*Carya ovata* (Mill.) K. Koch) exhibited one to four mistletoe clumps each.

The host tree species observed in this research were consistent with studies in nearby counties. The five main hosts at the Lexington-Blue Grass Army Depot in Madison County were black cherry, black walnut, American elm, white ash, and honey locust (Thompson 1992). This same host incidence was found at GBSP. Black cherry, black walnut, silver maple, and American elm were the four dominant host taxa in a survey of Berea, Kentucky, in Madison County (Thompson et al. 2008). In neighboring Garrard County, the primary host trees were black walnut, black cherry, American elm, black locust (*Robinia pseudoacacia* L.), and white ash (Thompson and Poindexter 2005). A mistletoe survey of Rockcastle County, contiguous to Pulaski County, revealed an occurrence of 12 host tree species with the most widespread hosts being black walnut and black cherry (Thompson and Noe, Jr. 2003). General Burnside State Park displays a nearly identical pattern of hemiparasite to preferred host infestation as these three south-central counties. Such affinities between these studies may be attributed to similarities in primary substrates and soils (limestone-based), as well as uniformity in available open habitats, topography, and host availability.

CONCLUSION

The forest vegetation of GBSP is a complex function of the limestone geology, substrate-derived soils, climate, topography, existing vegetation, and anthropogenic disturbance. The host trees for mistletoe-infestation at this island site favored calicoles. Host trees typically occurred in wooded groves, scattered bunches, or solitary trees in open habitats. These trees frequently were taller, older, full-crowned canopy trees in open sunlight. The total of 244 trees infested with eastern mistletoe on this 174 ha island exemplify the availability of host trees, physical site conditions, and bird vectors for its dispersal, establishment, and spread.

ACKNOWLEDGMENTS

We extend appreciation to Derick B. Poindexter, Appalachian State University, for a critical paper review, and to Melanie G. Bentley, Eastern Kentucky University, for Figure 1.

LITERATURE CITED

Jones, R. L. 2005. Plant life of Kentucky: an illustrated guide to the vascular flora. The University Press of Kentucky, Lexington, KY.

Kentucky State Parks. 2009. History of General Burnside Island State Park. (http://parks.ky.gov/findparks/recparks/ge/history).

Keys, J. E., Jr., C. A. Carpenter, S. L. Hooks, F. G. Koenig, W. H. McNab, W. E. Russell, and M.-L. Smith. 1995. Ecological units of the eastern United States, first approximation (colored map and booklet of map unit table). (Map scale 3,500,000). United States Department of Agriculture, Forest Service, Atlanta, Georgia.

Küchler, A. W. 1964. Potential natural vegetation of the conterminous United States (map and accompanying manual). American Geographical Society Special Publication No. 36, New York, NY.

Ross, J. C. 1974. Soil survey of Pulaski County, Kentucky. United States Department of Agriculture, Soil Conservation Service and Forest Service, Washington, DC.

Taylor, A. R., R. Q. Lewis, and J. H. Smith. 1975. Geologic map of the Burnside Quadrangle, south-central Kentucky, GQ-1253. (Map scale 1:24,000). United States Geological Survey, Washington, DC.

Thompson, R. L. 1992. Host occurrence of *Phoradendron leucarpum* in the Lexington-Blue Grass Army Depot, Blue Grass Facility, Madison County, Kentucky. Transactions of the Kentucky Academy of Science 53:170–171.

Thompson, R. L., and F. D. Noe, Jr. 2003. American mistletoe (*Phoradendron leucarpum*, Viscaceae) in Rockcastle County, Kentucky. Journal of the Kentucky Academy of Science 64:29–35.

Thompson, R. L., and D. B. Poindexter. 2005. Host specificity of American mistletoe (*Phoradendron leucarpum*, Viscaceae) in Garrard County, Kentucky. Journal of the Kentucky Academy of Science 66:40–43.

Thompson, R. L., K. Rivers Thompson, E. A. Fleming, R. D. Cooks, J. R. Price, M. N. Naseman, and A. J. Oles. 2008. Eastern mistletoe (*Phoradendron leucarpum*, Viscaceae) in the city of Berea, Kentucky: a high incidence of infestation and eight new hosts species for Kentucky. Journal of the Kentucky Academy of Science 69:3–10.

Woods, A. J., J. M. Omernik, W. H. Martin, G. J. Pond, W. M. Andrews, S. M. Call, J. A. Comstock, and D. D. Taylor. 2002. Ecoregions of Kentucky (color poster with map, descriptive text, summary tables, and photographs). (Map scale 1:1,000,000). United States Geological Survey, Reston, VA.

J. Ky. Acad. Sci. 70(1):17–28. 2009.

Annotated List of the Leaf Beetles (Coleoptera: Chrysomelidae) of Kentucky: Subfamily Galerucinae, Tribes Galerucini and Luperini

Robert J. Barney[1]

Community Research Service, Kentucky State University, Frankfort, Kentucky 40601

Shawn M. Clark

Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah 84602

and

Edward G. Riley

Department of Entomology, Texas A&M University, College Station, Texas 77843

ABSTRACT

An examination of leaf beetle specimens (Coleoptera: Chrysomelidae) in the largest beetle collections in Kentucky, recent inventory work in state nature preserves and other protected areas, and a review of the literature revealed thirty species of the tribes Galerucini and Luperini (Subfamily Galerucinae) present in Kentucky, thirteen of which are previously unreported for the state. Distribution maps and label data are presented for the thirty Kentucky species 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: *Derospidea brevicollis* (LeConte), *Monocesta coryli* (Say), *Trirhabda canadensis* (Kirby), *Galerucella nymphaeae* (L.), *Ophraella americana* (F.), *Ophraella cribrata* (LeConte), *Acalymma gouldi* Barber, *Acalymma vinctum* (LeConte), *Phyllecthris dorsalis* (Olivier), *Phyllecthris gentilis* LeConte, *Phyllobrotica limbata* (F.), *Phyllobrotica stenidea* Schaeffer, and *Metroidea brunnea* (Crotch).
KEY WORDS: Kentucky, leaf beetles, Chrysomelidae, biodiversity, new state records

INTRODUCTION

This paper is the fourth 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 protected locations. The first three papers presented information on the subfamilies Cassidinae (Barney et al. 2007), Donaciinae and Criocerinae (Barney et al. 2008a), and Chrysomelinae (Barney et al. 2008b).

Galerucinae is the largest leaf beetle subfamily with roughly 1000 genera and over 13,000 species described worldwide (Riley et al. 2002). Historically, this subfamily was separated into two subfamilies, Galerucinae (including Tribes Galerucini and Luperini) and Alticinae (Tribe Alticini). The Alticini, otherwise known as the flea beetles, is a very large and diverse group (470 described species in America north of Mexico, Riley et al. 2002), and will be treated in a subsequent paper in this issue of the Journal of the Kentucky Academy of Science.

Wilcox (1965) published a synopsis of the North American galerucines and provided keys to 212 species. Riley et al. (2002) reported that Galerucini contains 20 genera and 100 species while Luperini is represented by 23 genera and 133 species. Several luperines are of major agricultural importance and even recognized by home gardeners growing corn and curcubits (the corn rootworms and cucumber beetles of *Acalymma* and *Diabrotica*) and beans (bean leaf beetle, *Cerotoma*).

The purpose of this study is to present historical and current knowledge of the distribution, abundance, and plant associations of Galerucini and Luperini leaf beetles in Kentucky.

MATERIALS AND METHODS

To establish a historical perspective, leaf beetle specimens from the major insect

[1] Corresponding author e-mail: robert.barney@kysu.edu

collections in Kentucky (and from collections located in other states but known to contain Kentucky specimens) were examined, re-identified, and their label data recorded. The following collections were studied, with the timeframe of their Kentucky specimens listed:

CMC Cincinnati Museum Center, Cincinnati, OH 1871–1931
UKIC University of Kentucky Insect Collection, Lexington, KY 1889–1993
WKUC Western Kentucky University Collection, Bowling Green, KY 1958–2006
RJBC Robert J. Barney Collection, Frankfort, KY (private) 1983–present
BYUC Brigham Young University Collection, Provo, UT 1988–present
CWIC Charles Wright Collection, Frankfort, KY (private) 1991–present
KYSU Kentucky State University Collection, Frankfort, KY 2004–present

The Cincinnati Museum Collection, formerly known as the Cincinnati Museum of Natural History, houses the Charles Dury Collection, comprising approximately 75,000 specimens primarily collected in the Cincinnati/northern Kentucky area (Vulinec and Davis 1984). The University of Kentucky Collection contains the Charles V. Covell, Jr. Collection (emeritus professor of the University of Louisville).

The Kentucky State University Insect Collection is primarily 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 that are known for possessing uncommon plants and plant associations (Jones 2005) and have never been surveyed for leaf beetles. These sites are managed by the Kentucky State Nature Preserves Commission, The Nature Conservancy, and the United States Army at Fort Campbell Military Reservation (Baskin et al. 1994). Most specimens were collected by the senior author within five state nature preserves in 2004–2008 and Fort Campbell in 2008: 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) and Fort Campbell (Christian and Trigg Counties) in western Kentucky.

For each galerucine 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. Other pertinent information present on specimen labels, 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 28 species of Galerucini and 27 species of Luperini recorded in at least one of the seven states contiguous to Kentucky. However, only 17 species (7 Galerucini and 10 Luperini) were reported from Kentucky. An examination of 551 Galerucini and 1072 Luperini leaf beetle specimens from the major collections in the state and others known to contain Kentucky specimens revealed 30 species, including all of the 17 recorded in Riley et al. (2003), plus 13 new state records (Table 1).

The state collection at the University of Kentucky (UKIC) contains a total of 597 Galerucini and Luperini leaf beetles representing 17 species, including seven of the new state records reported herein. However, 86% of the galerucine specimens are pest species in three genera, *Acalymma*, *Diabrotica* and *Cerotoma*, reflecting the agricultural nature of this collection. The CWIC collection has 57 specimens representing eleven species. The WKUC has 44 specimens in five species. Recent collecting in state nature preserves and other protected area (KYSU) has produced 813 specimens of 19 species and four new state records. The RJBC contains 66 specimens in 16 species from Kentucky. An

Table 1. List of Galerucini and Luperini (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.

Tribe Galerucini	
Derospidea brevicollis (LeConte)	6 specimens: 1 county, 2007–2008 (new state record)
Monocesta coryli (Say)	24 specimens: 11 counties, 1906–2005 (new state record)
Trirhabda canadensis (Kirby)	5 specimens: 2 counties, 1971–2008 (new state record)
Trirhabda virgata LeConte	1 specimen: 1 county, 1968
Galerucella nymphaeae (L.)	1 specimen: 1 county, ca. 1900 (new state record)
Tricholochmaea tuberculata (Say)	3 specimens: 2 counties, 1990–1994
Xanthogaleruca luteola (Müller)	9 specimens: 5 counties, 1964–1987
Ophraella americana (F.)	176 specimens: 8 counties, 1971–2008 (new state record)
Ophraella communa LeSage	43 specimens: 7 counties, 1971–2008
Ophraella conferta (LeConte)	45 specimens: 17 counties, 1945–2008
Ophraella cribrata (LeConte)	201 specimens: 16 counties, 1971–2008 (new state record)
Ophraella notata (F.)	37 specimens: 6 counties, 1894–2008
Ophraella notulata (F.)	1 specimen: 1 county, ca. 1900
Tribe Luperini	
Acalymma gouldi Barber	1 specimen: 1 county, 2005 (new state record)
Acalymma vinctum (LeConte)	3 specimens: 1 county, 2006 (new state record)
Acalymma vittatum (F.)	84 specimens: 19 counties, 1889–2008
Diabrotica barberi R. Smith & Lawrence	51 specimens: 13 counties, 1899–1983
Diabrotica cristata (Harris)	97 specimens: 6 counties, 1915–2008
Diabrotica undecimpunctata howardi Barber	402 specimens: 58 counties, 1889–2008
Diabrotica virgifera virgifera LeConte	33 specimens: 19 counties, 1971–2008
Cerotoma trifurcata (Forster)	238 specimens: 30 counties, 1893–2008
Phyllecthris dorsalis (Olivier)	24 specimens: 6 counties, 1951–2000 (new state record)
Phyllecthris gentilis LeConte	29 specimens: 5 counties, 1890–2008 (new state record)
Scelolyperus cyanellus (LeConte)	2 specimens: 1 county, 1990
Scelolyperus liriophilus Wilcox	18 specimens: 7 counties, 1983–2006
Phyllobrotica circumdata (Say)	4 specimens: 3 counties, 1972–2002
Phyllobrotica lengi Blatchley	4 specimens: 1 county, 2004–2006
Phyllobrotica limbata (F.)	42 specimens: 8 counties, 1971–2008 (new state record)
Phyllobrotica stenidea Schaeffer	9 specimens: 1 county, 2006 (new state record)
Metroidea brunnea (Crotch)	31 specimens: 2 counties, 2004–2008 (new state record)

examination of the BYUC revealed 41 specimens in seven species. A new state record was found among the four specimens in four species of galerucine leaf beetles in the historical Dury Collection (CMC).

Derospidea brevicollis (J. L. LeConte) (Figure 1A) (new state record)

Kentucky County: Bullitt

Years: 2007 (3), 2008 (3)

Month: June (6)

Abundance: 6 specimens: 6-KYSU

Comments: All six specimens were recently collected at Apple Valley Glades Conservation Area on prickly ash, *Zanthoxylum americanum* P. Mill.

Monocesta coryli (Say) (Figure 1B) (new state record)

Kentucky Counties: Barren, Breathitt, Caldwell, Fayette, Fulton, Hardin, Hopkins, Logan, Powell, Russell, Warren

Years: 1906 (1), 1911 (2), 1913 (5), 1962 (1), 1965 (3), 1966 (1), 1968 (1), 1969 (1), 1972 (1), 1975 (1), 1978 (1), 1984 (3), 1997 (1), 2005 (2)

Months: June (3), July (16), August (5)

Abundance: 24 specimens: 1-CWIC, 5-RJBC, 14-UKIC, 4-WKUC

Comments: This species is often called the larger elm leaf beetle and has been recorded from various species of *Ulmus* (Ulmaceae) (Clark et al. 2004). Clark (1986) reported that this easily recognized species was not found in Ohio until 1979.

Trirhabda canadensis (Kirby) (Figure 1C) (new state record)

Kentucky Counties: Boone, Trigg

Years: 1971 (1), 2008 (4)

Months: June (4), July (1)

Abundance: 5 specimens: 4-KYSU, 1-UKIC

Comments: Clark et al. (2004) reported *Solidago* (Asteraceae) as the usual host plant for this species. Blake (1931) revised the

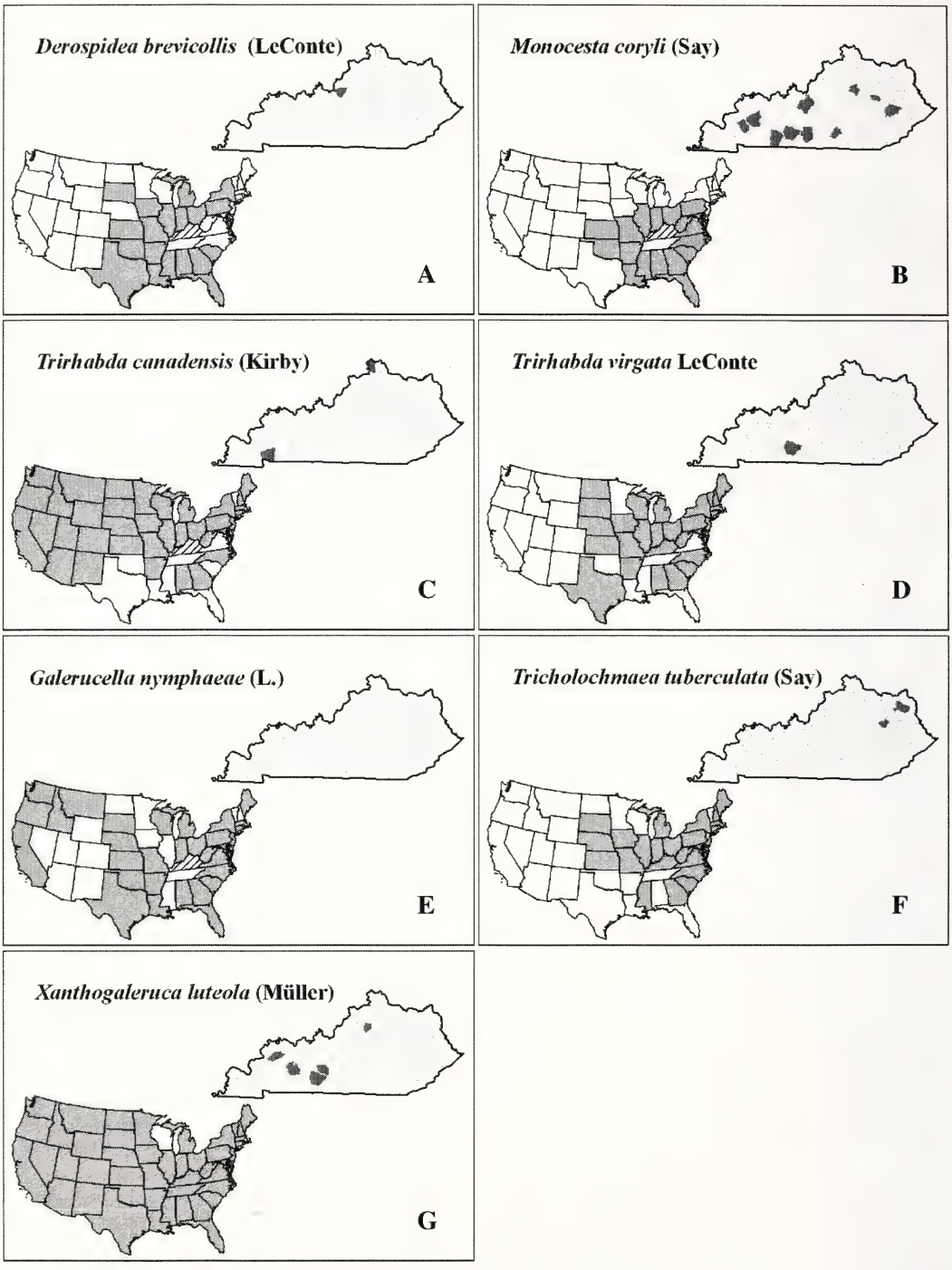


Figure 1. The known distribution of Galerucinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Trirhabda species for America north of Mexico, and additional unpublished information is included in the dissertation of Hogue (1970).

Trirhabda virgata J. L. LeConte (Figure 1D)

Kentucky County: Warren
Year: 1968 (1)
Month: July (1)
Abundance: 1 specimen: 1-WKUC
Comments: Clark et al. (2004) reported *Solidago* (Asteraceae) as the usual host plant for this species.

Galerucella nymphaeae (L.) (Figure 1E)

Abundance: 1 specimen: 1-CMC
Comments: One specimen was found in the Dury Collection with a label reading "Ky. near Cin. O.". *Brasenia* (Hydropeltidaceae), *Nuphar* (Nymphaeaceae), and *Polygomun* (Polygonaceae) are recognized as normal hosts of this species (Clark et al. 2004).

Tricholochmaea tuberculata (Say) (Figure 1F)

Kentucky Counties: Carter, Menifee
Years: 1990 (1), 1994 (2)
Month: May (3)
Abundance: 3 specimens: 3-BYUC
Comments: Hosts are species of *Salix* (Salicaceae) (Clark et al. 2004).

Xanthogaleruca luteola (Müller) (Figure 1G)

Kentucky Counties: Edmonson, Franklin, Muhlenburg, Warren, Webster
Years: 1964 (1), 1965 (1), 1967 (2), 1968 (2), 1969 (1), 1987 (2)
Months: March (1), May (2), June (1), July (1), August (1), October (3)
Abundance: 9 specimens: 2-RJBC, 7-WKUC
Comments: Often called the elm leaf beetle, this species is a pest of *Ulmus* (Ulmaceae).

Ophraella americana (F.) (Figure 2A) (new state record)

Kentucky Counties: Bullitt, Fleming, Hardin, LaRue, Lewis, Lincoln, Pendleton, Robertson
Years: 1971 (2), 2004 (3), 2005 (27), 2006 (35), 2007 (74), 2008 (35)
Months: April (1), May (15), June (94), July (65), August (1)
Abundance: 176 specimens: 173-KYSU, 1-RJBC, 2-UKIC
Comments: Almost all specimens were recently collected in protected, native-grassland habitats. Host plants are species of *Solidago* (Asteraceae) (Clark et al. 2004).

Ophraella communa LeSage (Figure 2B)

Kentucky Counties: Bullitt, Christian, LaRue, Lewis, Logan, Owsley, Trigg
Years: 1971 (2), 2005 (5), 2007(3), 2008 (33)
Months: May (1), June (29), July (11), August (2)
Abundance: 43 specimens: 41-KYSU, 2-UKIC
Comments: Almost all specimens were recently collected in protected, native-grassland habitats. LeSage (1986) listed this species from Campbell Co., Carroll Co., Florence (Boone Co.) and Henderson (Henderson Co.). Host plants are species of Asteraceae, especially *Ambrosia* (Clark et al. 2004).

Ophraella conferta (J. L. LeConte) (Figure 2C)

Kentucky Counties: Bell, Carter, Christian, Clark, Fayette, Grayson, Green, Hancock, Hardin, Jefferson, Kenton, LaRue, Lewis, Logan, McCreary, Pulaski, Whitley
Years: 1945 (1), 1981 (7), 1983 (6), 1990 (1), 1993 (1), 1999 (1), 2001 (1), 2003 (1), 2004 (1), 2005 (4), 2006 (11), 2007 (5), 2008 (5)
Months: May (21), June (15), July (9)
Abundance: 45 specimens: 2-BYUC, 6-CWIC, 21-KYSU, 15-RJBC, 1-UKIC
Comments: LeSage (1986) listed this species from Florence (Boone Co.) and Slade (Powell Co.). Host plants are species of *Solidago* (Asteraceae) (Clark et al. 2004).

Ophraella cribrata (J. L. LeConte) (Figure 2D) (new state record)

Kentucky Counties: Breckinridge, Bullitt, Christian, Edmonson, Franklin, Grayson, Hardin, Hart, Jefferson, LaRue, Lewis, Lincoln, Logan, Robertson, Russell, Trigg
Years: 1971 (1), 1981 (1), 1983 (1), 1985 (2), 1987 (1), 2004 (2), 2005 (22), 2006 (42), 2007 (42), 2008 (87)
Months: May (21), June (113), July (66), September (1)
Abundance: 201 specimens: 194-KYSU, 6-RJBC, 1-UKIC
Comments: Almost all specimens were recently collected in protected, native-grassland habitats. Host plants are species of *Solidago* (Asteraceae) (Clark et al. 2004).

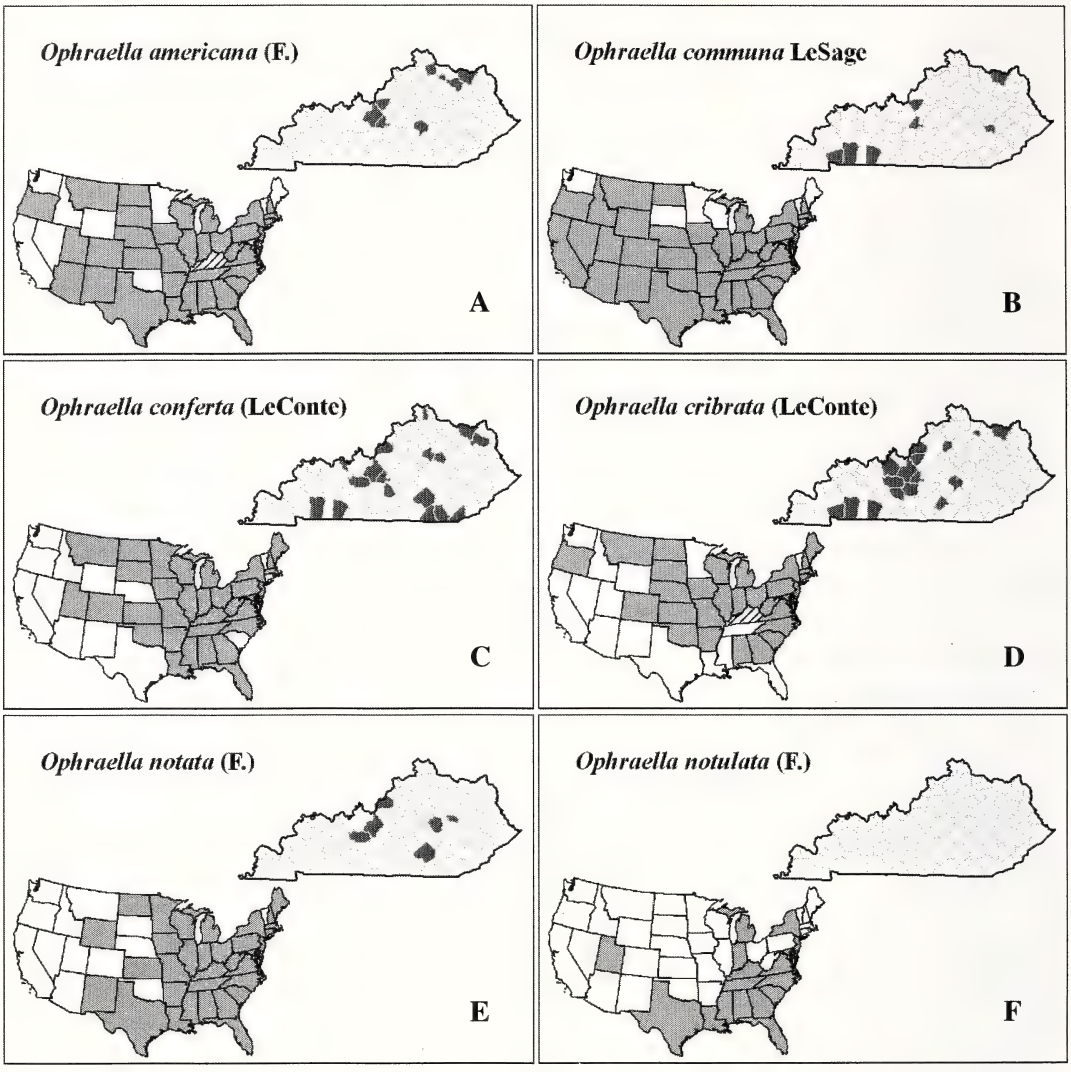


Figure 2. The known distribution of Galerucinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Ophraella notata (F.) (Figure 2E)

Kentucky Counties: Grayson, Hardin, Jefferson, Madison, Powell, Pulaski

Years: 1894 (1), 1939 (1), 1981 (1), 1983 (1), 2004 (10), 2005 (6), 2006 (8), 2007 (6), 2008 (3)

Months: May (10), June (10), July (11), August (3), September (3)

Abundance: 37 specimens: 32-KYSU, 3-RJBC, 2-UKIC

Comments: LeSage (1986) listed this species from Florence (Boone County). Almost of all the recently collected specimens were from Eastview Barrens State Nature Preserve on *Eupatorium perfoliatum* L. (Asteraceae), which was also listed as a host on the1894 specimen label.

Ophraella notulata (F.) (Figure 2F)

Abundance: 1 specimen: 1-CMC

Comments: One specimen was found in the Dury Collection with a label reading "Ky.". LeSage (1986) listed this species from Florence (Boone Co.). Host plants are species of *Iva* (Asteraceae) (Clark et al. 2004).

Acalymma gouldi Barber (Figure 3A) (new state record)

Kentucky County: Russell
Year: 2005 (1)
Month: July (1)
Abundance: 1 specimen: 1-KYSU
Comments: This species has been associated with Cucurbitaceae (Clark et al. 2004).

Acalymma vinctum (J. L. LeConte) (Figure 3B) (new state record)

Kentucky County: Warren
Year: 2006 (3)
Month: September (3)
Abundance: 3 specimens: 3-RJBC
Comments: This species has been associated with Cucurbitaceae (Clark et al. 2004).

Acalymma vittatum (F.) (Figure 3C)

Kentucky Counties: Barren, Carter, Casey, Daviess, Fayette, Franklin, Hardin, Hart, Henry, Jackson, Jefferson, Laurel, Nelson, Owsley, Russell, Scott, Trigg, Warren, Woodford
Years: 1889 (2), 1892 (2), 1894 (3), 1916 (1), 1919 (6), 1926 (1), 1938 (1), 1940 (1), 1941 (5), 1948 (2), 1950 (7), 1970 (5), 1971 (3), 1975 (1), 1976 (1), 1977 (3), 1978 (7), 1979 (1), 1981 (1), 1993 (1), 1994 (6), 1995 (1), 2004 (4), 2005 (13), 2006 (2), 2007 (3), 2008 (1)
Months: March (1), April (6), May (30), June (12), July (10), August (9), September (3), October (12), December (1)
Abundance: 84 specimens: 6-BYUC, 6-CWIC, 13-KYSU, 9-RJBC, 50-UKIC
Comments: This species, often called the striped cucumber beetle, has been associated with Cucurbitaceae (Clark et al. 2004).

Diabrotica barberi R. Smith & Lawrence (Figure 3D)

Kentucky Counties: Boone, Campbell, Daviess, Fayette, Fulton, Grayson, Hardin, Henderson, LaRue, Morgan, Owsley, Union, Washington
Years: 1899 (3), 1904 (1), 1908 (1), 1910 (2), 1913 (3), 1916 (5), 1920 (5), 1928 (1), 1941 (2), 1943 (1), 1946 (4), 1949 (1), 1965 (2), 1967 (2), 1971 (4), 1972 (1), 1974 (1), 1975 (1), 1979 (8), 1980 (1), 1983 (2)
Months: May (1), June (2), July (17), August (22), September (8), November (1)
Abundance: 51 specimens: 2-RJBC, 49-UKIC
Comments: The common name of this species is the northern corn rootworm. This agricultural pest has not been collected recently in natural areas.

Diabrotica cristata (Harris) (Figure 3E)

Kentucky Counties: Fayette, Grayson, Hardin, Henderson, Logan, Rowan
Years: 1915 (1), 1960 (23), 1983 (2), 2004 (5), 2005 (10), 2006 (42), 2007 (7), 2008 (7)
Months: June (34), July (39), August (24)
Abundance: 97 specimens: 2-CWIC, 67-KYSU, 4-RJBC, 24-UKIC
Comments: Butterfly milkweed, *Asclepias tuberosa*, was on a specimen label. This species is associated with Poaceae (Clark et al. 2004).

Diabrotica undecimpunctata howardi Barber (Figure 3F)

Kentucky Counties: Barren, Boone, Bourbon, Boyd, Boyle, Bracken, Caldwell, Calloway, Carlisle, Carter, Casey, Christian, Crittenden, Cumberland, Daviess, Fayette, Franklin, Fulton, Graves, Grayson, Greenup, Hardin, Harrison, Hart, Henderson, Hickman, Hopkins, Jefferson, Jessamine, Kenton, Letcher, Lewis, Lincoln, Livingston, Logan, Madison, Marion, Martin, Mason, McCracken, Meade, Mercer, Ohio, Powell, Robertson, Rockcastle, Rowan, Russell, Scott, Shelby, Simpson, Spencer, Todd, Trigg, Warren, Wayne, Wolfe, Woodford
Years: 1889 (53), 1890 (38), 1891 (34), 1892 (5), 1893 (9), 1894 (1), 1895 (1), 1897 (2), 1898 (1), 1901 (5), 1916 (2), 1924 (2), 1926 (1), 1928 (1), 1932 (1), 1933 (1), 1934 (1), 1936 (2), 1937 (4), 1938 (7), 1940 (6), 1941 (3), 1945 (3), 1946 (1), 1947 (8), 1948 (6), 1949 (8), 1950 (7), 1951 (4), 1952 (8), 1953 (3), 1954 (3), 1955 (4), 1956 (5), 1957 (1), 1958 (8), 1960 (2), 1962 (1), 1964 (1), 1965 (1), 1966 (2), 1967 (4), 1968 (2), 1969 (1), 1970 (16), 1971 (11), 1972 (6), 1974 (1), 1975 (7), 1976 (1), 1979 (2), 1995 (3), 1998 (6), 2001 (7), 2002 (2), 2003 (13), 2004 (18), 2005 (30), 2006 (9), 2007 (3), 2008 (4)
Months: May (14), June (47), July (86), August (64), September (115), October (71), November (5)
Abundance: 402 specimens: 9-BYUC, 22-CWIC, 42-KYSU, 5-RJBC, 299-UKIC, 25-WKUC
Comments: The common name of this species is spotted cucumber beetle or southern corn rootworm. This species is undoubtedly found in all counties but is often not

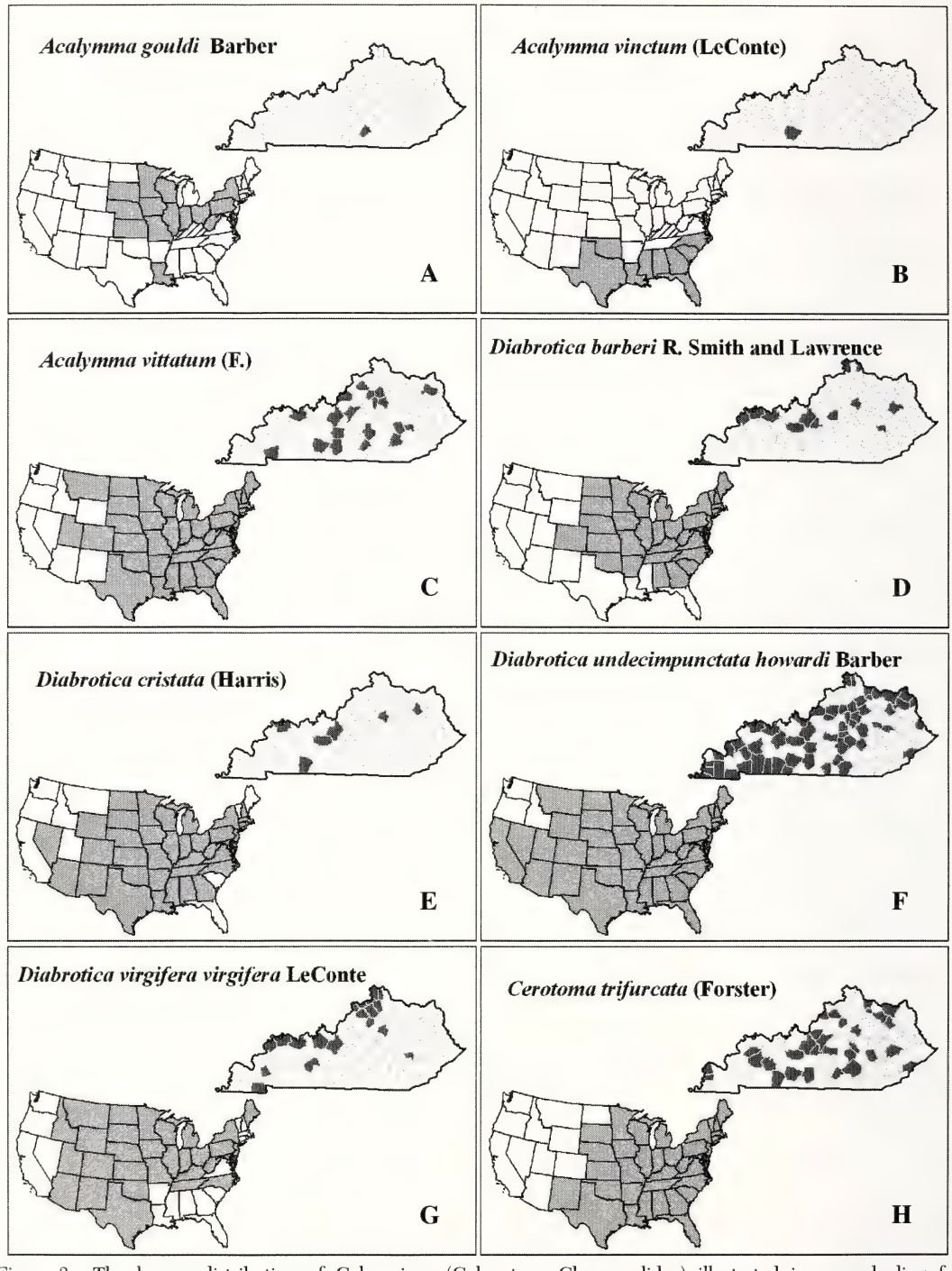


Figure 3. The known distribution of Galerucinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

retained by collectors, being regarded as too common to bother.

Diabrotica virgifera virgifera J. L. LeConte (Figure 3G)

Kentucky Counties: Boone, Breckinridge, Butler, Calloway, Carroll, Daviess, Fayette, Franklin, Gallatin, Grant, Hardin, Henderson, Henry, Kenton, Lyon, Owen, Owsley, Trimble, Union

Years: 1971 (1), 1979 (3), 1980 (24), 2003 (1), 2007 (1), 2008 (3)

Months: July (20), August (13)

Abundance: 33 specimens: 1-CWIC, 4-KYSU, 28-UKIC

Comments: The common name of this species is the western corn rootworm.

Cerotoma trifurcata (Forster) (Figure 3H)

Kentucky Counties: Ballard, Barren, Bracken, Breathitt, Bullitt, Carlisle, Fayette, Fleming, Franklin, Garrard, Grayson, Hardin, Henry, Hopkins, Jackson, LaRue, Lewis, Letcher, Lincoln, Logan, Marion, Muhlenburg, Nelson, Oldham, Pulaski, Robertson, Rowan, Russell, Warren, Webster

Years: 1893 (1), 1906 (2), 1910 (4), 1923 (3), 1924 (1), 1936 (3), 1938 (16), 1941 (1), 1943 (1), 1946 (1), 1947 (1), 1954 (3), 1962 (1), 1965 (1), 1967 (2), 1968 (3), 1970 (7), 1971 (4), 1972 (17), 1975 (22), 1982 (1), 1989 (1), 1994 (1), 1995 (2), 1998 (7), 1999 (1), 2001 (3), 2003 (4), 2004 (5), 2005 (31), 2006 (57), 2007 (20), 2008 (11)

Months: April (15), May (87), June (27), July (65), August (22), September (14), October (6), November (1)

Abundance: 238 specimens: 11-BYUC, 1-CMC, 12-CWIC, 117-KYSU, 2-RJBC, 88-UKIC, 7-WKUC

Comments: The common name of this species is the bean leaf beetle. This species is associated with Fabaceae (Clark et al. 2004). One specimen was found in the Dury Collection with a label reading "Ky. near Cin. O." Malaise trap was listed on several labels.

Phyllecthris dorsalis (Olivier) (Figure 4A) (new state record)

Kentucky Counties: Boyd, Breathitt, Knott, Lee, Perry, Pulaski

Years: 1951 (11), 1953 (6), 1962 (4), 1972 (2), 2000 (1)

Month: June (24)

Abundance: 24 specimens: 1-CWIC, 23-UKIC

Comments: This species is associated with Fabaceae (Clark et al. 2004).

Phyllecthris gentilis J. L. LeConte (Figure 4B) (new state record)

Kentucky Counties: Fayette, Grayson, Hardin, Pike, Robertson

Years: 1890 (1), 1891 (7), 1924 (1), 1955 (1), 1971 (1), 1983 (1), 2003 (2), 2005 (9), 2006 (2), 2008 (3)

Months: May (10), June (12), July (5), August (1)

Abundance: 29 specimens: 1-CMC, 2-CWIC, 14-KYSU, 1-RJBC, 11-UKIC

Comments: One specimen was found in the Dury Collection with a label reading "Ky.", with no date information. A UKIC specimen label documented a plant association with black locust. This species is associated with Fabaceae (Clark et al. 2004).

Scelolyperus cyanellus (J. L. LeConte) (Figure 4C)

Kentucky County: Elliott

Year: 1990 (2)

Month: May (2)

Abundance: 2 specimens: 2-BYUC

Comments: Clark (1996) reported *Phlox paniculata* L. (Polemoniaceae) as the host of this species.

Scelolyperus liriophilus Wilcox (Figure 4D)

Kentucky Counties: LaRue, Menifee, Muhlenburg, Perry, Pike, Rowan, Whitley

Years: 1983 (5), 1990 (2), 1993 (1), 1994 (7), 1995 (1), 2005 (1), 2006 (1)

Month: May (18)

Abundance: 18 specimens: 9-BYUC, 3-CWIC, 1-KYSU, 5-RJBC

Comments: A UKIC specimen label made a plant association with *Quercus*, and another with *Robinia pseudoacacia*.

Phyllobrotica circumdata (Say) (Figure 5A)

Kentucky Counties: Breathitt, Grayson, Hart

Years: 1972 (1), 1985 (2), 2002 (1)

Months: June (2), July (2)

Abundance: 4 specimens: 1-CWIC, 2-RJBC, 1-UKIC

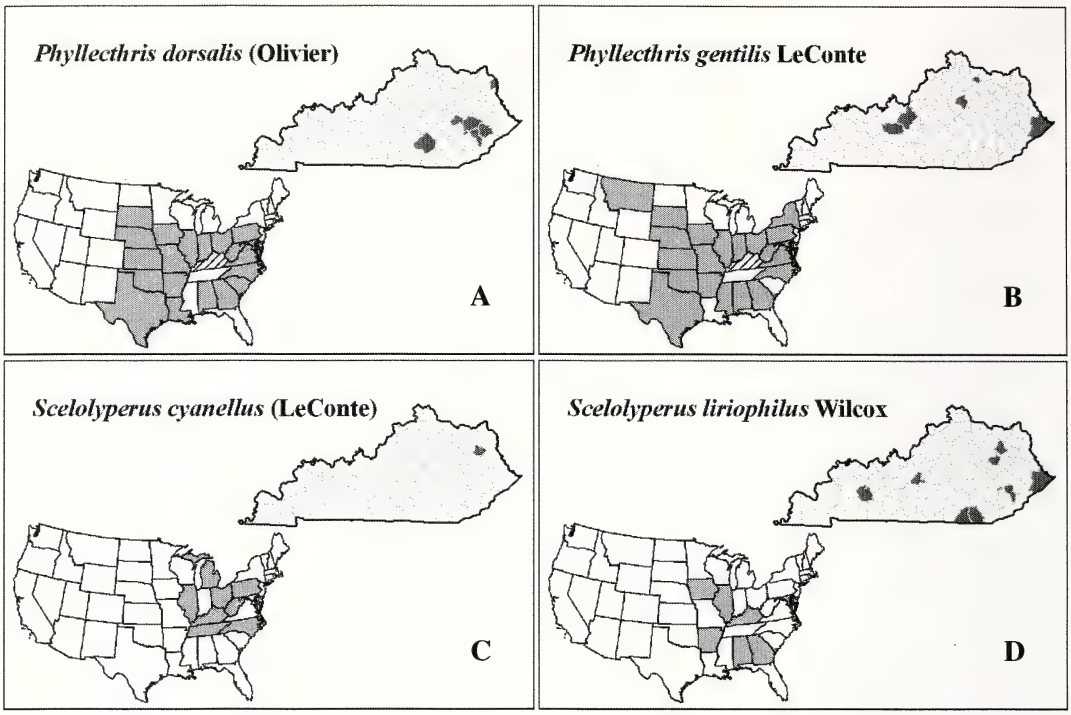


Figure 4. The known distribution of Galerucinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Comments: A UKIC specimen label made a plant association with *Scutellaria incana*.

Phyllobrotica lengi Blatchley (Figure 5B)

Kentucky County: Grayson
Years: 2004 (2), 2006 (2)
Months: May (3), June (1)
Abundance: 4 specimens: 4-KYSU
Comments: All specimens were recently collected from a very small railroad prairie. Clark et al. (2004) found this species feeding on *Scutellaria parvula* Michx. (Lamiaceae).

Phyllobrotica limbata (F.) (Figure 5C) (new state record)

Kentucky Counties: Breathitt, Bullitt, Grayson, Hardin, Henry, Lewis, Lincoln, Logan
Years: 1971 (1), 1983 (2), 2005 (13), 2006 (6), 2007 (9), 2008 (11)
Months: May (22), June (19), July (1)
Abundance: 42 specimens: 39-KYSU, 2-RJBC, 1-UKIC
Comments: Almost all specimens were recently collected in protected, native-grassland habitats. This species is associated with *Scutellaria* (Lamiaceae) (Clark et al. 2004).

Phyllobrotica stenidea Schaeffer (Figure 5D) (new state record)

Kentucky County: Robertson
Year: 2006 (9)
Months: May (3), June (6)
Abundance: 9 specimens: 9-KYSU
Comments: All specimens were recently collected from Blue Licks Battlefield State Resort Park.

Metroidea brunnea (Crotch) (Figure 5E) (new state record)

Kentucky Counties: Hardin, Logan
Years: 2004 (6), 2005 (10), 2006 (7), 2007 (1), 2008 (7)
Months: July (25), August (4), September (2)
Abundance: 31 specimens: 31-KYSU
Comments: All specimens were recently collected from Eastview Barrens SNP and Raymond Athey Barrens SNP.

DISCUSSION

We believe the data presented here are the most complete representation of the galerucine and luperine leaf beetles known from Kentucky. The large number of new state records

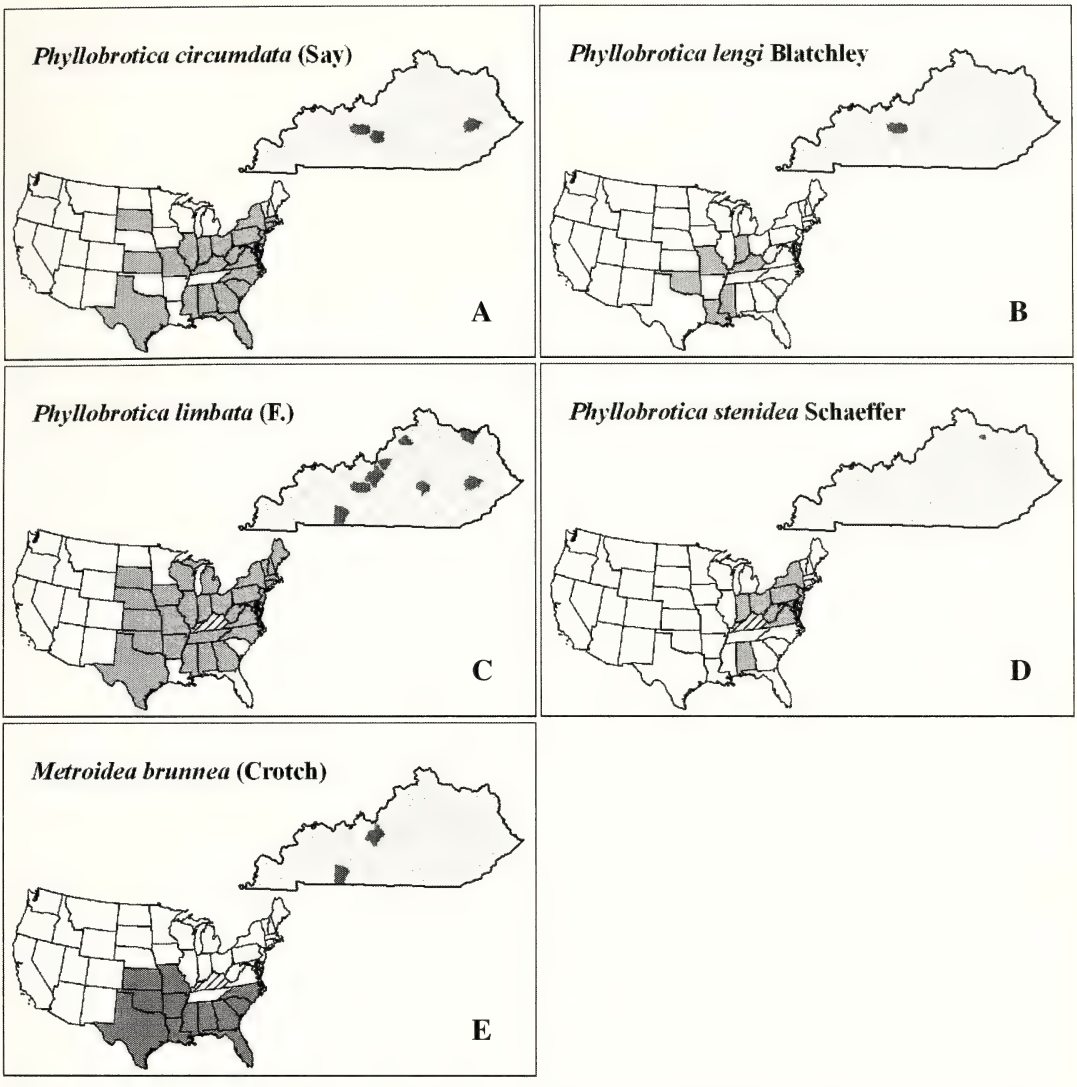


Figure 5. The known distribution of Galerucinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

documented here (13 of 30 species, or 43%) reflects a historical lack of leaf beetle collecting in Kentucky. Five of the thirteen new state records are the result of recent collecting efforts concentrated in protected, native-grassland habitats. These areas are actively managed with prescribed burning to preserve native grasses and forbs and appear to be remnant habitat 'islands' for many of these species.

ACKNOWLEDGMENTS

Thanks are extended to Michael Sharkey and Martha Potts (UKIC), Keith Philips (WKUC), Greg Dahlem (CMC), and Charles Wright (CWIC) for access to their collections. We thank the following people for granting access to the protected habitats they manage: Joyce Bender, Lane Linnenkohl and Zeb Weese, Kentucky State Nature Preserves Commission; Jeff Sole and John Burnett, The Nature Conservancy Kentucky Chapter; Steve McMillen, Kentucky Department of Fish and Wildlife; Andrew Leonard, Fort Campbell Fisheries and Wildlife Program; and Steve Bloemer, USDA Forest Service. We also thank Joyce Owens (KYSU) for sorting, organizing and transcribing, and Sarah Hall (KYSU) for creation of the distribution maps. This re-

search was supported by USDA-CSREES Project KYX-10-05-39P.

LITERATURE CITED

Barney, R. J., S. M. Clark, and E. G. Riley. 2007. Annotated list of the leaf beetles (Coleoptera: Chrysomelidae) of Kentucky: subfamily Cassidinae. Journal of the Kentucky Academy of Science 68:132–144.

Barney, R. J., S. M. Clark, and E. G. Riley. 2008a. Annotated list of the Subfamilies Donaciinae and Criocerinae. Journal of the Kentucky Academy of Science 69:29–36.

Barney, R. J., S. M. Clark, and E. G. Riley. 2008b. Annotated list of the leaf beetles (Coleoptera: Chrysomelidae) of Kentucky: subfamily Chrysomelinae. Journal of the Kentucky Academy of Science 69: 91–100.

Baskin, J. M., C. C. Baskin, and E. W. Chester. 1994. The Big Barrens Region of Kentucky and Tennessee: further observations and considerations. Castanea 59:226–254.

Blake, D. H. 1931. Revision of the beetles of the genus *Trirhabda* north of Mexico. Proceedings of the United States National Museum 79(no. 2868):1–36, pls. 1–2.

Clark, S. M. 1986. Occurrence of *Monocesta coryli* (Say) in Ohio (Coleoptera: Chrysomelidae). Ohio Journal of Science 86:213.

Clark, S. M. 1996. The genus *Scelolyperus* Crotch in North America (Coleoptera: Chrysomelidae: Galerucinae). Insecta Mundi 10:261–280.

Clark, S. M., D. G. LeDoux, T. N. Seeno, E. G. Riley, A. J. Gilbert, and J. M. Sullivan. 2004. Host plants of leaf beetle species occurring in the United States and Canada. The Coleopterists Society, Special Publication No. 2. 476 pp.

Hogue, S. M. 1970. Biosystematics of the Genus *Trirhabda* LeConte of America north of Mexico (Chrysomelidae: Coleoptera). Ph.D. Dissertation, University of Idaho. 212 pp.

Jones, R. L. 2005. Plant Life of Kentucky. University Press of Kentucky. 834 pp.

LeSage, L. 1986. A taxonomic monograph of the Nearctic galerucine genus *Ophraella* Wilcox (Coleoptera: Chrysomelidae). Memoirs of the Entomological Society of Canada no. 133:1–75.

Riley, E. G., S. M. Clark, R. W. Flowers, and A. J. Gilbert. 2002. Chrysomelidae Latreille 1802. Pages 617–691 in R. H. Arnett and M. C. Thomas (eds). American Beetles. CRC press.

Riley, E. G., S. M. Clark, and T. N. Seeno. 2003. Catalog of the leaf beetles of America north of Mexico. The Coleopterists Society, Special Publication No. 1. 290 pp.

Vulinec, K., and R. A. Davis. 1984. Coleoptera types in the Charles Dury Collection of the Cincinnati Museum of Natural History. The Coleopterists Bulletin 38: 232–239.

Wilcox, J. A. 1965. A synopsis of the North American Galerucinae (Coleoptera: Chrysomelidae). New York State Museum and Science Service Bulletin no. 400: iv + 226 pp.

J. Ky. Acad. Sci. 70(1):29–55. 2009.

Annotated List of the Leaf Beetles (Coleoptera: Chrysomelidae) of Kentucky: Subfamily Galerucinae, Tribe Alticini

Robert J. Barney[1]

Community Research Service, Kentucky State University, Frankfort, Kentucky 40601

Shawn M. Clark

Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah 84602

Edward G. Riley

Department of Entomology, Texas A&M University, College Station, Texas 77843

ABSTRACT

An examination of leaf beetle specimens (Coleoptera: Chrysomelidae) in the largest beetle collections in Kentucky, recent inventory work in state nature preserves and other protected areas, and a review of the literature revealed eighty-four species of the tribe Alticini (Subfamily Galerucinae) present in Kentucky, forty-five of which are unreported previously for the state. Distribution maps and label data are presented for the eighty-four Kentucky species 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: *Blepharida rhois* (Forster), *Luperaltica senilis* (Say), *Phyllotreta cruciferae* (Goeze), *Ceraltica insolita* (Melsheimer), *Glyptina brunnea* Horn, *Glyptina cyanipennis* (Crotch), *Glyptina spuria* LeConte, *Longitarsus acutipennis* Blatchley, *Longitarsus arenaceus* Blatchley, *Longitarsus melanurus* (Melsheimer), *Longitarsus pratensis* (Panzer), *Longitarsus testaceus* (Melsheimer), *Systena frontalis* (F.), *Systena hudsonias* (Forster), *Altica chalybea* Illiger, *Altica knabii* Blatchley, *Altica litigata* Fall, *Orthaltica copalina* (F.), *Orthaltica melina* Horn, *Epitrix humeralis* Dury, *Margaridisa atriventris* (Melsheimer), *Mantura floridana* Crotch, *Chaetocnema fuscata* R. White, *Chaetocnema quadricollis* Schwarz, *Disonycha admirabila* Blatchley, *Disonycha alternata* (Illiger), *Disonycha arizonae* Casey, *Disonycha caroliniana* (F.), *Disonycha fumata fumata* (LeConte), *Disonycha leptolineata* Blatchley, *Lupraea picta* (Say), *Parchicola iris* (Olivier), *Parchicola tibialis* (Olivier), *Capraita circumdata* (Randall), *Capraita scalaris* (Melsheimer), *Capraita sexmaculata* (Illiger), *Capraita subvittata* (Horn), *Kuschelina fimbriata* (Forster), *Kuschelina gibbitarsa* (Say), *Kuschelina miniata* (F.), *Kuschelina perplexa* (Blake), *Kuschelina suturella* (Say), *Dibolia sinuata* Horn, *Pseudodibolia opima* (LeConte), and *Psylliodes punctulatus* Melsheimer.

KEY WORDS: Kentucky, leaf beetles, Chrysomelidae, biodiversity, new state records

INTRODUCTION

This paper is the fifth 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 protected locations. The first four papers presented information on the subfamilies Cassidinae (Barney et al. 2007), Donaciinae and Criocerinae (Barney et al. 2008a), Chrysomelinae (Barney et al. 2008b), and tribes Galerucini and Luperini (Galerucinae) (Barney et al. this issue).

Leaf beetles in the galerucine tribe Alticini, commonly called the flea beetles due to the enlarged hind femora and flea-like jumping ability, are well known to many lay people with roughly 500 genera and over 10,000 species described worldwide (Riley et al. 2002). Several genera contain agricultural and garden pests such as the eggplant flea beetle, *Epitrix fuscula* Crotch and the sweet potato flea beetle, *Chaetocnema confinis* Crotch. Many of the genera have been revised, including *Altica* (in part, LeSage 1995), *Blepharida* (Furth 1998), *Capraita* and *Kuschelina* (as *Oedionychis*, Blake 1927), *Chaetocnema* (White 1996), *Crepidodera* (Parry 1986), *Dibolia* (Parry 1974), *Disonycha* (Blake 1933), *Distigmoptera* (Balsbaugh and Kirk 1968), *Epitrix* (in part, Gentner 1944), *Phyllotreta* (Chittenden 1927; in part, Smith 1985), and *Strabala* (Blake 1953).

[1] Corresponding author e-mail: robert.barney@kysu.edu

The purpose of this study is to present historical and current knowledge of the distribution, abundance, and plant associations of Alticini 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, but known to contain Kentucky specimens) were examined, reidentified, and their label data recorded. The following collections were studied, with the timeframe of their Kentucky specimens listed:

CMC Cincinnati Museum Center, Cincinnati, OH 1871–1931
UKIC University of Kentucky Insect Collection, Lexington, KY 1889–1993
WKUC Western Kentucky University Collection, Bowling Green, KY 1958–2006
RJBC Robert J. Barney Collection, Frankfort, KY (private) 1983–present
BYUC Brigham Young University Collection, Provo, UT 1988–present
CWC Charles Wright Collection, Frankfort, KY (private) 1991–present
KYSU Kentucky State University Collection, Frankfort, KY 2004–present

The Cincinnati Museum Collection, formerly known as the Cincinnati Museum of Natural History, houses the Charles Dury Collection, comprising approximately 75,000 insect specimens primarily collected in the Cincinnati/northern Kentucky area (Vulinec and Davis 1984). The University of Kentucky Collection contains the Charles V. Covell, Jr. Collection (emeritus professor of the University of Louisville).

The Kentucky State University Insect Collection is primarily 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 that are known for possessing uncommon plants and plant associations (Jones 2005) and have never been surveyed for leaf beetles. These sites are managed by the Kentucky State Nature Preserves Commission, The Nature Conservancy, and the United States Army at Fort Campbell Military Reservation (Baskin et al. 1994). Most specimens were collected by the senior author within five state nature preserves in 2004–2008 and Fort Campbell in 2008: 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) and Fort Campbell (Christian and Trigg Counties) in western Kentucky.

For each alticine 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. Other pertinent information present on specimen labels, 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 176 species of Alticini recorded in at least one of the seven states contiguous to Kentucky. However, only 41 species were reported from Kentucky. An examination of over 3500 alticine leaf beetle specimens from the major collections in the state and others known to contain Kentucky specimens revealed 82 species, including 38 (36 observed and two additional documented from the literature) of the 41 recorded in Riley et al. (2003), plus 45 new state records (Table 1). A breakdown of specimens, species and records by collection examined is presented in Table 2.

Blepharida rhois (Forster) (Figure 1A) (new state record)

Kentucky Counties: Calloway, Edmonson, Graves, Hardin, Hart, LaRue, McCreary, Whitley

Years: 1944 (7), 1964 (1), 1970 (1), 1983 (4), 1985 (1), 2004 (6), 2005 (14), 2006 (5), 2007 (4), 2008 (9)

Months: April (7), May (23), June (16), September (5), October (1)

Abundance: 52 specimens: 38-KYSU, 5-RJBC, 8-UKIC, 1-WKUC

Comments: We have hand picked this species from *Rhus* spp. Clark et al. (2004) reported this species associated with Anacardiaceae.

Luperaltica nigripalpis (LeConte) (Figure 1B)

Kentucky Counties: Hardin, Hopkins, Rowan

Years: 1995 (1), 2003 (4), 2004 (36), 2005 (5), 2007 (12), 2008 (2)

Months: July (21), August (18), September (21)

Abundance: 60 specimens: 1-BYUC, 4-CWC, 55-KYSU

Comments: Specimen labels reported this species collected from *Solidago* sp., *Coreopsis* sp., and *Liatris asperva* Michx.

Luperaltica senilis (Say) (Figure 1C) (new state record)

Kentucky County: unknown

Year: unknown

Month: unknown

Abundance: 1 specimen: 1-CMC

Comments: The only specimen examined is in the Dury collection and is labeled "Ky. near Cin O."

Phyllotreta bipustulata (F.) (Figure 1D)

Kentucky Counties: Fayette, Henry, Owen, Russell

Years: 1889 (2), 1916 (1), 1971 (1), 2005 (1), 2008 (1)

Months: May (2), June (1), July (1), August (1), September (1)

Abundance: 6 specimens: 2-KYSU, 4-UKIC

Comments: This species is primarily associated with Brassicaceae (Clark et al. 2004).

Phyllotreta cruciferae (Goeze) (Figure 1E) (new state record)

Kentucky Counties: Fayette, Franklin, Wolfe

Years: 1912 (1), 2000 (1), 2005 (18)

Months: May (7), June (6), July (7)

Abundance: 20 specimens: 1-CWC, 18-KYSU, 1-UKIC

Comments: The senior author collected this species from the following food plants: turnip greens, *Brassica rapa* v. *rapifera* L.; okra, *Abelmoschus esculentus*; collard, *Brassica oleracea* v. *acephala* L.; kale, *Brassica oleracea* v. *acephala* L.; mustard green, *Brassica juncea* (L.) Czern.

Phyllotreta liebecki Schaeffer (Figure 1F)

Kentucky Counties: Daviess, Webster

Years: 2004 (1), 2005 (2)

Month: May (3)

Abundance: 3 specimens: 3-CWC

Comments: This species is primarily associated with Brassicaceae (Clark et al. 2004).

Phyllotreta striolata (F.) (Figure 1G)

Kentucky Counties: Anderson, Bracken, Bullitt, Casey, Fayette, Franklin, Logan, Oldham

Years: 1912 (1), 1970 (4), 1974 (4), 1998 (2), 2003 (1), 2005 (16), 2006 (5), 2007 (3)

Months: May (2), June (19), July (13), August (2)

Abundance: 36 specimens: 2-BYUC, 2-CWC, 10-KYSU, 13-RJBC, 9-UKIC

Comments: This species is primarily associated with Brassicaceae (Clark et al. 2004).

Phyllotreta zimmermanni (Crotch) (Figure 1H)

Kentucky Counties: Breathitt, Bullitt, Caldwell, Clark, Clinton, Fleming, Franklin, Hancock, Laurel, Madison, Marshall, McCreary, Monroe, Perry, Webster

Years: 1889 (93), 1890 (2), 1891 (4), 1894 (1), 1895 (2), 1913 (1), 1920 (10), 1938 (2), 1966 (1), 1970 (1), 1971 (5), 1974 (2), 1988 (1), 1993 (4), 1995 (1), 2004 (2), 2005 (9), 2006 (1), 2007 (3)

Months: February (1), March (7), April (6), May (15), June (20), July (5), August (2), September (36), October (37), November (16)

Abundance: 145 specimens: 6-BYUC, 13-CWC, 2-KYSU, 124-UKIC

Comments: This species is primarily associated with Brassicaceae (Clark et al. 2004).

Ceraltica insolita (Melsheimer) (Figure 2A) (new state record)

Kentucky County: LaRue

Year: 2005 (1)

Month: May (1)

Abundance: 1 specimen: 1-KYSU

Comments: The single specimen was collected by the senior author at Thompson Creek Glades State Nature Preserve.

Glyptina brunnea Horn (Figure 2B) (new state record)

Table 1. List of Alticini (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.

Blepharida genus group	
Blepharida rhois (Forster)	52 specimens: 8 counties, 1944–2008 (new state record)
Luperaltica genus group	
Luperaltica nigripalpis (LeConte)	60 specimens: 3 counties, 1995–2005
Luperaltica senilis (Say)	1 specimen: 1 county, ca. 1900 (new state record)
Phyllotreta genus group	
Phyllotreta bipustulata (F.)	6 specimens: 4 counties, 1889–2008
Phyllotreta cruciferae (Goeze)	20 specimens: 3 counties, 1912–2005 (new state record)
Phyllotreta liebecki Schaeffer	3 specimens: 2 counties, 2004–2005
Phyllotreta striolata (F.)	36 specimens: 8 counties, 1912–2007
Phyllotreta zimmermanni (Crotch)	145 specimens: 15 counties, 1889–2007
Longitarsus genus group	
Ceraltica insolita (Melsheimer)	1 specimen: 1 county, 2005 (new state record)
Glyptina brunnea Horn	2 specimens: 2 counties, 1889–1972 (new state record)
Glyptina cyanipennis (Crotch)	5 specimens: 2 counties, 2003–2008 (new state record)
Glyptina spuria LeConte	41 specimens: 5 counties, 1889–2006 (new state record)
Longitarsus acutipennis Blatchley	2 specimens: 1 county, 1990 (new state record)
Longitarsus arenaceus Blatchley	1 specimen: 1 county, 1994 (new state record)
Longitarsus melanurus (Melsheimer)	15 specimens: 1 county, 2005–2008 (new state record)
Longitarsus pratensis (Panzer)	1 specimen: 1 county, 1995 (new state record)
Longitarsus testaceus (Melsheimer)	3 specimens: 2 counties, 1995–1998 (new state record)
Systena genus group	
Systena blanda Melsheimer	173 specimens: 21 counties, 1889–2008
Systena elongata (F.)	55 specimens: 18 counties, 1913–2008
Systena frontalis (F.)	42 specimens: 4 counties, 1910–2008 (new state record)
Systena hudsonias Forster	42 specimens: 6 counties, 1938–2008 (new state record)
Altica genus group	
Altica chalybea Illiger	73 specimens: 12 counties, 1891–2008 (new state record)
Altica knabii Blatchley	6 specimens: 3 counties, 2005–2008 (new state record)
Altica litigata Fall	57 specimens: 17 counties, 1890–2008 (new state record)
Altica subplicata LeConte	7 specimens: 5 counties, 2002–2008
Orthaltica genus group	
Orthaltica copalina (F.)	3 specimens: 1 county, 2003–2008 (new state record)
Orthaltica melina Horn	25 specimens: 6 counties, 1899–2008 (new state record)
Crepidodera genus group	
Crepidodera browni Parry	318 specimens: 36 counties, 1889–2008
Crepidodera longula Horn	157 specimens: 13 counties, 1971–2008
Crepidodera nana (Say)	3 specimens: 3 counties, 1994–1998
Crepidodera violacea Melsheimer	5 specimens: 1 county, 1995
Derocrepis aesculi (Dury)	1 specimen: 1 county, 1982
Derocrepis erythropus (Melsheimer)	125 specimens: 10 counties, 1892–2008
Epitrix brevis Schwarz	56 specimens: 12 counties, 1889–2008
Epitrix cucumeris (Harris)	27 specimens: 3 counties, 1921–2006
Epitrix fuscula Crotch	192 specimens: 17 counties, 1889–2008
Epitrix hirtipennis (Melsheimer)	224 specimens: 6 counties, 1889–1990
Epitrix humeralis Dury	70 specimens: 4 counties, 1889–1971 (new state record)
Margaridisa atriventris (Melsheimer)	19 specimens: 5 counties, 1889–2007 (new state record)
Trichaltica genus group	
Trichaltica scabricula (Crotch)	2 specimens: 2 counties, 2005–2008
Mantura genus group	
Mantura floridana Crotch	4 specimens: 2 counties, 1892–2004 (new state record)
Chaetocnema genus group	
Chaetocnema confinis Crotch	60 specimens: 6 counties, 1891–2007
Chaetocnema crenulata Crotch	1 specimen: 1 county, date unknown

Table 1. Continued.

Chaetocnema denticulata (Illiger)	57 specimens: 16 counties, 1889–2008
Chaetocnema fuscata R. White	3 specimens: 2 counties, 2005–2008 (new state record)
Chaetnocema minuta Melsheimer	1 specimen: 1 county, 1995
Chaetocnema pinguis LeConte	3 specimens: 3 counties, 1995–2007
Chaetocnema pulicaria Melsheimer	730 specimens: 23 counties, 1889–2008
Chaetocnema quadricollis Schwarz	1 specimen: 1 county, 1892 (new state record)
Disonycha genus group	
Disonycha admirabila Blatchley	102 specimens: 7 counties, 1983–2008 (new state record)
Disonycha alternata (Illiger)	1 specimen: 1 county, ca. 1900 (new state record)
Disonycha arizonae Casey	1 specimen: 1 county, 2006 (new state record)
Disonycha caroliniana (F.)	3 specimens: 3 counties, 1968–2005 (new state record)
Disonycha collata collata (F.)	6 specimens: 2 counties, 1890–1913
Disonycha discoidea (F.)	8 specimens: 6 counties, 1892–2008
Disonycha fumata fumata (LeConte)	10 specimens: 3 counties, 1889–2000 (new state record)
Disonycha glabrata (F.)	230 specimens: 25 counties, 1889–2008
Disonycha leptolineata Blatchley	5 specimens: 3 counties, 1984–1997 (new state record)
Disonycha triangularis (Say)	1 specimen: 1 county, 1915
Disonycha uniguttata (Say)	unknown
Disonycha xanthomelas (Dalman)	20 specimens: 6 counties, 1889–2006
Lupraea picta (Say)	5 specimens: 2 counties, 1970–2003 (new state record)
Strabala rufa rufa (Illiger)	8 specimens: 4 counties, 1983–2007
Parchicola genus group	
Parchicola iris (Olivier)	2 specimens: 2 counties, 1972–2006 (new state record)
Parchicola tibialis (Olivier)	3 specimens: 2 counties, 1998–2006 (new state record)
Oedionychis genus group	
Capraita circumdata (Randall)	13 specimens: 3 counties, 1990–2008 (new state record)
Capraita scalaris (Melsheimer)	1 specimen: 1 county, 2003 (new state record)
Capraita sexmaculata (Illiger)	14 specimens: 6 counties, 1894–2008 (new state record)
Capraita subvittata (Horn)	15 specimens: 3 counties, 1972–2008 (new state record)
Capraita thyamoides (Crotch)	52 specimens: 12 counties, 1972–2008
Kuschelina fimbriata (Forster)	1 specimen: 1 county, 1972 (new state record)
Kuschelina gibbitarsa (Say)	10 specimens: 6 counties, 1892–2004 (new state record)
Kuschelina miniata (F.)	1 specimen: 1 county, 1971 (new state record)
Kuschelina perplexa (Blake)	8 specimens: 3 counties, 2004–2008 (new state record)
Kuschelina petaurista (F.)	37 specimens: 11 counties, 1938–2008
Kuschelina suturella (Say)	4 specimens: 4 counties, 2005–2008 (new state record)
Kuschelina thoracica (F.)	9 specimens: 3 counties, 1889–2008
Kuschelina vians (Illiger)	15 specimens: 9 counties, 2001–2008
Sphaeronychus genus group	
Distigmoptera apicalis Blake	2 specimens: 1 county, 1920
Pachyonychus paradoxus Melsheimer	4 specimens: 3 counties, 1983–2004
Dibolia genus group	
Dibolia borealis Chevrolat	28 specimens: 10 counties, 1891–2008
Dibolia sinuata Horn	6 specimens: 1 county, 2007 (new state record)
Heikertingerella genus group	
Pseudodibolia opima (LeConte)	3 specimens: 3 counties, 2005–2008 (new state record)
Psylliodes genus group	
Psylliodes punctulatus Melsheimer	2 specimens: 1 county, 2006 (new state record)

Kentucky Counties: Allen, Fayette
Years: 1889 (1), 1972 (1)
Month: July (2)
Abundance: 2 specimens: 2-UKIC
Comments: This species has been reported from Euphorbiaceae (Clark et al. 2004).

Glyptina cyanipennis (Crotch) (Figure 2C) (new state record)

Kentucky Counties: Franklin, Woodford
Years: 2003 (1), 2006 (4)
Months: May (1), July (4)
Abundance: 5 specimens: 1-CWC, 4-RJBC

Table 2. The number of specimens, species and new Kentucky state records of Alticini flea beetles (Coleoptera: Chrysomelidae) found in the largest collections of leaf beetles collected from Kentucky.

Collection	Specimens	Species	Records
University of Kentucky Insect Collection	2425	48	20
Kentucky State University Collection	722	52	8
Charles Wright Collection	140	37	5
Robert J. Barney Collection	127	25	3
Brigham Young University Collection	124	32	5
Cincinnati Museum Center	20	15	4
Western Kentucky University Collection	10	6	0
Totals	3568	82	45

Comments: This species has been reported from *Euphorbia* spp. (Clark et al. 2004).

Glyptina spuria LeConte (Figure 2D) (new state record)

Kentucky Counties: Barren, Carter, Fayette, Henderson, Logan

Years: 1889 (3), 1890 (8), 1891 (12), 1892 (3), 1894 (3), 1895 (1), 1906 (1), 1916 (1), 1919 (3), 1920 (1), 1923 (1), 1968 (1), 1972 (1), 1994 (1), 2006 (1)

Months: March (1), April (5), May (12), June (13), July (2), August (2), September (2), October (3), December (1)

Abundance: 41 specimens: 1-BYUC, 1-KYSU, 39-UKIC

Comments: This species has been reported from Euphorbiaceae (Clark et al. 2004).

Longitarsus acutipennis Blatchley (Figure 3A) (new state record)

Kentucky County: Greenup
Year: 1990 (2)
Month: May (2)
Abundance: 2 specimens: 2-BYUC

Comments: This species is associated with *Eupatorium perfoliatum* L. (Asteraceae) (Riley and Enns 1979).

Longitarsus arenaceus Blatchley (Figure 3B) (new state record)

Kentucky County: Bath
Year: 1994 (1)
Month: May (1)
Abundance: 1 specimen: 1-BYUC

Comments: This species is reported to occur in association with *Opuntia humifusa* (Raf.) Raf. (Cactaceae) (Blatchley 1921).

Longitarsus melanurus (Melsheimer) (Figure 3C) (new state record)

Kentucky Counties: Bullitt, Hardin, Lewis, Logan

Years: 2005 (2), 2006 (3), 2008 (10)
Months: May (14), June (1)
Abundance: 15 specimens: 15-KYSU

Comments: All specimens were collected by the senior author in state nature preserves. This species feeds on Boraginaceae (Clark et al. 2004).

Longitarsus pratensis (Panzer) (Figure 3D) (new state record)

Kentucky County: Rowan
Year: 1995 (1)
Month: August (1)
Abundance: 1 specimen: 1-BYUC

Comments: This species has been reported from Plantaginaceae (Clark et al. 2004).

Longitarsus testaceus (Melsheimer) (Figure 3E) (new state record)

Kentucky Counties: Laurel, Lewis
Years: 1995 (1), 1998 (2)
Months: April (1), July (2)
Abundance: 3 specimens: 3-BYUC

Comments: Clark et al. (2004) reports *Eupatorium* as the normal host for this species.

Systena blanda Melsheimer (Figure 4A)

Kentucky Counties: Allen, Bath, Bracken, Breathitt, Caldwell, Fayette, Franklin, Hardin, Harlan, Henderson, Hickman, Jackson, Jefferson, LaRue, Letcher, Lincoln, Mason, Morgan, Pulaski, Trigg, Union

Years: 1889 (29), 1890 (10), 1891 (44), 1892 (4), 1893 (6), 1894 (13), 1897 (1), 1906 (7), 1913 (3), 1920 (2), 1934 (1), 1937 (3), 1938

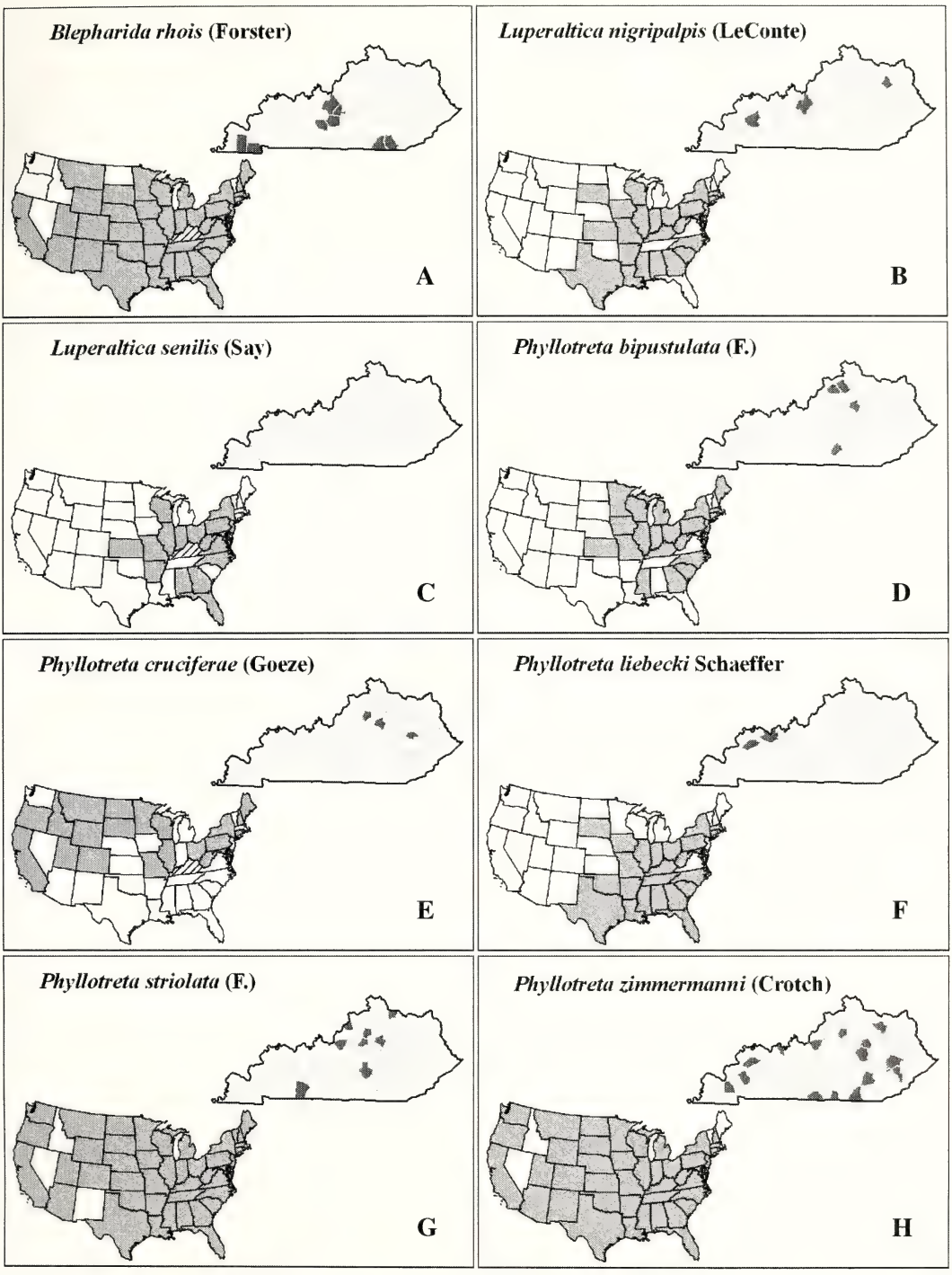


Figure 1. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

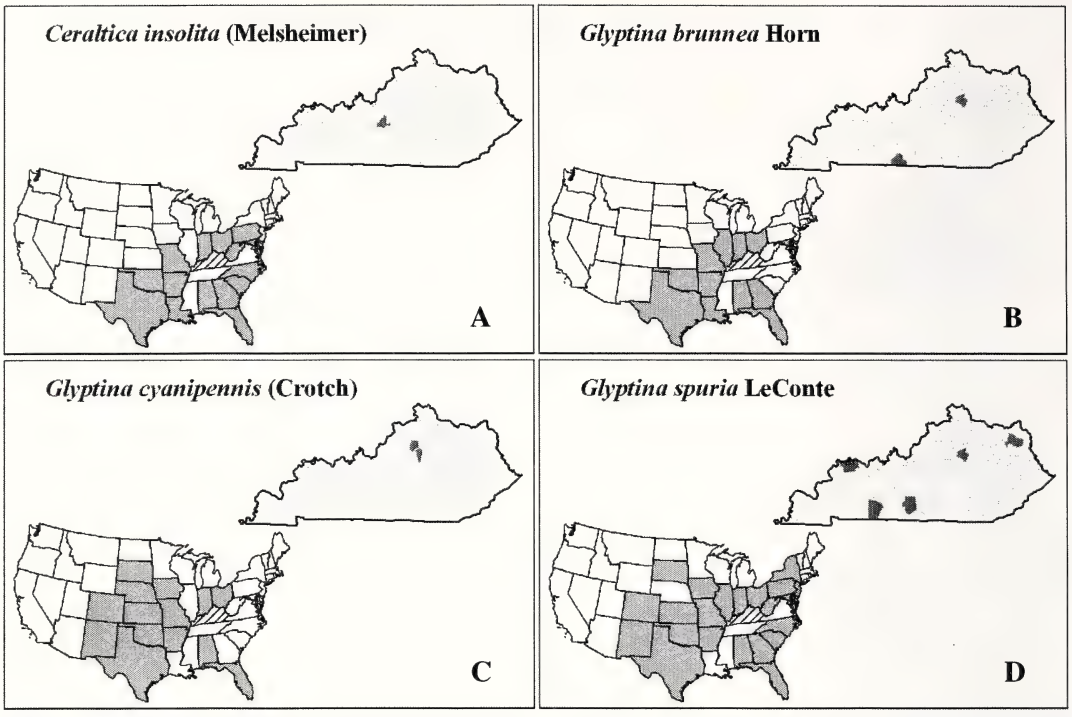


Figure 2. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

(4), 1942 (1), 1971 (10), 1972 (7), 1974 (4), 1975 (2), 1976 (3), 1989 (5), 1998 (6), 2003 (2), 2006 (1), 2007 (1), 2008 (3)

Months: March (3), May (13), June (51), July (39), August (40), September (21), October (1)

Abundance: 173 specimens: 11-BYUC, 1-CMC, 2-CWC, 4-KYSU, 4-RJBC, 151-UKIC

Comments: The CMC specimen was labeled as "Ky." The common name of this species is the pale-striped flea beetle.

Systena elongata (F.) (Figure 4B)

Kentucky Counties: Allen, Barren, Bath, Boyle, Bracken, Caldwell, Fayette, Franklin, Hardin, Henderson, Hopkins, Logan, Madison, Nelson, Russell, Union, Warren, Wayne

Years: 1913 (1), 1917 (5), 1937 (6), 1944 (2), 1963 (1), 1967 (1), 1968 (4), 1970 (3), 1972 (4), 1975 (3), 1992 (1), 1998 (1), 2005 (11), 2007 (6), 2008 (6)

Months: May (7), June (1), July (339), August (7), September (1)

Abundance: 55 specimens: 1-BYUC, 2-CWC, 22-KYSU, 28-UKIC, 2-WKUC

Comments: A series was collected on sweet potato, *Ipomoea batatas* L. Lam., and this species is reported from many crop plants (Clark et al. 2004).

Systena frontalis (F.) (Figure 4C) (new state record)

Kentucky Counties: Daviess, Fulton, Henry, Warren

Years: 1910 (36), 1968 (1), 2002 (1), 2007 (2), 2008 (2)

Months: July (40), September (1), October (1)

Abundance: 42 specimens: 1-CWC, 4-KYSU, 36-UKIC, 1-WKUC

Comments: Clark et al. (2004) reported a long list of plant associations.

Systena hudsonias (Forster) (Figure 4D) (new state record)

Kentucky Counties: Breathitt, Carter, Grayson, Hardin, Kenton, McCreary

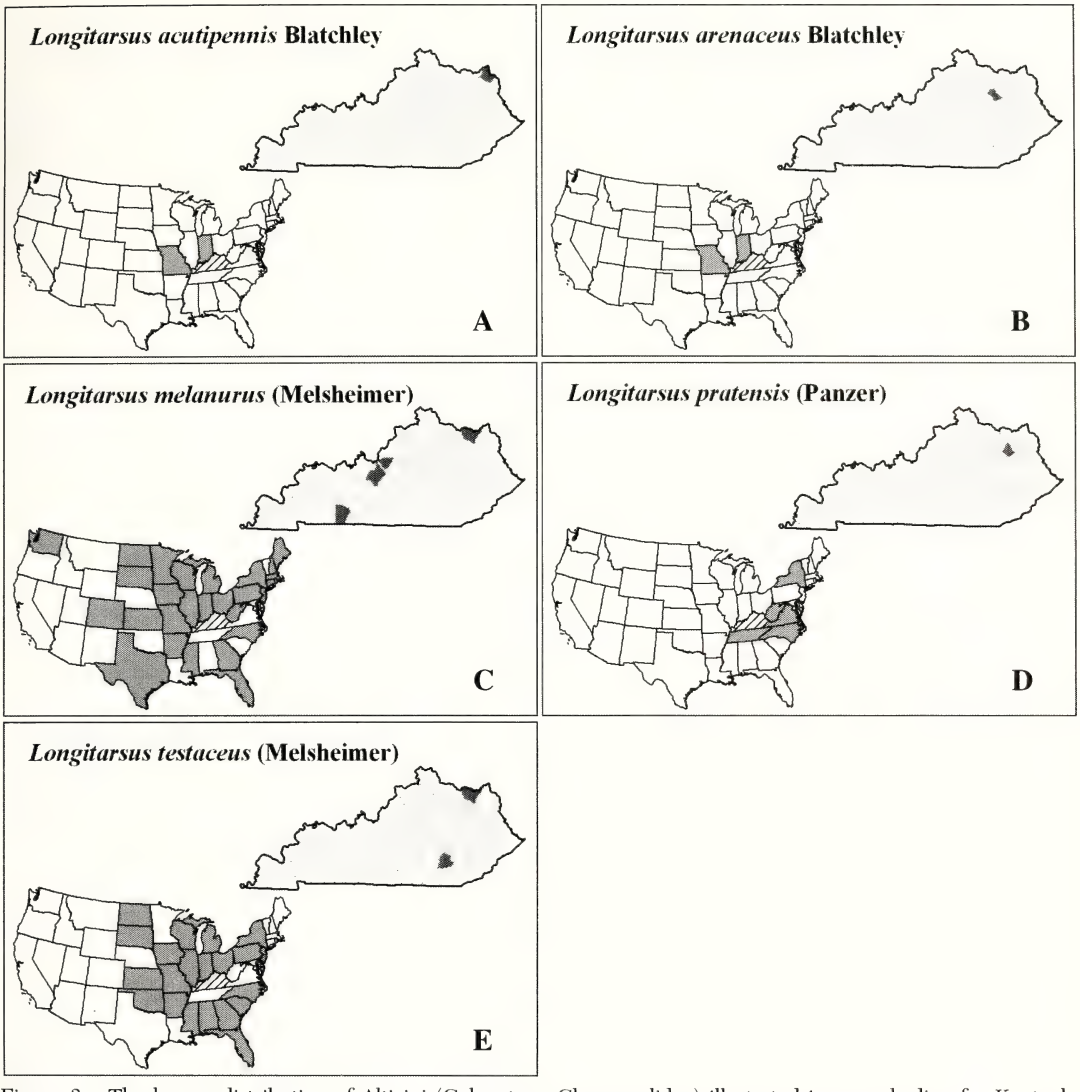


Figure 3. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Years: 1938 (2), 1969 (1), 1971 (3), 1972 (13), 1983 (7), 2006 (4), 2007 (1), 2008 (11)

Months: May (8), June (34)

Abundance: 42 specimens: 16-KYSU, 7-RJBC, 19-UKIC

Comments: The common name is smartweed flea beetle. As with the other *Systena* spp., Clark et al. (2004) reported a long list of plant associations.

Altica chalybea Illiger (Figure 4E) (new state record)

Kentucky Counties: Allen, Breathitt, Carter, Fayette, Franklin, Grant, Henderson, Jessamine, Menifee, Nelson, Perry, Trigg

Years: 1891 (2), 1892 (1), 1893 (1), 1894 (8), 1895 (3), 1896 (1), 1920 (13), 1940 (1), 1970 (2), 1971 (7), 1972 (2), 1973 (1), 1974 (2), 1975 (21), 1990 (1), 1993 (4), 1994 (2), 2008 (1)

Months: April (17), May (42), June (6), July (7)

Abundance: 73 specimens: 3-BYUC, 1-KYSU, 69-UKIC

Comments: LeSage (2002) reported specimens from Frankfort (Franklin County),

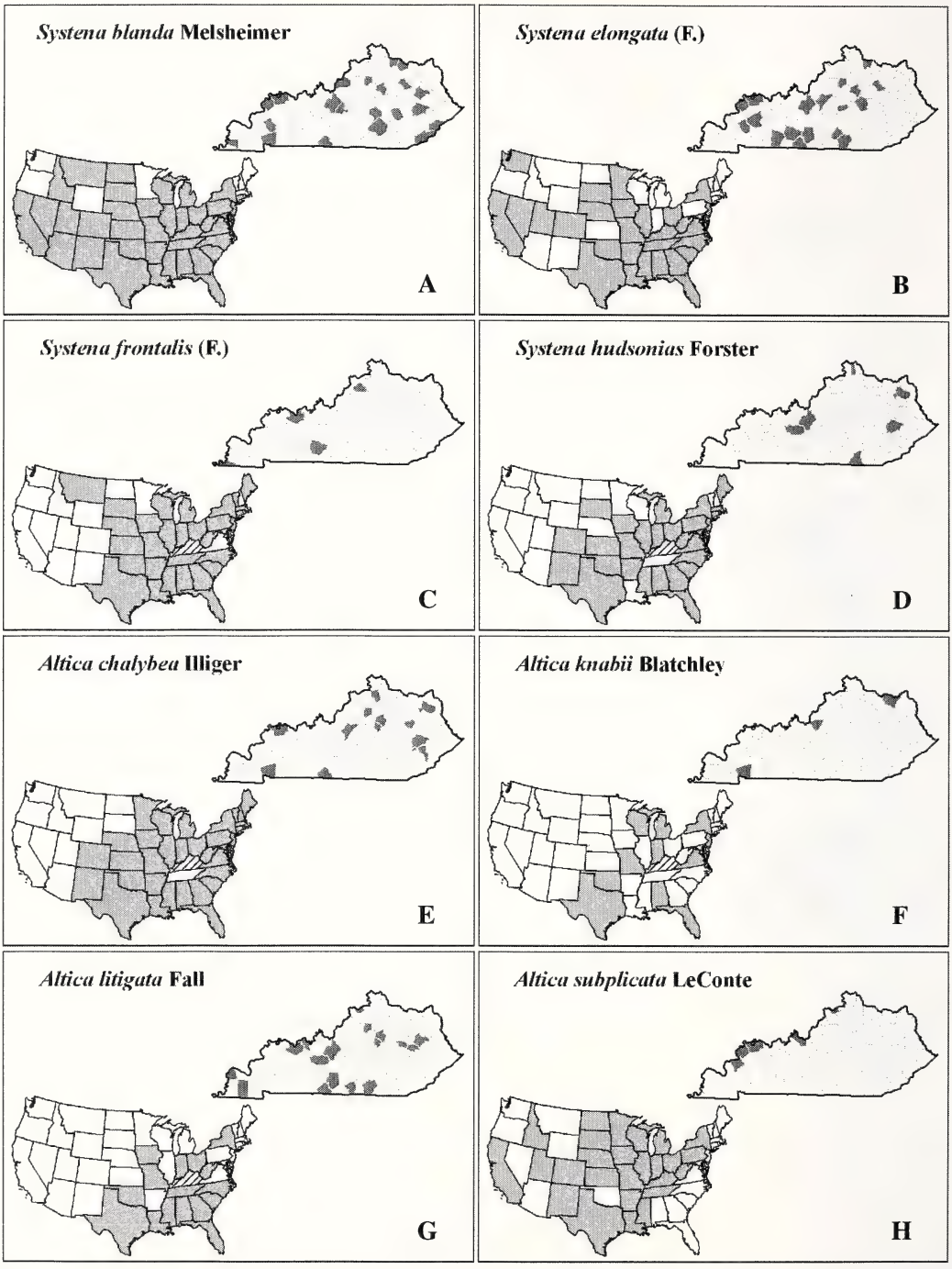


Figure 4. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Henderson (Henderson County), and Golden Pond (Trigg County). Association of this species with *Vitis* is well documented (Clark et al. 2004, LeSage 2002; LeSage and Zmudzinska 2004). One label noted material collected via Malaise trap.

Altica knabii Blatchley (Figure 4F) (new state record)

Kentucky Counties: Bullitt, Lewis, Trigg
Years: 2005 (2), 2007 (1), 2008 (3)
Months: June (5), July (1)
Abundance: 6 specimens: 6-KYSU
Comments: This species is associated with *Oenothera biennis* L. (Onagraceae) (Clark et al. 2004).

Altica litigata Fall (Figure 4G) (new state record)

Kentucky Counties: Allen, Ballard, Barren, Carroll, Cumberland, Daviess, Fayette, Franklin, Graves, Grayson, Hancock, Hardin, Jessamine, Morgan, Powell, Wayne, Wolfe
Years: 1890 (2), 1891 (1), 1920 (2), 1924 (7), 1958 (11), 1968 (1), 1969 (7), 1970 (1), 1971 (8), 1972 (1), 1974 (1), 1975 (2), 1983 (1), 1991 (1), 1995 (2), 1999 (1), 2004 (6), 2008 (2)
Months: April (7), May (19), June (25), July (3), August (1), September (2)
Abundance: 57 specimens: 7-CWC, 5-KYSU, 1-RJBC, 44-UKIC
Comments: This species is associated with Onagraceae (Clark et al. 2004). Labels reported collection via blacklight and Maliase trap.

Altica subplicata LeConte (Figure 4H)

Kentucky Counties: Carroll, Crittenden, Henderson, Meade, Union
Years: 2002 (3), 2006 (4)
Months: April (4), September (3)
Abundance: 7 specimens: 7-CWC
Comments: LeSage (1995) reported 40 specimens in the Cornell University Insect Collection from Carroll County, and one specimen in the University of Michigan Museum of Zoology from Henderson County. This species is associated with *Salix* (Salicaceae) (Clark et al. 2004).

Orthaltica copalina (F.) (Figure 5A) (new state record)

Kentucky County: Christian
Years: 2003 (2), 2008 (1)
Months: May (2), June (1)
Abundance: 3 specimens: 2-CWC, 1-KYSU
Comments: This species is associated with Anacardiaceae (Clark et al. 2004).

Orthaltica melina Horn (Figure 5B) (new state record)

Kentucky Counties: Campbell, Greenup, Hardin, Laurel, Logan, Union
Years: 1899 (8), 1970 (1), 1971 (1), 2003 (1), 2005 (1), 2006 (8), 2008 (4)
Months: May (2), June (21), July (1)
Abundance: 25 specimens: 1-CMC, 2-CWC, 12-KYSU, 10-UKIC
Comments: This species is associated with Anacardiaceae (Clark et al. 2004).

Crepidodera browni Parry (Figure 5C)

Kentucky Counties: Bracken, Breathitt, Breckinridge, Calloway, Carroll, Clay, Crittenden, Fayette, Fleming, Franklin, Fulton, Greenup, Hancock, Hardin, Henderson, Henry, Jefferson, Johnson, LaRue, Laurel, Lewis, Martin, Meade, Menifee, Muhlenberg, Nelson, Nicholas, Owen, Rockcastle, Rowan, Scott, Simpson, Spencer, Trigg, Warren, Webster
Years: 1889 (2), 1891 (9), 1893 (1), 1894 (5), 1895 (1), 1917 (7), 1969 (11), 1970 (43), 1971 (24), 1972 (74), 1974 (21), 1975 (2), 1981 (6), 1983 (3), 1990 (1), 1993 (3), 1994 (5), 1995 (1), 1998 (6), 2003 (4), 2005 (35), 2006 (26), 2007 (12), 2008 (16)
Months: April (35), May (54), June (134), July (62), August (30), September (2), October (1)
Abundance: 318 specimens: 13-BYUC, 8-CWC, 86-KYSU, 9-RJBC, 201-UKIC, 1-WKUC
Comments: The senior author repeatedly collected this species on *Salix* sp., often with *C. longula*. Parry (1986) reported this species from Louisville (Jefferson County) and Henderson (Henderson County).

Crepidodera longula Horn (Figure 5D)

Kentucky Counties: Breckinridge, Daviess, Fayette, Franklin, Hardin, Henderson, Henry, Jessamine, Lewis, Simpson, Spencer, Union, Webster
Years: 1971 (1), 1972 (94), 1973 (1), 1975 (1), 1992 (1), 2005 (23), 2006 (19), 2007 (9), 2008 (8)

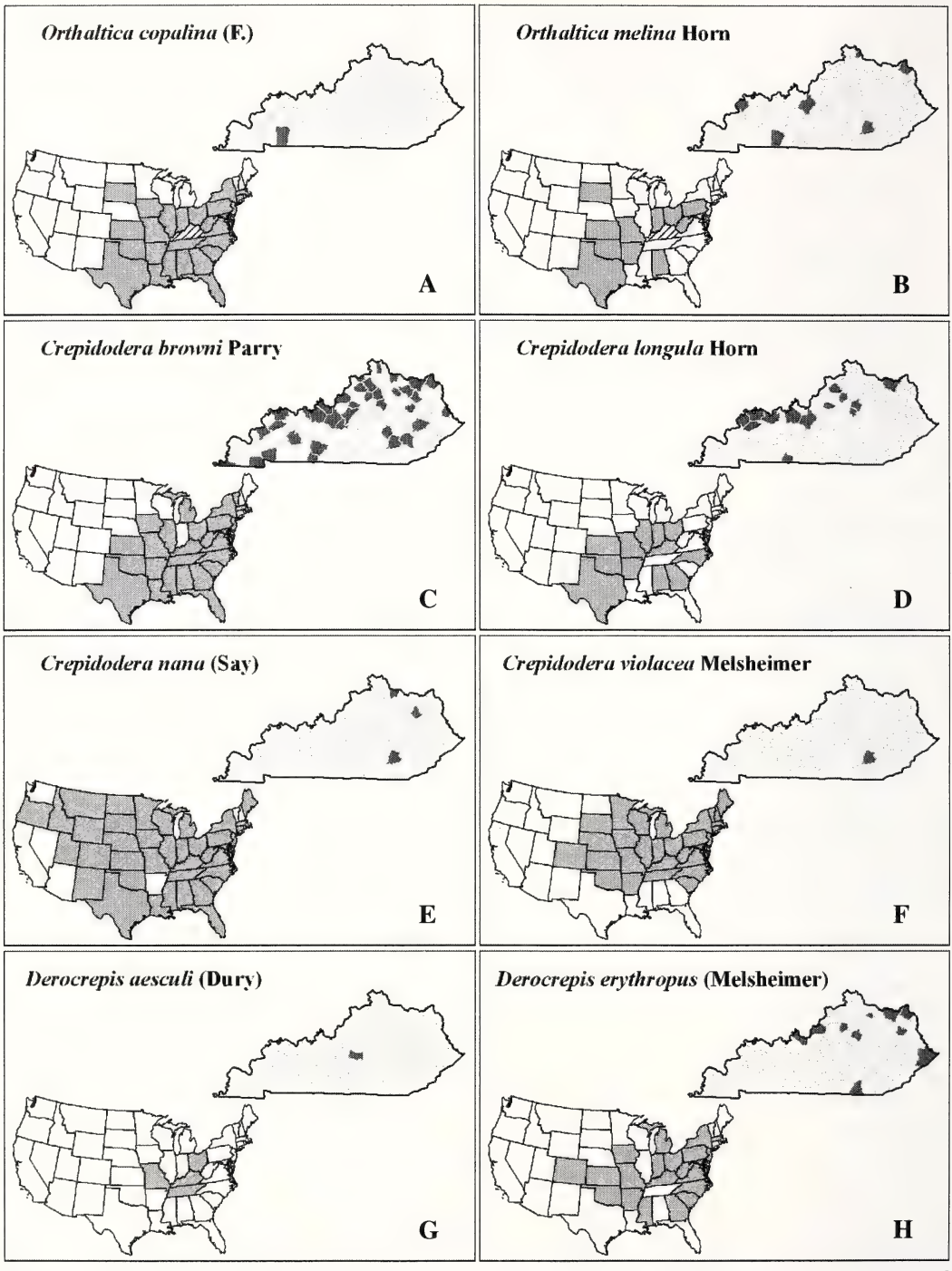


Figure 5. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Months: April (15), May (30), June (85), July (27)

Abundance: 157 specimens: 4-BYUC, 2-CWC, 54-KYSU, 97-UKIC

Comments: The senior author repeatedly collected this species on *Salix* sp., often with *C. browni*. Labels listed collections from *Juniperus* and *Populus*, but at least the occurrence on the first of these plants was probably incidental.

Crepidodera nana (Say) (Figure 5E)

Kentucky Counties: Bracken, Laurel, Rowan

Years: 1994 (1), 1995 (1), 1998 (1)

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

Abundance: 3 specimens: 3-BYUC

Comments: Parry (1986) reported this species from Morehead (Rowan County). This species is reported from *Salix* sp. (Clark et al. 2004).

Crepidodera violacea Melsheimer (Figure 5F)

Kentucky County: Laurel

Year: 1995 (5)

Month: April (5)

Abundance: 5 specimens: 5-BYUC

Comments: This species is reported from Rosaceae (Clark et al. 2004).

Derocrepis aesculi (Dury) (Figure 5G)

Kentucky County: Marion

Year: 1982 (1)

Month: May (1)

Abundance: 1 specimen: 1-BYUC

Comments: This species is associated with *Aesculus glabra* Willd. (Hippocastanaceae) (Clark et al. 2004).

Derocrepis erythropus (Melsheimer) (Figure 5H) (new state record)

Kentucky Counties: Elliott, Fayette, Franklin, Greenup, Jefferson, Lewis, McCreary, Meade, Pike, Robertson

Years: 1892 (11), 1897 (1), 1916 (1), 1917 (6), 1970 (40), 1971 (25), 1975 (12), 1981 (8), 1983 (2), 1990 (4), 1993 (7), 1995 (2), 2006 (5), 2008 (1)

Months: March (6), April (54), May (61), June (4)

Abundance: 125 specimens: 4-BYUC, 3-CWC, 4-KYSU, 11-RJBC, 103-UKIC

Comments: This species is reported from *Robinia* (Clark et al. 2004).

Epitrix brevis Schwarz (Figure 6A)

Kentucky Counties: Carter, Casey, Fayette, Franklin, Hardin, Laurel, Lewis, Logan, Madison, Pike, Russell, Union

Years: 1889 (5), 1890 (5), 1891 (5), 1892 (7), 1893 (1), 1917 (1), 1921 (11), 1928 (5), 1992 (1), 1995 (5), 1998 (1), 2003 (1), 2005 (5), 2006 (1), 2008 (2)

Months: March (1), April (5), May (4), June (9), July (13), August (12), September (11), December (1)

Abundance: 56 specimens: 7-BYUC, 2-CWC, 6-KYSU, 1-RJBC, 40-UKIC

Comments: This species is reported from Solanaceae (Clark et al. 2004).

Epitrix cucumeris (Harris) (Figure 6B)

Kentucky Counties: Fayette, Franklin, Russell

Years: 1921 (6), 2005 (14), 2006 (7)

Months: May (12), June (5), July (4), September (6)

Abundance: 27 specimens: 3-KYSU, 18-RJBC, 6-UKIC

Comments: The common name of this species is the potato flea beetle, and these insects are commonly collected on many Solanaceae (Clark et al. 2004).

Epitrix fuscula Crotch (Figure 6C)

Kentucky Counties: Breathitt, Casey, Clark, Fayette, Graves, Green, Greenup, Hardin, Henry, Laurel, Logan, Martin, Rowan, Russell, Simpson, Union, Webster

Years: 1889 (12), 1890 (8), 1891 (23), 1892 (13), 1893 (1), 1894 (2), 1896 (14), 1906 (2), 1910 (1), 1913 (2), 1915 (11), 1916 (5), 1921 (12), 1924 (5), 1925 (5), 1926 (4), 1927 (34), 1971 (4), 1972 (4), 1990 (2), 1995 (7), 2003 (5), 2005 (8), 2006 (1), 2008 (7)

Months: April (4), May (47), June (22), July (93), August (11), September (2), October (11), December (2)

Abundance: 192 specimens: 9-BYUC, 8-CWC, 13-KYSU, 162-UKIC

Comments: The common name of this species is the eggplant flea beetle, and these beetles are commonly collected on many Solanaceae (Clark et al. 2004).

Epitrix hirtipennis (Melsheimer) (Figure 6D)

Kentucky Counties: Clark, Elliott, Fayette, Jessamine, Madison, Warren

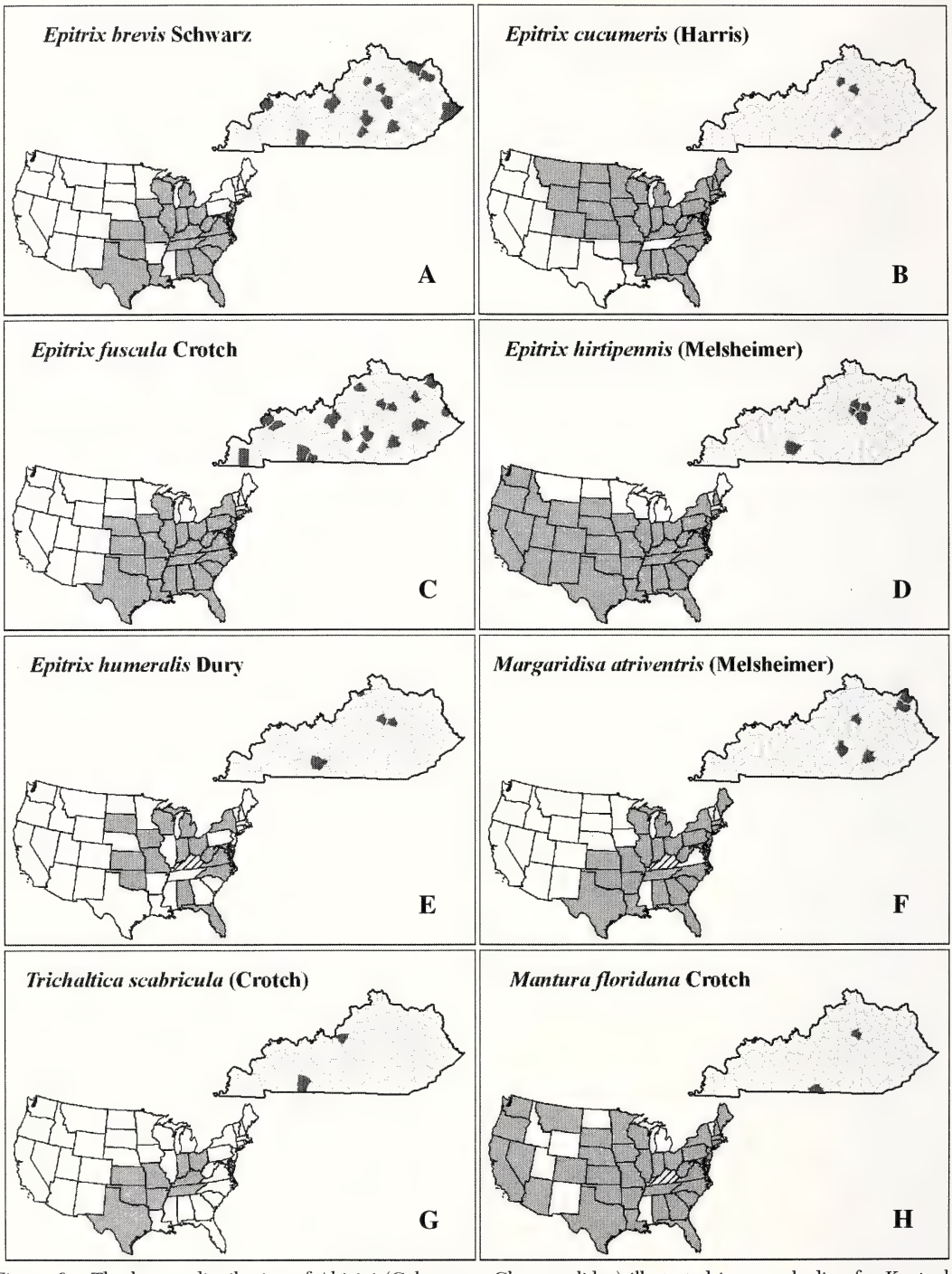


Figure 6. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Years: 1889 (12), 1890 (2), 1891 (9), 1892 (1), 1893 (6), 1894 (6), 1895 (3), 1905 (37), 1906 (2), 1913 (2) 1915 (2), 1917 (1), 1920 (44), 1921 (8), 1922 (48), 1923 (30), 1924 (6), 1970 (1), 1971 (2), 1975 (1), 1990 (1)

Months: April (3), May (3), June (111), July (38), August (7), September (11), October (40), November (10), December (1)

Abundance: 224 specimens: 1-BYUC, 223-UKIC

Comments: The common name of this species is the tobacco flea beetle, and these beetles are commonly collected on many Solanaceae (Clark et al. 2004).

Epitrix humeralis Dury (Figure 6E) (new state record)

Kentucky Counties: Carroll, Clark, Fayette, Warren

Years: 1889 (4), 1890 (2), 1891 (9), 1892 (10), 1893 (2), 1895 (1), 1915 (4), 1916 (2), 1917 (1), 1921 (16), 1922 (1), 1925 (2), 1926 (5), 1927 (1), 1928 (8), 1971 (2)

Months: April (2), May (22), June (3), July (20), August (7), September (15), October (1)

Abundance: 70 specimens: 70-UKIC

Comments: This species is associated with Solanaceae (Clark et al. 2004).

Margaridisa atriventris (Melsheimer) (Figure 6F) (new state record)

Kentucky Counties: Carter, Casey, Fayette, Greenup, Laurel

Years: 1889 (1), 1890 (1), 1891 (9), 1901 (2), 1992 (1), 1995 (2), 2003 (1), 2005 (1), 2007 (1)

Months: April (3), May (2), June (5), August (2), September (2), December (4)

Abundance: 19 specimens: 3-BYUC, 1-CWC, 2-KYSU, 13-UKIC

Comments: This species is associated with *Acalypha* (Euphorbiaceae) (Clark et al. 2004).

Trichaltica scabricula (Crotch) (Figure 6G)

Kentucky Counties: Bullitt, Logan

Years: 2005 (1), 2008 (1)

Months: May (1), June (1)

Abundance: 2 specimens: 2-KYSU

Comments: This species is associated with Oleaceae (Clark et al. 2004).

Mantura floridana Crotch (Figure 6H) (new state record)

Kentucky Counties: Fayette, Monroe

Years: 1892 (1), 1894 (1), 2004 (2)

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

Abundance: 4 specimens: 2-CWC, 2-UKIC

Comments: This species is associated with *Rumex* (Polygonaceae) (Clark et al. 2004).

Chaetocnema confinis Crotch (Figure 7A)

Kentucky Counties: Carter, Casey, Clark, Daviess, Fayette, Franklin, Henry, Jackson, Laurel, Lewis, Monroe, Owen, Russell, Simpson, Union, Webster

Years: 1891 (1), 1920 (3), 1922 (2), 1923 (1), 1972 (1), 1990 (3), 1993 (4), 1994 (7), 1995 (1), 1998 (4), 2003 (6), 2004 (4), 2005 (16), 2006 (1), 2007 (3)

Months: April (2), May (27), June (6), July (15), August (4), October (3)

Abundance: 57 specimens: 19-BYUC, 13-CWC, 16-KYSU, 1-RJBC, 8-UKIC

Comments: The common name of this species is the sweet potato flea beetle. White (1996) reported that adults damage the leaves of sweet potato and the larvae feed on the roots of bindweed and sweet potato. Labels report collection on the following plants: *Convolvulus* sp.; willow, *Salix* sp.; okra, *Abelmoschus esculentus*; sweet potato, *Ipomoea batatas*; and *Juniperus*. However, of these plants, only *Convolvulus* and *Ipomoea* are probably true hosts.

Chaetocnema crenulata Crotch (Figure 7B)

Kentucky County: Pulaski

Year: unknown

Month: unknown

Abundance: unknown

Comments: No specimens were observed but White (1996) reported this species from Burnside (Pulaski County).

Chaetocnema denticulata (Illiger) (Figure 7C)

Kentucky Counties: Breathitt, Caldwell, Carter, Casey, Clark, Clay, Fayette, Franklin, Graves, Hardin, Henderson, LaRue, Logan, Madison, Mercer, Robertson

Years: 1889 (4), 1890 (3), 1891 (2), 1895 (2), 1901 (1), 1907 (2), 1915 (1), 1916 (4), 1920 (1), 1928 (2), 1937 (1), 1969 (5), 1971 (9), 1972 (1), 1974 (3), 1994 (1), 1998 (1), 2004 (1), 2005 (3), 2006 (7), 2008 (3)

Months: February (1), March (2), April (2), May (15), June (11), July (17), August (4), September (3), October (1), November (2)

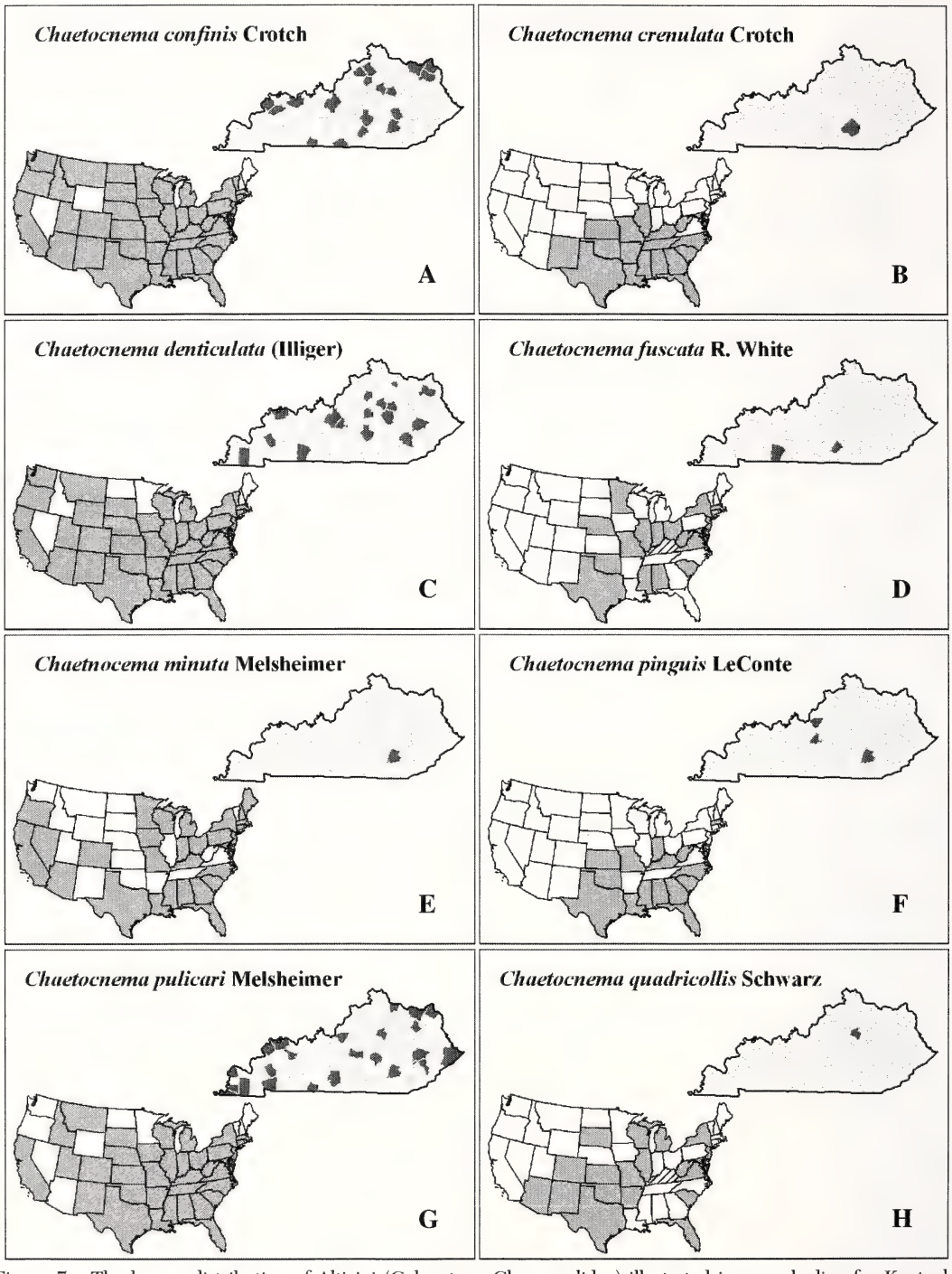


Figure 7. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Abundance: 57 specimens: 1-BYUC, 1-CMC, 1-CWC, 12-KYSU, 42-UKIC

Comments: This species is normally associated with Poaceae (Clark et al. 2004).

Chaetocnema fuscata R. White (Figure 7D) (new state record)

Kentucky Counties: Logan, Russell
Years: 2005 (2), 2006 (1)
Months: May (1), June (1), August (1)
Abundance: 3 specimens: 3-KYSU

Comments: White (1996) reported this species from *Lespedeza sericea* Benth. (Fabaceae) and *Andropogon gerardii* Vitman (Poaceae).

Chaetocnema minuta Melsheimer (Figure 7E)

Kentucky County: Laurel
Year: 1995 (1)
Month: April (1)
Abundance: 1 specimen: 1-BYUC

Chaetocnema pinguis LeConte (Figure 7F)

Kentucky Counties: Bullitt, LaRue, Laurel
Years: 1995 (1), 2005 (1), 2007 (1)
Months: April (1), June (1), July (1)
Abundance: 3 specimens: 1-BYUC, 2-KYSU

Comments: White (1996) reported material labeled from *Erigeron ramosus* Raf. (Asteraceae).

Chaetocnema pulicaria Melsheimer (Figure 7G)

Kentucky Counties: Ballard, Barren, Bracken, Breathitt, Bullitt, Caldwell, Fayette, Fulton, Graves, Greenup, Henderson, Hickman, Laurel, Lewis, Lincoln, Marion, McLean, Perry, Pike, Rowan, Simpson, Trigg, Union
Years: 1889 (148), 1890 (31), 1891 (124), 1892 (274), 1893 (6), 1894 (36), 1895 (2), 1896 (1), 1900 (4), 1906 (5), 1913 (13), 1915 (10), 1916 (9), 1919 (1), 1920 (2), 1921 (5), 1924 (4), 1925 (13), 1928 (4), 1968 (3), 1969 (1), 1970 (1), 1971 (3), 1972 (10), 1974 (1), 1982 (1), 1990 (1), 1994 (6), 1995 (3), 1998 (6), 2003 (1), 2006 (1), 2007 (3), 2008 (1)
Months: April (41), May (45), June (258), July (217), August (79), September (63), October (21), December (10)
Abundance: 734 specimens: 11-BYUC, 7-CWC, 5-KYSU, 711-UKIC

Comments: This species is a pest of corn, *Zea mays* L. and has been associated with other Poaceae.

Chaetocnema quadricollis Schwarz (Figure 7H) (new state record)

Kentucky County: Fayette
Year: 1891 (1)
Month: August (1)
Abundance: 1 specimen: 1-UKIC

Comments: This species is associated with Malvaceae (Clark et al. 2004).

Disonycha admirabila Blatchley (Figure 8A) (new state record)

Kentucky Counties: Grayson, Hardin, LaRue, Lewis, Lincoln, Logan, Robertson
Years: 1983 (1), 1985 (1), 2004 (10), 2005 (20), 2006 (17), 2007 (21), 2008 (32)
Months: May (14), June (36), July (50), August (2)
Abundance: 102 specimens: 95-KYSU, 7-RJBC

Disonycha alternata (Illiger) (Figure 8B) (new state record)

Kentucky County: probably northern Kentucky near Cincinnati
Year: ca. 1900
Month: unknown
Abundance: 1 specimen: 1-CMC

Comments: This Dury collection specimen was labeled 'Ky.'

Disonycha arizonae Casey (Figure 8C) (new state record)

Kentucky County: Franklin
Year: 2006 (1)
Month: June (1)
Abundance: 1 specimen: 1-RJBC

Disonycha caroliniana (F.) (Figure 8D) (new state record)

Kentucky Counties: Barren, LaRue, McCracken
Years: 1968 (1), 1972 (1), 2005 (1)
Months: April (1), July (2)
Abundance: 3 specimens: 1-KYSU, 2-UKIC

Disonycha collata collata (F.) (Figure 8E)

Kentucky Counties: Fayette, Franklin
Years: 1890 (3), 1892 (1), 1894 (1), 1913 (1)

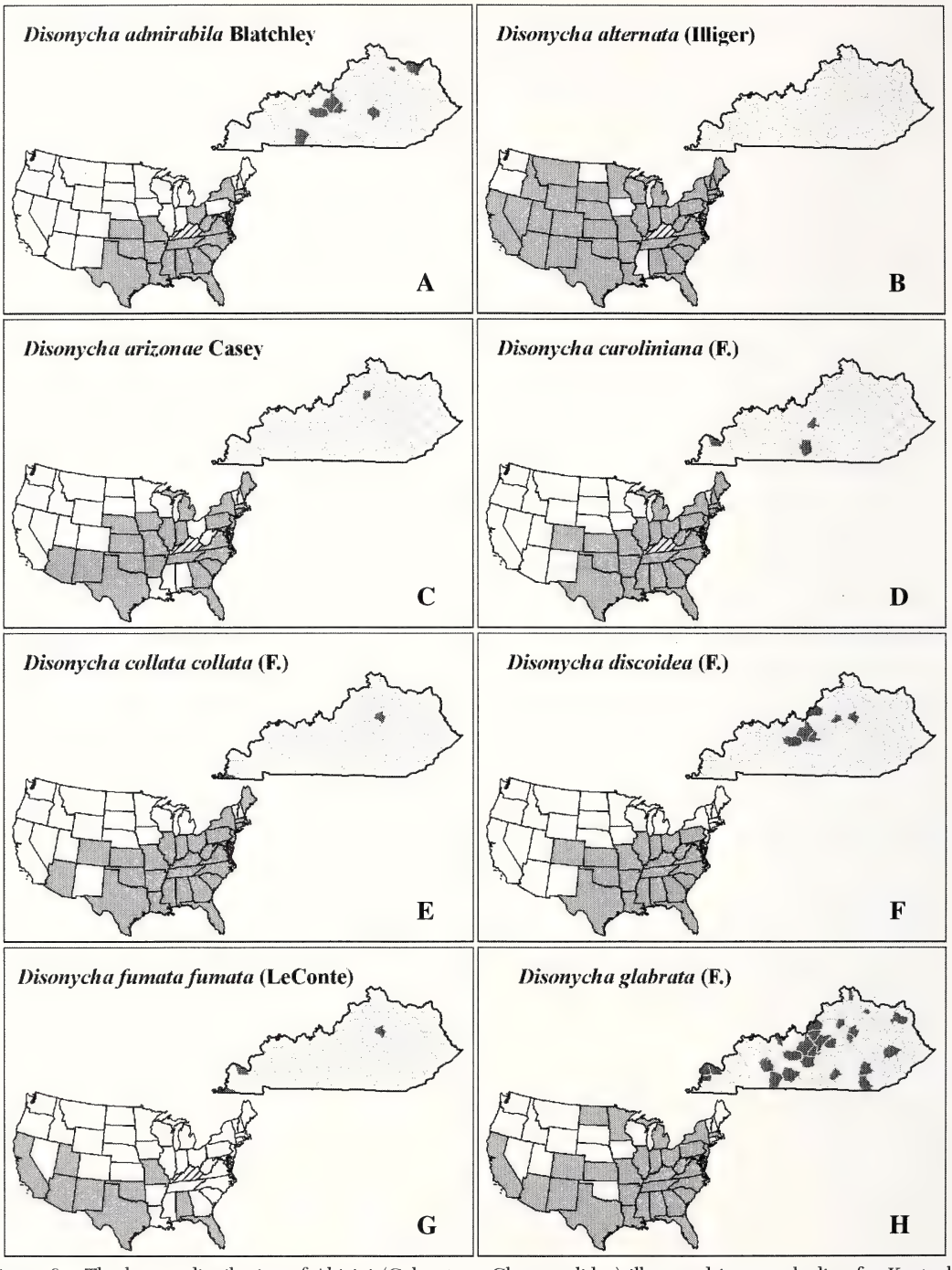


Figure 8. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Months: July (2), October (1), November (3)

Abundance: 6 specimens: 6-UKIC

Comments: Clark et al. (2004) reported Amaranthaceae, Caryophyllaceae, Chenopodiaceae and Portulacaceae as the likely true hosts.

Disonycha discoidea (F.) (Figure 8F)

Kentucky Counties: Anderson, Fayette, Grayson, Hardin, Jefferson, LaRue

Years: 1892 (1), 1949 (1), 1983 (1), 2005 (1), 2006 (1), 2008 (3)

Months: April (2), May (3), June (3)

Abundance: 8 specimens: 5-KYSU, 1-RJBC, 2-UKIC

Comments: Blake (1933) reported this species from Louisville (Jefferson County) and "near Cincinnati, Ohio." Recently collected specimens were caught in prairie remnants within nature preserves.

Disonycha fumata fumata (LeConte) (Figure 8G) (new state record)

Kentucky Counties: Fayette, Fulton, McCracken

Years: 1889 (2), 1891 (1), 1892 (1), 1894 (2), 1911 (1), 1916 (1), 1992 (1), 2000 (1)

Months: June (1), July (2), August (3), September (3), November (1)

Abundance: 10 specimens: 1-CMC, 2-CWC, 7-UKIC

Comments: This species is associated with Asteraceae (Clark et al. 2004).

Disonycha glabrata (F.) (Figure 8H)

Kentucky Counties: Ballard, Breathitt, Bullitt, Carlisle, Carter, Fayette, Franklin, Grayson, Hancock, Hardin, Hart, Jefferson, Jessamine, Kenton, LaRue, Laurel, Logan, McCracken, Muhlenberg, Nelson, Robertson, Russell, Warren, Washington, Whitley

Years: 1889 (16), 1890 (8), 1891 (5), 1892 (64), 1893 (2), 1894 (1), 1896 (5), 1913 (2), 1915 (7), 1932 (1), 1937 (1), 1938 (3), 1939 (1), 1941 (5), 1943 (1), 1944 (1), 1963 (1), 1964 (1), 1969 (1), 1970 (1), 1971 (4), 1972 (1), 1979 (1), 1984 (9), 1992 (1), 1993 (1), 1997 (1), 1999 (2), 2003 (1), 2004 (2), 2005 (31), 2006 (21), 2007 (11), 2008 (16)

Months: May (35), June (41), July (106), August (29), September (16), November (3)

Abundance: 230 specimens: 1-BYUC, 1-CMC, 13-CWC, 67-KYSU, 14-RJBC, 130-UKIC, 4-WKUC

Comments: Blake (1933) reported this species in material examined from Wickliffe, Kentucky (Ballard Co.). This species is common on redroot pigweed, *Amaranthus retroflexus*, an agricultural weed, and other Amaranthaceae.

Disonycha leptolineata Blatchley (Figure 9A) (new state record)

Kentucky Counties: Franklin, Powell, Whitley

Years: 1984 (1), 1988 (1), 1997 (1)

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

Abundance: 5 specimens: 1-BYUC, 2-CMC, 1-CWC, 1-RJBC

Comments: This species is associated with *Itea virginica* L. (Grossulariaceae) (Clark et al. 2004).

Disonycha triangularis (Say) (Figure 9B)

Kentucky County: Fayette

Year: 1915 (1)

Month: August (1)

Abundance: 1 specimen: 1-UKIC

Comments: Blake (1933) reported this species in material examined from "Kentucky." This species is associated with Chenopodiaceae (Clark et al. 2004).

Disonycha uniguttata (Say) (Figure 9C)

Kentucky County: unknown

Year: unknown

Month: unknown

Abundance: unknown

Comments: Blake (1933) listed "Kentucky" in her revision of this species, but no specimens have been found.

Disonycha xanthomelas (Dalman) (Figure 9D)

Kentucky Counties: Fayette, Franklin, Knox, LaRue, Mason, Warren

Years: 1889 (1), 1890 (2), 1892 (2), 1893 (1), 1913 (1), 1945 (1), 1955 (1), 1963 (1), 1970 (2), 1971 (1), 1984 (2), 1999 (1), 2002 (2), 2006 (2)

Months: May (3), June (3), July (7), August (2), September (3), October (2)

Abundance: 20 specimens: 3-CWC, 1-KYSU, 3-RJBC, 12-UKIC, 1-WKUC

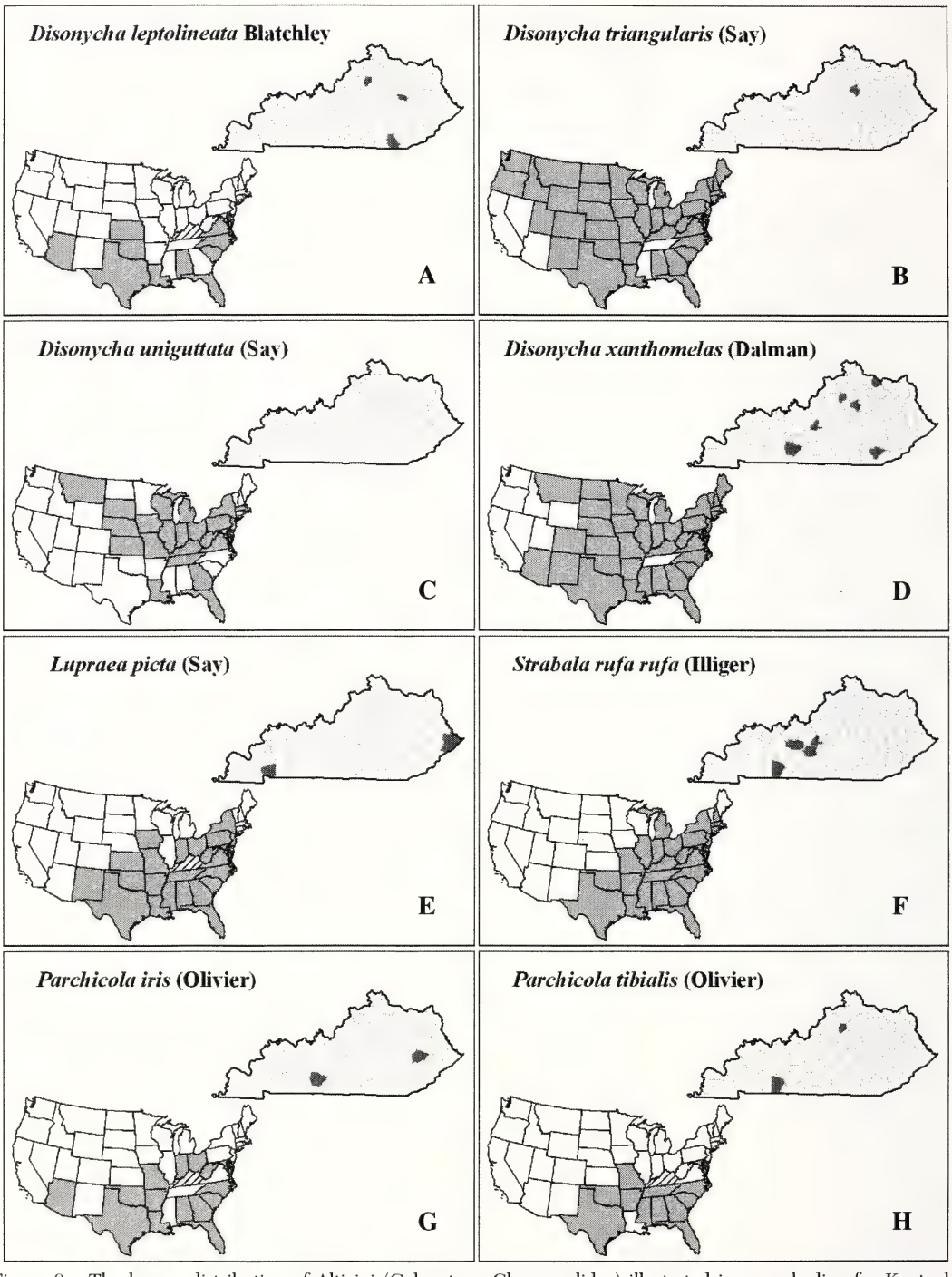


Figure 9. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

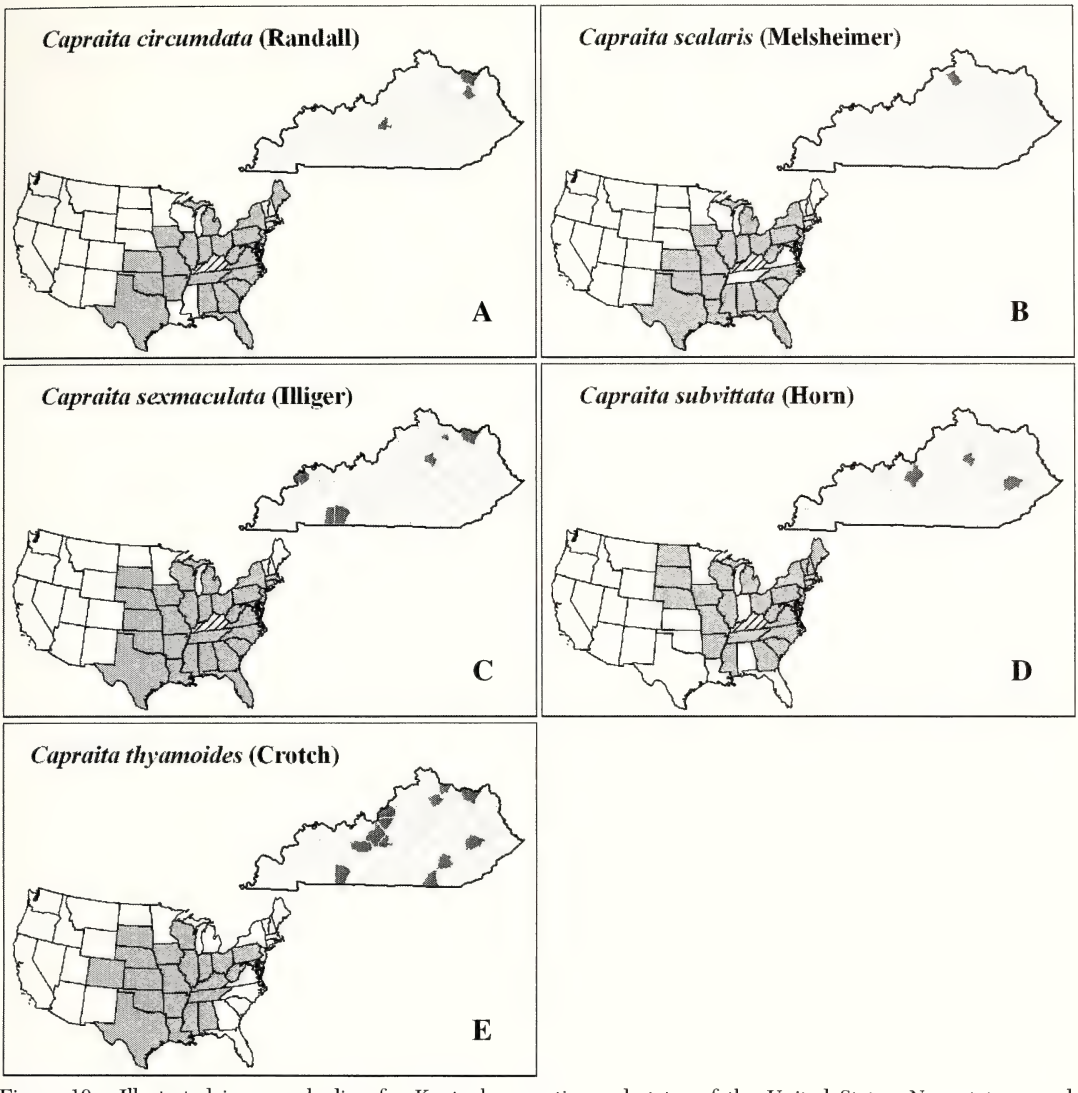


Figure 10. Illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Comments: The common name of this species is the spinach flea beetle.

Lupraea picta (Say) (Figure 9E) (new state record)

Kentucky Counties: Pike, Trigg
Years: 1970 (2), 2003 (1)
Month: June (3)
Abundance: 5 specimens: 2-CMC, 1-CWC, 2-UKIC
Comments: The Dury (CMC) specimens are labeled "Ky. near Cin. O."

Strabala rufa rufa (Illiger) (Figure 9F)

Kentucky Counties: Grayson, Hart, LaRue, Livingston
Years: 1983 (2), 2004 (1), 2005 (4), 2007 (1)
Months: April (1), May (2), June (2), July (3)
Abundance: 8 specimens: 2-CWC, 4-KYSU, 2-RJBC
Comments: This species feeds on *Diodia* (Rubiaceae) (Riley et al. 2002).

Parchicola iris (Olivier) (Figure 9G) (new state record)

Kentucky Counties: Breathitt, Warren
Years: 1972 (1), 2006 (1)

Months: July (1), September (1)
Abundance: 2 specimens: 1-RJBC, 1-UKIC
Comments: This species is associated with Passifloraceae (Clark et al. 2004).

Parchicola tibialis (Olivier) (Figure 9H) (new state record)

Kentucky Counties: Franklin, Logan
Years: 1998 (1), 1999 (1), 2006 (1)
Months: June (2), July (1)
Abundance: 3 specimens: 2-CWC, 1-KYSU
Comments: This species is associated with Passifloraceae (Clark et al. 2004).

Capraita circumdata (Randall) (Figure 10A) (new state record)

Kentucky Counties: LaRue, Lewis, Rowan
Years: 1990 (4), 2005 (4), 2006 (2), 2008 (3)
Months: April (1), May (10), June (2)
Abundance: 13 specimens: 4-BYUC, 4-CWC, 5-KYSU
Comments: Clark et al. (2004) reported a wide range of plant associations for this species.

Capraita scalaris (Melsheimer) (Figure 10B) (new state record)

Kentucky County: Owen
Year: 2003 (1)
Month: May (1)
Abundance: 1 specimen: 1-CWC
Comments: This species is associated with Ericaceae (Clark et al. 2004).

Capraita sexmaculata (Illiger) (Figure 10C) (new state record)

Kentucky Counties: Fayette, Lewis, Logan, Robertson, Todd, Union
Years: 1894 (1), 2005 (2), 2006 (3), 2007 (1), 2008 (2)
Months: May (2), June (5), July (1)
Abundance: 14 specimens: 2-CMC, 8-KYSU, 1-UKIC
Comments: This species is associated with Oleaceae (Clark et al. 2004).

Capraita subvittata (Horn) (Figure 10D) (new state record)

Kentucky Counties: Breathitt, Fayette, Hardin
Years: 1972 (2), 1975 (11), 2005 (1), 2008 (1)
Months: April (1), May (9), June (3), September (1), October (1)
Abundance: 15 specimens: 2-KYSU, 13-UKIC
Comments: This species is associated with *Eurybia divaricata* (L.) Nesom (Asteraceae) (Clark et al. 2004). A label listed a collection via Malaise trap.

Capraita thyamoides (Crotch) (Figure 10E)

Kentucky Counties: Bracken, Breathitt, Bullitt, Grayson, Hardin, Harrison, Jefferson, LaRue, Laurel, Lewis, Logan, McCreary
Years: 1972 (2), 1981 (1), 1983 (4), 1998 (1), 2005 (12), 2006 (7), 2007 (9), 2008 (16)
Months: April (2), May (23), June (13), July (14)
Abundance: 52 specimens: 1-BYUC, 44-KYSU, 5-RJBC, 2-UKIC
Comments: Clark et al. (2004) reported a wide range of plant associations for this species. A label listed a collection via Malaise trap.

Kuschelina fimbriata (Forster) (Figure 11A) (new state record)

Kentucky County: Warren
Year: 1972 (1)
Month: June (1)
Abundance: 1 specimen: 1-UKIC
Comments: A label listed a collection via Malaise trap.

Kuschelina gibbitarsa (Say) (Figure 11B) (new state record)

Kentucky Counties: Fayette, Grayson, Jefferson, Knox, Mason, Pike
Years: 1892 (1), 1895 (1), 1924 (1), 1976 (1), 1999 (1), 2003 (2), 2004 (1)
Months: March (2), May (1), June (3), July (2)
Abundance: 10 specimens: 2-CMC, 3-CWC, 1-KYSU, 1-RJBC, 3-UKIC
Comments: This species is associated with Lamiaceae (Clark et al. 2004).

Kuschelina miniata (F.) (Figure 11C) (new state record)

Kentucky County: Graves
Year: 1971 (1)
Month: July (1)
Abundance: 1 specimen: 1-UKIC

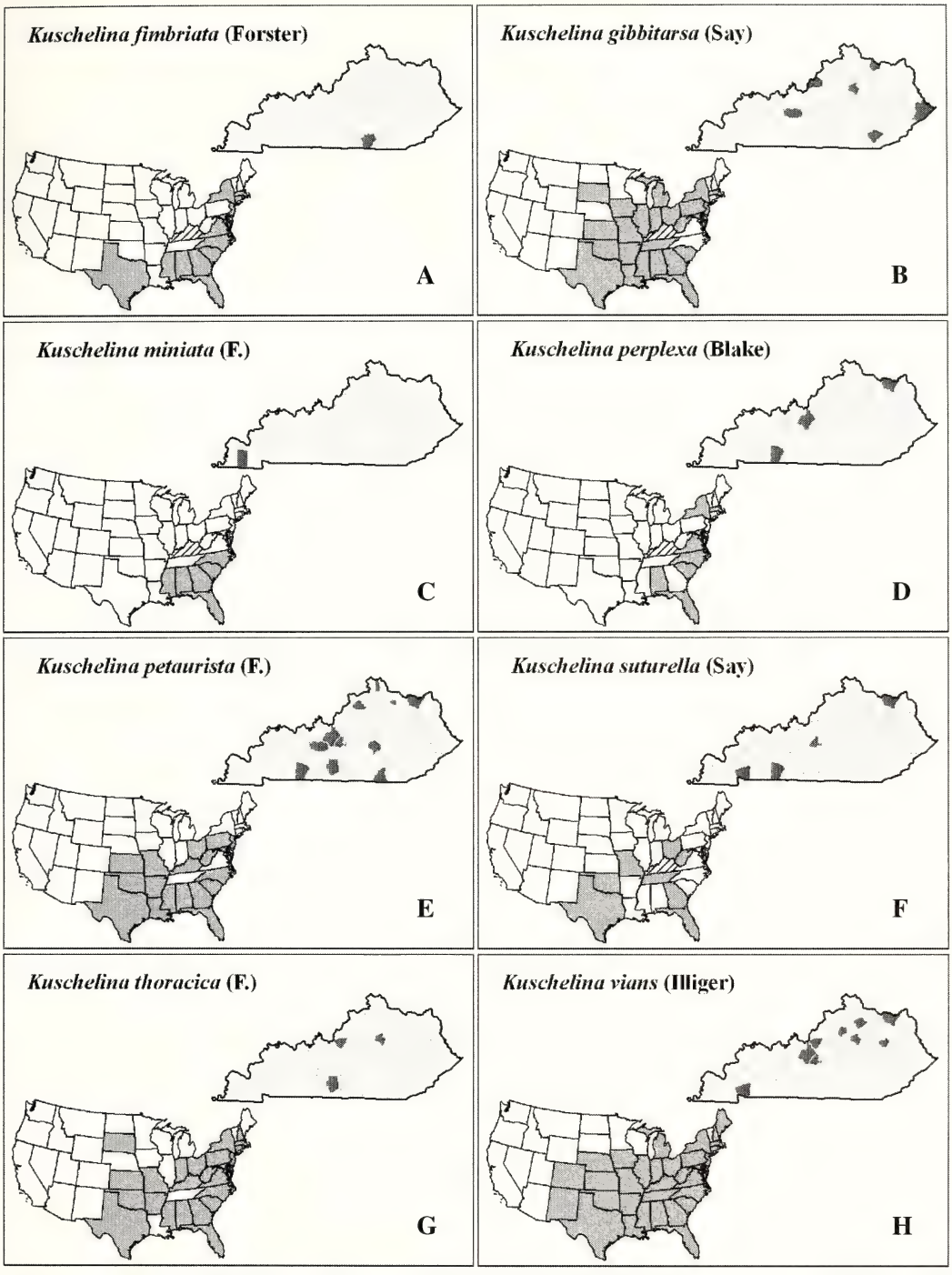


Figure 11. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Kuschelina perplexa (Blake) (Figure 11D) (new state record)

Kentucky Counties: Hardin, Lewis, Logan
Years: 2004 (1), 2005 (1), 2006 (4), 2008 (2)
Months: May (4), June (2), July (2)
Abundance: 8 specimens: 8-KYSU
Comments: All specimens were collected by the senior author in nature preserves.

Kuschelina petaurista (F.) (Figure 11E)

Kentucky Counties: Barren, Grayson, Hardin, Henry, Kenton, LaRue, Lewis, Lincoln, Logan, McCreary, Robertson
Years: 1938 (1), 1971 (1), 1983 (4), 1992 (1), 2005 (8), 2006 (13), 2007 (2), 2008 (6)
Months: May (17), June (17), July (2)
Abundance: 37 specimens: 1-CMC, 1-CWC, 29-KYSU, 4-RJBC, 2-UKIC

Kuschelina suturella (Say) (Figure 11F) (new state record)

Kentucky Counties: LaRue, Lewis, Logan, Trigg
Years: 2005 (2), 2006 (1), 2008 (1)
Months: June (2), July (1), August (1)
Abundance: 4 specimens: 4-KYSU
Comments: All specimens were collected by the senior author in nature preserves.

Kuschelina thoracica (F.) (Figure 11G)

Kentucky Counties: Barren, Bullitt, Fayette
Years: 1889 (2), 1892 (2), 1938 (2), 2008 (1)
Months: April (1), May (1), June (1), July (2)
Abundance: 9 specimens: 2-CMC, 1-KYSU, 6-UKIC
Comments: Several old specimen labels did not specify a month. This species is associated with Lamiaceae (Clark et al. 2004).

Kuschelina vians (Illiger) (Figure 11F)

Kentucky Counties: Bullitt, Fayette, Franklin, Hardin, Harrison, LaRue, Lewis, Menifee, Trigg
Years: 2001 (1), 2004 (3), 2005 (3), 2006 (2), 2007 (2), 2008 (1)
Months: April (2), May (2), June (5), July (2), October (1)
Abundance: 15 specimens: 3-CWC, 9-KYSU, 3-UKIC
Comments: This species is associated with Polygonaceae (Clark et al. 2004).

Distigmoptera apicalis Blake (Figure 12A)

Kentucky County: Fayette
Year: 1920 (2)
Month: June (2)
Abundance: 2 specimens: 2-UKIC

Pachyonychus paradoxus Melsheimer (Figure 12B)

Kentucky Counties: Hardin, Rowan, Whitley
Years: 1983 (1), 1994 (1), 2004 (1)
Months: May (2), June (1)
Abundance: 4 specimens: 1-BYUC, 1-CMC, 1-KYSU, 1-RJBC
Comments: This species is associated with Smilacaceae (Clark et al. 2004).

Dibolia borealis Chevrolat (Figure 12C)

Kentucky Counties: Boone, Bullitt, Fayette, Franklin, Greenup, Jackson, Lewis, Logan, McCreary, Robertson
Years: 1891 (1), 1924 (1), 1970 (1), 1974 (2), 1991 (1), 2001 (3), 2003 (2), 2005 (5), 2006 (8), 2008 (3)
Months: March (1), April (5), May (4), June (15), July (2)
Abundance: 28 specimens: 1-BYUC, 1-CMC, 5-CWC, 6-KYSU, 10-RJBC, 5-UKIC
Comments: We hand picked specimens of this species from buckhorn plantain, *Plantago lanceolata* L. (Plantaginaceae).

Dibolia sinuata Horn (Figure 12D) (new state record)

Kentucky County: Lewis
Year: 2007 (6)
Month: May (6)
Abundance: 6 specimens: 6-KYSU
Comments: All specimens were collected at Crooked Creek Barrens State Nature Preserve. This species is associated with Scrophulariaceae (Clark et al. 2004).

Pseudodibolia opima (LeConte) (Figure 12E) (new state record)

Kentucky Counties: Henry, LaRue, Lewis
Years: 2005 (1), 2008 (2)
Month: May (3)
Abundance: 3 specimens: 3-KYSU
Comments: This species is associated with Acanthaceae (Clark et al. 2004).

Psylliodes punctulatus Melsheimer (Figure 12F) (new state record)

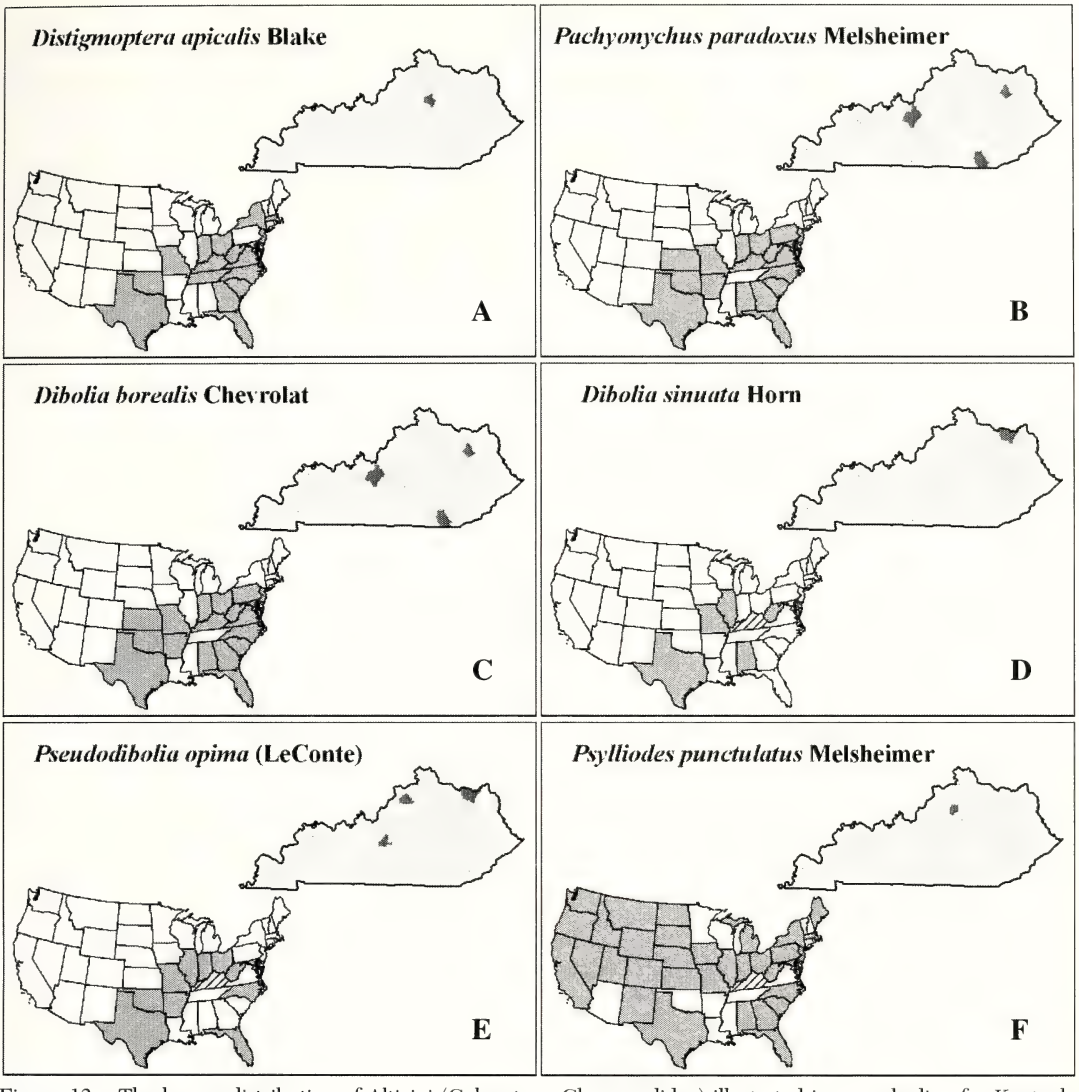


Figure 12. The known distribution of Alticini (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Kentucky County: Franklin
Year: 2006 (2)
Month: June (2)
Abundance: 2 specimens: 2-RJBC
Comments: This species is associated with Brassicaceae (Clark et al. 2004).

DISCUSSION

We believe the data presented here are the most complete representation of the alticine leaf beetles known from Kentucky. The large number of new state records documented here (45 of 84 species, or 54%) reflects a historical lack of leaf beetle collecting in Kentucky. This is reflected in the statement by Parry (1974) of "... the scarcity of material collected in the southern Midwest and in Kentucky," when referring to *Dibolia*.

Some of the records are not surprising, such as the one for the abundant, pestiferous species *Phyllotreta zimmermanni*, which is essentially found in all 48 contiguous states, while other records may extend the known range of species to the south (*Phyllotreta cruciferae*), to the east (*Longitarsus acutipennis*), to the west (*Kuschelina perplexa*), and to the north (*Kuschelina miniata*).

Many of these flea beetle genera are very difficult to determine to species (*Altica*, *Glyptina*, and *Longitarsus*), and several undetermined, additional species are present in the KYSU collection. Furthermore, many of these small, dark, jumping beetles are undoubtedly overlooked or uncatchable by non-Coleoptera collectors.

ACKNOWLEDGMENTS

Thanks are extended to Michael Sharkey and Martha Potts (UKIC), Keith Philips (WKUC), Greg Dahlem (CMC), and Charles Wright (CWC) for access to their collections. We thank the following people for granting access to the protected habitats they manage: Joyce Bender, Lane Linnenkohl and Zeb Weese, Kentucky State Nature Preserves Commission; Jeff Sole and John Burnett, The Nature Conservancy Kentucky Chapter; Steve McMillen, Kentucky Department of Fish and Wildlife; Andrew Leonard, Fort Campbell Fisheries and Wildlife Program; and Steve Bloemer, USDA Forest Service. We also thank Joyce Owens (KYSU) for sorting, organizing and transcribing, and Sarah Hall (KYSU) for creation of the distribution maps. This research was supported by USDA-CSREES Project KYX-10-05-39P.

LITERATURE CITED

Balsbaugh, E. U., and V. M. Kirk. 1968. Distributional and ecological notes on *Distigmoptera* and *Pseudolampsis* with a description of a new species *Distigmoptera* (Coleoptera: Chrysomelidae). Journal of the Kansas Entomological Society 41:243–247.

Barney, R. J., S. M. Clark, and E. G. Riley. 2007. Annotated list of the leaf beetles (Coleoptera: Chrysomelidae) of Kentucky: subfamily Cassidinae. Journal of the Kentucky Academy of Science 68:132–144.

Barney, R. J., S. M. Clark, and E. G. Riley. 2008a. Annotated list of the subfamilies Donaciinae and Criocerinae. Journal of the Kentucky Academy of Science 69:29–36.

Barney, R. J., S. M. Clark, and E. G. Riley. 2008b. Annotated list of the leaf beetles (Coleoptera: Chrysomelidae) of Kentucky: subfamily Chrysomelinae. Journal of the Kentucky Academy of Science 69:91–100.

Barney, R. J., S. M. Clark, and E. G. Riley. (this issue). Annotated list of the leaf beetles (Coleoptera: Chrysomelidae) of Kentucky: subfamily Galerucinae, tribes Galerucini and Luperini. Journal of the Kentucky Academy of Science.

Baskin, J. M., C. C. Baskin, and E. W. Chester. 1994. The Big Barrens Region of Kentucky and Tennessee: further observations and considerations. Castanea 59:226–254.

Blake, D. H. 1927. Revision of the beetles of the genus *Oedionychis* occurring in America north of Mexico. Proceeding of the United States National Museum 70(no. 2672), 1–44, pls. 1–2.

Blake, D. H. 1933. Revision of the beetles of the genus *Disonycha* occurring in America north of Mexico. Proceedings of the United States National Museum 82(no. 2969), 1–66, pls. 1–8.

Blake, D. H. 1953. The chrysomelid beetles of the genus *Strabala* Chevrolat. Proceedings of the United States National Museum 103(no. 3319), 121–134.

Blatchley, W. S. 1921. Notes on Indiana Halticini with characterization of a new genus and description of new species. Journal of the New York Entomological Society 29:16–27.

Chittenden, F. H. 1927. The species of *Phyllotreta* north of Mexico. Entomologica Americana 8:1–59.

Clark, S. M., D. G. LeDoux, T. N. Seeno, E. G. Riley, A. J. Gilbert, and J. M. Sullivan. 2004. Host plants of leaf beetle species occurring in the United States and Canada. The Coleopterists Society, Special Publication No. 2. 476 pp.

Furth, D. G. 1998. New World *Blepharida* Chevrolat 1936 (Coleoptera: Chrysomelidae: Alticinae). Memoirs of the Entomological Society of Washington 21:1–109.

Gentner, L. G. 1944. The black flea beetles of the genus *Epitrix* commonly identified as *cucumeris* (Harris). Proceedings of the Entomological Society of Washington 46:137–149.

Jones, R. L. 2005. Plant Life of Kentucky. University Press of Kentucky. 834 pp.

LeSage, L. 1995. Revision of the costate species of *Altica* Müller of North America north of Mexico (Coleoptera: Chrysomelidae). The Canadian Entomologist 127:295–411.

LeSage, L. 2002. Flea beetles of the genus *Altica* found on grape in northeastern North America (Coleoptera: Chrysomelidae). Journal of the Entomological Society of Ontario 133:3–46.

LeSage, L., and A. Zmudzinska. 2004. The immature stages of the grape flea beetles *Altica chalybea* Illiger and *A. woodsi* Isley (Coleoptera: Chrysomelidae). Pages 503–528 in P. Jolivet, J. A. Santiago-Blay and M. Schmitt (eds). New Developments in the Biology of Chrysomelidae. SPB Academic Publishing, The Hague, The Netherlands.

Parry, R. H. 1974. Revision of the genus *Dibolia* Latreille in America north of Mexico (Coleoptera: Chrysomelidae). Canadian Journal of Zoology 52:1317–1354.

Parry, R. H. 1986. The systematics and biology of the flea beetle genus *Crepidodera* Chevrolat (Coleoptera: Chrysomelidae) in America north of Mexico. Insecta Mundi 1:156–195.

Riley, E. G., S. M. Clark, R. W. Flowers, and A. J. Gilbert. 2002. Chrysomelidae Latreille 1802. Pages 617–691 in R. H. Arnett, M. C. Thomas, P. E. Skelley and J. H. Frank (eds). American Beetles. Volume 2. Polyphaga:

Scarabaeoidea through Curculionidae. CRC Press, Boca Raton, Florida.

Riley, E. G., S. M. Clark, and T. N. Seeno. 2003. Catalog of the leaf beetles of America north of Mexico. The Coleopterists Society, Special Publication No. 1. 290 pp.

Riley, E. G., and W. R. Enns. 1979. An annotated checklist of Missouri leaf beetles (Coleoptera: Chrysomelidae). Transactions, Missouri Academy of Science 13:53–83.

Smith, E. H. 1985. Revision of the genus *Phyllotreta* Chevrolat of America north of Mexico part 1. The maculate species. Fieldiana, Zoology (new series) no. 28:v + 168 pp.

Vulinec, K., and R. A. Davis. 1984. Coleoptera types in the Charles Dury Collection of the Cincinnati Museum of Natural History. The Coleopterists Bulletin 38: 232–239.

White, R. E. 1996. A revision of the genus *Chaetocnema* of America north of Mexico (Coleoptera: Chrysomelidae). Contributions of the American Entomological Institute 29:1–158.

J. Ky. Acad. Sci. 70(1):56–62. 2009.

Human Sex Ratio and Family Size for a Selected Sample from the India Population in 2007–2008

Archana Lakkaraju and Pramod R. Gupta

Department of Public Health

and

Elmer Gray[1]

Department of Agriculture, Western Kentucky University, Bowling Green, Kentucky 42101-3576

ABSTRACT

Students at nine colleges in Andhra Pradesh, India, were surveyed for size and gender composition of families in their parental, present, and projected generations. These data were used to calculate average family size, secondary sex ratios (males:100 females), impact of genders of existing children within families on eventual family size, and independence of sex ratios of successive births. For the parental, present, and projected generations, average number of children were 4.27, 2.99 and 2.10, and sex ratios were 101, 87, and 99; respectively. Gender differences, both in combinations and permutations, of existing children influenced parents' decisions to have additional children. Although son preference was evident, more families stopped having children with two, three, or four when both genders were present than when existing children were of same gender, including the all son combination. The most desired family consisted of two children, both genders, with the male born first. Realization of the average number of children (2.10) in families of the projected generation would result in a more stabilized population. Observed and expected combinations of genders in families with 2, 3, 4, and 5 children in the present generation differed significantly indicating a lack of independence. Also, the lack of independence was supported by significant negative correlations between gender compositions of successive births within families.

KEY WORDS: Human sex ratio, gender composition, human population, family size, India population

INTRODUCTION

The human sex ratio is of great interest especially in countries that are characterized by high populations and strong gender preferences. India epitomizes such countries by being the second most populated country in the world and by having a strong son preference. Mutharayappa et al. (1997) reported that elimination of son preference in India could lower the fertility rate by approximately 8% and be a major factor in reducing population growth. Strong preference for sons is a cultural ideal based upon economic and social values. The New Delhi Operations Research Groups reported that in 1991 approximately 72% of rural parents continued to have children until at least two sons were born (India 2006). Nath and Land (1994) stated that the desire of India families, regardless of economic status, to have more sons and to continue childbearing until reaching their goal was a major contributing factor to family size. Sex ratios vary among the states of India but overall the country shares a distinctive feature with South Asia and China, namely a deficit of women (India 2006). In China, the enforcement of government policies is resulting in a shortage of women leading to potentially disastrous social consequences (Qui and Mason 2006). In addition to son preference, parents in some cultures have preference for both genders (Gray and Lakkaraju 2008). Presence of both genders in the first two or three children resulted in fewer additional children as demonstrated by studies conducted in Britain (Thomas 1951), the United States (Loyd and Gray 1969; Gray 1972; Gray and Marrison 1974; Call and Gray 1996), and China (Gray et al. 1995).

Objectives of the present study were to characterize basic aspects of the human sex ratio and family size for a selected sample from the India population and to determine the impact of gender composition of existing children within families on the parents'

[1] Corresponding author email: elmer.gray@wku.edu

decision to have additional children. Also, the aim of the present study was to replicate a similar study conducted on a United States population by Gray and Lakkaraju (2008) and one being conducted on a China population.

METHODS

In 2007–2008, students at nine colleges in Andhra Pradesh, India, were surveyed for size and gender composition of their families. The 1190 respondents (595 of each gender) provided data on three generations (parental, present, and projected) resulting in information on family sizes and sex ratios for 4760 families. For the parental generation, data were combined for the mother's and father's families. For the present generation, data were obtained for each survey participant's family. The data included number of children and gender composition by order of birth, permitting calculations of average family size and sex ratios by order of birth. For the projected generation, survey respondents were asked to indicate their desired number and gender of children by order of birth. These data were utilized in calculating average family size and sex ratios. Independence of gender outcomes was examined by calculating linear correlation coefficients between sexes of consecutive and nonconsecutive births within families of the present generation and through comparisons of observed and expected binomial distributions of gender combinations for different family sizes in both the parental and present generations. The observed sex ratio for each generation was used in the binomial analysis. Chi-square as goodness of fit test was used to compare the observed and expected binomial distributions. Chi-square as test of independence was used to test the impact of gender composition of existing children on family size.

RESULTS

Average numbers of children decreased progressively from the parental (4.27), to the present (2.99), and to the projected (2.10) generation (Table 1). The decrease of 1.28 children from the parental to the present generation compared with a decrease of 1.46 (4.04 vs. 2.58) in a United States population (Gray and Lakkaraju 2008). The average desired number of children (2.10) compared with 2.45 in the United States study and coincided with the established 2.10 children per couple necessary for replacement in the India population (Mutharayappa et al. 1997).

Table 1. Average number of children and secondary sex ratio (males:100 females) for a selected sample from the India population in 2007–08.

Generation	No. of children	Sex ratio
Parental	4.27	101
Present	2.99	87
Projected	2.1	99

Because gender by order of birth was obtained for families in the present generation, it was possible to study the effect of gender composition of existing children on further births (Table 2). Whether the first child was female or male had no significant impact on further births. For two children families, significantly more families stopped with two when both genders were present. Gender order (male-female, female-male) had no significant effect. More families ceased having children following male-male than female-female combinations. Within families of three children, both combinations and permutations of gender had a significant impact on family size. Higher percentages of families stopped with three children when the existing children included both genders (2 males, 1 female 58.1% or 1 male, 2 females 56.9%) than when all children were of the same gender (3 males 33.3% or 3 females 45.0%). There were no significant differences between the two combinations including both genders or between the two combinations including only a single gender. When combinations were subdivided into permutations according to birth order, gender permutations differed significantly in their impact on parents' decision regarding additional children. Permutations including both sexes resulted in more families ceasing to have children. Families with female-female-male order were significantly less likely to have more children than those with any other permutation.

Comparisons of gender combinations within families of four children (Table 2) showed that for all female children 8.8% stopped, whereas for all male children 27.6% stopped.

Table 2. Influence of gender composition of sexes of existing children on family size in the present generation of Indian families sampled in 2007–08.

Gender combinations and permutations	Family stopped		Family increased		Total
	n	%	n	%	n
First child					
Female	43	6.3	636	93.7	679
Male	35	7.1	458	92.9	493
Total	78	6.7	1094	93.3	1172
First two children**					
Female-female	54	18.9	232	81.1	286
Female-male	145	41.1	208	58.9	353
Male-female	133	46.3	154	53.7	287
Male-male	54	32.1	114	67.9	168
Total	386	35.3	708	64.7	1094
First three children*					
Male-male-male	15	33.3	30	66.7	45
Female-female-female	41	45	50	54.9	91
Male-male-female	39	59.1	27	40.1	66
Male-female-male	41	64.1	23	35.9	64
Female-male-female	64	52.4	58	47.5	122
Female-female-male	84	61.8	52	38.2	136
Female-male-male	46	52.9	41	47.1	87
Male-female-female	54	55.7	43	44.3	97
Total	384	54.2	324	45.8	708
First 4 children* (combinations only)					
Four males	8	27.6	21	72.4	29
Three males, one female	24	52.2	22	47.8	46
Two males, two females	110	82.7	23	17.3	133
One male, three females	56	77.8	16	22.2	72
Four females	3	8.8	34	91.9	37
Total	201	63.4	116	36.6	317
>4 children	116				

*, ** indicate significance at the ($P \leq 0.05$) and ($P \leq 0.01$); respectively.

Combinations included both genders were significantly more likely to cease having children. Families with 2 males and 2 females were most likely to stop with four children. Of the 1172 families of the present generation, only 116 had more than four children.

Survey respondents were asked to provide information on their desired families indicating number of children and the combinations and permutations of genders. These data were used to characterize a projected generation (Table 3). Approximately 5% of respondents wanted no children. For the 15% wanting one child, males and females were essentially equal. Most respondents (769 or 68.8%) wanted two children. Desired combinations of sexes within the two-child families were 2.3% for two females, 3.8% for two males, and 93.9% for one female and one male. Within two-child families, 34.2% wanted a female, male order and 65.8% wanted a male, female order. The finding that the most desired family consisted of two children, both genders with the male born first and the female second is supported by other studies (Gray and Lakkaraju 2008). Approximately 8.5% of the respondents wanted three children with stronger preference for both genders over either all males or all females. Within the three-child families, preference for 2 females 1 male (67.4%) was significantly greater than that for 1 female-2 males (30.5%). Approximately 2% of the respondents desired more than four children. Overall, the average number of children for the projected generation was 2.10 (Table 1), the replacement levels for a stable population. To further elucidate results for desired families, responses from male and female respondents were analyzed separately. Males wanted an average of 2.19 children with a resulting sex ratio of 96; females wanted an average of 1.90 children with a resulting sex ratio of 100.

Table 3. Desired family size, combination and permutation of sexes of children in projected families for India respondents surveyed in 2007–08.

No. of children	Combinations and permutations	Respondents	
		n	%
0		60	5
1	One female	82	48.8
	One male	86	51.2
	Total	168	15.00
2	Two females	18	2.3
	One male one female	722	93.9
	Female male	247	34.2
	Male female	475	65.8
	Two males	29	3.8
	Total	769	68.6
3	Three females	1	1.0
	Two females one male	64	67.4
	Female-female-male	23	35.9
	Female-male-female	25	39.1
	Male-female-female	16	25
	One female two males	29	30.5
	Female-male-male	4	13.8
	Male-female-male	19	65.5
	Male-male-female	6	20.7
	Three males	1	1.0
	Total	95	8.5
4	Four males	0	0
	Three males one female	3	4.8
	Male-male-female-male	0	0
	Male-female-male-male	0	0
	Female-male-male-male	3	100
	Male-male-male-female	0	
	Two males two females	54	85.7
	Male-male-female-female	4	7.4
	Female-female-male-male	18	33.3
	Male-female-female-male	3	5.6
	Female-male-male-female	10	18.5
	Male-female-male-female	11	20.4
	Female-male-female-male	8	14.2
	One male three females	6	9.5
	Male-female-female-female	1	16.7
	Female-male-female-female	2	33.3
	Female-female-male-female	2	33.3
	Female-female-female-male	1	16.7
	Four females	0	0
	Total	63	
>4	Total	23	2

Sex ratios for the present and projected generations were analyzed by family size and birth order (Table 4). In the present generation, sex ratios were consistently higher for the last child of the family sizes further reflecting the son preference. Parents wanting sons were more likely to cease having children after a son was born. In the projected generation, the ratios for advancing births demonstrate the alternating of preferred genders (male first, female second), which has been reported in other studies (Gray and Lakkaraju 2008).

Independence of gender ratios and family size was analyzed by comparing observed and expected binomial distribution. Using the observed sex ratio for the parental generation, comparisons were made for sizes one through five. Frequencies of males and females in one-child families occurred as expected based upon the overall sex ratio. However, for family sizes two, three, four, and five observed frequencies differed significantly from expected. For two children, combinations of same gender (male-male and female-female) occurred less while the different gender (male-female) combinations occurred more frequently than expected. For three children, combinations including more males occurred less than expected and those including more females occurred more frequently than expected. In families of four and five children the greatest disparities between observed and expected occurred due to excesses of observed for 2 females-2 males and 3 females-2 males. In the present generation, observed and expected frequencies of males and females did not differ significantly for one child families. For two-child families observed frequencies were less than expected for female-female and male-male and greater than expected for male-female combinations. This same pattern held for three- and four-child families.

To further test independence of gender outcome, linear correlation coefficients (r) were calculated between genders of consecutive and nonconsecutive parities within families (Table 5). Coefficients were low in magnitude. For the first five parities, all coefficients were negative and those between parities 1 vs. 2, 1 vs. 3, 2 vs. 3, and 2 vs. 5 were highly significant, indicating inconsistencies in the gender balance among parities. Reported correlations between genders of parities within families have been low in magnitude, variable in sign, and generally non-significant (Loyd and Gray 1969; Gray and Lakkaraju 2008).

DISCUSSION

The human population is a function of both family size and number of families. Results of the present study indicated that average

Table 4. Sex ratios by family size and birth order in the present and projected generations for a selected sample from the India population in 2007–08.

Family size (no. of births)	n	Order of births 1	2	3	4	5	Over all births
Present generation		sex ratios (males:100 females)					
1	78	134					134
2	386	99	106				100
3	384	63	75	111			110
4	201	58	107	82	123		90
5	120	56	44	71	91	121	72
Overall sizes		75	88	97	112	121	87
Projected generation							
1	168	105					105
2	769	80	56				67
3	95	81	59	140			84
4	63	42	73	237	130		99
5	90	31	240	112	54	112	89
Overall sizes		142	59	136	112	112	99

family size in the sampled India population decreased over the generations studied. To the extent that this trend is true, the expected increase in India's population through 2060 (India 2006) will result largely from increasing numbers of families.

Results of the present study indicated that gender preferences of parents in Andhra Pradesh were evolving away from more sons and toward both sons and daughters. In the present generation more families stopped with two, three, or four children when both genders were present than when existing children were all sons or all daughters. The most desired family for the projected generation consisted of two children with both genders (93.9%) compared with all males (3.8%) or all females (2.3%). For desired families of three children, 97.9% of the respondents wanted both genders, 1.0% wanted all males, and 1.0% wanted all females. For desired families of four children, 85.7% of the respondents wanted two males-two females; none wanted all males or all females.

Table 5. Linear correlation coefficients (r) between genders of consecutive and nonconsecutive births of a selected sample from the India population in 2007–08.

Order of birth		Order of birth 2	3	4	5
1	r	−0.191**	−0.105**	−0.105	−0.36
	N	1094	708	324	123
2	r		−0.97**	−0.6	−0.384**
	N		708	324	123
3	r			−0.34	−0.12
	N			324	123
4	r				−0.007
	N				123

** indicates significance ($P \leq 0.01$).

This apparent change in parental gender preference is similar to changes reported from China. Merli and Smith (2002) reviewed changes in gender preferences within Chinese families during the post-1979 implementation of the One-Child Family Planning Policy. The earlier preference for two sons and one daughter was followed by preference for one son and one daughter. The preference for two children including both genders exceeded the preference for two sons. Bogg (1998) found that women in rural China wanted one son and one daughter; fewer than 4.0% wanted only sons. When limited to one child, those families preferring a son and applying practices to effectively alter gender outcome will imbalance the sex ratio and create social discord (Qui and Mason 2005). Preference for both genders of children is favorable for maintaining a more balanced sex ratio. However, any gender preference which causes parents to continue childbearing leading to that desired outcome will result in increased family size unless parents take recourse in gender selective practices.

The low sex ratio (87) indicating more females than males in the present generation is a conundrum. Further analyses of the ratio

by order of birth gave ratios of 75, 88, 97,112, and 121 for births one through five; respectively, and does not fit any recognizable pattern. Additional unexplained results were the negative correlations between genders of successive births and the disparities between expected and observed binomial distributions of genders among families.

Using college students as survey respondents is questionable but justifiable because they are at the age to both represent completed families of their parents and to have preferences for their own families. The college setting and sampling procedure permitted total anonymity as compared with on site census interviews with family planning representatives. Other studies (Gray et al. 1995; Gray and Lakkaraju 2008) have, likewise, used college students and their families. Bias in the data may result from failure of college students to be representative of different economic and cultural segments of the population. Higher levels of formal education of mothers have been found to be associated with fewer children in some developing countries (Gray and Bortolozzi 1977; Qu and Hesketh 2006). If the level of education of the female respondents had been a factor in the present study, it should have been evident in the projected generation. However, female and male preferences were comparable for family sizes and gender compositions. Also, the desired gender compositions in the projected generation coincided with observed combinations that reduced the likelihood of further family increases in the present generation.

The portion of India's population included in the present sample had some similarities and some dissimilarities with results of other studies, especially the parallel United States based study by Gray and Lakkaraju (2008). Similarities include decreases in family size from parental to present generations, higher percentages of families ceasing to have more children when both genders were present in existing children, and preference for two-child families, including both genders, with male being first born. Notable differences included lower secondary sex ratios for a population with a history of son preference, presence of significant negative correlations between genders of successive births within families, greater disparity between expected and observed binomial distribution of gender composition of families, lower desired family size than United States (2.10 vs. 2.45) (Gray and Lakkaraju 2008), but higher than China (2.10 vs. 1.65,) (Gray et al. 1995), and a more balanced desired sex ratio than United States (99 vs. 141) or China (99 vs. 110).

India is a dynamic cosmos of humanity. The government has a long history of explicit family planning policies. Policy makers accredit large family sizes as part and parcel to poverty. Development of strategic plans for moderating the population growth problem has been hampered by the vast diversity among India's cultures. Results of the present study indicate that family size is decreasing and that the strongly held son preference is waning.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Smt. N. Rajeshwari, Principle, Model High School, Andhra Pradesh, India, for coordinating the survey distribution and the official representatives and student respondents at the nine participating colleges. We thank Ogden College of Science and Engineering, especially the Department of Agriculture, and the College of Health and Human Services, especially the Department of Public Health, Western Kentucky University. Thanks are expressed to the office of the Provost for financial and other forms of support. Also, thanks are extended to Dr. Christine Nagy and Dr. John White for assistance with SPSS.

LITERATURE CITED

Bogg, L. 1998. Family planning in China: out of control? American Journal of Public Health 88:649–651.

Call, N. M., and E. Gray. 1996. Longitudinal studies of family size and the human sex ratio. Transactions of the Kentucky Academy of Science 57:101–105.

Gray, E. 1972. Influence of sex of first two children on family size. Journal of Heredity 63:91–92.

Gray, E., V. K. Hurt, and J. Y. Wu. 1995. Human sex ratio and factors influencing family size in Hunan, China. Transactions of the Kentucky Academy of Science 56:9–14.

Gray, E., and N. M. Morrison. 1974. Influence of combinations of sexes of children on family size. Journal of Heredity 65:169–174.

Gray, E., and A. Lakkaraju. 2008. Extended longitudinal studies of family size and the human sex ratio. Journal of Kentucky Academy of Science 69:50–55.

Gray, E., and J. Bortolozzi. 1977. Studies of the human sex ratio and factors influencing family size in Botucatu, Brazil. Journal of Heredity 68:241–244.

India. 2006. India - Population and family planning policy. Office of the Registrar General, Sample registration system 2000. New Delhi, India.

Loyd, R. C., and E. Gray. 1969. A statistical study of the human sex ratio. Journal of Heredity 60:329–331.

Merli, M. G., and H. L. Smith. 2002. Has the Chinese family planning policy been successful in changing fertility preferences? Demography 39:557–572.

Mutharayappa, R., M. K. Choe, F. Arnold, and T. K. Roy. 1997. Son preference and its effect on fertility in India. National Family Health Survey Subjects Reports 3:4–35.

Nath, C. D., and K. C. Land. 1994. Sex preference and third birth intervals in a traditional Indian Society. Journal of Biosocial Science 26:377–388.

Qi, Y., and W. M. Mason. 2005. Prenatal sex selective abortion and high sex ratio at birth in rural China. A case study in Henan Province. California Center for Population Research On-line Working series. CCPR-057-05.

Qu, J. D., and T. Hesketh. 2006. China's one child policy. BMJ 333:361–362. School of Public Health, University of California, Berkeley, CA.

J. Ky. Acad. Sci. 70(1):63–69. 2009.

Human Sex Ratio and Family Size for a Selected Sample from the China Population in 2008

Zheng Wang and Elmer Gray[1]

Department of Agriculture, Western Kentucky University, Bowling Green, Kentucky 42101-3576

ABSTRACT

Human fertility and sex ratio of China's population have attracted the interest of demographers and other scholars since the early twentieth century. The present study was initiated in 2008 to investigate recent changes in family sizes and sex ratios. Survey data were obtained from students enrolled at three universities located in Shenyang, China, (41.8 N, 123.4 E). Each student respondent supplied information on the number of children and gender composition for the parental, present, and projected generations. Average numbers of children were 4.5, 1.6, and 1.7, and secondary sex ratios (males:100 females) were 101.2, 108.3, and 107.1 for parental, present, and projected generations; respectively. In the parental generation, four children per family occurred most frequently, whereas in the present generation one child families were most frequent. In the projected generation, the most desired family consisted of two children with both sexes present and the male being born first. Binomial distribution and correlation analyses for the present generation demonstrated highly significant differences ($P < 0.01$) between observed and expected combination of sexes in two-child families. The response to China's One Child Family Planning Policy has resulted in an unmatched one generation reduction in family size from 4.5 to 1.6 children. These results indicated that there may be a waning of the historically strong son preference.

KEY WORDS: Family size, secondary sex ratio, combination of sexes, gender by order of birth

INTRODUCTION

Population issues continue to challenge demographers and sociologists to understand population dynamics not only of China, but also globally. Expansion of the human population continues to accelerate the threat of exceeding the natural resources available to meet human needs (Gray et al. 1995; Gray and Lakkaraju 2008). Facing the existing and growing jeopardy of excessive population and limited resources, it is imperative to restrict the population growth. China, with 25% of the world's total population must take effective and restrictive actions to control its population size and fertility rate. Researchers from diverse disciplines have studied the China's population from different aspects including fertility level, sex ratio, and missing girls (Greenhalgh and Bogaarts 1987; Hull 1990; Cai and Lavely 2003; Qu and Hesketh 2006). Also, Bogg (1998), Attane (2002), and Lutz et al. (2007) analyzed the fertility level and sex ratio, and made predictions on the future trend of China's population. Fewer research articles have focused upon individual family preference for future family size and sex ratio.

Objectives of the present investigation were to survey Chinese university students to gain further understanding of basic aspects of the human sex ratio and to study a sample of China's population to assess changes in family sizes and sex ratios.

POPULATION AND METHODS

The sample of 1050 students with equal numbers of males and females was selected from three universities (Shenyang Agricultural University, China Medical University, and Shenyang Institute of Chemical Technology) in the city of Shenyang, China in 2008. Survey results were received from 993 respondents resulting in 976 (488 males and 488 females) usable responses. Under anonymous and volunteer settings, respondents provided information on family sizes and genders for parental, present, and projected generations, and order of births in present and projected generations. Data were used in the calculations of average family sizes and secondary sex ratios for the three generations. The procedures and method of the present study followed the patterns of previous researches (Gray et al. 1995; Gray and Lakkaraju 2008).

For the parental and present generations, expected and observed binomial distributions

[1] Corresponding author e-mail: elmer.gray@wku.edu

Table 1. Average number of children and secondary sex ratios (males:100 females) for three generations in the selected sample from China's population in 2008.

Generation	Average family size	Sex ratio
Parental	4.5	101.2
Present	1.6	108.3
Projected	1.7	107.1

of gender composition within families of different sizes were compared using Chi-square. For the present generation, correlation coefficients were calculated between genders of consecutive and non-consecutive births within families. For the projected generation, respondents were requested to provide information on their desired number of children, and combinations and permutations of genders. Results of the study were analyzed with SPSS (Statistical Product and Service Solution version 16.0).

RESULTS AND ANALYSES

Average Family Size

Average numbers of children were 4.5 and 1.6 for parental and present generations; respectively (Table 1). For the parental family, 4.5 children per family was higher than the corresponding value reported for China in 1995 (Gray et al. 1995), but lower than the previous censuses data for China's population on fertility rate (Greenhalgh and Bongaarts 1987; Zhu 2003; Malcolm 2006). The fertility rate was relatively constant between 1950 and 1970, ranging from approximately 5.0 to 6.5 (Zhu 2003; Hesketh and Zhu 2006; Malcolm 2006). Reduction in number of children from 4.5 in the parental to 1.6 in the present

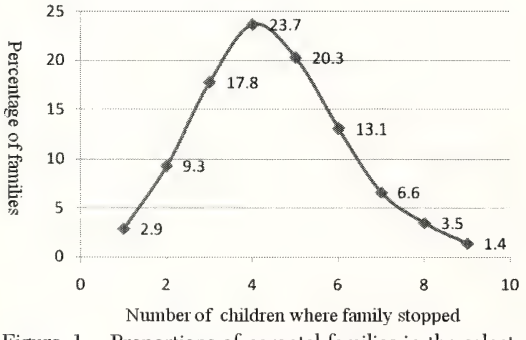


Figure 1. Proportions of parental families in the selected sample of China's population that stopped childbearing after having different number of children.

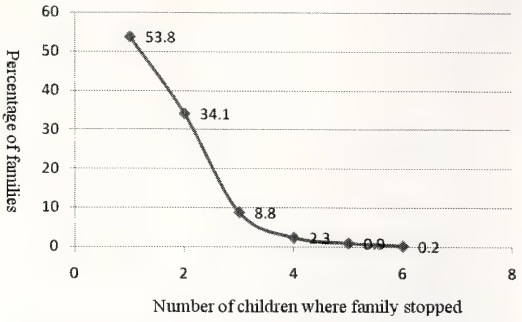


Figure 2. Proportions of present families in the selected sample of China's population that stopped childbearing after having different number of children.

generation surpassed the one generation reduction (4.32 to 3.36) for the earlier China study (Gray et al. 1995). Relationships between number of children and completion of childbearing in the parental and present generations were presented (Figures 1 and 2). The frequency distribution for the parental generation showed that the most frequent completed family size was four children (23.7%). At the extremes were 2.9% with one child and 1.4% with nine children (Figure 1). In contrast, for the present generation (Figure 2) more than one-half (53.8%) of the families stopped with one child and another one-third (34.1%) stopped with two children. Only 2.3% of families had four children which was the most frequent numbers of children in the previous generation.

Binomial Distribution and Correlations Between Sexes

Observed and expected binomial distributions were compared on family sizes 1 to 8 in the parental and 1 to 3 in the present generations using Chi-square goodness of fit test. Most present generation families had only one and two children (Figure 2). Consequently, binomial distribution analyses were limited to the first three children and permutations of sexes were applied only to the two-child families (Table 2). In the parental generation, there were distinct differences between observed and expected distribution in family size one to five except for five-child families (Table 3). For family sizes greater than five, the observed and expected distribution differed for sizes six and seven but not for eight-child families. The

Table 2. Composition of sexes of existing children and family size in the present generation in the selected sample of China's population in 2008.

Sex combinations	Family stopped			Family increased		
	No.	%	Sex ratio	No.	%	Sex ratio
First child$(ns)_1(ns)_2$						
f	162	57.0		122	43.0	
m	182	51.1		174	48.9	
Total	344	54.1	112.4	296	45.9	107.8
First two children**$(ns)_2$						
ff	37	72.6		14	27.4	
fm	55	78.6		15	21.4	
mf	90	72.0		35	28.0	
mm	36	72.0		14	28.0	
Total	218	73.8	99.1	78	26.2	121.7
First three children*$(ns)_2$						
mmm	3	100.0				
2f1m	16	64.0		9	36.0	
2m1f	33	73.3		12	26.7	
fff	4	80.0		1	20.0	
Total	56	70.8	118.2	22	29.2	166.7
More than three children	Only 22 families had more than three children					

$(ns)_1$, $P > 0.05$ that observed combinations are no significant compared with expected combinations, and * and **, $P < 0.05$ and 0.01 that distribution of observed combinations are significant to expected distribution. $(ns)_2$, $P > 0.05$ that increases in family size are independent of sexes of existed children.

divergence between observed and expected distribution resulted from deviations of certain combinations of sexes. For example, the combination of two females and two males in the four-child family was detected more frequently than expected. For the present generation, the disparities between observed and expected combinations occurred in both two-child ($P < 0.01$) and three-child ($P < 0.05$) families (Table 2). When analyzed for permutations of the two-child families, the permutation of "mf" was more frequent than expected. The observed distribution of combination of "2m1f" in three-child families occurred much more frequently than expected.

Correlation coefficients were calculated on the sexes of consecutive and non-consecutive children in the families of present generation (Table 4). The coefficients were calculated among the first three children for all families. All were negative, low in magnitude, and most

Table 3. Comparisons of observed and expected binomial distributions for combinations of sexes in the parental generation of the selected Chinese families with 1 to 5 children.

Family size		Binomial distribution						Chi-square
1	Combination	1m	1f					
	Expected	28.7	28.3					
	Observed	42	15					12.5**
2	Combination	2m	1m1f	2f				
	Expected	46.1	91.0	44.9				
	Observed	40	117	25				17.1**
3	Combination	3m	2m1f	1m2f	3f			
	Expected	44.2	130.9	129.3	42.6			
	Observed	25	144	158	20			28.0**
4	Combination	4m	3m1f	2m2f	1m3f	4f		
	Expected	29.6	116.9	173.2	114.1	28.2		52.8**
	Observed	18	89	246	96	13		
5	Combination	5m	4m1f	3m2f	2m3f	1m4f	5f	
	Expected	12.8	63.2	124.8	123.3	60.9	12.0	4.8 (ns)
	Observed	11	52	123	134	68	9	

** Observed binomial distribution is highly significant to expected distribution at 0.01 level.

Table 4. Correlation coefficients between sexes of children of different births in the present generation of the sample from China's population in 2008.

Family size and birth order	Number	Correlation coefficients
All families		
Birth 1 vs. birth 2	296	−0.293**
Birth 1 vs. birth 3	78	−0.210 (ns)
Birth 2 vs. birth 3	78	−0.113 (ns)
Families of two children		
Birth 1 vs. birth 2	218	−0.313**
Families of three children		
Birth 1 vs. birth 2	56	−0.116 (ns)
Birth 1 vs. birth 3	56	−0.240 (ns)
Birth 2 vs. birth 3	56	−0.119 (ns)

** Correlation is significant at 0.01 level.

of them were non-significant ($P > 0.05$); however, the correlation coefficients of birth 1 vs. birth 2 for all families and birth 1 vs. birth 2 for those consisting of two children were highly significant ($P < 0.01$). The significantly negative associations indicated that genders of the first two births were substantially opposite.

Compositions of Sexes

Combinations of existing children had no significant ($P > 0.05$) effect on family size (Table 2). The former China's study (Gray et al. 1995) showed that, although gender composition of the first two children had no significant impact on family sizes, genders of the first 3 and 4 children influenced the parents' decisions to have additional children. Similar results were reported for American Black and Appalachian populations (Gray and Morrison 1974). In the present study, parents were more concerned about regulations limiting family size to one child, or at the most two children, than about personal preferences.

Sex Ratio

Secondary sex ratios (males:100 females) were 101.2 and 108.3 for parental and present generations; respectively (Table 1). In the parental generation, all families except four- and five-child had more males than females resulting in overall sex ratio higher than 100.0. In the American study, the sex ratios for parental generations ranged from 102 to 105 in series of four studies conducted between 1968 and 2008 (Gray and Lakkaraju 2008). Sex ratios for the present generation were calculated for the first three family sizes by order of birth (Table 5). In the present generation, sex ratios by order of birth were 112.4, 99.1, and 118.2 for families with 1, 2, and 3 children; respectively (Table 5). There was an evident preference for more males in one-child and three-child families resulting in an alternating of sexes as a "high-low-high" pattern of sex ratio.

Whether families stopped or continued having children was independent of combinations and permutations of existing children for one-child and two-child families. For three-child families, the data were limited, but it appeared that more families stopped when both genders were present (Table 2).

Projected Generation

Respondents were asked to provide information on future family sizes and on combinations and permutations of sexes of their children (Table 6). Average family size was 1.7 children per family and the secondary sex ratio was 107.0 (Table 1). Percentages of respondents desiring 0, 1, and 2 children were 8.8%, 19.4%, and 65.6%; respectively (Figure 3). Only 19 of 973 respondents preferred more than 3 children (Table 6). The 8.8% of total respondents wanting no

Table 5. Sex ratios by order of birth within present family sizes resulting from combination and permutation of sexes of children in the present generation selected from China's population 2008.

Projected family size	No. of families	Order of birth			Overall births
		1	2	3	
1	344	112.4			112.4
2	218	137.0	71.7		99.1
3	56	133.3	64.7	194.7	118.2
>3*	Only 22 respondents desired more than 3 children				
Overall sizes	640	125.4	68.2	143.8	108.3

Table 6. Desired family size, combination and permutation of sexes of children in projected families of selected sample in China's population 2008.

No. of children	Combination of sexes	Respondents		Permutation of sexes	Respondents	
		No.	%		No.	%
0		86	8.8			
1	1f	71	7.3			
	1m	118	12.1			
	Total	189	19.4			
2	2f	12	1.2			
	1m, 1f	616	63.3	fm	160	16.4
				mf	456	46.9
	2m	10	1.0			
	Total	638	65.6			
3	3f	2	0.2			
	2f, 1m	17	1.8	ffm	2	0.2
				fmf	7	0.7
				mff	8	0.8
	2m, 1f	21	2.2	fmm	3	0.3
				mfm	9	0.9
				mmf	9	0.9
	3m	1	0.1			
	Total	41	4.2			
>3	Only 19 respondents desired more than 3 children					

child was similar to results reported by Gray and Lakkaraju (2008) and Gray et al. (1995). Preferences for family size shifted from one-child in present generation to two children with both sexes in the future generation (Table 2 and 6). This most desired family comprised of two children, both genders, male first born was also preferred by several other populations (Gray and Morgan 1976; Gray et al. 1980; Gray 1982). Sex ratio of the desired offspring of the respondents was 107.1 which was slightly lower than the number in the present generation (Table 1). However, the strong desire to have a boy for the first born remained in the future child plan, which would lead to further abnormal sex ratio. The "high-low-high" pattern of sex ratio by order of birth in projected generation continued, resulting in the overall sex ratio of 107.1 (Table 7).

DISCUSSION

One-Child Family Planning Policy

The China population was selected because of its uniqueness resulting from being the

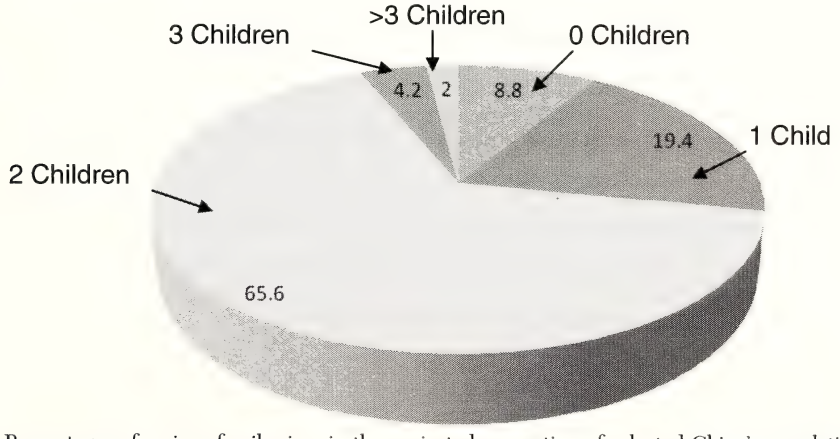


Figure 3. Percentages of various family sizes in the projected generation of selected China's population in 2008.

Table 7. Sex ratios by order of birth within desired family sizes resulting from combination and permutation of sexes of children in the projected generation of China's selected sample 2008.

Projected family size	No. of families	Order of birth			Overall births
		1	2	3	
1	189	166.2			166.2
2	638	270.9	36.3		99.4
3	41	192.9	95.2	57.7	101.6
>3*	Only 19 respondents desired more than 3 children				
Overall sizes	887	237.3	39.3	100.0	107.1

most populous country and by having the strictest population controls (Malcolm 2006). In 1979, the Chinese government launched one of the most important, restrictive, and unprecedented social policies called One Child Family Planning Policy in order to confine the rapid growth of the population in China (Malcolm 2006). The policy followed the principle of "Later, Longer, Fewer," implemented in the early 1970s. Its purpose was to educate people toward "Later marriage, Longer period between two children, and Fewer children as they could have" (Merli and Smith 2002; Zhu 2003).

The present study provided some insight on the effect of the policy on the population of China. The policy and its impact of only one child per family have been studied by many researchers including Greenhalgh and Bongaarts (1987), Kane and Choi (1999), Zhu (2003), Hesketh and Zhu (2006), and Qu and Hesketh (2006). University students selected for the survey were mostly in the 19–22 year-old range, being born between 1986 and 1989. Their parents were aware by the policy for nearly ten years. The parental generation with an average of 4.5 children (Table 1) was born when there were no restrictions on the number of children. The present generation with an average of 1.6 children (Table 1) was born after the policy's implementation. Previous data showed that the fertility rates in China through 1986 to 1989 were in the range of 2.24 to 2.59 (Lutz et al. 2007). This precipitous reduction in family size from the parental to present generation of approximately 3 children (4.5 vs. 1.6) was unmatched.

Through this unreflective action and a thousand-year tradition of son preference, China's population became distorted with extremely high sex ratio both at birth and existing population. Under the enforcement of the policy, most couples had to control their childbearing to cater to the policy without penalty. Paradoxically, they indeed preferred a boy, resulting in an abnormally high sex ratio. In the present study, high sex ratios occurred throughout, especially for first births, and exceeded 105.0 to 106.0, considered as the normal sex ratio for the globe (Hull 1990; Johansson and Nygren 1991; Coale and Banister 1994; Li 2007). Responding to the policy of one child while yielding to the son preference has resulted in sex-selective abortions and missing girls. Utilizations of ultra-sound and post-natal actions against female fetuses have led to the abnormally high sex ratio at birth (Li 2007). Couples have concealed the true numbers and combinations of children, especially girls, for fear of being punished by the authorized demographic departments (Johansson and Nygren 1991; Coale and Banister 1994; Merli and Raftery 2000; Cai and Lavely 2003). In the present study, respondents were nameless without pressure to participate. The results (Table 6) indicated that most respondents wanted two children with both genders, a preference that would work toward equalizing the sex ratio.

Li (2007) has reported that in the forthcoming 10–15 years, a new strategic plan will be implemented named "Care for Girls" in order to gradually equalize the biased sex ratio. The report estimated that the high sex ratio may steadily decline from 2011 to 2015 and remain at the normal level (106) from 2016 to 2020. Actually, hopes of neutralizing excessive males and minimizing the demographic impact of the policy have existed in some areas of China as early as 1980s (Merli and Smith 2002). Some citations were included in their report that a nine rural and suburban areas survey of Chinese provinces showed that a two-child family (one boy, one girl) was the most common preference.

Another survey, conducted in 1990s gave a replacement of one gender or two sons' preferences by two children with one son and one daughter. However, China's sex ratio and fertility rate are severe and complex, and unable to be normalized in a short period. Thus, accommodating the one child family while supporting a strong son preference will continue to lead to an unstable society, embodying that masculinization of births will easily result in huge amounts of "remain males," who are unmarried (Tuljapurkar et al. 1995).

ACKNOWLEDGEMENT

The authors gratefully acknowledge Dawei Guan, Degang Sun, and Qinghong Fang for their assistance and participations with data collecting and the Ogden College of Science and Engineering, especially the Department of Agriculture for supporting our research. Thanks are extended to the office of Provost for financial and other support.

LITERATURE CITED

Attane, I. 2002. China's family planning policy: an overview of its past and future. Studies in Family Planning 33:103–113.

Bogg, L. 1998. Family planning in China: out of control? American Journal of Public Health 88:649–651.

Cai, Y., and W. Lavely. 2003. China's Missing Girls: numerical estimates and effects on population growth. The China Review 3:13–29.

Coale, A. J., and J. Banister. 1994. Five decades of missing females in China. Demography 31:459–479.

Gray, E., and N. M. Morrison. 1974. Influence of combinations of sexes of children on family size. Journal of Heredity 65:169–174.

Gray, E., and D. K. Morgan. 1976. Desired family size and sex of children. Journal of Heredity 67:319–321.

Gray, E., D. Duckworth, and Y. Nakajima. 1980. The human sex ratio and factors influencing family size in Japan. Journal of Heredity 71:411–415.

Gray, E. 1982. Transgeneration analyses of the human sex ratio. Journal of Heredity 73:123–127.

Gray, E., V. K. Hurt, and J. Y. Wu. 1995. Human sex ratio and factors influencing family size in Hunan, China. Transaction of Kentucky Academic Science 56:9–14.

Gray, E., and A. Lakkaraju. 2008. Extended longitudinal studies of family size and the human sex ratio. Journal of Kentucky Academic Science 69:50–55.

Greenhalgh, S., and J. Bongaarts. 1987. Fertility policy in China: future options. Science 235:1167–1172.

Hesketh, T., and W. X. Zhu. 2006. Abnormal sex ratios in human population: causes and consequences. Proceeding of the National Academy of Sciences 103(36): 13271–13275.

Hull, T. H. 1990. Recent trends in sex ratios at birth in China. Population and Development Review 16:63–83.

Johansson, S., and O. Nygren. 1991. The missing girls of China: a new demographic account. Population and Development Review 17:35–51.

Kane, P., and C. Y. Choi. 1999. China's one child family policy. The British Medical Journal 319:992–994.

Li, S. Z. 2007. Imbalanced sex ratio at birth and comprehensive intervention in China. 4th Asia Pacific Conference on reproductive and sexual health and rights.

Lutz, W., S. Scherbov, G. Y. Cao, Q. Ren, and X. Y. Zhang. 2007. China's uncertain demographic present and future. Vienna Yearbook of Population Research 2007:37–59.

Malcolm, P. 2006. China's One Child Policy. The British Medical Journal 333:361–362.

Merli, M. G., and A. E. Raftery. 2000. Are births underreported in rural China? Manipulation of statistical records in response to China's population policies. Demography 37:109–126.

Merli, M. G., and H. L. Smith. 2002. Has the Chinese family planning policy been successful in changing fertility preferences? Demography 39:557–572.

Qu, J. D., and T. Hesketh. 2006. Family size, fertility preferences, and sex ratio in China in the era of the one child family policy: results from national family planning and reproductive health survey. The British Medical Journal 333:371–373.

Tuljapurkar, S., N. Li, and M. W. Feldman. 1995. High sex ratio in China's future. Science 267:874–876.

Zhu, W. X. 2003. The One Child Family Policy. Archives of Diseases in Childhood 88:463–464.

J. Ky. Acad. Sci. 70(1):70–74. 2009.

An Improved Route to Substituted Cyclopenta[*c*]thiophenes: Synthesis of 5-Alkyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophenes and Sulfone Ester Precursor

Chad A. Snyder,[1] Amber J. Bell, Vineet V. Karambelkar, Joseph B. Scott, Riley G. Jones, Paul J. Orosz, and Jessica M. Wilson

Chemistry Department, Western Kentucky University, Bowling Green, Kentucky 42101

and

Nathan C. Tice

Chemistry Department, Eastern Kentucky University, Richmond, Kentucky 40475

ABSTRACT

An improved route to substituted cyclopenta[*c*]thiophenes was accomplished by treating 1,3-dimethyl-5,6-dihydro-4*H*-cyclopenta[*c*]thiophene-5-one (**1**) with alkyl Grignard reagents to obtain the 5-alkyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophenes, 5-methyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophene (**2**) and 5-ethyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophene (**3**), in good yield (60% and 65%, respectively). An important cyclopenta[*c*]thiophene precursor, 5-carbomethoxy-5-phenylsulfonyl–1,3-dimethyl-5,6-dihydro-4*H*-cyclopenta[*c*]thiophene (**9**) was synthesized, in an alternate route, by treating 3,4-bis(chloromethyl)-2,5-dimethylthiophene (**7**) with methyl phenyl sulfonyl acetate (**8**).

KEY WORDS: Cyclopenta[*c*]thiophene, thiapentalene, methyl phenyl sulfonyl acetate, heterocycles, Grignard

INTRODUCTION

Heterocycles and their fused-ring aromatic analogs have been of interest for their electronic and biological applications. These include electrical conductors, nonlinear optical devices, photoresists, synthetic biological tissue, solar cells, and transistors (Katritzky and Rees 1984; Heeger 1986; Kanatzidis 1990; Burroughes and Friend 1991; Roncali 1997; Dallemagne et al. 2002; Dallemagne et al. 2003). Various heterocycles have been incorported into conducting polymers, with the ability to display semiconducting properties when doped. Polypyrrole (Figure 1A) and polythiophene (Figure 1B), and their anologs, have been the most thoroughly investigated conductive polymers owing to their unique properties (air-stable, tractable, and have a low band gap). Their stability is the result of their lone-pair electrons on the sulfur and nitrogen atoms, which tend to stabilize the positive charges in the p-doped polymers (Heywang and Jonas 1992).

Other thiophene derivatives include thiophene fused heterocycles, such as cyclopenta[*c*]thiophenes (Figure 1C), cyclopenta[*b*]thiophenes (Figure 1D). Cyclopenta[*c*]thiophenes, cyclopenta[*b*]thiophenes, and their corresponding η^5 complexes are utilized in a broad range of applications. For instance, cyclopenta[*c*]thiophenes exhibit significant anti-tumor properties (Dallemagne et al. 2002; Dallemagne et al. 2003). Heterocycle-fused cyclopenta[*c*]thienyl zirconium complexes have been shown to catalyze 1-alkene polymerization (Ewen et al. 1998; Ryabov et al. 2002). We have a long-term interest in the structure and electronic properties of cyclopenta[*c*]thiophenes and their incorporation into electronic devices and present here initial results of the synthesis and characterization of some 5-alkyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophenes and a novel sulfone ester precursor.

METHODS

All reactions were carried out using standard Schlenk techniques under a nitrogen atmosphere unless otherwise noted. $CDCl_3$ (Cambridge Isotopes) was used without further

[1] Corresponding author e-mail: chad.snyder@wku.edu

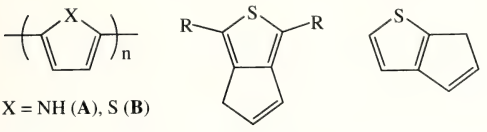


Figure 1. Structure of (A) Polypyrrole, (B) Polythiophene, (C) 1,3-Disubstituted-4*H*-cyclopenta[c]thiophene and (D) Cyclopenta[*b*]thiophene.

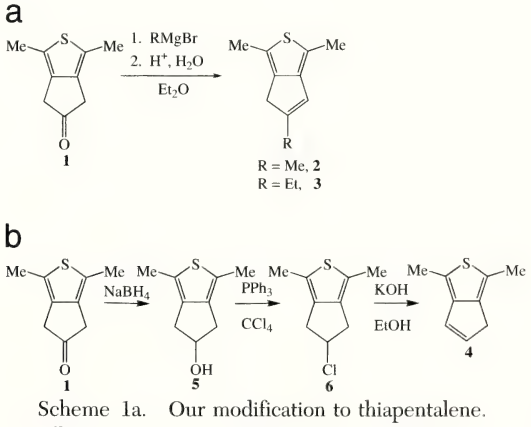
purification. 1,3-Dimethyl-5,6-dihydro-4*H*-cyclopenta[*c*]thiophene-5-one (**1**) precursors (2,5-dimethylthiophene, 3,4-bis(chloromethyl)-2,5-dimethylthiophene) were prepared according to literature methods (Cantrell and Harrison 1967). Paraformaldehyde (Eastman Chemicals), acetonyl acetone, P_4S_{10} (Acros), NaCN, DMF, and KH (Aldrich) were used without further purification. Grignard reagents methylmagnesium bromide and ethylmagnesium bromide (Aldrich) were used without further purification. Ethyl ether was dried over sodium benzophenone ketyl.

^{1}H and ^{13}C NMR spectra were recorded on a JOEL-500 NMR spectrometer at ca. 22°C and were referenced to $CDCl_3$. ^{13}C NMR spectra were listed as decoupled. Infrared spectra were recorded on Spectrum One FT-IR Spectrometer. Electron ionization (EI) mass spectra were recorded at 70 eV on a Varian Saturn GC/MS.

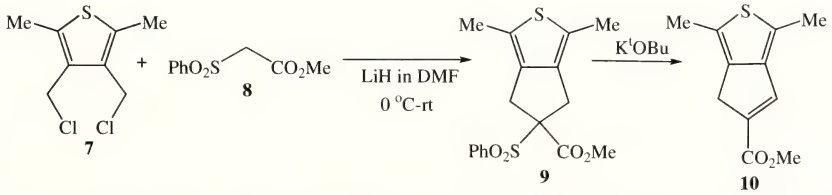
RESULTS

Ketone **1** was obtained according to previously reported methods (Wallace 1998). Compound **1** was then treated with an alkyl (R = Me, Et) Grignard reagent in dry ethyl ether, similar to those conditions employed by Ryabov and coworkers with 4,5-dimethyl-6*H*-cyclopenta[*b*]thiophene (Ryabov et al. 2002). In our case, ketone **1** (Scheme 1a) was treated with an alkylmagnesium bromide in ether at −70°C and then allowed to warm to room temperature. Stirring the solution for 10 min followed by acidic workup afforded the 5-alkyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophenes, 5-methyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophene (**2**) and 5-ethyl-1,3-dimethyl-4*H*-cyclopenta[*c*]thiophene (**3**), in good yield (60% and 65%, respectively) as amber oils.

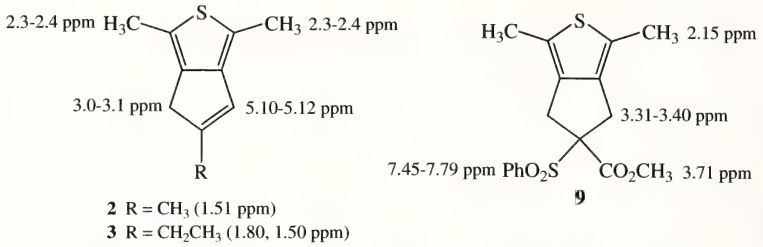
Dichloride **7** was synthesized according to previously reported methods (Wallace 1998). After two hours of stirring at 0°C, dichloride **7** was added to a solution of LiH and methyl phenyl sulfonyl acetate (**8**) in DMF and allowed to warm to room temperature (Scheme 2). These conditions are similar to



Scheme 1a. Our modification to thiapentalene.
Scheme 1b. Wallace and Selegue (Wallace 1998) modification to thiapentalene.



Scheme 2. Proposed route to 1,3-dimethyl 5-carbomethoxy-1,3-dimethyl-5,6-dihydro-4*H*-cyclopenta[*c*]thiophene (**10**).



2 R = CH_3 (1.51 ppm)
3 R = CH_2CH_3 (1.80, 1.50 ppm)

Figure 2. ^{1}H NMR chemical shifts for compounds **2**, **3**, and **9**.

those reported with 1,2-bis(chloromethyl) benzene (Scheme 3) (Palandoken et al. 1996). After stirring 48 hr, the reaction mixture was quenched with saturated NH_4Cl followed by aqueous workup to give the sulfone ester 5-carbomethoxy-5-phenylsulfonyl–1,3-dimethyl-5,6-dihydro-4*H*-cyclopenta[*c*]thiophene (**9**) in 76% crude yield. We currently are trying to optimize product isolation to improve the yield of sulfone ester **9**.

DISCUSSION

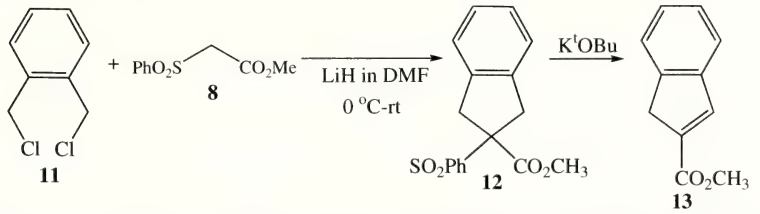
The synthesis of 4*H*-cyclopenta[*c*]thiophene, also known as 2-thiapentalene, and its 1,3-dichloro derivative was first reported by Skramstad (1969) via a 9-step synthesis. In 1998, Wallace and Selegue (Scheme 1b) improved the formation of 1,3-dimethyl-4*H*-cyclopenta[*c*]thiophene (**4**) (Wallace 1998), originally synthesized by Cantrell and Harrison (1967). Although we have been able to obtain thiapentalene **4** from 1,3-dimethyl-5,6-dihydro-4*H*-cyclopenta[*c*]-thiophene-5-one (**1**) using Wallace and Selegue's improved route, isolated yields for several steps were inordinately low. Although Wallace and Selegue reported high to nearly quantitative yields in each step, we were unable reproduce these yields, particularly for the ketone reduction step (30–35% at best). In an attempt to improve the methodology towards synthesizing substituted thiapentalenes, we reacted an alkyl Grignard reagent with ketone **1** (Scheme 1a). This reaction provides the thiapentalene in one step, as compared with three steps from compound **1** reported by Wallace and Selegue (Wallace 1998). Additionally, this system eliminates the low yielding reduction and chloro-substitution steps in favor of a higher yielding Grignard reaction.

NMR analysis of compounds **2** and **3** confirm the formation of thiapentalenes under the described conditions (Figure 2). The ^{1}H NMR spectra for thiapentalenes **2** and **3** display the thiophene methyl groups as two singlets at 2.3–2.4 ppm, as expected. As with thiapentalene **4**, the methylene protons are observed as singlets (3.0–3.1 ppm). The 5-methyl protons for **2** were observed as a singlet (1.51 ppm, 3H). Likewise, the ^{1}H NMR for **3** displayed a triplet (1.5 ppm, ^3J = 7 Hz, 3H) and quartet (1.8 ppm, ^3J = 7 Hz, 2H), corresponding to the ethyl group. The alkene protons for compounds **2** and **3** were observed as singlets at 5.10 and 5.12 ppm, respectively (Figure 2). In the ^{13}C NMR spectra for thiapentalenes **2** and **3** the thiophene methyl carbons were observed between 13.1–13.7 ppm. The methylene carbons were found at 29.7 ppm and 29.8 ppm, respectively. The vinyl and thiophene carbon resonances for **2** and **3** are observed between 120–140 ppm, typical for thiapentalenes reported (Snyder 2005; Tice 2006; Snyder et al. 2003; Snyder et al. 2005). For both thiapentalenes **2** and **3**, the absence of any resonances in the carbonyl region (180–200 ppm) indicated that the expected loss of the ketone substituent did occur. A summary of selected ^{1}H and ^{13}C NMR data can be found in Table 1. The IR spectrum showed loss of the carbonyl stretch (1668 cm^{-1}) for both thiapentalenes. IR analysis of compounds **2** and **3** showed the expected C_{sp3}-H stretches (2909, 2858 cm^{-1}) and C_{sp2}-H stretches (3050 cm^{-1}). All attempts to obtain an analytically pure

Table 1. Selected NMR data for thiapentalenes **2** and **3**.

Selected NMR data	**2**	**3**
δ_H ($SCCH_3$)[a]	2.32	2.40
δ_H (CH_2)[a]	3.00	3.01
δ_H (*CH*)	5.10	5.12
δ_C ($SCCH_3$)[a]	13.1, 13.7	13.4, 13.7
δ_C (CH_2)[a]	29.7	29.8
δ_C (*CR*CH)[a]	126.4, 140.8	126.5, 140.8

[a] $CDCl_3$.



Scheme 3. Synthesis of substituted indan from dichloride **11** (Palandoken et al. 1996).

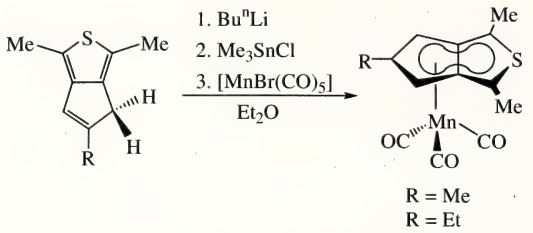
sample of compounds **2** and **3** were unsuccessful, as they readily decompose in air within minutes. Due to the low stability of **2** and **3** in air, we propose in the future to form these thiapentalenes and then *in situ* perform the metallation reactions. This will avoid any complications of exposing the free thiapentalenes to environmental conditions.

A proposed alternate route to 5-substituted cyclopenta[*c*]thiophenes involved 5,5′-fused thiophene ring synthesis in the first step beginning with 3,4-bis(chloromethyl)-2,5-dimethylthiophene (**7**) (Scheme 2). Our route was modeled after indan **13** synthesis where the authors report a two step synthesis beginning with dichloride **11** treated with activated methylene **8** to yield sulfone ester **12** (Palandoken et al. 1996). Compound **12** was then treated with K^{t}OBu to provide the indan **13** (Scheme 3). As part of the development of a general synthesis of cyclopenta[*c*]thiophenes, we attempted to dialkylate dichloride **7** with activated methylene **8** to give the sulfone ester **9** (Scheme 2).

Sulfone ester **9** was synthesized by C,C-dialkylation of dichloride **7** with activated methylene **8**, in good yield (76%). However, isolation of pure **8** has proved difficult and further attempts to isolate **9** are currently under way. In contrast to thiapentalenes **2** and **3**, ester **9** has high air and solution stability. As a result of this, we are confident that isolation of pure **9** should be attained and in good yield. Compound **9** (Scheme 2) was characterized by ^{1}H and ^{13}C NMR spectroscopy and displayed the expected chemical shifts. The ^{1}H NMR spectra for **9** showed a singlet (2.15 ppm, 6H) belonging to the thiophene methyl groups. Two doublets (3.31 and 3.40 ppm, both ^2J = 16.6 Hz, 4H) were observed belonging to both methylene groups. Palandoken et al. (1996) also reported two doublets (3.71 and 3.86 ppm, ^2J = 14.7 and 16.8 Hz, respectively) for their methylene protons. A second singlet (3.71 ppm, 3H) was observed belonging to the carbomethoxy group. Finally, benzylic protons were observed as two apparent triplets (7.54 ppm, ^3J = 7.45 Hz, 2H; 7.64 ppm, ^3J = 7.45 Hz, 1H) and a doublet (7.79 ppm, ^3J = 8.05 Hz, 2H) (Figure 2). ^{13}C NMR analysis of sulfone ester **9** showed a total of ten signals. A signal was observed δ32.9 belonging to the methylene carbon. The quaternary carbon was seen at 83.9 ppm. The methoxy carbon was found at 53.8 ppm, and the aromatic ppm values were observed at δ126.5, 128.8, 129.8, 134.2, 136.4, and 138.0. Finally the carbonyl carbon was observed at 168.5 ppm.

IR spectrum of **9** shows the expected carbonyl (1736 cm^{-1}), sulfone (1137, 1303 cm^{-1}), Csp^3-H (2920, 2848 cm^{-1}), and Csp^2-H (3073 cm^{-1}) stretches. GCMS analysis shows signals 318 (M^+-CH_4O), 290 (M^+-CO, CH_4O), and 152 (M^+-SO_2Ph, $C_2H_3O_2$).

Although it is known that free cyclopenta[*c*]thiophenes are unstable in solution and in the solid state, once complexed to a metal center these compounds display remarkable air and moisture stability (Wallace 1998; Snyder 2005; Snyder et al. 2005; Tice 2006). Our research goal is to eventually synthesize metal η^5-cyclopenta[*c*]thienyl complexes from thiapentalenes **3**, **4**, and **10**. Alkyl and aryl substituted cyclopenta[*c*]thienyl manganese complexes have already been synthesized and characterized according to Scheme 3. For the future, compounds **3**, **4**, and **10** will be deprotonated and then complexed to form the corresponding metallocenes. However, direct complexation with the lithium salts of these thiapentalenes has proven ineffective in the past. These lithium reagents are too strongly reducing to react directly with metal halides. For example, lithiated **4** reacts with [$MnBr(CO)_5$] to give mainly [$Mn_2(CO)_{10}$] and an oxidatively coupled bis(4H-cyclopenta [c]thiophene) (Wallace 1998; Snyder 2005; Snyder et al. 2005). In contrast, reaction of



R = Me
R = Et

Scheme 4. Proposed synthetic route for $[Mn(\eta^5\text{-}SC_7H_3\text{-}1,3\text{-}Me_2\text{-}5\text{-}R)(CO)_3]$ (Snyder et al. 2005).

lithiated **4** with Me_3SnCl forms the tin intermediate, $[SnMe_3(SC_7H_3\text{-}1,3\text{-}Me_2)]$, which react easily with $[MnBr(CO)_5]$ to give $[Mn(\eta^5\text{-}SC_7H_3\text{-}1,3\text{-}Me_2)(CO)_3]$ (Scheme 4) in 94% yield (Snyder et al. 2005). Thus, we plan to follow this same synthetic methodology in our metallation step as well. This should lead to thiapentalenyl complexes which display the same robust nature as those previously reported.

SUMMARY

Alkyl Grignard attack of ketone **1** afforded thiapentalenes **2** and **3**. The presence of the 5-alkyl substituted thiapentalenes was shown by ^1H, ^{13}C NMR and IR spectroscopy. Cyclopenta[*c*]thiophene precursor, sulfone ester **9**, was prepared in one step from dichloride **7** and activated methylene **8** in LiH in DMF. Compound **9** was isolated and characterized by ^1H and ^{13}C NMR spectroscopy, IR spectroscopy, and GCMS. Attempts to improve **3**, **4**, and **9** isolation conditions are being investigated.

ACKNOWLEDGEMENTS

We would like to acknowledge our sources of financial support, including Western Kentucky University's Chemistry department, their Office of Sponsored Programs, their Faculty Scholarship Council, and the WKU Materials Characterization Center.

LITERATURE CITED

Cantrell, T. S., and B. L. Harrison. 1967. 2-Thiapentalenyl anion. Tetrahedron Letters 45:4477–4480.

Dallemagne, P., L. P. Khanh, A. Alsaidi, O. Renault, I. Varlet, V. Collot, R. Bureau, and S. Rault. 2002. Synthesis and biological evaluation of cyclopenta[*c*] thiophene related compounds as new antitumor agents. Bioorganic & Medicinal Chemistry 10:2185–2191.

Dallemagne, P., L. P. Khanh, A. Alsaidi, I. Varlet, V. Collot, M. Paillet, R. Bureau, and S. Rault. 2003. Synthesis and biological evaluation of five-membered heterocycles fused to cyclopenta[*c*]thiophene as new antitumor agents. Bioorganic & Medicinal Chemistry 11:1161–1167.

Ewen, J. A., R. L. Jones, M. J. Elder, A. L. Rheingold, and L. M. Liable-Sands. 1998. Polymerization catalysts with cyclopentadienyl ligands ring-fused to pyrrole and thiophene heterocycles. Journal of the American Chemical Society 120:10786–10787.

Palandoken, H., W. T. McMillen, and M. H. Nantz. 1996. An improved synthesis of 2-(hydroxymethyl)indene. Organic Preparations and Procedures International 28:28702–704.

Ryabov, A. N., D. V. Gribkov, V. V. Izmer, and A. Z. Voskoboynikov. 2002. Zirconium complexes with cyclopentadienyl ligands involving a fused thiophene fragment. Organometallics 21:2842–2855.

Skramstad, J. 1969. Cyclopentathiophenes II. Synthesis of 4*H*-cyclopenta[*c*]thiophene. Acta Chemica Scandinavica 23:703–705.

Snyder, C. A. 2005. The synthesis, characterization, and structure of some cyclopenta[*c*]thiophenes and their manganese complexes. Unpublished dissertation, University of Kentucky.

Snyder, C. A., J. P. Selegue, E. Dosunmu, N. C. Tice, and S. Parkin. 2003. C,O-Dialkylation of Meldrum's Acid: synthesis and Reactivity of 1,3,7,7-Tetramethyl-4*H*, 10*H*-6,8,9-trioxa-2-thiabenz[*f*]azulen-5-one. Journal of Organic Chemistry 68:7455–7459.

Snyder, C. A., J. P. Selegue, N. C. Tice, C. E. Wallace, M. T. Blankenbuehler, S. Parkin, K. D. E. Allen, and R. T. Beck. 2005. Synthesis, Characterization, and Structure of Cyclopenta[*c*]thiophenes and Their Manganese Complexes. Journal of the American Chemical Society 125:15010–15011.

Tice, N. C. 2006. The synthesis, characterization, and reactivity of some organometallic-fused heterocycles. Unpublished dissertation, University of Kentucky.

Wallace, C. E. 1998. The synthesis, characterization, and reactivity of some ruthenocene-fused heterocycles. Unpublished dissertation, University of Kentucky.

J. Ky. Acad. Sci. 70(1):75–83. 2009.

The Upper Green River Barcode of Life Project

Jeffrey M. Marcus,[1] Devin D. Bell, Ashley N. Bryant, Emily C. Burden, Mollie E. Carter, Thomas J. Cataldo, Khrystin R. Clark, Heather E. Compton, Linze S. DeJarnette, V. Brooke Faulkner, Roger W. Gregory, Jason R. Hall, Lindsey N. Houchin, M. Elizabeth Hudson, Patrick F. Jenkins III, Jessica M. Jordan, Brandon K. Logan, Nicole R. Long, Hannah F. Maupin, Samantha R. McIntyre, J. Kaelen Mitchell, Justin K. Mobley, Allyson N. Nehus, Brittney N. Potts, Candace R. Read, K. Nicole Slinker, Chase E. Thompson, Tia M. Hughes, Douglas M. McElroy, and Robert E. Wyatt

Department of Biology, Western Kentucky University, Bowling Green, Kentucky 42101

ABSTRACT

The DNA barcoding initiative is an international effort to collect standardized DNA sequences from each Eukaryotic species to facilitate taxonomy and specimen identification. DNA barcoding experiments, because they are not technically difficult, are well suited to being used as investigative research experiences in a teaching laboratory. We have implemented a DNA barcoding exercise for our first year "Undergraduate Experience" students in which participants catch arthropods from our university field station, the Upper Green River Biological Preserve. The arthropod specimens were brought to the laboratory, mounted, photographed, and identified via keys and field guides based on morphological characters. This identification served as a working hypothesis for the identity of each specimen. A single leg was removed from each specimen, DNA was extracted, and a fragment of the *cytochrome oxidase I* gene was PCR amplified and sequenced. Then, using bioinformatics tools, the sequence for each specimen was compared to those in the Barcode of Life and Genbank nucleotide databases. A second species diagnosis based on DNA sequence matches was determined, which could be compared to the original morphological identification, serving as a test of that hypothetical species identity. In its first semester of implementation, 28 arthropod barcodes were produced, which will be augmented by the work of future classes.

KEY WORDS: Genetics education, DNA barcoding, Upper Green River Watershed, *cytochrome oxidase I*, arthropods

INTRODUCTION

The Barcode of Life Initiative is a worldwide consortium of researchers attempting to collect unique DNA sequence identifiers for all species of eukaryotic organisms to facilitate taxonomy and specimen identification (Hebert et al. 2003). The ultimate goal is to have multiple DNA sequence isolates from geographically diverse populations of each species of eukaryote on Earth. Thus every additional sequence, particularly from localities not well sampled by previous workers, represents a valuable addition to the Barcode of Life Initiative.

Thus far, work in animals has focused on a 658 base pair segment of mitochondrial *cytochrome oxidase I* gene (*COI*) for which nearly-universal polymerase chain reaction (PCR) primers are available (Folmer et al. 1994). Projects within this initiative generally have either focused on covering a taxonomic group (e.g., birds, fish) (Hebert et al. 2004; Marshall 2005; Kerr et al. 2007) or on creating an inventory of all of the species present in a particular locality (e.g., Great Smoky Mountains National Park, U.S.A.; Area de Conservación Guanacaste, Costa Rica (White 2005; Hajibabaei et al. 2006; Burns et al. 2007)).

We took the latter approach as a classroom exercise for our First Year "University Experience" students, initiating a DNA Barcode Project for the arthropods found at the Upper Green River Biological Preserve in Hart County, Kentucky, U.S.A. The preserve encompasses approximately 300 ha, lies 3 km up stream of Mammoth Cave National Park, and is home to 6 federally listed endangered species (none of which are terrestrial arthropods, and thus no endangered species were negatively affected by this project). University Experience courses at our institution usually are extended orientation and mentoring

[1] Corresponding author email: marcus@cc.umanitoba.ca

programs, but we felt that participation in a research project would considerably enrich the experience of the honors science majors enrolled in our sections of the course. A DNA barcoding project is ideally suited to first year undergraduates because it allows students to choose an organism that interests them, it is designed to include both fieldwork and laboratory work, the experimental techniques involved are not technically difficult, and yet the experimental protocols are sufficiently "high-tech" to motivate students.

MATERIALS AND METHODS

Student Participants

Two sections of first year college students (with enrollments of 12 and 14 students, respectively) conducted the experiments described here, under the direction of two instructors (J. M. Marcus and D. M. McElroy). All of the students enrolled in the course were participants in the Western Kentucky University Honors Program who had expressed an interest in becoming science majors, but not necessarily biology majors. Each student worked independently to identify an individual arthropod specimen. A more complete description of the student participants and a discussion of academic outcomes as a result of participating in the Upper Green River Barcode of Life Project can be found in Marcus et al. (In press).

Fieldwork

Field collections were conducted on 15–16 September 2006. Arthropods were collected using hand-held butterfly or sweep nets, and in a battery powered ultraviolet light trap which operated overnight (Winter 2000). Arthropods were selected because they are diverse, abundant, easy to capture, require no veterinary oversight, and a large library of taxonomic keys and field guides is available at our institution. No attempt was made for representative sampling of taxa, as the instructors anticipated that the students would be more excited about the project if they could chose which specimen to investigate themselves. Global Position System (GPS) coordinates were recorded for each specimen collected using a handheld Garmin GPS 12XL. Specimens were taken back to the laboratory, frozen at −20°C, mounted on insect pins, digitally photographed, identified based on morphology using keys and field guides, and a single leg from each specimen was removed and stored at −20°C for DNA extraction. The morphology-based species determinations made by each of the students served as the identification hypothesis that the students then tested via analysis of DNA sequence data. Voucher specimens from this project are maintained in the insect collection of Western Kentucky University.

Molecular Biology

DNA was extracted from legs with the QIAGEN DNEasy kit according to the manufacturer's instructions. DNA concentration and purity was determined spectroscopically using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). Final DNA concentration was approximately 50 ng/μL. We then prepared PCR reactions using 1 μL of each DNA sample, 1 μL each of forward and reverse primers (see below), 10 μL of Eppendorf Taq Polymerase Mastermix, and 12 μL of deionized distilled water. Unless otherwise specified, PCR reagents, primers, and conditions were as described in Marcus et al. (ms).

The primary PCR primer pair used was LCO1490 and HCO2198 (Folmer et al. 1994) which amplified a portion of the coding sequence of *cytochrome oxidase I (COI)* consisting of 710 base pairs (including primer sequences). PCR reaction conditions for these primers were 95°C for 5 min; 35 cycles of 94°C for 1 min, of 46°C for 1 min, 72°C for 1.5 min; and a 5 min final extension at 72°C before being placed on a 4°C hold in a BioRad MyCycler Thermocycler. If the initial PCR failed, it was repeated with the same primer. If the second PCR reaction also failed, as it did for 3 samples, alternate primer pairs Ron and Nancy or Tonya and Hobbes, which amplify an overlapping region of *COI*, were tried (Caterino and Sperling 1999; Monteiro and Pierce 2001). PCR reaction conditions for these primer pairs were 95°C for 5 min; 40 cycles of 95°C for 1 min, 46°C for 1 min, 72°C for 1.5 min; and 5 min final extension at 72°C before being placed on a 4°C hold. If these PCR reactions also failed more than once, we tried Jerry and Eva or Dick and Eva primers that amplify an adjacent region

spanning part of *COI* and a portion of *cytochrome oxidase II* (Blum et al. 2003). PCR conditions for these primer pairs were 4 cycles of 94°C for 30 sec, 48°C for 30 sec, 72°C for 1 min; followed by 29 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 1 min; and a 5 min final extension at 72°C before being placed on a 4°C hold.

Successful PCR amplifications were determined by 1% agarose gel electrophoresis in TAE buffer, followed by direct sequencing of PCR products in both directions with the amplification primers and the BigDye Terminator v3.1 Cycle Sequencing Kit and analyzed on an ABI 3130 capillary sequencer (Applied BioSystems). Sequences were edited in Sequencher 4.5 (Sequencher 2005) and then compared with sequences in the NCBI database by BLASTN (Altschul et al. 1997) and with sequences in the BOLD database (Ratnasingham and Hebert 2007) in order to identify each specimen on the basis of DNA sequence. Species identity was determined if there were a greater than 97% sequence match between a specimen in this study and a sequence in the database (Hebert et al. 2003), while a congeneric identification was made by a better than 90% sequence match (Hebert et al. 2004). DNA sequence-based identifications were then used to test the hypothesized species identification based on morphology.

Electronic Resources

For each specimen included in the project, students created a web page about the organism, including classification, digital photograph, GPS coordinates (with links to Google-Earth (2006)), maps, DNA sequence, BLASTN or BOLD identification results, a description of the biology of the organism, and references. To facilitate and standardize the web pages produced by the students, a set of ASP (Active Server Pages) applications was developed to allow the data entry, editing, and viewing of the pages in progress. The gateway to this on-line resource is available at: http://bioweb.wku.edu/faculty/Marcus/Barcode.html (Figure 1).

Phylogenetic analysis can assist in determining if newly sequenced species otherwise unrepresented in the databases can be assigned to the correct insect order. For the purposes of presentation here, 658 base pair *COI* sequences were aligned in Clustal W (Thompson et al. 1994) and most parsimonious trees were found in PAUP* 4.0610 (Swofford 1998) by 1000 replicate heuristic searches with random initial taxon arrangements. Bootstrap values were calculated for each node in the consensus tree from 1,000,000 fast stepwise addition heuristic search replicates, retaining compatible groupings <50%.

RESULTS

In the first implementation of the Upper Green River Barcode of Life project, sequences for 28 specimens were generated. Most specimens (25 of 28) could be amplified and sequenced successfully using the so-called "universal" *COI* primers, LCO1490 and HCO2198 (Folmer et al. 1994). The remaining 3 specimens (Bio175-09, Bio175-15, and Bio175-17) could not be amplified using LCO1490 and HCO2198, Ron and Nancy, or Tonya and Hobbes primers. The Jerry-Eva fragment of samples Bio175-09 and Bio175-15 and the Dick-Eva fragment of sample Bio175-17 were amplified and sequenced instead. Two samples that failed to amplify were exopterygote insects: *Spharagemon marmorata* (Harris) (Bio175-09, a grasshopper) and *Argia apicalis* (Say) (Bio175-15, a damsel fly). The third sequence (Bio175-17) was from a spider, which we tentatively identified as *Tetragnatha versicolor* Walckenaer based on morphology, but using BLAST to compare this sequence to the Genbank library showed its closest matches to beetles from the genus *Chaliognathus*. Sequences generated by this project have been deposited in Genbank accession numbers EU271647–EU271674.

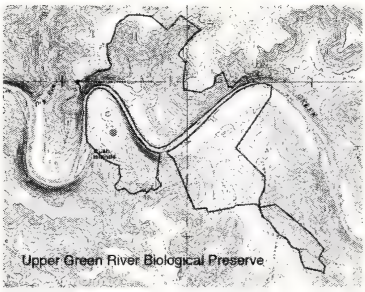
Of the 25 sequences that amplified successfully with the universal *COI* primers LCO1490 and HCO2198, 17 of them definitively matched sequences in the Genbank database, the BOLD database, or both, at 97% sequence identity or better, allowing identification at the species level by DNA sequence (Table 1). In 2006, when the initial analysis was made, two additional sequences for *Nicrophorus orbicollis* Say (Bio175-12, a burying beetle) and *Xylocopa virginica* (Linnaeus) (Bio175-24, a carpenter bee) matched

BIO 175 Honors - Upper Green River Barcode of Life Project

Back to the WKU Bar Code of Life (BoL) index

Collector	Nicole Long		
Collection Date	September 16, 2006	**Date added to DB:**	11/15/2006 11:15:56 AM
Scientific Name	*Apantesis phalerata*	Google search for *Apantesis phalerata*	
Common Name	Harnessed Tiger Moth		
Taxonomy	Superkingdom	Eukaryota	
	Kingdom	Animalia	
	Phylum	Arthropoda	
	Class	Insecta	
	Order	Lepidoptera	
	Family	Arctiidae	
	Genus	Apantesis	
	Species	phalerata	

Location map/ GPS Coordinates:



+37°14.669,-86°00.489 Google Maps Location

Collection Locality: Upper Green River Biological Preserve, gobbels Parcel, South Side, Hart County, Kentucky, USA

Organism Image/Size: *Apantesis phalerata*

Wingspan-4cm, Mass-.092g, Body length- 2.25cm

Organism Biology:

The Apantesis phalerata, harnessed tiger moth, is mostly black with cream-colored lines extending from the base and outlining the wings. There is often a pinkish color (sometimes orange) and black dots along the inner margins. The moth is found in Canada, and many areas in the United States. The seasons for the harnessed tiger moth cause adults fly from April to September in the south and May to August in the north.
Their diet consists of clover, cord grass, corn, dandelion, and plantain. (McLeod, 2005)

DNA Sequence:

AACATTATAT TTTATTTTTG GAATTTGAGC AGGTATAGTA GGAACATCTT TAAGATTATT AATTCGAGCA GAATTAGGAA ATCCCGGATC TTTAATTGGA GATGATCAAA
TTTATAATAC TATTGTAACA GCTCATGCTT TTATTATAAT TTTTTTTATA GTTATACCTA TTATAATTGG AGGATTCGGT AATTGATTAG TACCCCTTAT ATTAGGAGCA
CCTGATATAG CTTTTCCCCG AATAAATAAT ATAAGTTTTT GACTTTTACC CCCATCACTA ACTTTATTAA TTTCAAGAAG AATTGTAGAA AATGGAGCAG GAACAGGATG
AACCGTGTAC CCCCCACTTT CTTCTAATAT TGCTCATGGG GGGAGATCTG TCGATTTAGC TATTTTCTCC CTTCATTTAG CGGGAATTTC TTCAATTCTA GGAGCTATTA
ACTTTATTAC TACAATTATT AATATACGAT TAAATAATTT ATCATTTGAC CAAATACCTT TATTTGTTTG AGCGGTTGGA ATTACAGCTT TTTTATTACT CCTTTCACTC
CCTGTTTTAG CCGGAGCCAT TACTATATTA TTAACAGATC GAAATTTAAA TACATCCTTT TTTGATCCTG CAGGAGGGGG AGATCCTATT TTATATCAAC ATTTATTT

BLAST

Bar Code of Life Matches

Arthropoda Insecta Lepidoptera Arctiidae Apantesis phalerata 99.84
Arthropoda Insecta Lepidoptera Arctiidae Apantesis sp. 99.69
Arthropoda Insecta Lepidoptera Arctiidae Apantesis sp. 99.69
Arthropoda Insecta Lepidoptera Arctiidae Apantesis phalerata 99.69
Arthropoda Insecta Lepidoptera Arctiidae Apantesis sp. 99.64
Arthropoda Insecta Lepidoptera Arctiidae Apantesis phalerata 99.53
Arthropoda Insecta Lepidoptera Arctiidae Apantesis phalerata 99.53
Arthropoda Insecta Lepidoptera Arctiidae Apantesis phalerata 99.53
Arthropoda Insecta Lepidoptera Arctiidae Apantesis phalerata 99.38
Arthropoda Insecta Lepidoptera Arctiidae Apantesis carlotta 99.38

My assumption that the moth was an Apantesis phalerata (Harnessed Tiger Moth) was correct. The top matching result from The Barcode of Life supports this identification.

References

Bar Code of Life Data Systems. Accessed November 29, 2006.
www.barcodinglife.org

McLeod, Robin and Anta Gould. "Species Apantesis phalerata - Harnessed Tiger Moth -Hodges#8169".Bugguide. 15 Nov. 2006.

Back to the WKU Bar Code of Life (BoL) index

Figure 1. Example of a species web page produced by a student participant in the Upper Green River Barcode of Life Project.

Table 1. Specimen identification by comparison with *COI* DNA barcode sequences available in Genbank (NCBI) and the Barcode of Life Database (BOLD).

Specimen	NCBI blast maximum identity	BOLD specimen similarity	Final identification
Bio 175-2, 11, 20	*Apantesis nais* 95%, 95%, 95%	*Apantesis phalerata* 99.84%, 99.84%, 99.82%	*Apantesis phalerata* (Harris)
Bio175-15	*Erythromma najas* 84%	*Libellula luctuosa* 89.82%	*Argia apicalis* (Say)
Bio175-7, 13	*Argiope trifasciata* 98%, 98%	*Argiope trifasciata* 99.49%, 99.66%	*Argiope trifasciata* (Forsskål)
Bio175-6	*Battus philenor* 99%	*Battus philenor* 100%	*Battus philenor* (Linnaeus)
Bio175-21	*Heliothis proruptus* 91%	*Catocala piatrix* 100%	*Catocala piatrix* Grote
Bio175-14	*Catocala praeclara* 94%	*Catocala retecta* 99.62%	*Catocala retecta* Grote
Bio175-19	*Drosophila pilosa* 83%	*Phytomyptera sp.* 84.01%	*Chauliognathus pennsylvanicus* DeGeer
Bio175-10	*Chlorochlamys chloroleucaria* 99%	*Chlorochlamys chloroleucaria* 100%	*Chlorochlamys chloroleucaria* (Guenée)
Bio175-16	*Cupido osiris* 95%	*Cupido comyntas* 100%	*Cupido comyntas* (Godart)
Bio175-1, 27	*Grammia parthenice* 99%, 99%	*Grammia parthenice* 99.69%, 99.84%	*Grammia parthenice* (Kirby)
Bio175-4	*Halysidota tessellaris* 97%	*Halysidota harrisii* 100%	*Halysidota harrisii* Walsch
Bio175-28	*Junonia coenia* 99%	*Junonia coenia* 99.68%	*Junonia coenia* (Hubner)
Bio175-25	*Phalangium opilio* 76%	*Leuciacria olivei* 87.96%	*Leiobunum vittatum* Say
Bio175-18	*Neoconocephalus sp.* 91%	*Neoconocephalus sp.* 91.46%	*Neoconocephalus palustris* (Blatchley)
Bio175-12	*Nicrophorus orbicollis* 98%	*Nicrophorus sp.* 99.18%	*Nicrophorus orbicollis* Say
Bio175-22, 26	*Eurema mexicana* 92%, 92%	*Phoebis sennae* 99.85%, 100%	*Phoebis sennae* (Linnaeus)
Bio175-3	*Polistes metricus* 99%	*Polistes metricus* 99.85%	*Polistes metricus* Say
Bio175-5	*Urophora quadrifasciata* 85%	*Copecrypta sp.* 86.86%	*Promachus hinei* Bromley
Bio175-8	*Rabidosa punctulata* 93%	*Rabidosa rabida* 97.84%	*Rabidosa rabida* (Walckenaer)
Bio175-9	*Phlaeoba albonema* 87%	*Melanoplus marshalli* 90.53%	*Spharagemon marmorata* (Harris)
Bio175-23	*Spilosoma virginica* 97%	*Spilosoma virginica* 100%	*Spilosoma virginica* (Fabricius)
Bio175-17	*Chauliognathus sp.* 91%	No match	*Tetragnatha versicolor* Walckenaer
Bio175-24	*Xylocopa collaris* 93%	*Xylocopa virginica* 99.85%	*Xylocopa virginica* (Linnaeus)

at the level of genus (greater than 90% sequence identity), but were the first sequenced exemplars for their species. Since 2006, sequences from both species have been added to the databases, and species matches can now be made at the 97% sequence identity level. A further four specimens: *Chauliognathus pennsylvanicus* DeGeer (Bio175-19, a soldier beetle), *Neoconocephalus palustris* (Blatchley) (Bio175-18, a conehead katydid), *Promachus hinei* Bromley (Bio175-05, a robberfly), and *Leiobunum vittatum* Say (Bio175-25, a daddy-longleg spider) did not match any *COI* sequences at the 90% sequence identity threshold for matching to genus and are probably the first sequences for this part of the *COI* gene for their respective genera. Molecular identifications generally confirmed the students' identifications based on morphology, though in the case of several spiders and lepidopteran larvae, the molecular identifications suggested that the specimens belonged to different families than were originally hypothesized.

Phylogenetic analysis is an integral part of eukaryotic bar coding projects because it allows the verification of species identities and taxonomic relationships (Meusnier et al. 2008). Phylogenetic analysis of 658 base pairs in *COI* revealed 284 constant characters, 74 variable but parsimony uninformative characters, and 300 parsimony informative characters. A strict consensus of 4 most parsimonious phylogenetic trees showing the relationships of the 25 *COI* sequences produced by this study is shown in Figure 2. For the most part, phylogenetic analysis of the *COI* sequences assigns insects to a clade with other members of their order. The exceptions to this generalization are species in poorly sampled taxonomic groups in which relatively few *COI* sequences are available (e.g., beetles, flies, spiders) both in our data set and in the public databases. Rapidly evolving genes like *COI* reach saturation quickly, making them poor sequences for resolving deep phylogenetic nodes (Huet et al. 2002), and thus the bootstrap support for the relationships among and between members of these groups is very weak in our tree (Figure 2).

DISCUSSION

DNA barcoding experiments are extremely well suited to being used as exploratory research projects in a teaching laboratory. The experiment allows students to determine what organism they wish to study, providing an investigative component to this structured inquiry exercise (McKenzie and Glasson 1997). The exercise allows the students to go through an entire cycle of the scientific method (Gower 1996). First they use preliminary observations of the morphology of an organism to generate a hypothetical species assignment. Then, they conduct an experiment (PCR and sequencing) that allows them to collect data to test their hypothesis. Then, the students interpret the data by comparing the DNA sequences that they generated with those that other researchers have collected to come up with a DNA-based species assignment. Comparing the two species determinations allows them to test their morphology-based hypothesis. If the two species assignments disagree, then the students need to account for the disagreement by generating a further hypothesis. Finally, through their efforts, the students collected data that has been deposited in Genbank and will be useful to the community of researchers who are interested in creating a library of genetic barcodes with broad geographic and taxonomic representation.

When morphological and molecular diagnoses disagreed substantially, students needed to think critically about their data. For example, it was determined that the *COI* sequence obtained for a spider identified on the basis of morphology as *Tetragnatha versicolor* (Bio175-17) most closely matched *COI* sequences in the database from the beetle genus *Chaliognathus*. A portion of *COI* from *T. versicolor* has been sequenced by another laboratory and submitted to Genbank (Agnarsson and Blackledge 2008). On the basis on this submitted sequence, we have been able to determine that none of our primer pairs would be expected to produce a PCR amplification product from this spider because it has a divergent *COI* sequence. *Chaliognathus* beetles were among the most abundant insects at the Upper Green River Biological Preserve on the days that we made collections, so it is not unreasonable to suspect that a predatory spider may have captured a beetle as prey, and while manipulating the prey item, may have had some beetle hemo-

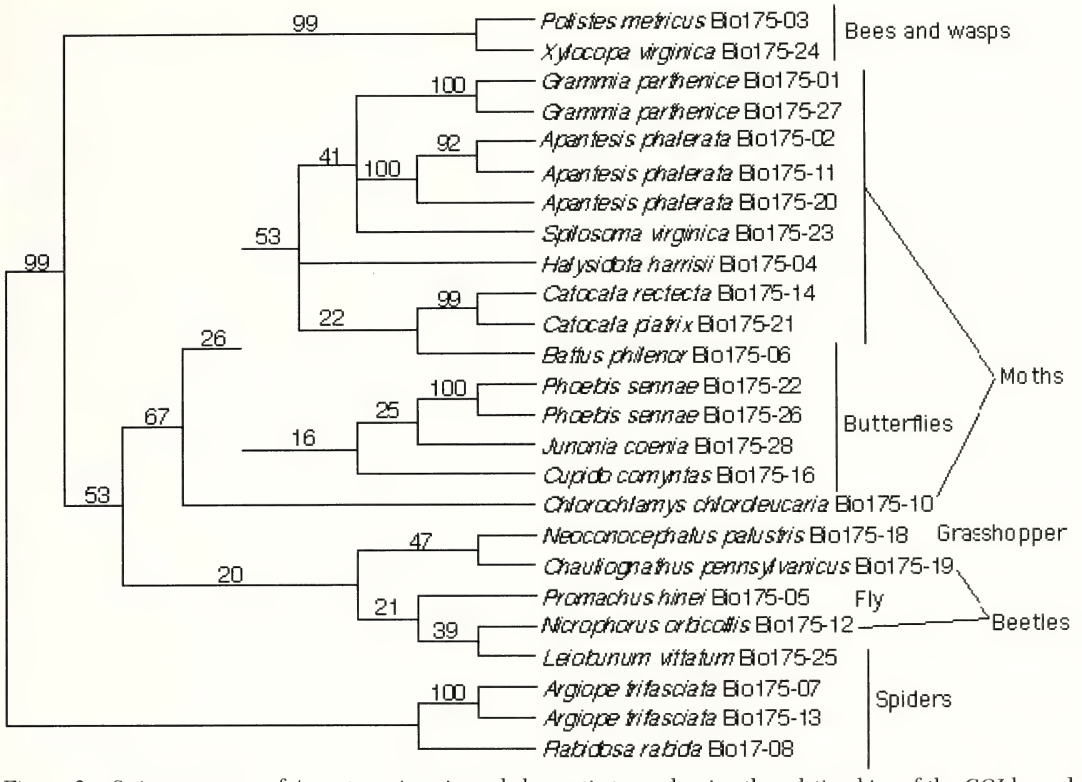


Figure 2. Strict consensus of 4 most parsimonious phylogenetic trees showing the relationships of the *COI* barcode sequences obtained from this study. Numbers above each node show the bootstrap support associated with that node. Specimens from the same species form monophyletic groups. In general, specimens from the same insect order also grouped together in monophyletic groups. The only exceptions to this generalization are associated with nodes with only low bootstrap support.

lymph (blood) adhere to its legs, one of which we later used for DNA barcoding analysis. It is perhaps noteworthy that while we were able to amplify a substantial part of the coding sequence of *COI* from *Chauliognathus pennsylvanicus* DeGeer (Bio175-19) from a beetle specimen, but we were only able to amplify a small and non-overlapping portion of *Chaliognathus COI* from the *T. versicolor* spider specimen, using an alternate primer pair. This is consistent with the presence of degraded *Chaliognathus* DNA molecules being present on the legs of the spider, which was then amplified by the primers which did not match *T. versicolor COI* sequences.

There are several potential problems that others may encounter if they wish to conduct a DNA barcoding project. The most significant to these is that no PCR primers are truly universal and there will always be samples that fail to amplify with any given primer pair. Courses that implement a barcode of life project will either have to set aside class time in which students can participate in troubleshooting exercises, or they will have to devote time and trained personnel outside of class to deal with samples that fail to amplify. In our experience, the endopterygote insects are more likely to amplify successfully with the standard LCO1490 and HCO2198 primers (Folmer et al. 1994) used for barcoding than less derived insects and spiders. It is also very important to have on hand as diverse an array of keys and field guides to the local fauna as possible for student use. Some regions and some organisms have better coverage than others, and instructors may wish to steer students towards organisms that are easier to identify on the basis of morphological characters. Similarly, DNA barcode sequencing efforts have also been uneven. Many more sequences are available for some taxa (e.g.,

birds, fish, butterflies and moths), than for others (e.g., flies, beetles, spiders). The best verifications of morphological identifications will be in those taxa for which much sequence data is already available, but the greatest contributions students can make towards the international barcoding initiative (Hebert et al. 2003) will be in those taxa for which few sequences are now available.

At the local level, through the Upper Green River Barcode of Life project, we are creating an electronic field guide to the arthropod fauna of the Upper Green River Preserve. In the future, we plan to coordinate this project with other faunal surveys in our region such as Kentucky Butterfly Net (Covell et al. in press). We also plan to add sequence data and web pages for additional species, creating a rich resource for researchers at the Upper Green River Biological Preserve and elsewhere for specimen identification and species distribution information.

ACKNOWLEDGEMENTS

Thanks to Scott Grubbs, Albert Meier, Ouida Meier, and Mike Stokes for facilitating our work at the Upper Green River Biological Preserve. Anonymous reviewers and David White provided useful comments on an earlier draft of this manuscript. We also thank Ann Cutler, Bruce Grant, and Joanne Seiff for helpful suggestions. This project received financial support from the Western Kentucky University Honors Program, the Department of Biology, and the Ogden College of Science and Engineering. J. M. M. and T. M. H. are supported by grants from National Science Foundation and the Commonwealth of Kentucky (EPS-0132295 and 0447479), a grant from the US Environmental Protection Agency (X796463906-0), and a grant from the National Institutes of Health and National Center for Research Resources (P20 RR16481).

LITERATURE CITED

Agnarsson, I., and T. A. Blackledge. 2008. Can a spider web be too sticky? Tensile mechanics constrains the evolution of capture spiral stickiness in orb weaving spiders. Submission to Genbank Accession. FJ525317.

Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25:3389–3402.

Blum, M. J., E. Bermingham, and K. Dasmahapatra. 2003. A molecular phylogeny of the neotropical butterfly genus *Anartia* (Lepidoptera: Nymphalidae). Molecular Phylogenetics and Evolution 26:46–55.

Burns, J. M., D. H. Janzen, M. Hajibabaei, W. Hallwachs, and P. D. N. Hebert. 2007. DNA barcodes of closely related (but morphologically and ecologically distinct) species of skipper butterflies (Hesperiidae) can differ by only one to three nucleotides. Journal of the Lepidopterists' Society 61:138–153.

Caterino, M. S., and F. A. H. Sperling. 1999. *Papilio* phylogeny based on mitochondrial *cytochrome oxidase I* and *II* genes. Molecular Phylogenetics and Evolution 11:122–137.

Covell, C. V., B. D. Marcus, and J. M. Marcus. In press. KY Butterfly Net: an Interactive Web Database to Facilitate Lepidoptera Research and Education in Kentucky. Journal of the Lepidopterists Society.

Folmer, O., M. B. Black, W. Hoch, R. A. Lutz, and R. C. Vrijehock. 1994. DNA primers for amplification of mitochondrial *cytochrome c oxidase subunit I* from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294–299.

GoogleEarth. 2006, Mountain View, CA: Google.com.

Gower, B. 1996. Scientific Method: a Historical and Philosophical Introduction. Routledge, New York.

Hajibabaei, M., D. H. Janzen, J. M. Burns, W. Hallwachs, and P. D. N. Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences USA 103:968–971.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B-Biological Sciences 270:313–321.

Hebert, P. D. N., M. Stoeckle, T. S. Zemlak, and C. M. Francis. 2004. Identification of birds through DNA barcodes. Public Library of Science Biology 2:1657–1663.

Huet, F., J. T. Lu, K. V. Myrick, L. R. Baugh, M. A. Crosby, and W. M. Gelbart. 2002. A deletion-generator compound element allows deletion saturation analysis for genomewide phenotypic annotation. Proceedings of the National Academy of Sciences USA 99:9948–9953.

Kerr, K. C., M. Y. Stoeckle, C. J. Dove, L. A. Weigt, C. M. Francis, and P. D. N. Hebert. 2007. Comprehensive DNA barcode coverage of North American Birds. Molecular Ecology Notes 7:535–543.

Marcus, J. M., A. L. Harper, T. M. Hughes, M. R. Johnson, A. B. Maupin, A. B. Polen, T. B. Powell, T. H. Shehan, D. B. Ritland, and C. V. Covell. ms. Phylogenetics and hybridization in the North American butterfly genus *Limenitis* (Nymphalidae) and the origins of the aberrant *Limenitis* form *rubidus* (Strecker). Submitted to BioMed Central Evolutionary Biology.

Marcus, J. M., T. M. Hughes, D. M. McElroy, and R. E. Wyatt. In press. Engaging First Year Undergraduates in Hands-On Research Experiences: the Upper Green River Barcode of Life Project. Journal of College Science Teaching.

Marshall, E. 2005. Will DNA bar codes breathe life into classification? Science 307:1037.

McKenzie, W. L., and G. E. Glasson. 1997. Investigative Learning in Undergraduate Freshman Biology Laboratories. Journal of College Science Teaching 27:189–193.

Meusnier, I., G. A. C. Singer, J.-F. Landry, D. A. Hickey, P. D. N. Hebert, and M. Hajibabaei. 2008. A universal DNA mini-barcode for biodiversity analysis. BioMed Central Genomics 9:214 doi:10.1186/1471-2164-9-214.

Monteiro, A., and N. E. Pierce. 2001. Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from *COI*, *COII* and *EF-1a* gene sequences. Molecular Phylogenetics and Evolution 18:264–281.

Ratnasingham, S., and P. D. N. Hebert. 2007. BOLD: the Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7:355–364.

Sequencher. 2005. Version 4.5.4.5. Gene Codes Corporation, Ann Arbor, MI.

Swofford, D. L. 1998. PAUP*, Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

White, P. S. 2005. Chairman's Message: Paul Hebert and the DNA barcodes of life. All Taxa Biological Inventory Quarterly 6:2.

Winter, W. D. 2000. Basic Techniques for Observing and Studying Moths and Butterflies. The Lepidopterists' Society, Los Angeles, CA.

J. Ky. Acad. Sci. 70(1):84–93. 2009.

Pre-service Teacher Education Online: Student Opinions from a Science Methods Course

Wilson J. González-Espada[1]

Department of Earth and Space Sciences, Morehead State University, Morehead, Kentucky 40351

ABSTRACT

Because of increased technology access and convenience, as well as potentially lower costs, distance education and internet courses are becoming more commonplace in higher education. As the popularity of these remote modes of instruction increases, it is important to carefully consider to what extent pre-service teachers can be adequately prepared for their role as educators via the internet compared with pre-service teachers that complete their degrees traditionally. An examination of the science education literature demonstrates serious research gaps regarding the long-term preparation of educators through online courses. The purpose of this paper was to provide a critical perspective on the teaching of undergraduate science methods courses online from the viewpoint of the students' opinions provided through course evaluations. Areas of interest included the dichotomy theory vs. practice, the role of field experiences, students' perceived satisfaction with online courses, inquiry, and the difficulties of valid assessment.

KEY WORDS: Distance education, online courses, science, science methods, teacher preparation

INTRODUCTION

No one can argue that a shortage of qualified science and mathematics teachers can result in many students without the tools they need to achieve scientific literacy (Lavoie 1997). The push for more and better science teachers has produced a shift from traditional courses to those offered online (Barab et al. 2001; Garrison and Anderson 2003; Schrum et al. 2007). Online education delivers courses in remote sites or off-campus in a variety of natural and social science disciplines (Burguess 1997; Collins and Pascarella 2003). One of the promises of distance education is to reach rural underserved science teachers who are isolated from other colleagues (Lynch 2000; Annetta and Shymansky 2006).

Statistics describing online learning present a changing paradigm in higher education. According to Allen and Seaman (2007), in 2009 more than 4.2 million students will be taking at least one online class, with most demand for associate and masters degrees. The nontraditional nature of many students explains why distance education has skyrocketed (Roach and Lemasters 2006). Other reasons for the growth of online education include convenience, limited local availability of traditional courses, and scheduling conflicts (Barab et al. 2001; Rowe and Asbell-Clarke 2008).

Most of the literature related to online education focuses on professional development (Lavoie 1997; Annetta and Shymanksky 2006; Jaffe et al. 2006; Whitehouse et al. 2006; Rowe and Asbell-Clarke 2008), graduate courses (Barab et al. 2001; Smith-Strickland and Butler 2005; Spooner et al. 1999), and science content courses (Collins 1997; Cannon 2002; Harlen and Altobello 2003; Rowe and Asbell-Clarke 2008). A much smaller portion of the literature focuses on science education courses at the graduate level (Rodrigues 1999; Harlen 2004).

Many of these studies compared traditional and online courses and concluded that there is no significant difference between traditional and online delivery modes (Barry and Runyan 1995; Navarro and Shoemaker 1999; Sujo de Montes and Gonzales 2000; Cannon 2002; Collins and Pascarella 2003). For instance, a recent meta-analysis conducted by Zhao et al. (2005) found that about two thirds of the studies favored distance education whereas the rest showed the opposite. This study agrees with the general belief that online programs and courses vary wildly in quality (Talvitie-Siple 2006). Conversely, some researchers have pointed out that technology cannot replace the human factor in learning and that technology is not nearly as important as other variables, such as learning tasks,

[1] w.gonzalez-espada@moreheadstate.edu

learner characteristics, student motivation, and the role of the instructor (Merisotis and Phillips 2001).

The conclusion that online courses are just as good as traditional courses must be taken with the proverbial grain of salt (Schrum et al. 2007). Researchers have pointed out methodological shortcomings in the literature, such as anecdotal accounts, a lack of control for extraneous variables, non-randomly selected subjects, participant's self-reporting and self-selection, using instruments with questionable reliability and validity, and not adequately controlling for the context, feelings, and attitudes of the students and faculty studied (Cannon 2002; Collins and Pascarella 2003; Dede et al. 2006; Whitehouse et al. 2006). Furthermore, many studies fail to demonstrate cause and effect to propose predictive models (Merisotis and Phillips 1999) or to measure long-term effects in changing teacher behaviors and their impact on student learning (Whitehouse et al. 2006; National Research Council 2007).

Specifically in science education, online education has been identified as a rapidly growing phenomenon (Rowe and Asbell-Clarke 2008). As recently as September 2008, the National Science Teachers Association (NSTA) issued a position statement in which e-learning is supported and encouraged "for preK–16 science students, as well as for science educators engaging in professional development in the traditional, informal, or distance learning environment." In addition, NSTA supports an integration of traditional and online learning: "Traditional classroom instruction that incorporates the planned and effective use of collaborative and/or interactive digital tools and resources, blended learning experiences that incorporate various combinations of technology-mediated and traditional classroom instruction, and distance delivered courses or programs." (National Science Teachers Association 2008).

In the case of novice "practice-oriented" courses, such as medicine and science methods, the literature is almost nonexistent. In medicine, for example, the literature emphasizes successful online professional development, never beginning physician training (Beitz and Snarponis 2006; Chumley et al. 2002; Cuellar 2002; Curran et al. 2006; Fordis et al. 2005; Ruiz et al. 2006; Wutoh et al. 2004; Winters and Winters 2007). Similarly, many studies assessing online science education courses describe professional development or graduate courses. Articles reporting on pre-service science methods courses using a blended approach (Bodzin and Park 2000) or delivered fully online were "nonexistent" just a few years ago (Cannon 2002; Wallace 2003).

The only research study that included pre-service teachers and their perception of online science methods courses was published by Noh (2004). In this study, the author concluded that although many preferred online courses, the preference was not as strong compared with inservice teachers. This author concluded that traditional/online blends might work best for pre-service science teachers.

The fact is that little is known about best practices in the design and implementation of online science methods courses (Whitehouse et al. 2006) and whether these courses are consistent with proven methods for conceptual change in teacher education, such as actively engaging teachers, modeling appropriate inquiry, and envisioning teachers as lifelong learners (Loucks-Horsley et al. 1998; Barab and Duffy 2000).

The purpose of this study is to report students' opinions of an online science methods course for elementary teachers offered at a regional, rural, public university in the South and, from these perceptions, to discuss implications for online undergraduate teacher training. This analysis was framed from the perspective of end-of course student evaluations and the informed viewpoint of the instructor who designed and taught the course. The course is called Science Education in the Elementary School (SEES) and was described in the university catalog as follows:

> [This course is] an overview of the most recent and research-based strategies and techniques for planning, teaching, and assessing elementary science. Inquiry-based methods and other constructivist approaches as described in the National Science Education Standards will be emphasized. Design and execution of learning activities for an elementary school setting are required.

Pre-requisites for the course included junior standing, the completion of the courses

Introduction to Early Childhood Education and Field-Based Experience Seminar in Early Childhood, and at least six hours of college science courses.

SEES uses Peters and Stout (2006) as the main textbook. However, a number of supplementary resources are used including the Essential Science for Teachers and Case Studies in Science Education video series from Annenberg/CPB (1997, 2004a, 2004b), an online inquiry videocases module (Lesson Lab Research Institute 2008), and a variety of readings on the nature of science (McComas 1998), laboratory safety (Arkansas Science Teachers Association 1999), teaching evolution and the nature of science (National Academy of Science 1998), assessment (Atkin et al. 2001), the Arkansas K-8 science curriculum frameworks (Arkansas Department of Education 2005), and multicultural issues in the science classroom (Northwest Regional Education Laboratory 1997). In addition, between four and six 30-minute field experiences in an elementary science classroom were required.

Assessments for SEES consisted on weekly reports on the various readings and videos, two closed-book partial tests based on the material covered in Peters and Stout (2006), and a comprehensive final test. The field experiences were assessed based on an evaluation form completed by the guest teacher at the field site, the lessons submitted prior to teaching, and a critical self-reflection of the experience.

The research questions that guided this study were (a) is there a difference in the quantitative end-of-course evaluation of online and traditional versions of the same science methods course and (b) what course components were perceived by students as more or less effective in helping them learning the course material? The study is important for two main reasons. First, it addresses the implementation and evaluation of an online pre-service science methods course, an area where a gap in the literature has been identified (Cannon 2002; Wallace 2003). Second, by describing the course in the context of a rural, public university, this study helps to understand better the needs of rural pre-service science teachers, a group that is underserved in terms of access and resources for quality teaching (Annetta and Shymansky 2006; Lynch 2000).

METHODS

The study's data came from end-of-course evaluation forms, a source of information previously used in similar publications (Spooner et al. 1999). A total of 101 participants, mostly junior and senior female students, took SEES with the same instructor between 2003 and 2008 and completed end-of-course evaluation forms. Of those, 41 students were enrolled in on-campus sections and 60 were enrolled in online sections.

The end-of-course evaluation forms included a Likert-scale section with the following 10 statements. The word or phrase in parentheses is the abbreviation used in the findings section:

1. The instructor was knowledgeable in the subject matter of the course (knowledge).
2. The instructor effectively presented the content of the course (effectiveness)
3. The instructor was well prepared for each class (preparation).
4. The class time was a valuable experience in helping my understanding (class time).
5. The instructor was available during scheduled office hours (availability).
6. The instructor fairly evaluated my work in this course (fair grading).
7. The textbook required for the course was useful (textbook).
8. The instructional aides, e.g., audio visual, web, etc., were beneficial (teaching aides)
9. The instructor is fluent in English (English).
10. LThe instructor's overall performance as a teacher was excellent (excellence).

Students had to evaluate each statement in a scale from "1" (strongly disagree) to "5" (strongly agree).

In addition, a section for students to express themselves about the strengths of the course, suggestions for improvement, and general comments was available. Not all students who completed the Likert-scale section of the evaluation form included written comments.

Unfortunately, only aggregated quantitative data for each question was available. As a consequence, it will be analyzed using descriptive statistics. The qualitative data from

Table 1. Pooled end-of-course evaluation scores for traditional students (on-campus) and online students.

Evaluation statement	Traditional (n = 41)	Online (n = 60)	Difference
The instructor was knowledgeable in the subject matter of the course	4.64	4.55	0.09
The instructor effectively presented the content of the course	4.36	4.15	0.21
The instructor was well prepared for each class	4.63	4.46	0.17
The class time was a valuable experience in helping my understanding	4.11	4.00	0.11
The instructor was available during scheduled office hours	4.34	4.40	−0.06
The instructor fairly evaluated my work in this course	4.55	4.22	0.33
The textbook required for the course was useful	3.42	3.81	−0.39
The instructional aides, e.g., audio visual, web, etc., were beneficial	4.15	3.94	0.21
The instructor is fluent in the English language	3.98	4.28	−0.30
The instructor's overall performance as a teacher was excellent	4.43	4.07	0.36

students enrolled in the online SEES is more abundant and will provide a better context for understanding how students were experiencing the online science methods course. This data was transcribed and prepared for analysis using qualitative techniques (Merriam 2001; Rubin and Rubin 1995). The data were classified according to each of the three main areas addressed in the end-of-course evaluation form, that is, strengths of the course, suggestions for improvement, and other comments. The students' responses were compared and contrasted, a process that revealed both common responses and contradicting perspectives, which created the categories and themes presented in the results section. Representative quotes from the students were used to present the emerging themes. The methodology is consistently used in qualitative educational research (Creswell 1998).

RESULTS

It is important to emphasize that the following data represent the students' opinions after completing the online science methods course as expressed in their end-of-course evaluations. Because this study does not have data from an objective, external observer, generalizations should be carefully constructed by the reader.

The quantitative data (Tables 1, 2) suggest that the students enrolled in traditional sections of SEES perceived that the instructor was more knowledgeable of the content taught in the science methods course (92.8% vs. 91.0%), that the content of the course was presented more effectively (87.2% vs. 82.8%), that the instructor was better prepared (92.6% vs. 89.2%), and that the time engaged in the class was more valuable in helping the students understand the course content

Table 2. Average end of course scores for each evaluation statement. S = spring semester; F = fall semester; D = distance (online) section; T = traditional (on-campus) section; n = sample size.

Evaluation statement	S2008D (n = 5)	F2007D (n = 21)	S2007D (n = 7)	F2006D (n = 7)	S2005D (n = 20)	S2003T (n = 18)	F2003T (n = 23)
The instructor was knowledgeable in the subject matter of the course	4.20	4.50	4.63	4.43	4.70	4.67	4.61
The instructor effectively presented the content of the course	4.20	4.32	4.50	4.29	3.80	4.61	4.17
The instructor was well prepared for each class	4.20	4.55	4.14	4.43	4.55	4.65	4.61
The class time was a valuable experience in helping my understanding	3.20	4.23	4.00	4.43	3.80	4.11	4.11
The instructor was available during scheduled office hours	4.00	4.45	4.5	4.43	4.40	4.41	4.28
The instructor fairly evaluated my work in this course	4.00	4.14	4.50	4.00	4.35	4.61	4.50
The textbook required for the course was useful	4.20	4.05	4.50	3.71	3.25	2.82	3.89
The instructional aides, e.g., audio visual, web, etc., were beneficial	4.00	3.91	4.50	4.29	3.65	4.35	4.00
The instructor is fluent in the English language	3.80	4.41	4.5	4.71	4.05	4.17	3.83
The instructor's overall performance as a teacher was excellent	4.00	4.09	4.63	3.86	3.95	4.41	4.44

(82.2% vs. 80%). In addition, traditional students perceived that the instructor evaluated their work more fairly (91% vs. 84.4%), that the instructional aides were more beneficial (79.6% vs. 78.3%), and that the instructor's overall performance as a teacher was more excellent (88.6% vs. 81.4%). On the other hand, students enrolled in the online sections of SEES perceived that the instructor was more accessible during office hours (86.8% vs. 88.0%), that the textbook was more useful (68.4% vs. 76.2%), and that the instructor was more fluent in English (79.6% vs. 85.6%).

Although there are percent differences between the evaluation scores from the traditional and online settings, the type of data available are not suitable for establishing statistically significant differences. A visual inspection of the data suggests that the traditional and online versions of SEES were evaluated roughly in the same way, with slightly higher marks for the traditional version. The qualitative data showed in more detail what aspects of the online course were viewed positively and negatively by students.

Generally, students expressed their liking of the online SEES. For example, several students said, "The course was great, I learned a lot," "I think the course was great overall," and "I really enjoyed the class." One student stated that, "before this course, I had never written a lesson plan. I believe that because I had this course I can now teach science with confidence." Another one commented that, "I did learn a lot from this class and I really enjoyed having you as a teacher."

One aspect where students were particularly positive was the microteaching experience. One student said, "I think that the microteaching exercises were very helpful to me." Another one stated: "The microteachings were very beneficial. I have learned more from those six, thirty minutes than I have in the whole class." A third student pointed out that, "The microteachings helped me out the most, and was the most fun assignment of all of them." One student suggested scheduling the microteachings later in the semester so that the course can give them a better foundation in science methods, planning, and assessment.

Another component of the course that received generally positive feedback was the video reflections based on Case Studies in Science Education and Essential Science for Teachers. One student said, "The video reflections were interesting. They gave me some good ideas." Another student mentioned that, "the videos that were used are great for helping teacher understanding of the [course] content and also in helping to determine how the children respond to those areas."

Several students mentioned the fact that the instructor was easily accessible by email and provided prompt feedback. Two students stated, "[The instructor] is always very prompt in replying to e-mails and answering any questions we may have" and "the teacher was always willing to help and be there if there was anything needed." Another student said, "[The instructor] was great at getting grades posted promptly and providing feedback to questions. Props to him."

On the other hand, a number of students perceived their experience with the online SEES in a somewhat negative fashion. For example, students considered the amount of material covered in the class as excessive, arguing that they were working full-time or had other responsibilities. Two students said, "I think this is the hardest course I have ever taken. I feel that [the instructor] gave us a little too much work" and "some people have to take other classes along with this one and there is no way to get all the work done." A student stated, "I am a single parent and this class literally stressed me out. I feel that I have neglected my children and my job." Not all students were of the opinion that the course covered too much material, although. A student wrote, "There is sufficient time to complete assignments. The instructor always e-mailed reminders to us about upcoming assignments and tests." Another one said, "Instructions for assignments are very well defined. I always knew what was expected of me."

One of the components of the course that students apparently did not like was the amount of material for each test and the test's length. One student said, "The test had 107 questions; I got to a point where my eyes were blurring up on me. The test was entirely too long. You should have more tests but make them shorter." The testing process on Blackboard seemed to be difficult for some,

especially those with low connectivity speed. One student stated, "[I need] more time for test taking ... it took my computer 45 seconds to load a question, leaving little time [to answer it]. It was always difficult because, unlike a paper test, we could not go back and correct answers." Other students disagreed on the amount of points allocated for lesson plans, teaching evaluations, textbook reports, and self-reflection.

A group of comments were explicitly addressing the limitations of online science methods courses. A student felt disappointed by the inconsistencies between what is taught and how it is taught: "The instructor taught us that we should teach by letting students work hands-on. He also said that we should use lecture sparingly. Then in the course we did hands-on learning but the majority of the grade came from lectures, readings, and memorizing facts for testing purposes. This is exactly what he was telling us not to do when we teach our students." This student's appreciation is correct in that, with the exception of the microteachings, the course was heavily geared towards science teaching knowledge building rather than the application of this knowledge and the development of science inquiry skills.

The issue of convenience in online courses was patent when the instructor, after discovering that some students were using their textbooks, the Internet and other print resources for their closed-book tests, decided to schedule the final test on the same day and time in one of three colleges in the region. Some students expressed their disagreement with the instructor's decision. One of them said, "I was disappointed in the final exam schedule. In every other online class I have been able to take it at my local college. [The instructor] had only a few set sites. I am taking an online course so it works around my schedule. I had to take a day off [work] to take the test." A similar reaction to the in-site final test was expressed by another student, "All instructors should take into consideration that this is an online class and that some of us can't take off work during the day to take proctored tests. It should be offered over a 2 or 3 day period and after 3:00 P.M. also."

DISCUSSION

The Likert-scale data, taken as a whole, shows a total score of 42.6 points (about 85%) for the traditional SEES, compared with 41.9 points (about 84%) for the online SEES. This difference in the evaluation of the traditional and online versions of the course is insignificant, suggesting that the reason why students take the online course might not be directly linked to the content itself, but more with convenience and access.

One of the lowest scores, and the largest difference in scores, about 7.8%, corresponds to the usefulness of the science methods textbook. Although the online students evaluated the textbook as more useful, it is apparent that a better selection of textbook will result in a more positive experience for many students. Another relatively large difference, about 7.2%, was reported for the overall performance of the instructor. Online science methods instructors, who never directly "teach" students, might be at a disadvantage compared with instructors in regular classrooms when the course is evaluated. The personal connection and the modeling of good science teaching practices is a learning experience that many online students might not be able to observe. This finding is consistent with comments from the qualitative section, where students pointed out the small correlation between what science methods teachers model and the content covered in online courses. A possible alternative to reduce this gap in the student preparation might be to include live or pre-recorded webcasts where the instructor can demonstrate different science teaching strategies.

An interesting finding was that online students evaluated the English knowledge of the instructor higher than traditional students. This difference comes from the fact that the author and SEES instructor is of Hispanic descent and has an accent. Apparently the use of online education might help student to focus on the course content rather that the instructor's ethnic background. This is definitely an interesting area for further research.

The written comments present a very informative picture of the students' perceptions of the online version of SEES. It is obvious that course assessments where

students have to be actively involved (field experiences) or where students can see science teachers in action and students developing science content knowledge (videos) were preferred over reports and tests based on readings or the textbook. This is consistent with research on how much learning occurs when the instructional experiences are direct, simulated, or vicarious, compared with visual or verbal ones (Victor et al. 2008). This finding implies that online science methods courses must strive to engage as many senses as possible in the learning process, possibly by increasing the amount of audiovisual resource and, especially, field experiences.

The data suggests that students might feel less intimidated by the instructor in an online course and are willing to communicate more frequently with him, which provides the instructor an excellent way to establish rapport with students. These students perceive that the answer to any of their questions is just an email away, as opposed to traditional instructors who might not be available in the evenings or weekends. Based on this finding, instructors must keep the communication lines open as much as possible, making an effort to check electronic messages frequently and providing detailed feedback and comments. A word of caution, though; because of the absence of body language when using email, there is an increased possibility of miscommunication (Kato and Akahori 2004).

The fact that many of the students who decided to take SEES online are non-traditional, it was not surprising that they perceived the amount of material covered as excessive. It is important to point out that the amount of material covered in the traditional version of SEES is similar. Unfortunately, this is an area where accommodations for online students might not be possible. A watered-down version of SEES will probably not prepare them as well for teacher licensure tests, such as Praxis II and III, and the professional demands and responsibilities of science teaching, which could increase teacher attrition, currently estimated at around 50% after five years of teaching experience (Alliance for Excellent Education 2005). It is suggested that instructors provide as much information up-front about course requirements, assessments, deadlines, etc. as possible so that students can plan their schedules to fulfill both academic and professional/personal responsibilities.

Taking tests on Blackboard seems to be stressful for many online SEES students, especially given the fact that the instructor's tests are closed-book and cheating-prevention strategies are in place. Based on the students' apparent unfamiliarity with these strategies, it is possible to conclude that they are likely assessed with open-book tests in other courses. The whole idea of valid and reliable assessment in online courses is still a debated topic and, potentially, its most problematic "Achilles' heel." As described in the literature review, long-term research on the effectiveness of online science methods courses in assuring lasting learning, as opposed to "passing a class," is much needed. Overall, the use of multiple ways of assessment, including traditional and performance-based, are recommended to reduce test anxiety among online science methods students.

It was precisely the difficulty of valid and reliable assessment, along with the discovery of students "copy-and-pasting" information from unauthorized internet resources on tests, that lead to the decision of a final test not offered online. From the instructor's perspective assessment validity and reliability trumps convenience, especially if it is for only one day out of the whole semester. Based on the data, and despite possible negative comments from some students, proctored tests should be scheduled whenever possible to ensure accurate evaluation of the students' learning in online science courses. Testing centers at nearby college campuses or adult education centers are two possible options for offering proctored tests.

SUMMARY

The first research question asked whether there was a difference in the quantitative end-of course evaluation of online and traditional versions of the same science methods course. Data suggest that students evaluated both versions of SEES similarly. The second research question asked what course components were perceived by students as more or less effective in helping them learning the course material? Although most students

reported liking the online SEES, especially its use of field experiences, science education videos and frequent communication between the instructor and the students, other students found the testing experience stressful and the amount of material they were exposed to as excessive.

Because this study only explores one course and one instructor, it is difficult to differentiate effects related to the course itself, the instructor, the student population, or the way the course was delivered. Although this study adds to the literature on the implementation and effectiveness of pre-service science methods courses delivered online, only long-term studies that avoid limitations such as participant self-selection, lack of comparison groups, and self-reporting data, will provide a better perspective on this important topic. In general, and based on the data in this study and the literature, it can be concluded that students perceive traditional and online courses as equivalent. Although students might like the convenience of an online course, it is still an open question whether this delivery mode translates into a qualified science teacher.

LITERATURE CITED

Allen, I. E., and J. Seaman. 2007. Online nation: five years of growth in online learning. The Sloan Consortium, Needham, MA.

Alliance for Excellent Education. 2005. Teacher attrition: a costly loss to the Nation and the States. Alliance for Excellent Education, Washington, DC. http://www.all4ed.org/files/archive/publications/TeacherAttrition.pdf. Date accessed 10/25/2008.

Annenberg/CPB. 2004a. Essential science for teachers: physical sciences. Harvard-Smithsonian Center for Astrophysics, Washington, DC.

Annenberg/CPB. 2004b. Essential science for teachers: earth and space sciences. Harvard-Smithsonian Center for Astrophysics, Washington, DC.

Annenberg/CPB. 2004c. Case studies in science education. Harvard-Smithsonian Center for Astrophysics, Washington, DC.

Annetta, L. A., and J. A. Shymansky. 2006. Investigating science learning for rural elementary school teachers in a professional development project through three distance education strategies. Journal of Research in Science Teaching 43:1019–1039.

Arkansas Department of Education. 2005. K-8 science curriculum frameworks. Arkansas Department of Education, Little Rock, AR.

Arkansas Science Teachers Association. 1999. Laboratory safety guide for Arkansas K-12 schools. Arkansas Department of Education, Little Rock, AR. http://arkansased.org/teachers/pdf/labsafe.pdf. Date accessed 10/13/2008.

Atkin, J. M., P. Black, and J. Coffey. 2001. Classroom assessment and the national science education standards. National Research Council, Washington, DC.

Barab, S. A., and T. Duffy. 2000. From practice field to communities of practice. Pages 25–56 in D. Jonassen and S. M. Land (eds). Theoretical foundations of learning environments. Lawrence Erlbaum, Mahwah, NJ.

Barab, S. A., M. K. Thomas, and H. Merrill. 2001. Online learning: from information dissemination to fostering collaboration. Journal of Interactive Learning Research 12:105–143.

Barry, M., and G. B. Runyan. 1995. A review of distance learning studies in the U.S. military. The American Journal of Distance Education 9:37–47.

Beitz, J. M., and J. A. Snarponis. 2006. Strategies for online teaching and learning. Nurse Educator 31:20–25.

Bodzin, A. M., and J. C. Park. 2000. Dialogue patterns of preservice science teachers using asynchronous computer-mediated communications on the World Wide Web. Journal of Computers in Mathematics and Science Teaching 19:161–194.

Burguess, W. E. 1997. The Oryx guide to distance learning. A comprehensive listing of electronic and other media-assisted courses. Oryx Press, Phoenix, AZ.

Cannon, J. R. 2002. Distance learning in science education: practices and evaluation. Pages 243–265 in J. W. Altschuld and D. D. Kuman (eds). Evaluation of science and technology at the dawn of the new millennium. Kluwer Academic Publishers, New York, NY.

Chumley, H. S., A. Dobbie, and C. L. Alford. 2002. Web-based learning: sound educational method or hype? A review of the literature. Academic Medicine 77: S86–S93.

Collins, J., and E. T. Pascarella. 2003. Learning on campus and learning at a distance: a randomized instructional experiment. Research in Higher Education 44:315–326.

Collins, M. 1997. Developing and running a WWW biology course. The American Biology Teacher 59:594–596.

Creswell, J. W. 1998. Qualitative inquiry and research design: choosing among five traditions. Sage Publications, Thousand Oaks.

Cuellar, N. 2002. The transition from classroom to online teaching. Nursing Forum 37:5–11.

Curran, V., J. Lockyer, J. Sargeant, and L. Fleet. 2006. Evaluation of learning outcomes in web-based continuing medical education. Academic Medicine 81:S30–S34.

Dede, C., D. Jass-Ketelhut, P. Whitehouse, L. Breit, and E. McCloskey. 2006. Research agenda for online

teacher professional development. Harvard Graduate School of Education, Cambridge, MA.

Fordis, M., J. E. King, C. M. Ballantyne, P. H. Jones, K. H. Schneider, S. J. Spann, S. B. Greenberg, and A. J. Greisinger. 2005. Comparison of the instructional efficacy of Internet-based CME with live interactive CME workshops: a randomized controlled trial. Journal of the American Medical Association 294:1043–1051.

Garrison, D. R., and T. Anderson. 2003. E-learning in the 21st century: a framework for research and practice. Routledge Falmer, New York, NY.

Harlen, W. 2004. Can teachers learn through enquiry online? Studying professional development in science delivered on-line and on-campus. International Journal of Science Education 26:1247–1267.

Harlen, W., and C. Atobello. 2003. An investigation of "Try Science" studied online and face-to-face. Technical Education Research Centers (TERC), Cambridge, MA.

Jaffe, R., E. Moir, E. Swanson, and G. Wheeler. 2006. Online mentoring and professional development for new science teachers. Pages 89–116 in C. Dede (ed). Online professional development for teachers: Emerging models and methods. Harvard Education Press, Cambridge, MA.

Kato, Y., and K. Akahori. 2004. E-mail communication versus face-to-face communication: perception of other's personality and emotional state. Pages 4160–4167 in L. Cantoni and C. McLoughlin (eds). Proceedings of World Conference on Educational Multimedia, Hypermedia and Telecommunications. Association for the Advancement of Computing in Education, Chesapeake, VA.

Lavoie, D. R. 1997. Delivering university science content/education courses to high school science teachers via telecommunications: An evaluation. Electronic Journal of Science Education 1(4). http://ejse.southwestern.edu/original%20site/Manuscripts/v1n4/ issue.html. Date accessed 10/11/2008.

Lesson Lab Research Institute. 2008. Videocases for science teaching analysis. Pearson, Santa Monica, CA.

Loucks-Horsley, S., P. W. Hewson, N. Love, and K. E. Stiles. 1998. Designing professional development for teachers of science and mathematics. Corwin Press, Thousand Oaks, CA.

Lynch, S. 2000. Equity and science education reform: listening to our better angles. Lawrence Erlbaum, Mahwah, NJ.

McComas, W. F. 1998. The principal elements of the nature of science: dispelling the myths. In W. F. McComas (ed). The nature of science in science education: Rationales and Strategies. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Merisotis, J., and R. Phillips. 1999. What's the difference: Outcomes of distance education vs. traditional classroom-based learning. Change 31:13–17.

Merriam, S. B. 2001. Qualitative research and case study applications in education. Jossey-Bass Publishers, San Francisco.

National Academy of Science. 1998. Teaching about evolution and the nature of science. National Academies Press, Washington, DC.

National Research Council. 2007. Enhancing professional development for teachers: potential uses of information technology. National Academies Press, Washington, DC.

National Science Teachers Association. 2008. Position Statement: the role of e-learning in science education. http://www.nsta.org/pdfs/PositionStatement_E-learning.pdf. Date accessed 10/10/2008.

Navarro, P., and J. Shoemaker. 1999. The power of cyberlearning: an empirical test. Journal of Computing in Higher Education 11:29–57.

Noh, T. 2004. Perceived professional needs of Korean science teachers majoring in chemical education and their preferences for online and on-site training. International Journal of Science Education 26: 1269–1289.

Northwest Regional Education Library. 1997. Science and mathematics for all students: it's just good teaching. Northwest Regional Education Library, Portland, OR.

Peters, J. M., and D. L. Stout. 2006. Methods for teaching elementary school science. Pearson/Prentice Hall, Upper Saddle River, NJ.

Roach, V., and L. Lemasters. 2006. Satisfaction with online learning: a comparative descriptive study. Journal of Interactive Online Learning 5:317–332.

Rodrigues, S. 1999. Evaluation of an online masters course in science teacher education. Journal of Education for Teaching 25:263–270.

Rowe, E., and J. Asbell-Clarke. 2008. Learning science online: What matters for science teachers? Journal of Interactive Online Learning 7:75–104.

Rubin, H. J., and I. S. Rubin. 1995. Qualitative interviewing: the art of hearing data. Sage Publications, Thousand Oaks.

Ruiz, J. G., M. J. Mintzer, and R. M. Leipzig. 2006. The impact of e-learning in medical education. Academic Medicine 81:207–212.

Schrum, L., M. DBurbank, and R. Capps. 2007. Preparing future teachers for diverse schools in an online learning community: perceptions and practice. Internet and Higher Education 10:204–211.

Smith-Strickland, J., and J. Butler. 2005. Establishing guidelines for determining appropriate courses for online delivery. Journal of Interactive Online Learning 4:129–140.

Sujo de Montes, L. E., and C. L. Gonzales. 2000. Been there, done that: Reaching teachers through distance education. Journal of Technology and Teacher Education 8:351–371.

Talvitie-Siple, J. 2006. Best practices applied in online science teacher education. The Online Journal of Adult Workforce and Education 1(4). Retrieved on October 10, 2008 from http://welcome.coe.jmu.edu/vertex.

Victor, E., R. D. Kellough, and R. H. Tai. 2008. Science K-8: an integrated approach. Pearson Merrill Prentice Hall, Upper Saddle River, NJ.

Wallace, R. M. 2003. Online learning in higher education: a review of research on the interactions among teachers and students. Education, Communication and Information 3:241–280.

Wenger, E. 1997. Communities of practice: learning, meaning, and identity. Cambridge University Press, New York, NY.

Whitehouse, P., L. Breit, E. McCloskey, D. Ketelhut, and C. Dede. 2006. An overview of current findings from empirical research on online teacher professional development. Pages 13–30 in C. Dede (ed). Online professional development for teachers: emerging models and methods. Harvard Education Press, Cambridge, MA.

Winters, J. M., and J. M. Winters. 2007. Videoconferencing and telehealth technologies can provide a reliable approach to remote assessment and teaching without compromising quality. Journal of Cardiovascular Nursing 22:51–57.

Wutoh, R., S. A. Boren, and E. A. Balas. 2004. E-learning: a review of Internet-based continuing medical education. Journal of Continuing Education in the Health Professions 24:20–30.

Zhao, Y., J. Lei, B. Y. Chun-Lai, and H. S. Tan. 2005. What Makes the Difference? A Practical Analysis of Research on the Effectiveness of Distance Education. Teachers College Record 107(8), 1836–1884.

J. Ky. Acad. Sci. 70(1):94–96. 2009.

NOTES

Documenting the Distribution of *Portulaca oleracea* in Kentucky—*Portulaca oleracea* L. is a cosmopolitan succulent weed, preferring places such as eroding or otherwise disturbed bare ground, sidewalk cracks, and cultivated areas (Matthews et al. 1993; Mitich 1997; Matthews 2003). Its native range is unclear. While it often has been considered one of the many U.S. weeds introduced from Europe, pre-Colombian age seeds found in samples from New World archaeological sites argue against it being a recent introduction (Byrne and McAndrews 1975; Matthews 2003).

Despite its wide range and weedy nature, the occurrence of purslane in Kentucky has been characterized as infrequent (Jones 2005). The USDA (2009) also lists purslane as specifically occurring only in a handful of Kentucky counties, which made us wonder how easy it would be to get distribution data for the other counties. We decided to use this as a test case to determine how well our herbaria and state floristic literature actually reflect the abundance and range of weedy species such as purslane. One assumes that, for common plants, distributional data should be abundant. Our goal was to gain a better understanding of the actual range of *P. oleracea* in Kentucky by surveying the floristic literature and local herbarium collections.

Plant Life of Kentucky (Jones 2005) and *Flora of North America* (Matthews 2003) both list *P. oleracea* as occurring throughout Kentucky but do not provide individual county listings. A literature search produced listings for 20 counties: Barren, Boone, Bullitt, Calloway, Edmonson, Hardin, Hart, Henry, Jefferson, Jessamine, Lyon, McLean, Meade, Nelson, Oldham, Pike, Shelby, Spencer, Trigg, and Warren (Price 1893; Nelson 1918; Greenwell 1935; Davies 1955a, 1955b; Gunn 1959; Sisk and Sisk 1966; Gunn 1968a, 1968b; Johnson 1981; Campbell and Meijer 1989; Cranfill 1991; Meijer 1992; Chester 1993; Medley 1993 (Figure 1).

We focused on floristic studies that were likely to include habitats where purslane would be encountered. Of the more than 35 journal articles, county floras, and other sources consulted, 14 listed *P. oleracea*. However, no mention of the species was made in treatments covering Casey, Hickman, Laurel, Letcher, or Rockcastle counties, although these studies seemed to cover areas where one would expect to find some *P. oleracea* (Murphy 1970; Sole et al. 1983; Thompson et al. 1984; Grubbs and Fuller 1991; Thompson and Wade 1991; Thompson and Fleming 2004).

Our survey of *P. oleracea* in Kentucky herbaria (BEREA, EKU, KNK, MDKY, MUR, UK, WKU) showed a total of 19 specimens from the following counties: Allen, Calloway, Campbell, Fulton, Henry, Kenton, Lewis, Livingston, Madison, Marshall, McLean, Pike, Rowan, and Warren (Figure 1). We collected specimens from 11 more counties in Northern Kentucky: Boone, Bourbon, Bracken, Carroll, Franklin, Gallatin, Grant, Harrison, Owen, Pendleton, and Scott. These specimens are now archived in the John W. Thieret Herbarium at Northern Kentucky University.

Based on our herbarium survey, specimens currently in Kentucky collections represent only 14 of our 120 counties, or just under 12% of the state. However, in two days we collected *P. oleracea* in 11 counties. For 10 of these, we found no previous reports of *P. oleracea* being

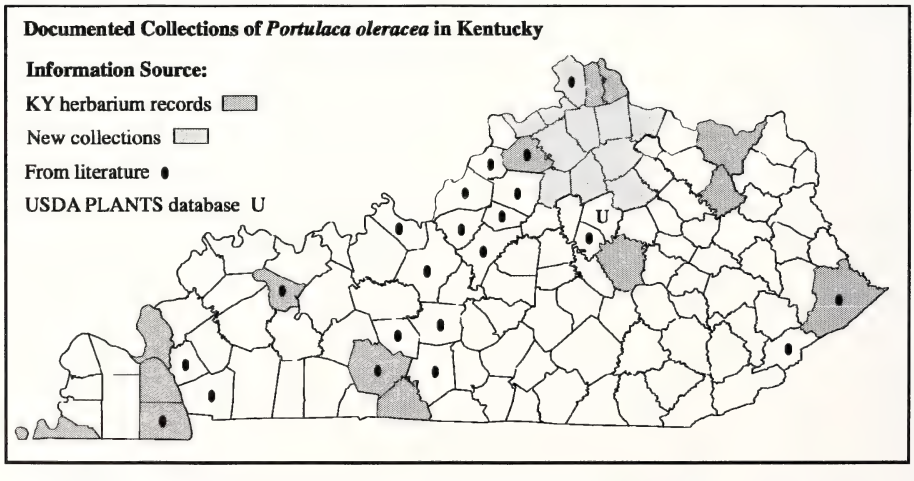


Figure 1. Distribution of *Portulaca oleracea* in Kentucky based on literature citations and collections in Kentucky herbaria. "New collections" were those made specifically for this study. The USDA (2008) listed *P. oleracea* from several counties, but here we cite only listings not duplicated by other sources.

collected there, either via herbarium specimens or literature citations (Figure 1).

Based on our collections, *P. oleracea* is indeed common here, but under-collected. That records in the literature outnumber actual herbarium specimens supports this idea. The succulent nature of *P. oleracea* plants may explain the dearth of collections because attractive dried specimens are difficult to produce. However, mention of *P. oleracea* is sporadic in the many county floras and checklists that have been published for Kentucky. Perhaps its weedy status and preference for highly human-disturbed habitats also discourages collectors from bothering with it.

Portulaca oleracea is not entirely limited to human-disturbed areas. It also has been reported in cedar glades (Baskin and Baskin 2003), on drying, exposed mud along river banks (Meijer 1992), and on natural rock outcrops (Campbell and Meijer 1989). Because *P. oleracea* is an economically important weed whose origins are still somewhat unclear, further collections would be worthwhile. As our study shows, knowing that a plant is common is not a guarantee that our herbaria contain ample material for study. That the range of a plant is well-known is not the same as well-documented, and our study should serve to demonstrate why additional herbarium collections of both common and unusual plants are still needed.

We would like to thank the staff and curators of the following herbaria for taking the time to look up and e-mail records: Berea College, Eastern Kentucky University, University of Kentucky, Morehead State University, Murray State University, and Western Kentucky University. Julian Campbell and Max Medley helped provide inspiration for this project by generously supplying us with a draft of their *Illustrated Atlas of Vascular Plants in Kentucky: a First Approximation*. Cynthia Cain and Sabine Zacate helped collect plants, and Richard Boyce provided valuable suggestions for improving early versions of this manuscript.

LITERATURE CITED. Baskin, J. M., and C. Baskin. 2003. The vascular flora of cedar glades of the southeastern United States and its phytogeographical relationships. Journal of the Torrey Botanical Society 130:101–118. Byrne, R., and J. H. McAndrews. 1975. Pre-Colombian purslane (*Portulaca oleracea* L.) in the New World. Nature 253:726–727. Campbell, J. J. N., and W. Meijer. 1989. The flora and vegetation of Jessamine Gorge, Jessamine County, Kentucky: a remarkable concentration of rare species in the Bluegrass Region. Transactions of the Kentucky Academy of Science 50:27–45. Chester, E. W. 1993. Vascular flora of Land Between the Lakes, Kentucky and Tennessee: an updated checklist. Journal of the Tennessee Academy of Science 68:1–14. Cranfill, R. 1991. Flora of Hardin County, Kentucky. Castanea 56:228–267. Davies, P. A. 1955a. A preliminary list of the vascular plants of Meade County, Kentucky. Transactions of the Kentucky Academy of Science 16:88–97. Davies, P. A. 1955b. A preliminary list of the vascular plants of Mammoth Cave National Park. Castanea 20:107–127. Greenwell, R. A. 1935. A flora of Nelson County, Kentucky, with a selected list of economically important plants. Nazareth College, Louisville, KY. Grubbs, J. T., and M. J. Fuller. 1991. Vascular flora of Hickman County, Kentucky. Castanea 56:193–214. Gunn, C. R. 1959. A flora of Bernheim Forest, Bullitt County, Kentucky. Castanea 24:61–98. Gunn, C. R. 1968a. A check list of the vascular plants of Bullitt County, Kentucky. Castanea 33:89–106. Gunn, C. R. 1968b. The flora of Jefferson and seven adjacent counties, Kentucky. Annals of the Kentucky Society of Natural History 2:1–322. Johnson, G. P. 1981. An unreported cedar glade in Warren County, KY. Transactions of the Kentucky Academy of Science 42:101–105. Jones, R. L. 2005. Portulacaceae. Pages 474–475. Plant life of Kentucky, an illustrated guide to the vascular flora. The University Press of Kentucky, Lexington, KY. Matthews, J. F. 2003. *Portulaca*. Pages 496–501 in Flora of North America Editorial Committee (eds). 2003. Flora of North America. Vol 4. Magnoliophyta: Caryophyllidae, Part 1. Oxford University Press, New York. Matthews, J. F., D. W. Ketron, and S. F. Zane. 1993. The biology and taxonomy of the *Portulaca oleracea* L. (Portulacaceae) complex in North America. Rhodora 95:166–183. Medley, M. E. 1993. An annotated catalogue of the known or reported vascular flora of Kentucky. Ph.D. Dissertation. University of Louisville, Louisville, KY. Meijer, W. (ed). 1992. Herbaceous spring-summer flora of Kentucky. KNPS Wildflower Weekend, 1–3 May 1992 ed. University of Kentucky, Lexington, KY. Mitich, L. W. 1997. Intriguing world of weeds: common purslane (*Portulaca oleracea*). Weed Technology 11:394–397. Murphy, G. W. 1970. A preliminary survey of the flora of Casey County, Kentucky. Castanea 35:118–131. Nelson, J. C. 1918. Plants of Boone County, Kentucky. Proceedings of the Indiana Academy of Science 28:125–143. Price, S. F. 1893. Flora of Warren County, Kentucky. C. F. Carr, New London, Wisconsin. Sisk, J. S., and M. E. Sisk. 1966. Edible wild spermatophytes of Calloway County, Kentucky. Transactions of the Kentucky Academy of Science 27:5–15. Sole, J. D., S. Lassetter, and W. H. Martin. 1983. The vascular flora of Lilley Cornett Woods, Letcher County, Kentucky. Castanea 48:174–188. Thompson, R. L., and C. A. Fleming. 2004. Vascular flora and plant communities of the John B. Stephenson Memorial Forest State Nature Preserve (Anglin Falls Ravine), Rockcastle County, Kentucky. Castanea 69:125–138. Thompson, R. L., W. G. Vogel, and D. D. Taylor. 1984. Vegetation and flora of a coal surface-mined area in Laurel County, Kentucky. Castanea 49:111–126. Thompson, R. L., and G. L. Wade. 1991. Flora and vegetation of a 12-

year-old coal surface-mined area in Rockcastle County, Kentucky. Castanea 56:99–116. USDA, NRCS. 2009. The PLANTS Database. http://plants.usda.gov. Date accessed (07/03/2007).—**Maggie Whitson, Laura Trauth, and Angela Tullis,** Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099. Corresponding author email: whitsonma@nku.edu

NOTES

First Report of Oak Mistletoe [*Phoradendron leucarpum* (Raf.) Reveal & M.C. Johnston] on the Invasive Liana, Oriental Bittersweet (*Celastrus orbiculatus* Thunb.)—On 26–27 January 2009, a severe ice storm affected large parts of east-central Kentucky and brought down numerous trees. While removing a large, ice-felled wild black cherry (*Prunus serotina* Ehrh.) from a residential yard in an older part of Berea, the first author cut a large tangle of Oriental bittersweet (*Celastrus orbiculatus* Thunb.) out of the upper limbs. A portion of the liana (woody vine) had unusual swollen cankers of 1.2–2.0 cm in diameter. Several of the cankers had green shoots 4.0–8.0 mm long (largest at 1.5 cm) with opposite leaves 3.0–7.0 mm growing out of it. Closer examination of these shoots revealed the presence of oak mistletoe [*Phoradendron leucarpum* (Raf.) Reveal & M.C. Johnston, Viscaceae]. Lianas were 8.0–9.0 mm in diameter above and below the haustorial swellings. The crown of the wild black cherry had heavy infestations with 25–35 clumps of oak mistletoe. Several other trees in the neighborhood, e.g., black walnut (*Juglans nigra* L.), silver maple (*Acer saccharinum* L.), and American elm (*Ulmus americana* L.) also had heavy infestations of mistletoe (see Thompson et al. 2008).

The base of the Oriental bittersweet liana was over 9.0 cm in diameter, which indicated it was several years old. The wild black cherry at 7.0 m from the root collar was aged at 70 years. It is speculated that the close proximity of the liana to infested branches of the black cherry provided the opportunity for viscid mistletoe fruits and seeds to fall and adhere to the vine by action of birds, i.e., bill wiping or defecation and/or gravity. The long association of the liana with the cherry tree gave ample opportunity for deposition of fruits and seeds on the woody vine. Mistletoe shoots on the bittersweet were chlorotic (yellowish-green) compared with the dark green shoots on the black cherry. Several swollen cankers did not yet have shoots protruding and some mistletoe shoots were broken off in the felling and handling of the tree.

In Berea, oak mistletoe has been reported from other non-native species including Bradford pear (*Pyrus calleryana* Decne.), Siberian elm (*Ulmus pumila* L.), Lavallee's hawthorn (*Crataegus* × *lavallei* Herincq. ex Lavallee), and Amur honeysuckle [*Lonicera maackii* (Rupr.) Herder] by Thompson et al. (2008). Examination of Kuijt (2003) and an extensive literature review did not reveal oak mistletoe associated with any exotic vines as hosts.

Our report documents the first North American occurrence of *Phoradendron leucarpum* on *Celastrus orbiculatus* and the first record of oak mistletoe hemiparasitism on a non-native liana for the continental United States. The voucher specimen is deposited at Berea College Herbarium (BEREA). Kentucky: Madison County: Berea, in the back yard of 410 Center Street, hemiparasitic on *Celastrus orbiculatus* liana in the top of a 20 m tall *Prunus serotina* felled by the ice storm of 26–27 January 2009. Wild black cherry had heavy infestation of ca. 25–35 clumps. Base of Oriental bittersweet was 9.0 cm in diameter; 6 March 2009, *David D. Taylor 18752* (BEREA).

LITERATURE CITED. Kuijt, J. 2003. Monograph of *Phoradendron* (Viscaceae). Systematic Botany Monographs 66:1–643. Thompson, R. L., K. Rivers Thompson, E. A. Fleming, R. D. Cooks, J. R. Price, M. N. Naseman, and A. J. Oles. 2008. Eastern mistletoe (*Phoradendron leucarpum*, Viscaceae) in the city of Berea, Kentucky: a high incidence of infestation and eight new host species for Kentucky. Journal of the Kentucky Academy of Science 69:2–10.—**David D. Taylor**, USDA Forest Service, 1700 Bypass Road, Winchester, KY 40391 and **Ralph L. Thompson**, Berea College Herbarium, Biology Department, Berea College, Berea, KY 40404. Corresponding author emails: dtaylor02@fs.fed.us; ralph_thompson@berea.edu.

J. Ky. Acad. Sci. 70(1):98–101. 2009.

NOTES

Population Parameters for the Allegheny Woodrat (*Neotoma magister* Baird) at Two Sites in Eastern Kentucky—The historical range of the Allegheny woodrat (*Neotoma magister* Baird) extended from southeastern New York and western Connecticut to the Tennessee River in northern Alabama (Newcombe 1930). Over the last three decades there have been major population declines in the northern portion of the animal's range (Hicks 1989; Hayes 1990; Beans 1992; Sciascia 1993). Numerous reasons have been offered for the apparent decline, e.g., habitat loss (Balcom and Yahner 1996), reduced winter food supply (Beans 1992), and parasitic infection (Beans 1992; Sciascia 1993; LoGiudice 2000). The uncertainty surrounding the range-wide stability of the species has prompted population monitoring studies in a number of states, e.g., Indiana (Johnson 2002), Pennsylvania (Balcom and Yahner 1996; Hassinger et al. 1996), Kentucky (Thomas 2003), West Virginia (Castleberry 2000; Wood 2001), and Maryland and Virginia (Ford et al. 2006). Some have assumed the woodrat population is stable in Kentucky (Hicks 1989; Beans 1992), but prior to the initiation of this and other studies the question had not been addressed. The goal of our study was to provide baseline population information concerning two colonies of the Allegheny woodrat in eastern Kentucky.

The Daniel Boone National Forest (DBNF) is located in eastern Kentucky along the western edge of the Cumberland Plateau (Martin et al. 1993). Two colonies of *Neotoma magister* in the Morehead Ranger District, DBNF, Menifee County, KY, were chosen for monitoring based on U.S. Forest Service records of woodrat presence. The Murder Branch and Ratliff study sites consisted of broken clifflines and rocky outcrops typical of Allegheny woodrat habitat (Rhoads 1903; Newcombe 1930; Gottschang 1981; Balcom and Yahner 1996). Sandstone formations dominated each site and were characterized by shallow caves, rock houses, ledges, and extensive areas of breakdown. Both study sites were accessible by Forest Service roads and separated from public use areas by 1–2 km.

Trap locations in each woodrat study colony were defined as one of 20 pre-chosen locations along the cliffline and rock outcrop habitat. The minimum distance between trap locations at a study site was determined based on the average approximate radius of woodrat home range size, i.e., 28 m (Thomas 2003). Random values between 14 and 28 m were chosen to serve as minimum distances between trap locations within a site. Actual distances between trap locations were greater than 56 m in some instances because the cliffline habitat was intermittent and specific trap locations were selected based on the presence of woodrat sign.

Monthly two-night mark and recapture sessions were conducted at each woodrat study site between May 1997 and April 1998. Tomahawk live traps (two per trap location) baited with apple slices were covered with boards, supplied with polyester batting during inclement weather, and placed near identifiable woodrat sign (order of sign preference from high to low: fresh cut green vegetation, nest, latrine, stick midden, food cache). Specific placement of two traps per trap location was determined based on the location of sign.

Captured woodrats were assigned to size classes based on mass: 175 g for juveniles, 175–224 g for subadults, ≥225 g for adults. Reproductive condition (scrotal vs. nonscrotal testes for males, and perforate vs. imperforate vaginal opening and enlarged mammae for females) was recorded for each captured individual. Captured individuals had numbered aluminum Monel #1 eartags placed in each ear. Animals were released near the point of capture; an attempt was made to keep handling time to a minimum.

A chi-square contingency table procedure (McClave and Dietrich 1991) was used to test for independence between Allegheny woodrat sex ratio and site. Average mass for adult males and females at each site was determined using the first mass recorded for each individual for every month it was captured as an adult. Monthly age-class structure was derived from data for each captured individual in each month of the study period.

Two criteria were used to estimate the duration of the breeding season for Allegheny woodrats in eastern Kentucky. The first was reproductive condition of captured woodrats. Male and female woodrats were considered reproductively active if they had scrotal testes or a perforate vaginal opening. External characteristics of the genitalia have been used by other researchers to determine woodrat reproductive activity (Patterson 1933; Fitch and Rainey 1956; Monty 1997). The second factor used to suggest breeding season was derived from the mass of juvenile woodrats captured; taking into account when they appeared in the population, growth rate, average mass at birth (Poole 1940), and gestation period (Poole 1940; Zambernardi 1956).

Each Allegheny woodrat study site was monitored monthly between May 1997 and April 1998 (January 1998 was omitted at Murder Branch and February 1998 at Ratliff due to inaccessibility to trap sites). Annual trap success for 880 trap-nights was 22% at Murder Branch and 16% at Ratliff. A total of 42 and 27 individual woodrats were marked at Murder Branch and Ratliff, respectively.

Male:female sex ratios of the two marked woodrat populations differed from equilibrium at both sites (1:1.4 at Murder Branch, 1.4:1 at Ratliff). Sex ratio and site were independent of each other ($X^2_{0.05} = 3.84$); hence the sex ratio of a colony was not dependent on the location of the colony. Recruitment varied markedly between sites. Of the 12 juveniles marked at Murder Branch, only 4 reappeared in one or more months following initial

capture (recruitment = 33%), while all 7 juveniles marked at Ratliff reappeared in the months following initial capture (recruitment = 100%). The male:female juvenile sex ratio was skewed toward females at Murder Branch (1:3), and near equilibrium at Ratliff (1.0:1.1).

Mean mass of adult male Allegheny woodrats at the Murder Branch and Ratliff sites was 286 ± 24 g and 279 ± 41 g, respectively; adult females, 269 ± 27 g and 262 ± 28 g, respectively. In general, males and females at Ratliff weighed least in winter (Dec.–Feb.) and were heaviest in spring (March–May). At Murder Branch, both sexes weighed least in spring; mass of females peaked in summer (June–Aug.), and males peaked in fall (Nov.). Due to small sample sizes, mean daily increase in mass was calculated over the entire trapping period (rather than within each month or season) for juveniles and subadults. Juvenile and subadult woodrats in this study grew at an average rate of 1.0 g/day (n = 12) and 0.6 g/day (n = 11), respectively.

Allegheny woodrat age class structure within each colony was determined for each month of the study period (Table 1). Juveniles were present only in late spring and early summer at Murder Branch but were present from early summer to mid-winter at Ratliff. Some transient woodrats became residents (immigration) at both study sites. At Murder Branch, the immigrants included 5 males (4 adults, 1 subadult); while at Ratliff there were 8 total immigrants [6 males (4 adults, 2 subadults) and 2 females (1 adult, 1 subadult)].

Based on external characteristics, Allegheny woodrats in this study were reproductively active at least from March to October and in December. The animals appeared to be sexually reproductive at a minimum of 6 to 7 months of age. Utilizing the determined growth rate of 1.0 g/day for juveniles, an average mass at birth of 15 g, and average gestation of 30–36 days, conception occurred during breeding bouts from January to June (and possibly in part of December). Woodrats in the area encompassed by this study demonstrated the potential to breed year-round. Both adult and subadult females were perforate in March in both colonies. Perforate subadult females were captured in April, May, August, and September. Reproductively active females captured late in the fall and winter were all adults. Three adult females showed evidence of being polyestrous within the breeding season (alternating states of vaginal perforation in consecutive months). No females captured in January, February, or November showed signs of reproductive activity. There was a single perforate adult female captured at Ratliff in December. One subadult male had scrotal testes in March at Ratliff. All other scrotal males captured at both colonies in all months were in the adult age class. There were no reproductively active males captured at either study site in October, November, January, or February. There was a single male with partially scrotal testes captured at Ratliff in December.

Table 1. Monthly age class structure at two Allegheny woodrat study sites in the Daniel Boone National Forest, Menifee County, KY, May 1997–April 1998.

	Murder Branch study site			Ratliff study site		
	Adults	Subadults	Juveniles	Adults	Subadults	Juveniles
May	7	2	3	No captures		
June	9	1	10	4	3	
July	9	4	2	5	1	3
August	10	4		5	4	
Sept.	9	2		6	4	2
Oct.	14	3		3	4	2
Nov.	14	2		9	3	2
Dec.	7	1		4	3	1
Jan.	*	*		5	3	
Feb.	2	1		*	*	
March	3	1		4	2	
April	3			7	1	

* trap sites inaccessible due to weather.

Allegheny woodrat populations whose sex ratios differed from equilibrium have been observed in other parts of the species' range (Cudmore 1984; Myers 1997; Wood 2001; Thomas 2003). Rainey (1956) described a cycle in male body weight of *Neotoma floridana* in Kansas. In contrast to the trend noted in this study, Rainey (1956) found male weights peaked in early spring, declined during the summer, and increased again during fall and winter. Thomas (2003) reported male Allegheny woodrats in Kentucky exhibit a biennial cycle in mean body weight; with winter weights alternating between high point and low point from one year to the next. Fitch and Rainey (1956) stated adult *N. floridana* weight is influenced largely by season and individual differences, and seasonal trends vary from year-to-year. Although the calculated juvenile growth rate was based on a small sample size (n = 12), the authors felt it was more meaningful to use data from these colonies rather than published growth rates for different species in different geographical localities. Therefore a juvenile growth rate of 1.0 g/day was used to extrapolate time to sexual maturity and months of breeding activity.

Poole (1940) reported captive Allegheny woodrats in Pennsylvania produced 2 or 3 litters per year. Males in West Virginia had sperm in the tubules of the epididymys in February and December (Patterson 1933). Myers (1997) suggested that *N. magister* in West Virginia produced 3 or 4 litters per year between early January and late August (breeding occurred from December to late July). Zambernardi (1956) noted woodrats in Alabama produced 2 or 3 litters per year, between March and September (breeding occurred from February to August). Barbour and Davis (1974) indicated woodrats in Kentucky probably have multiple litters per year which are born beginning in March (conception as early as February). In subsequent monitoring at one of the sites surveyed in this study (i.e., Murder Branch), Thomas (2003) reported capturing two juvenile woodrats, and having a female give

birth to 3 pups in a live trap, in March. These studies support our findings that the Allegheny woodrats monitored in this study may be polyestrous and capable of breeding year-round.

The determination of breeding season and related population parameters for Allegheny woodrats in eastern Kentucky cannot be arrived at definitively based on a single year of sampling. Time to sexual maturity in *N. magister* should be explored to much greater depths than in the current study. Our results offer suggestive, but not conclusive, indicators of the potential duration of the breeding season and possible age of initial reproductive activity. Perhaps determining if *N. magister* females have year-round estrous cycles, as determined for *N. floridana* in Kansas (Chapman 1951), would bring researchers closer to determining the factors that influence the duration and onset of the breeding season in Kentucky. With the decline or disappearance of *Neotoma magister* in portions of its historic range (Castleberry 2000), it becomes paramount that the dynamics of still viable populations be understood. The results of this study represent baseline data for use in monitoring Allegheny woodrat populations in eastern Kentucky.

Financial support for this project was provided by the Kentucky Department of Fish and Wildlife Resources, U.S. Forest Service — Daniel Boone National Forest, and Eastern Kentucky University's Department of Biological Sciences.

LITERATURE CITED. Balcom, B. J., and R. H. Yahner. 1996. Microhabitat and landscape characteristics associated with the threatened Allegheny woodrat. Conservation Biology 10:515–525. Barbour, T. W., and W. H. Davis. 1974. Mammals of Kentucky. The University Press of Kentucky, Lexington. Beans, B. E. 1992. Without a trace: the puzzling demise of the Allegheny woodrat. Audubon 94:32–34. Castleberry, S. B. 2000. Conservation and management of the Allegheny woodrat in the central Appalachians. Ph.D. dissertation, West Virginia University, Morgantown. Chapman, A. O. 1951. The estrous cycle in the woodrat, *Neotoma floridana*. The University of Kansas Science Bulletin 34:267–299. Cudmore, W. W. 1984. The present distribution and status of the eastern woodrat, *Neotoma floridana*, in Indiana. Proceedings of the Indiana Academy of Science 94:621–627. Fitch, H. S., and D. G. Rainey. 1956. Ecological observations on the woodrat, *Neotoma floridana*. University of Kansas Publications, Museum of Natural History 8:499–533. Ford, W. M., S. B. Castleberry, M. T. Mengak, J. L. Rodrigue, D. J. Feller, and K. Russell. 2006. Persistence of Allegheny woodrats (*Neotoma magister*) across the mid-Atlantic Appalachian Highlands landscape, USA. Ecography 29:745–754. Gottschang, J. L. 1981. A Guide to the Mammals of Ohio. Ohio State University Press, Columbus. Hassinger, J., C. Butchkoski, J. Hart, and D. Diefenbach. 1996. Eastern woodrat surveys. Project Annual Job Report, Pennsylvania Game Commission, Bureau of Wildlife Management, Research Division, Harrisburg, PA. Hayes, J. P. 1990. Biogeographic, systematic, and conservation implications of geographic variation in woodrats of the eastern United States. Ph.D. dissertation, Cornell University, Ithaca, NY. Hicks, A. 1989. The decline and fall of a native New Yorker: Whatever happened to the Allegheny woodrat? The Conservationist 43:34–38. Johnson, S. A. 2002. Reassessment of the Allegheny woodrat (*Neotoma magister*) in Indiana. Proceedings of the Indiana Academy of Science 111:56–66. LoGiudice, K. 2000. *Baylisascaris procyonis* and the decline of the Allegheny woodrat (*Neotoma magister*). Ph.D. dissertation, Rutgers University, New Brunswick, NJ. Martin, W. H., S. G. Boyce, and A. C. Echternacht. 1993. Biodiversity of the Southeastern United States: Upland terrestrial communities. John Wiley and Sons, Inc., New York, NY. McClave, J. T., and F. H. Dietrich. 1991. Statistics. 5th edition. Dellen Publishing Co., San Francisco, CA. Monty, A. 1997. The eastern woodrat (*Neotoma floridana*) in southern Illinois: Population assessment and genetic variation. Ph.D. dissertation, Southern Illinois University, Carbondale. Myers, R. T. 1997. Microhabitat and ecology of the Allegheny woodrat in northcentral West Virginia. M.S. thesis, West Virginia University, Morgantown. Newcombe, C. L. 1930. An ecological study of the Allegheny cliff rat (*Neotoma pennsylvanica* Stone). Journal of Mammalogy 11:204–211. Patterson, R. C. 1933. Notes on *Neotoma pennsylvanica* with special reference to the genital organization. Proceedings of the West Virginia Academy of Science 6:38–42. Poole, E. L. 1940. A life history sketch of the Allegheny woodrat. Journal of Mammalogy 21:249–270. Rainey, D. G. 1956. Eastern woodrat, *Neotoma floridana*: Life history and ecology. University of Kansas Publication, Museum of Natural History 8:535–646. Rhoads, S. N. 1903. Mammals of Pennsylvania and New Jersey. Wickersham Printing Co., Lancaster, PA. Sciascia, J. C. 1993. Raccoons may be linked to eastern woodrat decline. Nongame News, New Jersey Endangered and Nongame Species Program, Trenton, N.J. Thomas, S. C. 2003. Allegheny woodrat monitoring. Final Performance Report, Kentucky Department of Fish and Wildlife Resources, Frankfort, KY. Wood, P. B. 2001. Characteristics of Allegheny woodrat (*Neotoma magister*) habitat in the New River Gorge National River, West Virginia, Final Project Report. West Virginia Cooperative Fish and Wildlife Research Unit, West Virginia University, Morgantown. Zambernardi, J. 1956. Woodrats of the genus *Neotoma* in Alabama. M.S. thesis, University of Alabama, Tuscaloosa.—**Bree Enderle McMurray**, Environ-

mental Unit-Design Division, Missouri Department of Transportation, P.O. Box 270, Jefferson City, MO 65102; **Steven C. Thomas**, National Park Service, Cumberland Piedmont Network, P.O. Box 8, Mammoth Cave, KY 42259; and **Charles L. Elliott**, Department of Biological Sciences, Eastern Kentucky University, Richmond, KY 40475. Corresponding author email: Charles.Elliott@eku.edu

J. Ky. Acad. Sci. 70(1):102–103. 2009.

Additional Abstracts of Some Papers Presented at the 2008 Annual Meeting of the Kentucky Academy of Science

Edited by Robert J. Barney

AGRICULTURAL SCIENCES

The Influence of Light on Annonaceous Acetogenin Activity in Pawpaw (*Asimina triloba*) Stem and Leaf Tissue. EMERALD W. GATES*, JEREMIAH D. LOWE, KIRK W. POMPER, and SHERI B. CRABTREE, Land Grant Program, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

The pawpaw [*Asimina triloba* (L.) Dunal] is a native Kentucky tree-fruit which contains Annonaceous acetogenins in the twigs and fruit which display antitumor and pesticidal effects. This tree is usually found in the forest understory and prefers growing in low-light conditions. Our working hypothesis was that high light levels stress the pawpaw plant and induce high acetogenin activity in the stem and leaf tissue. Higher extractable acetogenin levels would be desirable for future product development. The objective of this study was to determine if there was a positive correlation between increased light level and acetogenin activity in the stems and leaves of pawpaw seedlings. Three month old greenhouse grown seedlings were subjected to three light treatments using no shade cloth (100% ambient light), 35% shade cloth (65% ambient light), and 80% shade cloth (20% ambient light). A randomized block design was used in the experiment with three replicate seedlings in each treatment in three replicate blocks (3 plants × 3 treatments × 3 blocks) for a total of 27 plants. The plants were destructively harvested after 6 weeks; stems and leaves were dried at 50°C, ground, and extracted with 95% ethanol. The Brine Shrimp Test (BST) bioassay was employed to assess acetogenin activity of the pawpaw extracts. Brine shrimp mortality at 0, 5, 10, 50, and 100 ppm of extract after 24 hours was used to determine the LC_{50} for each treatment. A negative correlation between extract LC_{50} and shade was found and we rejected our working hypothesis.

Soluble Solids Content Varies by Pawpaw (*Asimina triloba*) Variety. SHERI B. CRABTREE*, ANTHONY MCCORMICK, CHARLENE DANIELS, and KIRK W. POMPER, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

The pawpaw [*Asimina triloba* (L). Dunal] is the largest tree fruit native to the United States and is in the initial stages of commercialization as a unique, high-value fruit crop for fresh-market sales or processing. As the satellite site for the USDA National Clonal Germplasm Repository for *Asimina* species, priorities of the Kentucky State University (KSU) pawpaw research program include description and classification of unique germplasm. Soluble solids content (SSC), or Brix, is a measure of the approximate sugar content of fruits, vegetables, juices, and wines. SSC has not been previously examined for major pawpaw selections. The objective of this study was to determine SSC (°Brix) in 31 pawpaw selections. Five ripe fruit were harvested from 31 different pawpaw selections at the KSU Research Farm in September 2006, skin and seeds removed, and flesh pureed and frozen. Three ~2 ml samples of each selection were thawed, and °Brix was determined using a refractometer. Differences in SSC among pawpaw selections were observed. The selections Potomac, K8-2, 9-47, Susquehanna, 5-5, Overleese, and Taytwo had the highest SSC (Brix >23). The selections 3-21, Mitchell, and PA-Golden had the lowest SSC (Brix <17). Brix can be correlated with perceived sweetness in fruits, which can affect consumer taste preference. Classifying pawpaw varieties by SSC could improve cultivar recommendations for pawpaw growers and aid the Repository in description of fruit characteristics of germplasm material.

Assessment of Variation in Annonaceous Acetogenin Activity in Pawpaw (*Asimina triloba*) Cultivars. JEREMIAH D. LOWE*, KIRK W. POMPER, SHERI B. CRABTREE, and JESSICA DURHAM, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601.

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit that has potential as a new niche crop for small farmers in the eastern United States. Pawpaw contains Annonaceous acetogenins, which are promising new anti-tumor and pesticidal agents, present in extracts of twigs, fruit, seeds, roots, and bark of pawpaw. Ripe fruit potentially represent a large source of biomass for the extraction of acetogenin compounds. Identification of pawpaw cultivars displaying a high acetogenin activity would be beneficial for farmers wishing to grow pawpaw as a source of these compounds. The objective of this study was to assess the variation in acetogenin activity of 16 different pawpaw genotypes. Five ripe fruit were harvested from each of the pawpaw cultivars Middletown, Mitchell, NC-1, Potomac, Sunflower, Susquehanna, Taylor, Taytwo, Wabash, Wells, and Zimmerman as well as the advanced selections 2-10, 3-11, 10-35, 11-13, and K2-7. Fruit pulp was homogenized, placed in ziplock bags, and stored at −15°C until extraction. Pulp was extracted with 95% ethanol and the Brine Shrimp Test (BST) bioassay was employed to assess acetogenin activity. The BST identified acetogenin activity in the pulp of all cultivars examined. The ripe fruit pulp of the cultivar NC-1 had the highest activity while the cultivars Sunflower and Wells displayed the lowest

activity. Other cultivars showed activity levels that were intermediate. BST can serve as a rapid screening method in identifying high acetogenin pawpaw genotypes.

Clonality of Pawpaw (*Asimina triloba*) Patches in Kentucky. KIRK W. POMPER, JEREMIAH D. LOWE, LI LU, SHERI B. CRABTREE, and LAUREN A. COLLINS, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

Pawpaw [*Asimina triloba* (L.) Dunal] is a fruit tree native to the southeastern region of the United States. As part of Kentucky State University USDA Pawpaw Repository efforts, assessing genetic diversity across the pawpaw's native range is a high priority. Pawpaw is usually found in large patches in the understory of hardwood forests. Because root suckering is often observed, these patches are believed to be clonal in nature. In this study we wished to test the hypothesis that native pawpaw patches are clonal. The objective of this study was to utilize inter-simple sequence repeat (ISSR) DNA-PCR fingerprinting techniques to determine if DNA fingerprint patterns indicated pawpaw patches contained genetically different trees (seedlings) in a patch. DNA was extracted from leaf samples collected from 20 trees each from six native patches in central Kentucky. Two ISSR primers yielded three polymorphic markers, 841T-1470, 841C-2800, and 841C-750, and six monomorphic markers, 841T-1380, 841T-670, 841C-1945, 841C-1830, 841C-1550, and 841C-1480 in the six patches (A–F). Patches B, C, and D did not display any polymorphic markers in each patch, suggesting these patches were clonal. However, Patches A, E, and F did show polymorphic markers within each patch, indicating these patches were not clonal and contained trees of at least two genotypes within each patch. With 50% of the pawpaw patches that we examined not being clonal, we reject of our hypothesis that native patches are clonal. This study suggests that to assess the genetic diversity of populations, more intensive sampling strategies will be required.

SCIENCE EDUCATION

Molecular Biology and Biotechnology Courses and Opportunities at 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.

Modern molecular biology and biotechnology impact multiple areas of biology and chemistry, such as genetics, biochemistry, cell biology, medicine, and agriculture. Training in biotechnology and molecular biology techniques is critical for students who wish to pursue careers in the life sciences and agriculture. In 2005, a USDA 1890 Institution Capacity Building Grant titled "Development of Biotechnology Courses to Enhance Aquaculture and Life Science Programs and Recruit Students to Kentucky State University" 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 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. About 50 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, and bacterial transformations. In the course "Advanced Techniques in Biotechnology" (re-named as "Advanced Molecular Biotechnology"), the students received additional training in modern techniques including purification of DNA from agarose gel; ligation of DNA fragments to create new constructs; tissue culture and transformation of model plant *Arabidopsis*; and usage of bioinformatics databases and software, such as Genbank, EMBL, and BLAST.

J. Ky. Acad. Sci. 70(1):104–105. 2009.

Abstracts of Some Notable Papers Presented at the 2008 Meeting of the Junior Kentucky Academy of Science

Edited by Ruth Beattie

Got Mercury? A Two Year Spectrophotometric Analysis of Fish Tissue Utilizing Diphenylcarbazone. KRISTIN L. FIELDS and ASHLEY C. FIELDS, Ballard High School, Louisville, KY 40243.

Levels of Hg in tuna have been examined over the past two years. This year fresh Ahi tuna from a local fish marked samples were examined for levels of Hg. The control was a standard solutions of 1×10^{-3} M $Hg(NO_3)_2$. This solution was diluted through a serial dilution down to 10^{-7} M and a plot was made of the resulting data. 18 samples of fish were digested in a nitric/sulfuric (70/30) solution. After the digestion process the solution was tested utilizing a Perkin Elmer UV-Vis Spectrophotometer and a diphenylcarbozone (DPC) indicator. All samples had measureable amounts of Hg.

The Effect of the S2 Pyocin on the Growth and Biofilm Formation of *Pseudomonas aeruginosa*. LINDSEY E. HASTINGS, DuPont Manual High School, Louisville, KY 40208.

Pseudomonas aeruginosa is a gram-negative bacterium that has a 6.3 Mbp highly mutable genome. As a result, it has become resistant to many types of antibiotics typically used to treat infections in cystic fibrosis patients. It is a possible alternative or aid to competitor toxin, used to treat the biofilms often found *in vivo* in cystic fibrosis patients. We determined if the introduction of the S2 pyocin to *P. aeruginosa* biofilms would result in less biofilm growth and development than in the controls. The S2 pyocin was produced by applying oxidative stress in the form hydrogen peroxide and iron starvation to several *P. aeruginosa* colonies. The pyocins were the purified and tested against 16-hr biofilms grown at 37 °C and 8-hr biofilms grown at 50 °C. The biofilms were visualized using dye and microscope assay. ComState™ and MatLab™ were used for data and statistical analysis. The average control biomass was considerably higher ($\approx$0.94 μm^2) than that of the pyocin treated biofilms ($\approx$0.2 $\mu m^3/\mu m^2$). Paired *t*-test showed the data to be significant with a P value of 0.0186. Fluorescence analysis with Fluoview™ software also confirmed these data. It was found that foreign S2 pyocins greatly reduce biofilm formation and cause substantial cell death in *P. aeruginosa* biofilms.

Exacting Value from Waste. KARTIK MALHOTRA, DuPont Manual High School, Louisville, KY, 40208.

An estimated 5 to 9 billion pounds of poultry feathers are produced each year in the US alone. A small portion is reprocessed and fed back to farm animals as meal; however, a significant amount is put into landfills. This project explored several different possible applications for chicken feathers. The first application was erosion control using hydro-seeding mats. It was hypothesized that including feathers in the mat would improve their water resistance. The hypothesis was fully supported, and water resistance increased dramatically while tensile decrease slightly. Hydro-seeding mats that contain feathers are now being tested for commercial use. The second application explored was flocculation, in which the feathers were evaluated as a flocculating aid. They helped to some extent. The final application was filtration. The ability of feathers to absorb salts of metals was evaluated and it was demonstrated that feathers could effectively filter heavy metal ions such as chromium, lead and arsenic from water. The absorption efficiency was further improved by sonicating the feathers to reduce ions to ppb levels below allowable potable limits. These studies identified two value added applications to deal with real world problems by using a waste product. They provide possible alternatives to current methods that have a negative effect on the environment and/or are expensive. In contrast, the approaches that where identified in this project are environmentally friendly, cost effective, and can contribute to sustainable development.

Metals in Plants. URVI PATWARDHAN, Winburn Middle School, Lexington, KY 40511.

The purpose of this project was to determine if plants would increase metals uptake if provided with water that contained leached metals. Different metals were added to 5 different water containers and allowed to leach for one week. The amount of metals leached in water was measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES). Mustard seeds were planted in different containers and watered using specific metal leached water for 10 days. The amount of metal uptake by mustard plants was also measured by ICPOES. All metals, except stainless steel, leached in water. Copper and iron leached the most. Plants did take up metals that were present in the water but in different amounts. Iron was taken up most followed by copper. The presence of certain metals decreased the uptake of other metals; plants that received water with leached aluminum had smaller amount of copper uptake than plants that received control water. This property could be used to decrease content of undesired metals in plants.

Tracking Cosmic Ray Muons Using a Cloud Chamber. LEAH WILSON* and ABBY LENHART, Math, Science and Technology Magnet, DuPont Manual High School, Louisville, KY 40208.

The muon is a type of cosmic ray. The purpose of this project was to measure the muon rate at Louisville,

Kentucky for the first time. At Bellarmine University, a cloud chamber was assembled using a basketball display case modified to uniformly produce an environment where muon tracks could be detected. Felt pads inside of the chamber were soaked with 91% isopropyl alcohol to create a supersaturated atmosphere of alcohol. The chamber was set on a block of dry ice to create the required environment for muon detection. In a dark room, with a constant beam of light shining through a side, cosmic ray muons were observed striking through the "alcohol fog". In the cloud chamber, the effective area of observation was about 100 cm^2. During a 20-minute period in 5 different trials, an average of 119 muons were observed. The muon flux (number of muons hitting the earth's surface per cm^2) was calculated to be 0.06 events per minute per cm^2 for the Louisville area. The muon flux count was also consistent (adjusted for the method of collection) with the muon flux data obtained from SLAC's Cosmic Ray Detector.

J. Ky. Acad. Sci. 70(1):106. 2009.

Kentucky Academy of Science
Business Meeting
University of Kentucky Student Center
October 31, 2008

The 2008 KAS Annual Business Meeting was called to order by Dr. John Mateja at 4:00 P.M. Twelve board members and KAS members were in attendance. Dr. Mateja welcomed the membership.

Report from Heritage Land Conservation Fund Board KAS representative William Martin.

William Martin shared with KAS members the goals and objections as well as activities of this important board.

President Elect Report

Robin Cooper stated planning of the 2008 Meeting is complete and we are expecting a successful meeting.

Vice President's Report

Nancy Martin announced the Superlative Award winners:

Outstanding College/University Teacher: D. Joseph Hagerty, U of L

Outstanding Academy Service: Susan Templeton, KSU

Distinguished College/University Scientist: Diane Snow, UK

Distinguished Professional Scientist (in non academic setting) Daniel Phelps

No 2008 Outstanding Secondary School teacher award.

Treasurer's Report (handout filed with minutes)

Ken stated the Wachovia account has lost 26% YTD compared to 32% for the S&P 500. The US Bank/Athey Trust account balanced has had less volatility due to the more conservative asset allocation.

Executive Director's Report

2008 KAS total membership to date is 1382.

501 individuals have pre registered for the 2008 KAS annual meeting.

Program Director's Report

Bob Creek reported there are 357 total research presentations at the 2008 annual meeting with 188 oral presentations (85 URC and 44 GRC) and 169 poster presentations (103 URC). Bob strongly encouraged everyone to attend the 2008 KAS Symposium later this evening.

2009 KAS Board Member Election Results

David Olson, Chair of the Nominations Committee was not able to attend the Annual Meeting. He forwarded the election results prior to the Annual Business Meeting and John Mateja announced the 2009 KAS Board members:

— Social Science Representative—Sean Reilley; Morehead State University
— Physical Sciences Representative—KC Russell; Northern Kentucky University
— Vice President—Barbara Ramey, Eastern Kentucky University

KAS Centennial Celebration

John Mateja reported this event will be in 2014. He will speak with incoming VP Barbara Ramey regarding initiating plans for the KAS Centennial Celebration.

Recognition of Outgoing Board Members

John Mateja thanked Nigel Cooper, Scott Nutter, and David Olson for their service to KAS. Plaques were presented to the outgoing board members present at the meeting.

President Elect—Robin Cooper

Robin Cooper thanked John Mateja for his outstanding service over the past year and presented a plaque from the Academy. John will forward the KAS gavel to Robin, and presented the traditional $100 gift to the President's Fund as his final act of service to KAS.

Meeting Adjourned at 4:20 P.M.

Respectfully submitted,

Rob Kingsolver, KAS Board Secretary
Jeanne Harris, KAS Executive Director

CONTENTS