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Cover: Two spider species collected at the Morgan-Monroe/Yellowwood State Forest Ecoblitz. The ecoblitz was hosted by the Indiana Forest Alliance between 2014 and 2017. Left: *Neoscona crucifera*, an orb-weaver spider in the family Araneidae, was frequently encountered at night in the bottomland at the ecoblitz site. The upper surface of the abdomen is brown and hairy; the legs display alternating light and dark brown bands; and the undersurface of the abdomen is black, with two white spots (not visible in this image). (Photo copyright Jeffrey Belth, used with permission.) Right: *Dolomedes albineus*, a large fishing spider in the family Pisauridae, is a new distribution record in Indiana. Also called White-banded fishing spider do to the white band along the 'face' (clypeus). This species was found in bottomland and slope woods at night. The female in this photo is guarding an eggcase. (Photo by Brian Foster) For more information on the spider fauna from Indiana, see the article entitled "A Survey of Spider diversity in Morgan-Monroe/Yellowwood State Forest" in this issue.

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Figures 1–4.—Right chelicerae of species of *Centruroides* from Timbuktu. 1. Dorsal view; 2. Prolateral view of moveable finger; 3. *Centruroides* holotype male; 4. *Centruroides* female. Scale = 1.0 mm.

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CRANIOMETRIC INDICATORS OF ANCESTRY AMONG FRENCH AMERICANS

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ABSTRACT. The accidental discovery of human remains washed out of the Wabash River bank in northwestern Indiana has led to attempts to identify them. The individuals are thought to be associated with Fort Ouiatenon, a historic French fur trading post constructed in 1717. The contents of the site (12T1198), found approximately 1.6 km from the fort, include human remains and associated coffin nails. The human remains studied (n = 3) were fragmented and incomplete. This study attempts to determine ancestry of the individuals using metric indicators, following procedures laid out in Standards for Data Collection of Human Skeletal Remains (Buikstra & Ubelaker 1994) and analyzed utilizing SPSS version 23. The study built a database of individuals of French, non-French European, and African ancestries. Analysis was conducted using discriminant function analysis to cluster and predict ancestries. The results of the study were successful in differentiating French ancestry, but the individuals of 12T1198 could not be confidently placed within this group. However, post priori analysis suggests a large amount of gene flow occurring early in the Americas causing individuals of French American ancestry to plot within different groups. The individuals of 12T1198 align with this discovery by plotting into multiple groups. The ultimate designation of these burials as European points to a possible association with Fort Ouiatenon, meaning they may be among the first French settlers in the area.

Keywords: Ancestry, craniometrics, French American

INTRODUCTION

In 2011, human remains were discovered washed out of the bank of the Wabash River in Tippecanoe County, Indiana, approximately 1.6 km from Fort Ouiatenon. Recovery efforts, conducted by the Indiana University-Purdue University Archaeological Survey (IPFW-AS), discovered four individuals in various levels of completeness, as well as historic coffin nails. Deemed site 12T1198, the remains were sent to the University of Indianapolis for bioarchaeological analysis.

The question quickly arose, are these individuals associated with the fort? If so, they would represent some of the oldest historic remains in the state of Indiana. The analysis that followed sought to extract as much information from the remains as possible, including age, sex, and ancestry. However, when attempting to determine ancestry, it was not enough to state whether these individuals were European; a more precise designation was necessary. This was done by collecting craniometric data from French, non-French European, and African populations. Discriminant function analysis (DFA) was conducted to determine if groups could be separately classified. The DFA classification was applied to the individuals from 12T1198 to determine if the individuals classified as French.

Determination of ancestry.—One of the fundamental concerns for those who study skeletal remains is the establishment of the biological profile, which includes age, sex, ancestry, stature, and other idiosyncrasies (Reichs 1986; Byers 2011; White et. al. 2012). The term ancestry refers to one's biological heritage and used to be termed "race." However, while the term race sees continued use, especially in the general public, it is considered antiquated by anthropologists because it refers to both ethnic and anatomical indicators of heritage.

The concept of race may also be conflated with geographic variation (Caspari 2010). Anthropologists agree that variation exists across geographic areas, but the question is whether these variations can be seen in the phylogeny of local and regional populations. Livingstone (1962) argues that variation is best understood through clines and the distribution of individual morphologic and genetic traits, as opposed to racial categories which display a non-concordance of

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many traits and obscures the migration and gene flow that causes the observed variation.

Unfortunately, the concept of race still exists due to the social categorization of people and the visible physical characteristics on which these racial categories are assigned by the public. To aid the public in understanding the problems with the concept of race, the American Association of Physical Anthropologists (AAPA) issued a statement declaring that all humans belong to a single species (*Homo sapiens*) and, while regional populations exist, all groups descend from a common ancestral group (AAPA 1996). Additionally, the AAPA states there is more genetic diversity within, than between, populations. The AAPA then goes on to add that there are obvious physical differences between groups living in different parts of the world with varied traits that researchers have sought to classify for centuries.

Carl Linnaeus performed some of the earliest classification when he divided humans into four subspecies: *Homo sapiens europaeus, Homo sapiens asiaticus, Homo sapiens afer*, and *Homo sapiens americanus* (Blumenfield 2011). Generally, biological anthropologists today designate races within three major groups: White, Black, and Asian/Native American. It is within these three groups that the most research has been conducted. However, these racial categories continue the problem of conflating ethnic and anatomical indicators (DiGangi & Hefner 2013). Thus, herein, the term ancestry will be used in place of 'race' since race is not a biologically useful term .

Anthroposcopy, defined as the "visual inspection of the human body... for the purpose of identifying traits of a qualitative nature" (Byers 2011, p. 14), became the basis for the earliest studies to differentiate between groups (Giles & Elliott 1963). These studies have since been reworked and built upon and the use of anthroposcopic traits is still commonly used to determine ancestry in unknown remains (Buikstra & Ubelaker 1994; Gill 1998; Byers 2011; White et. al. 2012). However, researchers are often frustrated by the ambiguity of anthroposcopic traits, especially as form is seen as variation across a continuum, as opposed to discrete groupings. This has led to efforts to quantify anatomical differences using standardized measurements of the skeleton (Brues 1990).

One of the earliest studies, conducted by Giles & Elliott (1963), sought to differentiate between Whites and Blacks. The study focused on American White and Black crania from the Hamann-Todd collection and used nine measurements to establish discriminant function equations able to separate unknown individuals into one of the two ancestral groups. W.W. Howells (1973) expanded on these early studies by analyzing crania from around the world. Howells (1973, 1989) observed the variation among populations and sought to record measurements of groups from Europe, Africa, Asia, Oceania, North America, and South America.

French Colonial history in North America.—In 1534, Jacques Cartier was sent by the French king to explore the coast of Newfoundland and the St. Lawrence River, prompting fur traders and settlers to begin immigrating to these areas (Gascoigne 2001). In 1608, Samuel de Champlain founded Quebec, then Montreal three years later, thereby establishing the region that would become New France. After the founding of the city of Montreal, immigration was primarily composed of French citizens, especially single males (Vigeant 2012). After 1680, immigration to New France shifted and, while France retained the majority of arrivals, immigrants from the British Isles and other European countries were not uncommon.

In the 1670s Louis de Buade, Comte de Frontenac et de Palluau was appointed governor general of New France (Eccles 1983). Frontenac made it his personal mission to expand both the area controlled by France and the fur trade. He began by building forts within the areas already controlled by New France, which would provide protection for French traders in the region. Then, in 1673, Louis Jolliet found the mouth of the Mississippi River, opening up vast new areas to the French and the fur trade.

In 1717, Fort Ouiatenon was established on the Wabash River near the present-day city of West Lafayette, Indiana (Tippecanoe County Historical Society, No Date). Named for the Ouiatenon (Wea) Native American village located nearby, the trading post became an important site for the regional fur trade.

Territorial disputes between the French and English led to the French and Indian War in 1754. At the conclusion of the war, the French lost all their lands in North America, including Fort Ouiatenon, which was soon garrisoned by English troops. However, a lack of trust in the English and a growing number of settlers streaming across the Appalachian Mountains, caused the Native American groups to rebel. In 1763, an Ottawa

uprising led to the capture of eight frontier posts, including Fort Ouiatenon. It was here that the opposing groups met to discuss a peace treaty.

Terms of the treaty allowed the British to peaceably maintain forts in the West, but all settlers had to return east of the Appalachian Mountains. The British abandoned Fort Ouiatenon and the post returned to a small, French settlement until after the Revolutionary War when Native American groups again became alarmed at the increasing number of white settlers and skirmishes broke out across the region. Fort Ouiatenon became a staging ground by Native Americans for raids into the surrounding area. Fearing for their lives, the remaining French settlers abandoned the post in 1780. Efforts to secure the fort proved fruitless and, in 1791, President Washington ordered the post to be destroyed and the Native groups dispersed. Over time, the fort was obliterated from the landscape and, eventually, forgotten. Then, in 1967, after decades of searching and mistaken locations, the fort was rediscovered (Noble 1982, 1991).

In 2011 excavations were conducted by IPFW-AS after human remains were discovered washed out of the bank of the Wabash River in Tippecanoe County, Indiana, approximately 1.6 km from the fort (Williams-Draeger, Pers. Comm.). The site, 12T1198, contained the remains of four individuals as well as associated historic coffin nails. After excavation, the remains were sent to the University of Indianapolis Indiana Prehistory Laboratory, where they remain today. Analysis of the individuals was conducted by the author (H. Miller) and the three individuals complete enough for analysis were determined to be males of young to middle adult age (Miller 2014, 2015).

Anthroposcopic and craniometric ancestral analysis were conducted on two individuals, Burial 1 and Burial A, but was deemed inconclusive as a preponderance of indicators could not be achieved. In an effort to estimate ancestry and determine if the individuals are associated with the fort, a study was designed to utilize cranial measurements to predict French ancestry. The authors recognize that France is a European country and not a homogenous racial, ethnic, or ancestral group. However, based on the previous discussion of clinal variation (Livingstone 1962), the authors hypothesized that France may be a distinct enough population, with variation from both peninsular separation and access to Mediterranean trade, to create a statistically distinct group. This could allow discrimination between and prediction into groups utilizing DFA.

MATERIALS AND METHODS

Sample and data.—Measurements were chosen for analysis based on the availability of data within the samples used. Measurements were chosen to keep the highest number of individuals in the sample. As a general rule, 7–10 measurements are typically used in DFA. Therefore, seven measurements were selected to keep a meaningful sample size. In the case of all samples, reported measurements were transferred into the measurement system used in Standards for Data Collection from Human Skeletal Remains (Buikstra & Ubelaker 1994) to promote consistency and allow for comparison among groups. The seven measurements used were Maximum Cranial Length (GOL), Maximum Cranial Breadth (MCB), Nasal Height (NLH), Nasal Breadth (NLB), Orbital Breadth (OBB), Orbital Height (OBH), and Frontal Chord (FRC). Groups were created based on data collected and mined from a number of archaeological sites and databases.

Materials.—The first Montreal parish church, the Notre-Dame church, began construction in 1672 with church records indicating the cemetery was in use from 1691 through at least 1796 (Vigeant 2012). The majority of individuals from Notre-Dame cemetery were of French ancestry, with more than half being born in Montreal. The Ville of Sainte-Marie was founded in 1736 (Municipality of Sainte-Marie 2014). The religious parish was founded just after the city in 1737 with the burial ground in use from 1748 to 1879. The Notre-Dame and Sainte-Marie collections are housed at the University of Montreal with analysis led by Dr. Isabelle Ribot. In addition, a Parisian sample was used that represents 19th century Parisians. This database was shared by Dr. Marie Danforth of Mississippi State University and originally came from Dr. Alain Frement at the University of Maryland.

Fort Biloxi was established in present day Mississippi in April 1699 (Carter et. al. 2004; Danforth 2011) and became the capital in 1719. Full excavation of the Moran site began in May 2007, led by Dr. Marie Danforth of Southern Mississippi University. Analysis conducted on the burials determined the individuals to be European, with French ancestry as the mostly likely

ancestral determination based on skeletal analysis, diet, and analysis of associated artifacts.

The use of comparative samples is essential for the aims of this research and the data collected by Howells (1973, 1989, 1995) establishes an extremely useful database from which to begin. Two European groups were chosen: Norse and Berg, as well as two African groups: Egypt and Dogon. The data used were compiled by Howells between 1965 and 1980 and the authors acquired it from the William W. Howells Craniometric Data Set provided by the University of Tennessee, Knoxville (Auerbach 2014).

Excavations began in 1990 on the west side of Chicago to save what was left of the earliest Dunning Cemetery (Grauer & McNamara 1995; Grauer et al. 1998). The portion excavated was believed to be associated with the Cook County poor farm, the almshouse, and the insane asylum. Historical resources suggest this portion of the cemetery was in use from 1851 until 1869. Sex estimation indicated the presence of 19 adult males with osteological analysis conducted by faculty and students at Loyola University in Chicago with data provided to the authors by Dr. Anne Grauer. Ancestral analysis was conducted by the author (H. Miller) utilizing FORDISC (Ousley & Jantz 1996) to assign individuals to an ancestral category. A posterior probability of 0.80 was chosen for a confident designation. Of the 19 adult males, three were confidently assigned to the European ancestral group and two were assigned to the African ancestral group and placed in the West African sample. The remaining individuals were not used in analysis due to a lack of recordable craniometrics and confident ancestral designation.

The Wright and Rhoads cemeteries were excavated as part of cultural resource management mitigation projects near Indianapolis, Indiana (Nawrocki et al. 1998, 2010). Osteological analysis of both cemeteries was conducted at the University of Indianapolis Archaeology Forensic Laboratory led by Dr. Stephen Nawrocki. Historical research of the Rhoads cemetery determined that the property was owned by the Rhoads family from either 1821 or 1822 until sometime after 1906, but before 1928. Excavations uncovered 46 burials and one cremation urn within the delineation of the cemetery. Excavation of the Wright/Whitesell/Gentry [Wright] cemetery uncovered 33 burials. Available indicators of individuals from both cemeteries suggest the interred are of European ancestry.

In sum, the sample consists of 316 individuals separated into four groups: French, non-French European, North African, and West African populations. The French group ($n = 85$) consists of individuals from the Notre Dame and Saint Marie cemeteries of Montreal, and the Moran site from Biloxi, Mississippi, as well as measurements mined from the Parisian craniometric database. The non-French European sample ($n = 124$) consists of individuals from the Dunning Poorhouse Cemetery, the Wright and Rhoads Cemeteries, and Howells' Norse and Berg populations. The North African sample ($n = 58$) is comprised of Howells' Egypt population, and the West African sample ($n = 49$) is comprised of Howells' Dogon population plus two individuals from the Dunning Poorhouse. For the purposes of this study, only adult males with positive ancestral determination were used.

Methods.—For the sample used in this study, linear discriminant analysis was conducted using SPSS version 23. Initial analysis was conducted to test whether individuals of French ancestry could be classified as a group separate from the non-French European ancestry. The four groups: French, European, North African, and West African were chosen as the grouping variables with cranial measurements chosen as the independent variables. A second analysis was conducted to observe where the individuals from 12T1198 would plot into the groups created in the DFA. The individuals from 12T1198 were added to the database as ungrouped cases thereby excluding them from the analysis to create groups, but allowing group membership to be predicted.

Validation of classification was accomplished in two ways. First a leave-one-out cross-validation test was conducted during the initial DFA. Second, a quadratic discriminant analysis was conducted using the statistical language R.

RESULTS

Discriminant function analysis.—The total sample consisted of 316 individuals, with 32 individuals excluded from analysis due to missing discriminating variables (craniometric measurements) for an analysis sample size of 264 individuals. The assumption of homogeneity of covariance was assessed using a Box's M Test with an alpha level used in this analysis of 0.001. The *p*-value of this test is 0.000, meaning there is no evidence of difference between the covariance structures (Table 1). This violates

Table 1.—Results of the Box's M Test of Homogeneity.

Box's M		500.166
F	Approx.	5.658
	df1	84
	df2	104417.285
	Sig.	0.000

the assumption of homogeneity. However, this test is sensitive to departure from normality. Due to the violation of assumption and the sensitivity of the test, a quadratic discriminant analysis was conducted to confirm the linear discriminant analysis (see below).

The analysis of Eigenvalues proves the functions created are effective (functions 1 and 2) and moderately effective (function 3) at discriminating between variables (Table 2). In addition, the Wilks' Lambda Test of Assumptions displays a *p*-value of less than 0.001 for all the functions, thus rejecting the null hypothesis that the functions have no discriminating ability (Table 3).

The discriminate functions were then used to classify individuals into groups based on the grouping variables (Table 4). Of the original groupings, 89.7% of French individuals, 66.7% of non-French European individuals, 70.7% of North African individuals, and 93.6% of West African individuals were correctly placed into the predicted groups (French, European, North African, and West African, respectively). Overall, this analysis saw 77.5% of original grouped cases correctly classified into Predicted Groups. The bottom half of the table displays the results of the leave-one-out cross-validation test. This test is conducted to confirm the classification performance of the analysis. Overall, 76.1% of cross-validated grouped cases were correctly classified.

As mentioned previously, the quadratic DFA (Table 5) was conducted after the results of the Box's M test came back significant, indicating there was evidence of differences between covariance structures. The top portion of the chart displays the prior probabilities, the bottom portion of the chart displays the classification table. When compared against the output from SPSS, the data were almost identical. Thus, the quadratic discriminant analysis was successful in correctly classifying individuals into the predetermined groups, thereby confirming the linear discriminant analysis.

Output 2.—A second analysis was conducted to observe where the individuals from 12T1198 would plot into the groups created in the original DFA. All classification groups remained the same. The individuals from 12T1198 were added to the database as ungrouped cases thereby excluding them from the analysis to create groups, but allowing group membership to be predicted. Since the individuals of 12T1198 were not included in the classification, the underlying output (Table 6) is almost identical to that of the first analysis and will not be further discussed.

The individuals from 12T1198 were analyzed as ungrouped cases and are placed into predicted groups based on the groups created from the other samples. In the result of interest, one 12T1198 individual was placed in the Predicted European Group, and one was included in the Predicted North African Group.

DISCUSSION

By saving the classification results to the dataset, it can be seen that Burial 1 was classified as North African and Burial A was classified as European. Interestingly, this is the opposite of what was expected. Burial 1 displayed anthroposcopic traits more often associated with Europeans while Burial A displayed highly mixed traits, including a guttered nasal sill and wide nasal aperture displayed often in individuals of African ancestry. This surprising classification could reflect the anthroposcopic ancestral assessment which saw an amalgamation of traits in both individuals. It could also be attributed to the presence of admixture.

However, when referring back to the classification output, some French individuals were also classified as North African, with others classified as non-French European and West African. These same misclassified individuals were primar-

Table 2.—Results of the Analysis of Eigenvalues.

Function	Eigenvalue	% of variance	Cumulative %	Canonical correlation
1	1.801	51.7	51.7	0.802
2	1.384[a]	39.8	91.5	0.762
3	0.296[a]	8.5	100.0	0.478

Table 3.—Results of the Wilks' Lambda Test of Assumptions.

Test of function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	0.116	598.769	21	0.000
2 through 3	0.324	312.974	12	0.000
3	0.772	71.884	5	0.000

ily from the French populations in North America. Therefore, a new hypothesis was posed that the individuals of 12T1198 would plot to the same group as French American individuals. In a third analysis, the individuals of French American ancestry were removed from the French group and left uncategorized to test into which group they would be classified (Table 7). In this classification result five of the nine uncategorized individuals were classified as North African, with only one as European, and three as West African.

Why are these individuals being classified as North African? This may be due to the atypicality of the North African group, comprised solely of Egyptian individuals from the Howells dataset and dated to 600–200 B.C. This dataset, as we learned after our initial analysis, has potential issues with inclusion of individuals from other areas of the world, including Greece. Thus, the Egyptian dataset was excluded from further analysis and interpretation.

After removing the North African group from analysis, all of the uncategorized individuals that were previously classified as North African were then classified as non-French European (Table 8). In addition, the percentage of correctly classified grouped cases rises to 95%, with 94.1% of cross-validated grouped cases correctly classified.

The DFA confidently discriminated between ancestral groups and accurately placed individuals within predicted ancestral groups. In particular French ancestry could be determined through craniometric analysis. Furthermore, the post priori tests indicated that the individuals of 12T1198 and the French American individuals computed very similarly, first as North African and then as non-French European. Further analysis showed that French Americans, while a small sample size, do not create a homogenous group, instead clustering into multiple groups. This is likewise reflected in the individuals of 12T1198.

Perhaps DFA is detecting the disappearance of French traits in the Americas. This hypothesis has been posed previously by Gore (2008), who posited that geography and genetic drift were more influential on facial morphology and biological variation than genetic ancestral groups.

Table 4.—Classification Table showing predicted classification of individuals.

			Predicted Group Membership				
		GroupComb	French	European	N. African	W. African	Total
Original	Count	French	61	1	4	2	68
		European	4	74	31	2	111
		N. African	3	10	41	4	58
		W. African	0	0	3	44	47
	%	French	89.7	1.5	5.9	2.9	100.0
		European	3.6	66.7	27.9	1.8	100.0
		N. African	5.2	17.2	70.7	6.9	100.0
		W. African	0.0	0.0	6.4	93.6	100.0
Cross-validated	Count	French	61	1	4	2	68
		European	4	71	34	2	111
		N. African	3	10	41	4	58
		W. African	0	0	4	43	47
	%	French	89.7	1.5	5.9	2.9	100.0
		European	3.6	64.0	30.6	1.8	100.0
		N. African	5.2	17.2	70.7	6.9	100.0
		W. African	0.0	0.0	8.5	91.5	100.0

77.5% of original grouped cases correctly classified.
76.1% of cross-validated grouped cases correctly classified.

Table 5.—Output of Quadratic DFA using R.

	French	European	N. African	W. African
Prior probabilities of groups:	0.2394366	0.3908451	0.2042254	0.1654930
Predicted Group Membership				
French	61	4	4	0
European	0	68	5	1
N. African	4	37	47	1
W. African	3	2	2	45

This hypothesis is most clearly reflected at the Moran site, where all three complete individuals were categorized as West African, despite firm genetic evidence that categorizes them as European.

The results from the 12T1198 site may be illustrating a mixing of characteristics due to the amount of gene flow between groups that occurred within a few hundred years of the appearance of Europeans in the Americas.

In this analysis, we can see the individuals from site 12T1198 and those from the French American samples were classified into a variety of groups (Table 9). This is not surprising as the dates of the cemeteries used for the French American group would have allowed time for genetic flow to occur, but could still represent more recent immigration patterns. This analysis supports our hypothesis that gene flow in the Americas worked to remove distinct European and French traits, thereby

Table 6.—Output 2 Classification Table including individuals of site 12T1198.

				Predicted group membership				
			GroupComb	French	European	N. African	W. African	Total
Cases Selected	Original	Count	French	61	1	4	2	68
			European	4	74	31	2	111
			N. African	3	10	41	4	58
			W. African	0	0	3	44	47
		%	French	89.7	1.5	5.9	2.9	100.0
			European	3.6	66.7	27.9	1.8	100.0
			N. African	5.2	17.2	70.7	6.9	100.0
			W. African	0.0	0.0	6.4	93.6	100.0
	Cross-validated	Count	French	61	1	4	2	68
			European	4	71	34	2	111
			N. African	3	10	41	4	58
			W. African	0	0	4	43	47
		%	French	89.7	1.5	5.9	2.9	100.0
			European	3.6	64.0	30.6	1.8	100.0
			N. African	5.2	17.2	70.7	6.9	100.0
			W. African	0.0	0.0	8.5	91.5	100.0
Cases Not Selected	Original	Count	French	0	0	0	0	0
			European	0	0	0	0	0
			N. African	0	0	0	0	0
			W. African	0	0	0	0	0
			12T1198	0	1	1	0	2
		%	French	0.0	0.0	0.0	0.0	100.0
			European	0.0	0.0	0.0	0.0	100.0
			N. African	0.0	0.0	0.0	0.0	100.0
			W. African	0.0	0.0	0.0	0.0	100.0
			12T1198	0.0	50.0	50.0	0.0	100.0

77.5% of selected original grouped cases correctly classified.
76.1% of selected cross-validated grouped cases correctly classified.

Table 7.—Results of Classification Analysis of French American sample.

			Predicted group membership				
		GroupComb	French	European	N. African	W. African	Total
Original	Count	French	61	1	1	0	63
		European	5	72	32	2	111
		N. African	3	10	43	2	58
		W. African	0	0	3	44	47
		French American	0	1	5	3	9
	%	French	96.8	1.6	1.6	0.0	100.0
		European	4.5	64.9	28.8	1.8	100.0
		N. African	5.2	17.2	74.1	3.4	100.0
		W. African	0.0	0.0	6.4	93.6	100.0
		French American	0.0	11.1	55.6	33.3	100.0
Cross-validated	Count	French	60	2	1	0	63
		European	5	71	33	2	111
		N. African	3	10	41	4	58
		W. African	0	0	4	43	47
	%	French	95.2	3.2	1.6	0.0	100.0
		European	4.5	64.0	29.7	1.8	100.0
		N. African	5.2	17.2	70.7	6.9	100.0
		W. African	0.0	0.0	8.5	91.5	100.0

78.9% of original grouped cases correctly classified.
77.1% of cross-validated grouped cases correctly classified.

creating a more homogenized 'American' ancestral group.

The aims of this project sought to understand ancestry of the individuals at 12T1198. Instead, we stumbled across evidence regarding morphological changes in the Americas. This research can help improve our understanding of variation in the Americas, especially that of French Americans. The historical background proves that French individuals had significant contact with disparate groups in the new world, and we, as researchers, must consider gene flow from French, Native America, African, and other European groups when assessing morphological variation in the Americas.

Utilizing craniometrics and discriminant function analysis, we were able to successfully separate French individuals from the European macro-

Table 8.—Results of Classification Analysis without North African group.

			Predicted group membership			
		GroupComb	French	European	W. African	Total
Original	Count	French	61	2	0	63
		European	4	103	4	111
		W. African	0	1	46	47
		French American	0	6	3	9
	%	French	96.8	3.2	0.0	100.0
		European	3.6	92.8	3.6	100.0
		W. African	0.0	2.1	97.9	100.0
		French American	0.0	66.7	33.3	100.0
Cross-validated	Count	French	61	2	0	63
		European	5	101	5	111
		W. African	0	1	46	47
	%	French	96.8	3.2	0.0	100.0
		European	4.5	91.0	4.5	100.0
		W. African	0.0	2.1	97.9	100.0

95.0% of original grouped cases correctly classified.
94.1% of cross-validated grouped cases correctly classified.

Table 9.—Results of Classification Analysis of American groups.

			Predicted group membership				
		GroupFinal	1.0	2.0	4.0	5.0	Total
Original	Count	Fr. American	2	0	1	2	5
		Parisian	0	61	2	0	63
		European	0	4	103	4	111
		W. African	0	0	0	47	47
		12T1198	0	0	2	0	2
	%	Fr. American	40.0	0.0	20.0	40.0	100.0
		Parisian	0.0	96.8	3.2	0.0	100.0
		European	0.0	3.6	92.8	3.6	100.0
		W. African	0.0	0.0	0.0	100.0	100.0
		12T1198	0.0	0.0	100.0	.0	100.0
Cross-validated	Count	Fr. American	1	0	2	2	5
		Parisian	0	61	2	0	63
		European	0	5	101	5	111
		W. African	1	0	1	45	47
	%	Fr. American	20.0	.0	40.0	40.0	100.0
		Parisian	0.0	96.8	3.2	0.0	100.0
		European	0.0	4.5	91.0	4.5	100.0
		W. African	2.1	0.0	2.1	95.7	100.0

94.2% of original grouped cases correctly classified.
92.0% of cross-validated grouped cases correctly classified.

group. In addition, site 12T1198 was found to be not inconsistent with French, especially American French individuals. More importantly, we uncovered morphological variation within American French and non-French European groups that points to rapid gene flow within the Americas. The individuals of site 12T1198 appear to reflect this complex gene flow.

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A COMPARISON OF PHYTOCHEMICALS PRESENT IN MIDWESTERN MEDICINAL PLANT EXTRACTS

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ABSTRACT. Plants consumed as medicines are thought to exert their physiological effects in part through the activity of their secondary metabolites, which include molecules with antioxidant activities. In this study, the concentrations of total phenolic, flavonoid, and anthocyanin compounds of eight medicinal plant extracts collected from Earlham College in Richmond, Indiana were examined. The collected data showed a wide range of variation in the concentration of antioxidant compounds in the eight examined plant extracts; the locally-collected gingko samples had the highest total phenolic content and plantain had the highest flavonoid content, while the black raspberry and mulberry samples had by far the highest anthocyanin content. In addition to describing the chemical composition of medicinal plants valued in the Midwestern United States, we compared different sources (Earlham College vs. purchased) and preparations (acetone vs. hot water extraction) of gingko leaves and compared the chemical composition of extracts that underwent an unintended additional freeze-thaw cycle. Gingko leaf extracts have the highest phenolic content of all extracts examined, and the purchased gingko teas and powder had higher levels of phenolic contents than all the locally collected, acetone extracts. Additionally, among the Midwestern species tested, no significant changes were observed in the concentrations of compounds measured in the extracts that underwent an additional freeze-thaw cycle. This study not only compares the phenolic compound composition of medicinal plant extracts but also provides pertinent information on the collection, preparation and storage of plant extracts to conserve these phenolic compounds.

Keywords: Midwestern medicinal plants, extracts, phenolic content, flavonoid, anthocyanin

INTRODUCTION

Phenolic compounds are naturally found in plants, including those commonly used as foods and medicines. These compounds are known to have multiple biological effects, including high antioxidant activity. In biochemistry and food industry research, there is increasing interest in the crude extracts of medicinal fruits, herbs, and other plant materials with phenolic compounds for a variety of applications. The abundance of phenolic compounds not only prevents the oxidative degradation of plant-based foods and materials but also improves the quality and nutritional value of such products (Löliger 1991). There is also interest in the role of flavonoid and anthocyanin compounds as "functional foods" in the prevention of coronary heart disease and cancer as well as in the overall maintenance of health (Serafini et al. 1998; Pandey & Rizvi 2009; Reis et al. 2016).

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The following plants were selected for this study because they are commonly used as traditional medicines throughout the world, including Indiana: goldenrod (*Solidago canadensis*), sassafras (*Sassafras albidum*), plantain (*Plantago* sp.), dandelion (*Taraxacum officinale*), ginkgo (*Ginkgo biloba*), black raspberry (*Rubus occidentalis*), and mulberry (*Morus alba*) (Fig. 1).

The leaves of gingko (also known as maidenhair tree) are highly regarded in traditional medicine and by the pharmaceutical industry due to their high concentration of phenolic compounds, specifically flavonoids (Pereira et al. 2013; Isah 2015). There are many narratives, oral traditions, and scientific studies recording the uses and values of this tree. Recently, gingko extracts were demonstrated to improve cerebral and peripheral blood circulation, especially in the lower limbs (Meston et al. 2008; Mashayekh et al. 2011). Moreover, these extracts have been shown to protect the nervous system against the effects of aging such as short-term memory and vertigo

Figure 1.—Local medicinal plants used in this study. Illustration of plants used, listed from left to right with common name, scientific name, parts of the plant used, and known traditional uses of each plant (Mashayekh et al. 2011; Isah 2015): dandelion (*Taraxacum officinale*; root; anti-inflammatory), goldenrod (*Solidago canadensis*; root, leaves; anti-inflammatory), plantain (*Plantago sp.*; leaves; lung related issues), sassafras (*Sassafras albidum*; root; fever, diarrhea), and ginkgo (*Ginkgo biloba*; leaves; memory aid). Figure by research lab member, Evelyn Sanchez.

(Meston et al. 2008; Pandey & Rzivi 2009; Mashayekh et al. 2011).

While multiple freeze-thaw cycles are generally believed to impact the chemical concentration of medicinal extracts, the limited research studies available report varying impacts of this temperature change on the concentrations of phenolic compounds in medicinal plants. One study showed that temperature fluctuations had significant impacts on the change in the anthocyanin concentrations of fruit (Gustafson et al. 2012). However, another study reported that the change in temperature and an additional free-thaw cycle had no significant effects on antioxidant concentration (Ellnain-Wojtaszek et al. 2001).

The current study aimed to describe the chemical differences between valued, medicinal plants, focusing on the phenolic compounds thought to influence the health of humans when consumed. Specifically, we compared the concentrations of total phenolic compounds (containing hydroxyls, -OH, group covalently bonded to an aromatic hydrocarbon ring), total flavonoids (yellow pigment, class of phenolic compound), and anthocyanins (purple/red/blue pigment depending on pH, class of flavonoid compounds) in the samples. Additionally, the role of extract preparation (dried sample with water vs. wet sample with acetone extraction), season of collection (summer vs. fall), and sex of the plant (male vs. female) on gingko leaf extract chemistry were examined.

MATERIALS AND METHODS

Plant collection.—The following plants were collected on the Earlham College back campus (unless otherwise noted) in Richmond, Indiana at the times indicated: goldenrod leaves and root (May 2017), sassafras root (May 2017), ginkgo leaves (mixed male and female trees, May 2017), dandelion root (May 2017), plantain leaves (May 2017), black raspberry (June 2017; Boston, Indiana forest), mulberry (June 2017; Boston, Indiana forest), ginkgo leaves from female tree (September 2017), and ginkgo leaves from male tree (September 2017). Soil was removed from the root samples by gently shaking in the field and rinsing with tap water. All plants were air dried (surface-level) on a counter for several hours before storage. The collected plants were frozen at -20° C until extraction. The two purchased gingko products are as follows: ginkgo tea bag (Budda Organic Herbal Teas – Living Wellness Partner, LLC) and Ginkgo Supplemental Powder (Organic Ginkgo Leaf Powder – Nature Vibe Botanicals). All extracts and the abbreviations used for this in the figures are listed in Table 1.

80% acetone botanical extraction.—The plants collected in Indiana were thawed and

Table 1.—Abbreviations used for each extract and the initial stock concentrations.

Abbreviation	Name of the extracts	Stock concentration (g/mL)
GOL	goldenrod leaves	0.592
GOR	goldenrod roots	0.774
SAR	sassafras roots	0.6001
GIL	ginkgo leaves male & female summer	1.045
DAR	dandelion root	0.296
PIL	plantain leaves	0.34
BLR	black raspberry	1.083
MUL	mulberry	1.614
GIFF	ginkgo leaves female fall	3.73
GIFM	ginkgo leaves male fall	3.837
Tea	ginkgo tea	0.005448
Powder	ginkgo powder	0.00631

extracted using a rotary evaporator and 250 mL 80% acetone in double distilled water. The plant matter and 80% acetone mixture was blended and filtered. Then, 250 mL of filtrate was roto-vaped in a 1L round bottom flask until the acetone was evaporated. The final extract concentration was calculated using the mass of plant used and the final volume (Table 1). Extracts were aliquoted for storage into clearly labeled tubes and stored in -20° C freezer for use in this study and in -80° C freezer for long term storage. Samples used in the extra freeze-thaw comparison of this study underwent the additional thaw from -80° C to room temperature and back to -80° C due to an unplanned ultralow freezer failure in the department in 2017. Not all samples were impacted by this catastrophe.

Water extraction.—The ginkgo tea and supplemental powder were extracted using the directions provided on the containers. One tea bag or 2.5 mL of gingko powder was mixed into 250 mL of boiling DI water, allowed to steep for five min, and filtered. Extracts were aliquoted into clearly labeled tubes and stored in -20° C freezer for use in this study.

Total phenolic content assay (Folin–Coicalteu Method).—The following protocol was based on Herald et al. (2012) with some alterations: 8.5 mL of Folin-Coicalteu (F-C) reagent was freshly prepared for every assay by mixing 2.5 mL F-C reagent with 2.5 mL DI water. Gallic acid standards were prepared using serial dilutions of 10 mL of a 800 μg/mL gallic acid solution and stored at 4° C for reuse for up to three weeks. Next, 100 mL of 75 g/L Na_2CO_3 was prepared and stored in a labeled bottle at room temperature for up to three months. Each 96-well plate included three replicates of each of the following: samples (diluted by the same dilution factor, which was selected such that the absorbance measurements did not max out the instrument and fell within the standard curve); gallic acid standards (five concentrations: 800 g/mL, 400 g/mL, 200 g/mL, 100 g/mL, 50 g/mL); water blank; and one replicate of sample control per extract (sample without F-C reagent and Na_2CO_3). The extracts were thawed and filtered into a clean Eppendorf tube. Sample dilutions were prepared if necessary. According to designed plate layout, 75 μL distilled water was added to each well of the 96-well plate to be used, followed by 25 μL of standard or sample solution. Except for the sample control wells, 25 μL of 1:1 F-C prepared reagent was added to all wells. The plate was then covered with parafilm, mixed by shaking 30 sec, and allowed to sit for six min. One-hundred μL of Na_2CO_3 was added to each well and the plate was mixed again and covered entirely with aluminum foil for 90 min. After 90 min, the plate was shaken for 60 sec and absorbance was measured at 765 nm. Total phenolic concentration was estimated using the gallic acid standard curve, and expressed in terms of mg gallic acid equivalents (GAE) per gram sample to allow for comparisons.

Total flavonoid content assay (Spectrophotometric Method).—The following method was based on Herald et al. (2012) with some alterations: 100 mL each of 50 g/L $NaNO_2$, 100 g/L $AlCl_3$, and 1 mol/L NaOH were prepared in the fume hood and stored at room temperature in clearly labeled containers for up to three months. The catechin standards were stored at 4° C to reuse for up to three weeks. Experiments included three replicates of each of the following on one 96-well plate: samples (with the same dilutions factor for total phenolic content assay); catechin standards (five concentrations: 1.5 mg/mL, 0.75 mg/mL, 0.375 mg/mL, 0.1875 mg/mL, 0.09375 mg/mL); DI water blank. The extracts were thawed and filtered into a clean Eppendorf tube. Sample dilutions were prepared if necessary. All supplies were moved to the fume hood for safety before starting. One-hundred μL distilled water was added to each well of the 96-well

plate to be used, followed by 10 μL of 50 g/L $NaNO_2$ to all of the wells. Then, 25 μL of standard or sample solution was added to the wells, and the plate was incubated for 5 min. Next, 15 μL of 100 g/L $AlCl_3$ was added to all wells, followed by incubation for 6 min. Finally, 50 μL of 1 mol/L NaOH followed by 50 μL of distilled water was added to all of the wells. Before reading the absorbance at 510 nm, the plate was shaken for 30 sec. The total flavonoid concentration was calculated from the catechin standard curve and was expressed in terms of mg catechin equivalent (CE) per gram sample.

Total anthocyanin content assay (pH Differential Method).—The following method was adapted from Zhang et al. (2006) with some alterations. First, 100 mL of 0.025 M KCl/HCl (pH 1.0) and 100 mL of 0.025 M (pH 4.5) CH_3COONa/HCl was prepared and stored at room temperature. All extracts were diluted to the same concentration for easy comparison of the anthocyanin content. Each experiment included three replicates of the following two combinations for each botanical extract: 30 μL sample with 120 μL KCl buffer; and 30 μL sample with 120 μL CH_3COONa buffer. The plate was incubated on the shaker at room temperature for 20 min. The absorbance measurement was determined at 510 nm and 700 nm, and the anthocyanin concentration was estimated using the difference in absorbance measurement at each pH and Beer's Law, using the following formula:

$$\text{mg/L cyanidin 3-glucoside cation (a specific anthocyanin)} = \frac{\text{A*MW*Df*1000}}{(\varepsilon\text{*L})}$$

where:

A (absorbance in nm) = $(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH\ 4.5}$
MW (molecular weight of cyanidin 3-glucoside cation) = 449 (g/mol)
Df (dilution factor) = 5
ε (molar absorbance of cyanidin 3-glucoside cation) = 26900 (L mol^{-1} cm^{-1})
B (cell path length) = 1 (cm)

Data analysis.—Each analytical experiment was repeated at least three times with freshly thawed samples, each in triplicate in the 96-well plate. The values for each measurement were compared using one-way ANOVA and Tukey post-hoc test using SPSS (Statistical Package for the Social Sciences).

RESULTS

Medicinal plant extracts of Indiana plants contain a range of phenolic compounds.—In the broadest chemical comparison in this study, the total phenolic compound concentration, the extracts collected in Indiana fell into three main statistical groups. The ginkgo leaf extract (mixed sexes, collected at Earlham) showed the highest phenolic content (6800 ± 200 μg GAE/g sample; column c in Fig. 2; one-Way ANOVA, $p < 0.01$). The extracts of goldenrod leaves, goldenrod root, sassafras root, plantain leaves, and black raspberry were all statistically similar (concentration range from 2500 to 5000 μg GAE/g sample; Fig. 2). The mulberry and dandelion root extracts had the lowest phenolic content (from 1000 to 1500 μg GAE/g sample; Fig. 2).

Interestingly, when comparing the flavonoid (a class of phenolic compounds) concentration of extracts prepared from Indiana medicinal plants, different statistical groups appear. Plantain leaf extract has the highest flavonoid concentration with (4 ± 2 mg CE/g; Fig. 3). Most of the other extracts fall into two statistically similar groups regarding flavonoid content: group (b) included goldenrod roots, goldenrod leaves, sassafras root, and ginkgo, ranging from 2.0 to 3.5 mg CE/g sample; and group (c) included dandelion root, black raspberry, and mulberry extracts, ranging from 0.5 to 2.0 mg CE/g sample (Fig. 3).

While the berry extracts (black raspberry and mulberry) had lower flavonoid and total phenolic content than the other extracts, they had the highest anthocyanin content. However, given their dark purple color, this striking result was not surprising. Black raspberry extracts had by far the highest anthocyanin load with 190 ± 11 μg anthocyanin/L sample, followed by mulberry extracts with 40 ± 2 μg (Fig. 4). All other extracts had anthocyanin values much lower than the berries, and separated into three statistical groups: group (a) ranged from 1 to 6 μg anthocyanin/L sample and included goldenrod leaves, goldenrod roots extracts; group (e) included extracts contents below 1 μg anthocyanin/L sample and included sassafras root and ginkgo leaf extracts; and the remaining dandelion root and plantain leaf extract belong to group (b) and contained 6 to 12 μg anthocyanin/L sample (Fig. 4).

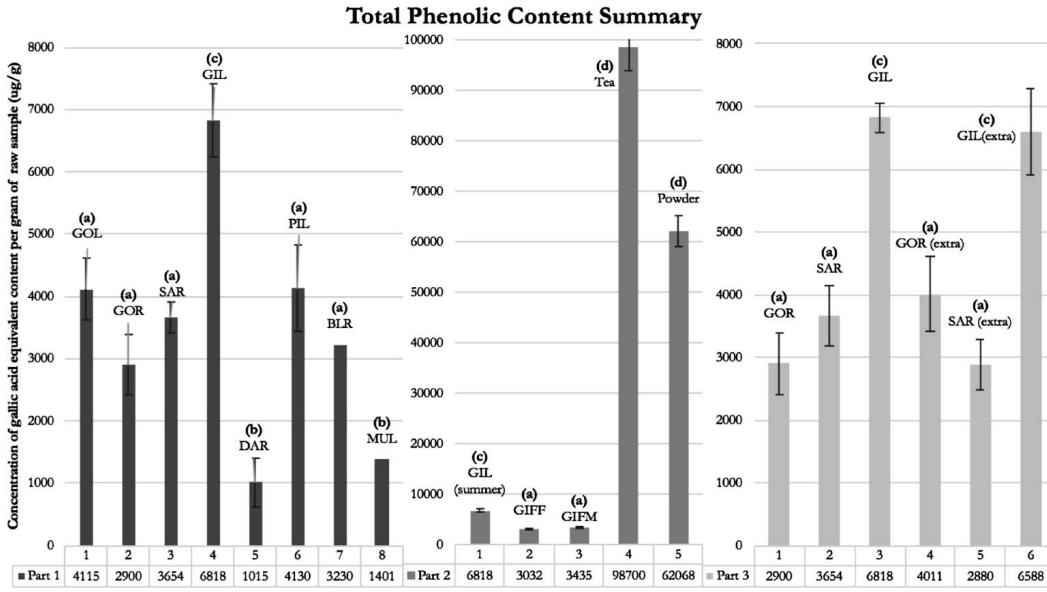

Figure 2.—Total phenolic content of medicinal plant extracts. Total phenolic content measured using Folin-Coicalteu method from Herald et al. (2012). Darkest gray bars represent plants collected in Indiana and extracted with 80% acetone, middle gray bars show comparison of all gingko extracts (note that the GIL bar is the same data as in the darkest gray category), and lightest gray bars show the comparison of original extracts (data not marked "extra" are the same as in darkest gray category) with the same extract preparation that underwent an additional freeze-thaw cycle (labeled "extra"). Bars represent the phenolic content average based on gallic acid equivalence over six independent trials, each with fresh samples. Error bars represent standard deviation between trials. Numbers across the bottom of the graphs are the average value for that extract. Each replicate had a gallic standard curve with $R^2 = 0.9909–0.9955$.

Impact of extract preparation and season of collection on the chemical composition of gingko.—Both purchased gingko products extracted with hot water had higher phenolic and flavonoid content than any acetone-extracted gingko sample in this study. The purchased ginkgo tea had the highest phenolic content (97000 ± 4000 μg GAE/g sample), followed by the purchased ginkgo powder (56000 ± 1000 μg GAE/g sample; Fig. 2). The mixed male and female tree gingko extract collected in the summer had the third highest concentration, followed by the male and female tree extracts collected in the fall, each having much lower phenolic content (concentration range from 2500 to 5000 μg GAE/g sample; Fig. 2). The purchased ginkgo tea and powder also had the two highest flavonoid contents: 9 ± 2 mg CE/g sample and 5 ± 3 mg CE/g sample, respectively (Fig. 3). Interestingly, the acetone extracts of male ginkgo leaves collected in the fall had the highest anthocyanin concentration (2 ± 2 μg anthocyanin/L; Fig. 4). The other gingko extracts, i.e., ginkgo leaves in the summer, ginkgo female leaves in the fall, ginkgo tea, and powder, belonged to group (e) in Fig. 4, which included extracts with contents below 1 μg anthocyanin/L sample.

No difference in extracts experiencing an additional freeze/thaw cycle.—Several acetone-extracts experiencing an unexpected extra freeze/thaw cycle (due to an ultralow freezer failure), provided the opportunity to test the impact of this thaw on the phenolic compounds in the extracts. No significant change was observed in the total phenolic and flavonoid content of goldenrod root, sassafras root, or ginkgo leaves following an additional freeze-thaw cycle (Fig. 2). However, the anthocyanin concentration for these extracts did show a slight difference, although not statistically significant.

DISCUSSION

Many plants are used medicinally throughout the world, and their varying impacts on

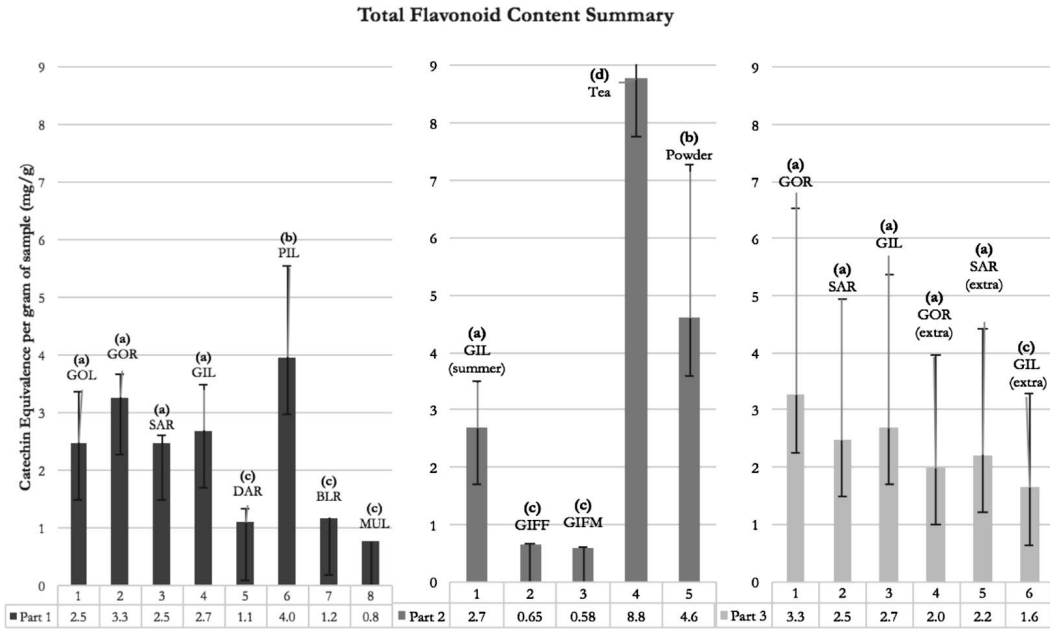

Figure 3.—Total flavonoid content summary. Total flavonoid content measured using spectrophotometric method from Herald et al. (2012). Darkest gray bars represent plants collected in Indiana and extracted with 80% acetone, middle gray bars show comparison of all gingko extracts (note that the GIL bar is the same data as in the darkest gray category), and lightest gray bars show the comparison of original extracts (data not marked "extra" are the same as in darkest gray category) with the same extract preparation that underwent an additional freeze-thaw cycle (labeled "extra"). Bars represent the content average based on catechin equivalence over seven independent trials. Error bars represent standard deviation between trials. Numbers across the bottom of the graphs are the average value for that extract. Each replicate had a catechin standard curve with $R^2 = 0.9641–0.998$.

physiology and health may be described by comparing their chemical components. Here, we describe the similarities and differences in phenolic compound concentrations of eight medicinal plants found in Indiana. The unique chemical mixtures present in medicinal plant extracts likely contribute to their biological action. Thus, due to their varying traditional uses (Gray 2011; Alfs 2013), it is not surprising that we found variation in the total phenol, total flavonoid, and anthocyanin content in acetone extracts of these plants. Among the Indiana plant extracts, we observed different rankings and relationships between plant extracts when measuring total phenol, flavonoid, and anthocyanin concentration, suggesting that other phenolic compounds are involved in the bioactivity of many of these extracts. Future work should relate the concentration of these classes of phenols within extracts to the bioactivity of those phenols (using extract fractionation). Importantly, other phenolic compounds (e.g., tannins) and non-phenolic compounds (e.g., alkaloids such as caffeine) not examined in this study are also known to exert biological effects when consumed.

The comparison between the extracts (sassafras, dandelion, gingko) that underwent an extra freeze-thaw cycle extracts did not show any significant changes in phenolic compound content. It is interesting to note that while the concentration of these compounds did not change, other chemical compounds may be affected by additional freeze-thaw cycles, which may impact bioactivity of these extracts. Future work should explore whether additional freeze-thaw cycles of the (unextracted) fruit or leaf impacts phenolic content and/or bioactivity, mimicking what could happen in a consumer's kitchen (i.e., intended or unintended freezing and thawing of collected or purchased fruit).

The gingko extracts, both the locally-collected and the purchased dried samples, have among the

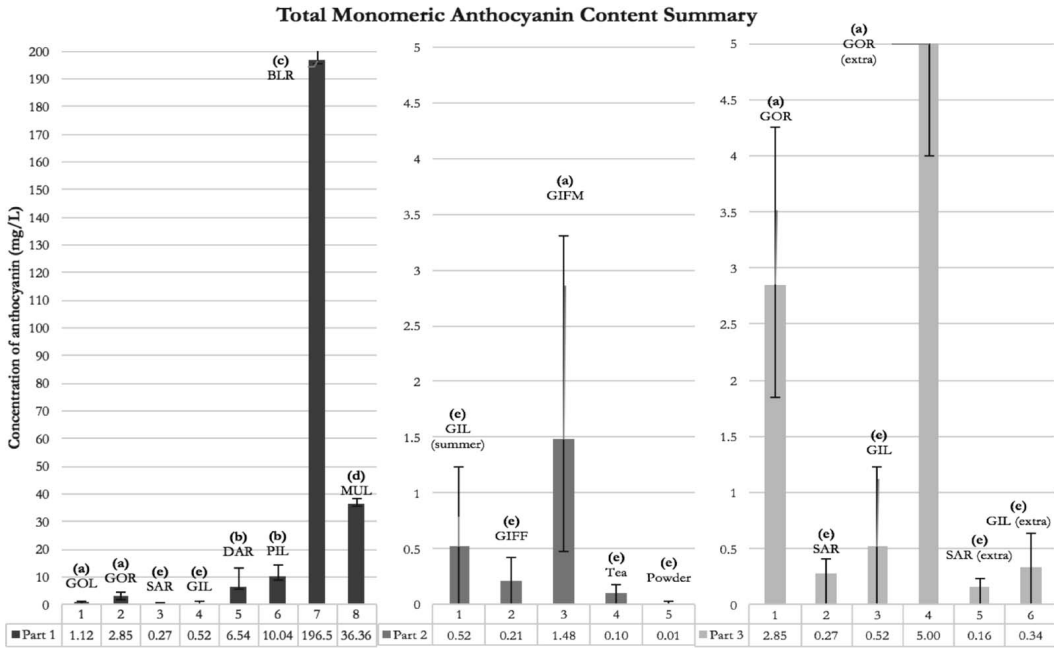

Figure 4.—Total monomeric anthocyanin summary. Total anthocyanin content measured using pH differential method from Zhang et al. (2006). Darkest gray bars represent plants collected in Indiana and extracted with 80% acetone, middle gray bars show comparison of all gingko extracts (note that the GIL bar is the same data as in the darkest gray category), and lightest gray bars show the comparison of original extracts (data not marked "extra" are the same as in darkest gray category) with the same extract preparation that underwent an additional freeze-thaw cycle (labeled "extra"). Bars represent the average concentration of anthocyanin content over four independent trials. Error bars represent standard deviation between trials.

highest values of total phenolic and flavonoid content compared to other extracts. The ginkgo leaves (combined male and female trees) collected in the summer had much higher phenol and flavonoid content than the individual male and female leaf samples collected in the fall. However, there was not a significant difference in phenolic and flavonoid concentration in the ginkgo leaves collected from the female tree and those collected from the male tree. Interestingly, the ginkgo leaves collected from the male tree had a much higher anthocyanin concentration than those from the female tree. This may be due to slightly different environmental exposures (the trees are approximately 3 m apart) or due to biological differences among the two trees; samples from additional pairs of trees should be collected and examined to explore the mechanism behind this difference in anthocyanin content. The purchased ginkgo tea and powder extracts also showed interesting results; both had higher phenolic and flavonoid contents than the other locally-collected ginkgo extracts. However, the anthocyanin content in both ginkgo tea and powder is significantly lower than the concentration in other ginkgo acetone extracts. This may be the result of differences in the acetone and hot water preparations. Moreover, this increase in antioxidant concentration could also be caused by the drying process of the commercial-obtained tea and powder, which removed most of the moisture content to enhance shelf-stability (i.e., the phenolic compounds were more concentrated in the dried, purchased samples than in the fresh, locally-collected samples). Further research should be conducted to evaluate this hypothesis in a more controlled setting, where only one variable is monitored: similar methods of preparation on various samples, or various sample preparation methods on the same sample. In summary, the phenolic content of gingko leaf extracts, and potentially as a consequence, the antioxidant activity and medicinal value of these products, is influenced be season of collection, extract preparation procedures, and appropriate temperature storage.

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EFFECT OF NATIVE AND NON-NATIVE PLANTINGS IN URBAN PARKING LOT ISLANDS ON DIVERSITY AND ABUNDANCE OF BIRDS, ARTHROPODS, AND FLOWER VISITORS

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ABSTRACT. Redesigning urban landscapes so that they better support biodiversity has the potential to reduce well-documented losses of pollinators and birds. This study tested whether small-scale native and non-native ornamental urban plantings affect either arthropod or bird diversity. Birds, floral visitors, and arthropod diversity were monitored in six parking lot islands landscaped with native plants and six parking lot islands with non-native ornamental plantings on the Indiana University South Bend campus located in South Bend, St. Joseph County, Indiana. Higher bird species richness and four times higher bird abundance was observed in parking lot islands with native plantings compared to non-native plantings. Abundance of flower visitors was significantly higher in native areas compared to non-native areas. There was no significant difference in arthropod order richness between the two types of parking lot islands. However, arthropod abundance was significantly higher in native plantings compared to non-native ornamental plantings. Overall, including native plants in small-scale landscaping increases biodiversity by supporting higher abundances of arthropods, flower visitors, and birds.

Keywords: Native plants, non-native plants, biodiversity, arthropods, birds, urban landscapes

INTRODUCTION

The amount of area covered with non-native turf grasses used in lawns is estimated to be 163,812 km^2 (± 35,850 km^2) – an area three times larger than any irrigated crop in the United States (Milesi et al. 2005). In addition to non-native turf grasses, non-native plant species are commonly used in urban landscaping and urban sprawl favors the spread of non-native plant species (Concepción et al. 2016). Habitats with few native plants are associated with a decrease in native herbivore species, lower insect abundance, and reduced diversity and abundance of native bird species (Burghardt et al. 2009; Helden et al. 2012; Concepción et al. 2016). Over large spatial scales, urban landscapes are associated with homogenization and an increase in generalist insect and bird species (Aronson et al. 2014; Thomas 2016; Jokimaki et al. 2018). Although urbanization is generally thought to contribute to loss of biodiversity and increased homogeneity of communities, studies done at different spatial scales show mixed effects on biodiversity (Murthy et al. 2016; Jokimaki et al. 2018). Bird diversity at a local scale (level of trees) and landscape scale has been compared and both were important in determining bird distribution in urban areas (Melles et al. 2003). At intermediate to large spatial scales, species richness can increase with human population density due to heterogeneous landscapes that promote diversity (Murthy et al. 2016). For example, higher biodiversity and better connectivity between patches can occur in areas that mix vegetation present in residential (gardens, yards, street spaces) and non-residential (parks and other green space) areas (Smith et al. 2014). There is also evidence that structural complexity (the vertical distribution of vegetation) in a community is more important towards increasing bird diversity and abundance compared to plant species composition (Atauri & de Lucio 2001; Tews et al. 2004). Determining how biodiversity is affected at different scales (local patches, habitat, landscape, and global levels) is of theoretical interest in identifying factors that have the greatest effects on biodiversity and has practical interest in designing urban landscapes that support biodiversity and ecosystem function.

Specificity of plant-insect interactions is one factor that can contribute to small spatial scale effects on biodiversity. Approximately 90% of all insect herbivores can only reproduce and survive on plant lineages with which the insects have a shared evolutionary history (Southwood et al.

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1982). This specialization can be extreme with over 50% of 64 hemipteran species tested showing preference for wild-propagated plants over cultivars of the same species (Poythress & Affolter 2018). Even generalist insects that should be able to survive on a broad range of plant hosts have poor survival on non-native plant species (Tallamy et al. 2010). Therefore, areas dominated by non-native plant species will likely differ in arthropod abundance and diversity compared to areas with primarily native plants. In addition, indirect factors, such as competition between native and non-native plant species, can reduce the visitation rates of floral visitors to native plants, resulting in lowered reproductive success for native plants (Stubbs et al. 2007).

Since birds rear about 96% of their young on insect protein or use insect protein as food sources during different seasons, changes in insect abundance and diversity may also affect bird abundance and diversity (Dickinson 1999). For instance, insectivore bird species have been shown to decline with increasing urbanization while omnivore bird species increase in abundance (Burghardt et al. 2009; Helden et al. 2012; Strohbach et al. 2013). These studies compared insect and bird diversity in areas with mostly native plants or mostly non-native plants separated by more than 1.5 km or compared insects and birds associated with native and non-native trees. The objective of our study was to test whether there were differences in arthropod and bird diversity and abundance in small-scale urban plantings using either native plants or non-native ornamental plants that are located in parking lot islands.

We hypothesized that due to the evolutionary history of plant-insect interactions, native plants would support more arthropods and attract more birds due to greater food resources. An alternative hypothesis is that areas with similar vegetation structure would attract and support similar bird diversity and abundance. To test these hypotheses, richness and abundance of arthropods, floral visitors, and birds were compared in parking lot islands that were similar in number of trees (thus similar vertical vegetation structure), but were planted with either non-native ornamental plants or plants native to the midwestern United States over three months.

METHODS

Site description.—Indiana University South Bend is an urban campus located along the St. Joseph River in South Bend, Indiana, population approximately 100,000. New parking lot islands were constructed as rain gardens and landscaped with plants native to Midwestern prairies and savannas in 2012 when the Education and Arts building was renovated. Older parking lot islands have traditional landscaping dominated by non-native ornamental plants and small native trees (cultivars of *Amelanchier* sp. and *Crataegus* sp.). In 2015, we selected six parking lot islands with non-native ornamental plants (defined as plant species not present in the area before European settlers) and six parking lot islands with mostly native plantings (defined as plant species that have historically occurred in the region; Fig. 1). Parking islands with traditional landscaping (referred to as non-native areas) were dominated by non-native plants and native plant parking lot islands (referred to as native areas) were dominated by native plant species (Table 1). To make the area sampled in native and non-native plots more similar, five of the non-native plots were two adjacent islands (Figure 1). None of the sites contained bird feeders, water sources, or other structures that could affect arthropod and bird abundance.

Traffic was low due to summer hours, and was the same across the parking lot. Number of trees per site was counted, and the amount of herbaceous plant cover, plant species present, and average plant height was estimated using 1 m^2 quadrats. A transect tape was laid through the parking lot island and a stratified random design was used so that quadrats were sampled randomly within each 5 m section. The average distance between the native and non-native parking lot islands was 12.5 $\pm$ 8.08 meters, and the average ($\pm$ standard deviation) size of native areas at 157 $\pm$ 81.1 m^2 compared to 28 $\pm$ 11.6 m^2 in non-native areas (see Fig. 1). Percent plant cover was estimated using the Daubenmire scale ($<$ 5%, 5–25%, 25–50%, 50–75%, 75–95%, and $>$ 95%), and the midpoint values were used for calculations (Elzinga et al. 1998). Percent cover was calculated per plant species and due to overlapping of individuals, plant cover could exceed 100%.

Arthropods.—Arthropods were collected from each site using 355 mL (12 oz) yellow solo bowls as pan traps. Pan traps were filled with water and a drop of dawn dish soap. Three bowls were placed randomly within each site once per week, weather permitting, from

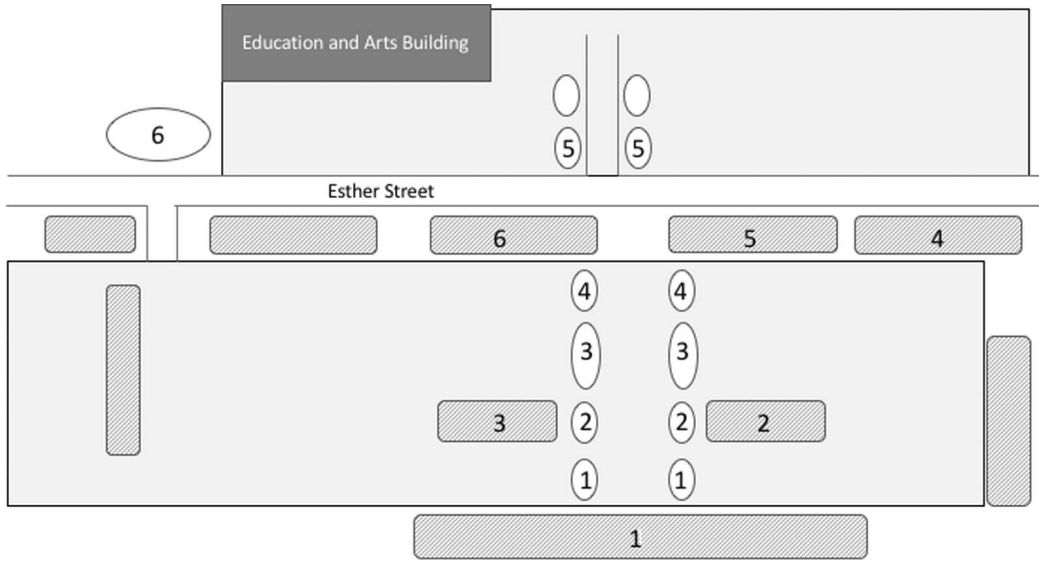

Figure 1.—Parking lot islands landscaped with either native or non-native plant species on the Indiana University South Bend Campus (EA = Education and Arts Building). Parking lot islands with native plants are shown in shaded rectangles (numbered 1–6) and parking lot islands with non-native ornamental plants shown in solid circles (numbered 1–6). Drawing shows location of plots, but is not drawn to scale.

June–August 2015 for a total of 11 days of sampling. All sites were sampled on the same days and bowls were left out for the same amount of time on each sampling date (16–18 hr). Arthropods were identified to order (Milne & Milne 1995) and abundance was recorded.

Floral visitors.—Floral visitors were collected from each site using 104 mL (3.5 oz) solo cups following protocols from Ksiazek et al. (2014). These cups were painted with Krylon Fluorescent Lemon Yellow paint to attract floral visitors, and each cup was filled with water and a drop of dawn dish soap to trap visitors (Ksiazek et al. 2014). Cups were left out for 24 hr and were placed on the ground 5 m apart from one another. Only Hymenoptera (bee and wasp) abundance was counted. Due to the difference in area among parking lot islands, there were more cups in the native areas; therefore, results are reported as average number of individuals per cup. Floral visitors were sampled only once per month, on days with no rain, for July and August to reduce risk of depleting their populations. As with arthro-

Table 1.—Vegetation characteristics for parking lot islands landscaped with native plants or non-native plants (sd = standard deviation, ns = not significantly different). Asterisk (*) indicates significant difference between parking lot island types.

Vegetative characteristics	Native plant areas (mean ± sd) n = 6 sites	Non-native plant areas (mean ± sd) n = 6 sites	Statistics
Total plant cover per m^2 (%)	148.1 ± 0.1*	107.5 ± 0.1	T = 4.5, df = 10, $P < 0.001$
Native cover (%)	106.7 ± 0.1*	18.9 ± 0.1	T = 7.5, df = 10, $P < 0.001$
Non-native cover (%)	41.3 ± 0.2	88.5 ± 0.1	
Number of plant species			
Native	20.8 ± 3.7*	2.0 ± 0. 9	T = 6.6, df = 10, $P < 0.001$
Non-native	6.6 ± 2.7	3.8 ± 1.8	T = 1.7, df = 10, $P = 0.06$
Average plant height (cm)	77.7 ± 6.9*	52.0 ± 6.7	T = 6.0, df = 10, $P < 0.001$
Average number trees per site	2.2 ± 3.0	2.2 ± 1.6	ns

Table 2.—Arthropod abundance, floral visitor abundance, and arthropod diversity and community similarity indices for parking lot islands with native and non-native plants. sd = standard deviation. Asterisk (*) indicates significant difference between parking lot island types.

Metric	Native plant areas	Non-native plant areas	Statistics
Arthropod abundance (individuals per bowl) mean ± sd	12.7 ± 7.2*	7.0 ± 3.8	Mann Whitney $U_{8,8} = 54$, $P = 0.02$
Floral visitor abundance (number floral visitors per cup) mean ± sd	2.6 ± 1.2*	1. 5 ± 0.8	$T = 4.29$, df = 22, $P < 0.001$
Arthropod order richness	15	16	$T = 0.60$, df = 20, $P = 0.59$
Shannon's Diversity Index (H) (Arthropod orders)	1.77	1.89	
Bray-Curtis Index for arthropod community similarity			70%

pod collection, each site was sampled on the same day.

Birds.—Birds were monitored three days per week from June to August for a total of 28 days of observations. Preliminary observations showed that there was more bird activity in the morning than at other times during the day, thus birds were monitored for 1.5 hr between 7:00–9:00 am. The sites were observed using 10-minute rotations, and the order of observations was randomly rotated each day. Bird species were identified (Sibley 2003; Cornell Lab of Ornithology 2015) and abundance of each species was recorded. To minimize double counting individual birds, during rotations between sites the observer noted birds traveling between sites and only recorded these birds once for the area where they were first observed.

Statistical analysis.—Species richness and Shannon's Diversity Index was calculated for arthropod orders and birds present in native and non-native plantings. A two-tailed t-test assuming unequal variances was used to test whether there were significant differences in vegetative characteristics, flower visitor, or in bird abundance. The % plant cover data was arcsine square root transformed to meet distribution assumptions. Variances did not meet the assumption of homogeneity of variances in the arthropod abundance data set, so a Mann-Whitney U-test was used to test for differences in arthropod abundance between native and non-native plantings.

Community similarity for arthropod orders and bird species in native and non-native plantings was calculated using the Bray-Curtis Index. The Bray-Curtis index includes both richness and evenness and varies from 0% (meaning no species are similar between two areas) to 100% (all species present in similar abundance in each area).

RESULTS

Vegetative characteristics.—Mean percent plant cover was 1.3 times higher in parking lot islands with native plants compared to parking lot islands with non-native (Table 1). Plant species richness was 3.1 times higher in areas with native plantings compared to non-native plantings (Table 1, Appendix 1). Average herbaceous plant height was approximately 25 cm higher in native areas than non-native areas (Table 1). However, the number of trees per site was similar, thus vertical vegetation structure was similar between native and non-native sites (Table 1).

Arthropod abundance and richness.—There were on average 5.7 more arthropods per bowl in native areas (Mann-Whitney $U_{8,8} = 54$, $P < 0.05$) compared with the number of individuals per bowl in non-native areas (Fig. 2, Table 2). A total of 15 arthropod orders were identified (Fig. 2), and there was no significant difference between sites in arthropod richness at the taxonomic level of order (Table 2). Shannon's diversity index (H) was slightly higher in non-native areas compared to native areas (Table 2).

The Bray-Curtis Index for arthropod orders showed 70% community similarity between the parking lot islands with native vs. non-native landscaping (Table 2). Floral visitor abundance was significantly higher in native plant areas with on average 57% more floral visitors per cup in native areas compared to non-native areas (Table 2).

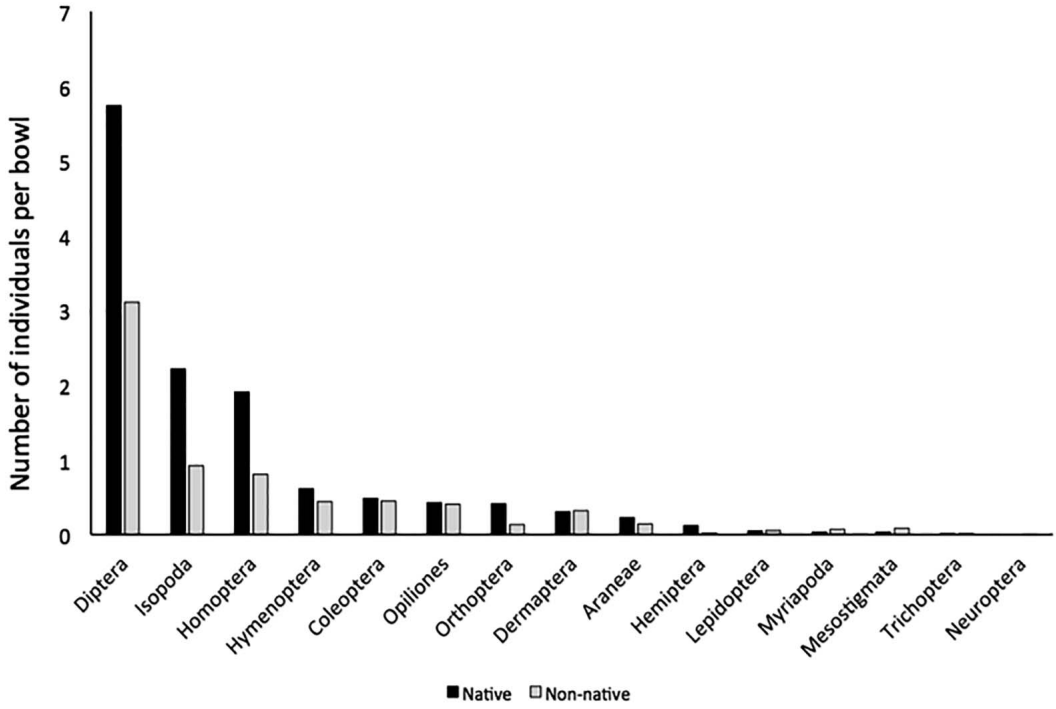

Figure 2.—Mean number of arthropods per bowl in native and non-native areas for June-August categorized by order (n = 11 sampling days).

Bird abundance and richness.—Bird abundance was four times greater in native areas than in non-native areas (Table 3). Significantly more bird species were observed in the parking lot islands with native plants compared to areas with non-native plants (Table 3; native sites = 12 species of birds, non-native sites = 8 species of birds). The bird species were mostly omnivores and herbivores (Table 4), and the European starling and house sparrow were the only two species that were non-native to this region. More European starlings were observed in non-native areas (3 in native vs. 18 in non-native), whereas more house sparrows were observed in native areas (68 in native vs. 17 in non-native). In addition, two insectivore bird species were only seen in native areas. Shannon's diversity index was slightly higher in non-native areas than native areas (Table 3). Unlike the arthropod data, the bird community only showed a 31.4% Bray-Curtis similarity between native and non-native areas (Table 3). Overall, parking lot islands with native plant species had a greater abundance and diversity of bird species.

DISCUSSION

In this study, we compared two hypotheses that may affect arthropod and bird diversity in urban landscapes. The first hypothesis states that due to

Table 3.—Comparison of bird abundance and diversity indices and community similarity in native and non-native parking lot areas. Asterisk (*) indicates significant difference between parking lot island types.

Bird abundance and diversity	Native areas	Non-native areas	Statistics
Bird abundance (individuals/area)	441 *	100	$T = 5.03$, df = 54, $P < 0.0001$
Bird richness	12 *	8	$T = 5.42$, df = 54, $P < 0.0001$
Shannon's Diversity Index (H)	1.75	1.89	
Bray-Curtis Index for community similarity			31.4%

Table 4.—Bird species observed in native and non-native areas for June–August 2015 and their diet (n = 28 days of observations for 1.5 hours in the morning). **Bold** indicates bird species only observed in areas with native plantings. All other bird species were seen in both native and non-native areas.

Bird species (common/scientific name)	Diet	Native/Non-native
Goldfinch (*Spinus tristis*)	Herbivore	Native
House Finch (*Haemorhous mexicanus*)	Herbivore	Native
Cedar Waxwing (*Bombycilla cedrorum*)	Omnivore	Native
Chipping Sparrow (*Spizella passerina*)	Omnivore	Native
Mourning Dove (*Zenaida macroura*)	Omnivore	Native
Robin (*Turdus migratorius*)	Omnivore	Native
European Starling (*Sturnus vulgaris*)	Omnivore	Non-Native
House Sparrow (*Passer domesticus*)	Omnivore	Non-Native
Brewer's Blackbird (*Euphagus cyanocephalus*)	**Omnivore**	**Native**
Cardinal (*Cardinalis cardinalis*)	**Omnivore**	**Native**
House Wren (*Troglodytes aedon*)	**Insectivore**	**Native**
Black Capped Chickadee (*Poecile atricapillus*)	**Insectivore**	**Native**

the evolutionary history of plant-insect interactions, native plants would support more arthropods and attract more birds due to greater food resources. The second hypothesis was that areas with similar vegetation structure would attract and support similar bird diversity and abundance. The parking lot islands had similar vegetation structure in terms of number of trees, but differed in diversity and abundance of native plant species. Our results provide greater support for the first hypothesis that use of native plants in landscaping can increase the abundance of arthropods, abundance of floral visitors, and abundance and diversity of birds.

Non-native plant species negatively affect the biodiversity and abundance of native herbivore species in urban areas (Southwood et al. 1982; Burghardt et al. 2009; Tallamy et al. 2010; Helden et al. 2012; Aronson et al. 2014; Concepción et al. 2016). Larger landscape scale studies have shown that the abundance of moths and butterflies was five times greater and bird diversity was higher in residential areas with predominantly native plants compared to areas with non-native ornamental plants separated by 1.5 or more km (Burghardt et al. 2009). A similar result was observed in our small-scale study where native and non-native areas were located only a few meters away from each other. Arthropod abundance was two times greater in native sites and there was a 57% increase in floral visitors per bowl in native sites compared to non-native sites. In contrast to arthropod and floral visitor abundance, overall richness and Shannon Diversity's index at the level of arthropod order was similar between native and non-native areas for arthropods. The dominance of Diptera in our study increased species evenness (Fig. 2). As a result, the insect diversity index differed little between native and non-native areas.

Four more bird species were observed in the parking lots with native vegetation compared to parking lots with ornamental non-native vegetation. Two of these bird species were insectivores, which may reflect more abundant food sources in the areas with native plants. Studies of urbanization often show that omnivore bird species tend to increase in abundance while insectivore bird abundance decreases (Burghardt et al. 2009; Helden et al. 2012; Strohbach et al. 2013). The greater number of insectivorous birds in our study and in the Burghardt et al. (2009) study suggests that increasing the use of native plants in landscaping would help reverse this homogenization of bird species and loss of insectivorous birds. Perhaps most surprising in our study was that bird abundance was four times greater in the parking lot islands with native plants compared to parking lot islands with traditional landscaping. Little difference was observed in the Shannon diversity index between the two areas, but this could have been due to the dominance of goldfinch and house sparrows skewing the diversity index. Interestingly, for parking lot islands that were only meters apart, the Bray Curtis index for bird community composition showed only 31% similarity between site types. Thus, small-scale differences in landscaping may have large effects on the abundance and diversity of birds.

Plant diversity and plant height were significantly greater in the parking lot islands with native vegetation. This may have biased our

results in providing more plant material for arthropods to feed on (bias towards hypothesis 1). This also could have increased the structural heterogeneity of the native habitats, which according to Tews et al. (2004) allows for more diverse niches and increased resources, thus increasing species diversity. To better test the degree to which native plants versus use of non-native plants in landscaping affect arthropod and bird abundance, one would need to plant areas controlling for plant diversity and plant height. We were not able to control this aspect of our study.

Another limitation of this study was that we did not identify all of the arthropods to species, which may mask differences in arthropod diversity between sites (bias towards hypothesis 2). For example, specialist insects were observed on *Asclepias tuberosa* and *Asclepias syriaca*, i.e., *Lygaeus kalmii* (small milkweed bug), *Oncopeltus fasciatus* (large milkweed bug), *Tetraopes tetrophthalmus* (red milkweed beetle), and *Danaus plexippus* (monarch caterpillars and adult butterflies). All of these species are restricted to feeding on species of *Asclepias*, and were not present in areas with non-native ornamental plant species. This is just one example of how native plants can support a more diverse insect community and provide more food resources for higher trophic levels.

Additionally, the presence of small native trees in the parking lot islands with non-native ornamental plants reduced differences in bird abundance between the two site types. For instance, the presence of ripe berries on Serviceberry trees (*Amelanchier* sp.) during the month of June attracted Cedar Waxwings and other omnivore birds to non-native areas during this study. Although four times more birds were observed in parking lot islands with native vegetation, the difference in bird abundance may have been even greater if native trees had been absent from the areas landscaped with non-native ornamentals.

In summary, our results clearly show that landscaping choices, even on a small scale, have large effects on the arthropod and bird community. Although vegetation structure (small understory trees and herbaceous plants) was similar between the parking lot islands in our study, future studies that control plant species richness and height between native and non-native plantings would provide more information on the degree to which native plants drive differences in biodiversity in small-scale urban plantings.

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Appendix 1.—Plant species present in native and non-native parking lot areas. Species with a "+" were found at the site type and species with a "–" were not found at the site type.

Plant species	Native sites	Non-native sites
Acer saccharum Marshall	+	+
Achillea millefolium L.	+	+
Agrimonia gryposepala Wallr.	+	–
Ambrosia artemisiifolia L.	+	–
Amelanchier sp. Medik.	–	+
Andropogon gerardii Vitman	+	+
Anemone virginiana L.	+	–
Aquilegia canadensis L.	+	–
Arnoglossum plantagineum Raf.	+	–
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	+	–
Asclepias syriaca L.	+	–
Asclepias tuberosa L.	+	–
Bouteloua curtipendula (Michx.) Torr.	+	–
Cirsium arvense (L.) Scop.	+	–
Cirsium vulgare (Savi) Tenn.	+	–
Conyza canadensis (L.) Cronquist	+	+
Coreopsis grandiflora Sweet	+	–
Coreopsis tripteris L.	+	–
Crataegus sp. Tourn. ex L.	–	+
Cyperus esculentus L. var. *leptostachyus* Boeckeler	–	+
Dianthus barbatus L.	–	+
Echinacea purpurea (L.) Moench	+	–
Equisetum hyemale L.	+	+
Eryngium yuccifolium Michx.	+	–
Eutrochium purpureum (L.) E.E. Lamont	+	–
Festuca sp. L.	+	–
Festuca glauca Vill.	–	+
Geranium maculatum L.	+	–
Helianthus annuus L.	+	–
Helianthus occidentalis Riddell	+	–
Hemerocallis fulva (L.) L.	–	+
Heuchera americana L.	+	–
Lactuca serriola L.	+	–
Lathyrus odoratus L.	+	–
Lavandula angustifolia Mill.	–	+
Lespedeza capitata Michx.	+	–
Liatris aspera Michx.	+	–
Medicago lupulina L.	+	+
Monarda fistulosa L.	+	–
Myrica pensylvanica Mirbel	–	+
Nepeta × *faassenii* Bergmans ex Stearn	–	+
Oenothera biennis L.	+	–
Physalis virginiana Mill.	–	+
Poa pratensis L.	–	+
Prunus serotina Ehrh.	+	–
Pycnanthemum virginianum (L.) T. Dur. & B.D. Jacks. ex B.L. Rob. & Fernald	+	–
Rudbeckia hirta L.	+	–
Schizachyrium scoparium (Michx.) Nash	+	–
Securigera varia (L.) Lassen	–	+
Silphium laciniatum L.	+	–
Silphium terebinthinaceum Jacq.	+	–
Solidago canadensis L.	+	–
Sorghastrum nutans (L.) Nash	+	–
Spiraea japonica L.f.	–	+
Symphyotrichum novae-angliae (L.) G.L. Nesom	+	–
Taraxacum officinale (L.) Weber ex F.H. Wigg.	+	+
Taxus baccata L.	+	–
Toxicodendron radicans (L.) Kuntze	+	–
Tradescantia ohiensis Raf.	+	–
Tragopogon pratensis L.	+	+
Trifolium repens L.	+	+

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INVERTEBRATES FROM PORCUPINE (*ERETHIZON DORSATUM*) ROCK DENS FROM GREENE COUNTY, CATSKILL MOUNTAINS, NEW YORK

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ABSTRACT. Invertebrates were collected from four ground-level porcupine dens. These dens are used mostly in winter. They were under flat rocks or in rock crevices but the openings were typically large enough that one could crawl into them. A number of kinds of invertebrates were found, some of the more interesting being the mites *Acotyledon paradoxa, Bakerdania* sp., *Calvolia* sp., *Coccotydeus* sp., *Eucheyletia bishoppi*, and *Dermacarus* sp. and related glycyphagids. These invertebrates are entirely different from parasites found on the porcupine, and must have entered directly from the environment or were present all year.

Keywords: Porcupine, *Erethizon dorsatum*, invertebrates, mites, Acarina

INTRODUCTION

Although porcupines spend much time in trees, they often use underground dens. The dens studied were mostly under slab rock, and the openings were large enough that a person could climb into them. The porcupines in this study had been studied by Roze (1989, 2009) for several years. Roze had placed radio transmitters on them to trace them to their dens. On the floor of the dens were detritus and porcupine feces. These dens appeared to produce an environment favorable for various kinds of invertebrates; therefore collections were made from four of the dens for analysis. The fauna of porcupine underground dens from New York State had not been previously studied. Calder & Bleakney (1965) have studied the microarthropod fauna of a porcupine cave in Nova Scotia.

The objectives of this paper were to determine the invertebrate fauna of these dens and to observe if seasonal differences occurred. Additionally, we wanted to determine if parasites found on porcupines were also found in dens.

MATERIALS AND METHODS

The dens were from Prattsville, Greene County, in the Catskill Mountains of New York, the same area where Roze (1989, 2009) did his studies on this species. Six collections were made: one in December 1991; one in January 1992; and two in May, one in July, and one in August, all in 1992. Greater numbers of samples were taken in winter when the porcupines most often use their dens. All four dens were sampled in midwinter, and two of the four were sampled in spring and summer (Table 1). A pint of nest material was gathered each time a den was visited.

Material collected was sifted through a Berlese funnel, and the smaller items were mounted on microscope slides in PVA and ringed with Euparal. Individuals on slides were identified using a 20 to 70 power zoom dissecting microscope. After initial identification by Whitaker, some slides were sent to Alex Fain (Institut Royal des Sciences naturelles de Belgique, Brussels, Belgium) for verification/identification as needed.

Large numbers of mites were collected and many were not identified. Those mites at least identified to family or genus and the other invertebrates are indicated in Table 1.

RESULTS

Most organisms in samples from den floors of *Erethizon dorsatum* were Acarina, mostly mites and one tick (Table 1). Also found were one spider, one flea, one fly larva (Chironomidae), and 23 springtails (Collembola). Some of the more interesting invertebrates are indicated below.

***Acotyledon paradoxa* (Acaridae, Rhizoglyphinae).**—Oudemans (1903) described the genus

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Table 1.—Invertebrates from porcupine dens from Prattsville, Greene County, Catskill Mountains, New York.

	Dec/Jan	May/July	Total
Mites			
Acotyledon paradoxa Oudemans 1903	18	0	18
Eucheyletia bishoppi Baker, 1949	0	8	8
Amerosiidae	12	18	30
Androlaelaps sp.	0	1	1
Bakerdania sp.	1	0	1
Calvolia sp.	7	0	7
Coccotydeus sp.	103	0	103
Dermacarus sp. and related forms (Glycyphagidae)	16	3	19
Hypoaspis	9	18	27
Laelapidae, and other larger mites	0	72	72
Oribatoidea	29	47	76
Tarsonemus sp.	23	2	25
Ticks			
Ixodes sp.	0	1	1
Insects			
Collembola (Springtails)	23	0	23
Coleoptera: Staphylinidae	0	2	2
Diptera: larvae (Chironomidae)	0	1	1
Hymenoptera: Ant	0	2	2
Flea (Siphonaptera)	0	1	1
Other Coleoptera	0	4	4
Beetle larvae	0	2	2
Other Invertebrates			
Nematodes	0	150+	150+
Spider	0	1	1

Acotyledon for a new species, *A. paradoxa*. It was described from a single hypopus (non-feeding form) from a bat from Russia. Zachvatkin (1941) discovered additional hypopi and also protonymphs of this species.

This genus has undergone a complex series of name changes and many species have been placed in this genus, but finally it appears that *Acotyledon* is a good genus with *A. paradoxa* as the type. The genus has been a catch-all that included many species belonging to a number of other genera. *Acotyledon*, now, besides *A. paradoxa* and *A. neotoma*, probably includes a few species described in the Russian literature from stored products (Ashfaq et al. 1986).

Fain & Philips (1978) indicate habitats of the species include wheat and granaries, nests of owls including a nest box containing a screech owl and red squirrel (*Tamiasciurus hudsonicus*), and nests of *Peromyscus leucopus*. Barry OConnor (Pers. Comm.) has observed this species a number of times in tree holes (with or without vertebrate hosts) and in habitats such as hay, straw, and grain. Thus, it is not surprising that this species is associated with porcupine dens. Among the mites of this species were 11 hypopi, four tritonymphs, two males, and one female. All were found only in winter, probably because this is the season that porcupines used the dens.

Amerosiidae.—Mites of this family inhabit many terrestrial and above-ground substrates such as manure, moss, compost, forest humus, rotting wood, bracken fungi, stored foods, and the nests of mammals, birds, and social insects (Krantz & Walter 2009). It is not surprising that amerosiids are found in porcupine dens. Some amerosiids feed on fungi whereas others have mouthparts with which they can feed on pollen and nectar. Some species occur on insects. Many species are phoretic.

***Androlaelaps* sp. (Laelapidae).**—Many species of this genus are mammal parasites, while some are free-living. One, *A. fahrenholzi*, is found on more North American hosts than any other ectoparasite.

***Bakerdania* sp. (Pygmephoridae).**—This genus has a relatively large number of species, many described by S. Mahunka. Only one

species of *Bakerdania* has been described from North America, although we have a number of undescribed species found on various mammal hosts.

***Calvolia* sp. (Winterschmidtiidae).**—One species, *C. lordi*, is virtually cosmopolitan (Krantz & Walter 2009). Among the *Calvolia* were one tritonymph, three males, and one female.

***Coccotydeus* sp. (Tydeidae).**—These mites are very small and are plant feeders (Fain, Pers. Comm.). All 103 individuals were found in winter.

***Dermacarus* sp. (Glycyphagidae).**—Most members of this genus live as hypopi on various species of mammals, whereas the adults usually live in nests or detritus. A number of members of this genus and related forms were found among the material from porcupine dens.

***Eucheyletia bishoppi* (Cheyletidae).**—This mite has been found on species of *Microtus, Sorex*, and *Tamias*. It seemed unexpected to find this species in porcupine dens, but other mammal species may have entered the dens.

***Hypoaspis* sp. (Laelapidae).**—This genus has had a confused history and has been divided into several closely related genera. Members of the genus *Hypoaspis* may be found in litter or soil substrates, but also in mammal or arthropod nests or directly associated with insects. The mites in the dens may belong in one of the new genera.

Oribatoidea.—There are many species of oribatoid mites in several families. They live in many situations, but most species live in the soil, and the detritus in the den is essentially soil.

***Tarsonemus* sp. (Tarsonemidae).**—The Tarsonemidae constitute a very large family of which about half the species are in the genus *Tarsonemus*. One species of *Tarsonemus* is found under the elytra of beetles. *Tarsonemus* species are common in house dust.

DISCUSSION

Parasites found previously from the porcupines themselves were *Ischrypoda armatus* (Laelapidae) and three species of chigger mites (Trombiculidae): *Euschongastia decipiens* (Allred & Beck 1966; Wrenn & Loomis 1974), *E. radfordia* (Brennan & Beck 1955), and *Neotrombicula harperi* (Lawrence et al. 1965). Porcupine ectoparasites encountered in the NY study area include the scabies mite, *Sarcoptes scabiei* (Payne & O'Meara 1958; Roze 1989, 2009), the tick *Ixodes cookei*, and the louse *Eutrichophilus setosus* (Roze 1989, 2009). None of these species were found in the porcupine dens we examined.

As might be expected, the fauna of the porcupine dens included a wide variety of forms, especially mites, but also insects and other invertebrates. Many of the mites were fungivores. There was essentially no relation between the items in the dens and those on the animals. Finding a single chironomid in this situation seems peculiar, as chironomid larvae are usually numerous when they occur.

Amerosiidae, *Dermacarus* and relatives, *Hypoaspis*, oribatoids, Coleoptera (including larvae), and Nematoda were found in all seasons. These latter forms are likely present throughout the year. The following were found only in winter: *Acotyledon*, *Bakerdania*, *Calvolia*, Coccotydeidae, and Collembola; whereas *Eucheyletia*, *Androlaelaps* and the one tick were found only in the warm season. *Tarsonemus* was found almost entirely in winter (23) with only two in the warm season.

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INHABITANTS OF THREE NESTS OF THE THIRTEEN-LINED GROUND SQUIRREL, *ICTIDOMYS* (FORMERLY *SPERMOPHILUS*) *TRIDECEMLINEATUS* (MITCHILL), FROM INDIANA

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ABSTRACT. The fauna was examined from three nests of the thirteen-lined ground squirrel, *Ictidomys tridecemlineatus*, from Terre Haute, Vigo County, Indiana. The most abundant mite was *Sertitympanum separationis*, mostly females. Immature and male individuals of this species were also found, both for the first time. *Androlaelaps fahrenholzi* was the second most abundant mite. Thirty-eight mites including at least three new species of the genus *Bakerdania* were present. In small numbers were mites of the species *Macrocheles mesochthonius* (Krantz & Whitaker 1988). The most abundant insects in the nests were larvae and adults of the flea *Opisocrostis bruneri*, and an unidentified staphylinid beetle, *Atheta* sp. Other species of invertebrates occurred in lower numbers.

Keywords: Nest fauna, ground squirrel, invertebrates, Indiana, *Ictidomys tridecemlineatus*

INTRODUCTION

Most publications on ectoparasites of *Ictidomys tridecemlineatus* (Mitchill, 1821) involve information concerning the ectoparasites found on the host. The ectoparasites of this host have been studied in Indiana (Whitaker 1972). Little is known about the inhabitants of the nest community. We were particularly interested in the presence or absence of several organisms, as follows. In 1969 a staphylinid beetle, *Atheta* sp., was found living on a few specimens of *I. tridecemlineatus* captured on September 20 and 22 in Vigo County, Indiana. Members of this genus have parasitic or at least commensal relationships with Central and South American rodents, thus its relationship to this host was of particular interest. The ameroseiid mite *Sertitympanum separationis* was described by Elsen & Whitaker (1985), primarily from *I. tridecemlineatus* from the present study area. Previously only females had been described of this mite, and little is known of the biology of the species. *Macrocheles* sp. was reported on this host from Indiana by Mumford & Whitaker (1982), and has been described as *M. mesochthonius* by Krantz & Whitaker (1988). Hypopi (deutonymphs of Astigmata) associated with mammals either are simply phoretic, e.g., *Xenoryctes latiporus* and "*Dermacarus*" *reticulosus;* or they are parasites, e.g. *Aplodontopus micronyx*. These examples have all been found on this host from Indiana. Adults of hypopi presumably occur in the nests, but adults have not been found for any of these species.

There is no previous study of mites from the nests of *I. tridecemlineatus*, although Hendricks (1967) previously reported other organisms from the nests of this host, including fleas *Opisocrostis bruneri, Ctenophthalmus pseudagyrtes,* and *Oropsylla arctomys*; the tick *Ixodes sculptus*; flea larvae; larvae of the dipteran family Anthomyiidae; pupae of the moth *Pseudaletia unipuncta*; and dipluran adults of the family Japygidae.

The objectives of this study were to look for the species mentioned above and to determine other inhabitants of the nest community of *I. tridecemlineatus*, in Terre Haute, Vigo County, Indiana.

METHODS AND MATERIALS

A reconnaissance of burrows in Terre Haute, Vigo County, Indiana sought to determine which might contain nests. Suspect burrows were excavated, and three nests were located in three separate burrows on 5 June 1988. The nests were removed and immediately taken to the lab in plastic bags. Invertebrates, mostly mites, were

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Table 1.—Inhabitants of three nests of the Thirteen-lined Ground Squirrel, *Ictidos tridecemlineatus*, from Terre Haute, Vigo County, Indiana.

Organism	Number nests occurrence	Total number	Notes
	MITES (ACARINA)		
Sertitympanum separationis	3	159	110 females, 16 males, 33 immatures
Androlaelaps fahrenholzi	3	105	49 females, 24 males, 32 immatures
Bakerdania sp.	2	38	apparently at least 3 new species
Stratiolaelaps scimitus	2	13	2 females, 3 males, 8 immatures
Alloparasitus n.sp.	1	5	4 females, 1 male
Macrocheles mesochthonius	3	5	5 females
Proctolaelaps pygmaeus	1	3	3 immatures
Cheyletus eruditus	1	2	2 immatures
Dendrolaelaps sp.	1	4	4 immatures
Chigger	1	1	
Oribatid mites	3	24	24 immatures
Glycyphagids	2	24	24 immatures
	FLEAS (SIPHONAPTERA)		
Opisocrostis bruneri	3	68	5 adults, 63 immatures
	INSECTS AND SPIDERS		
Staphylinidae, rove beetles, *Atheta* sp.	2	5	5 adults
Formicidae, ants	3	10	10 adults
Collembola, springtails	2	3	3 adults
Beetle, unidentified	1	1	1 adult
Spider	1	1	

collected from the nest material by means of a Berlese funnel. They were preserved in alcohol or were stained in Nesbitt's solution containing acid fuchsin, mounted in Hoyer's solution, and coverslips were ringed with euparal. Large numbers of mites were removed from each of the three nests and smaller subsamples were taken from each.

RESULTS AND DISCUSSION

Seventeen categories of nest fauna were included in the sample, totaling 447 invertebrates. Of these, 360 were mites, 68 were fleas, mostly larvae, and 21 were miscellaneous invertebrates (Table 1). The most abundant nest inhabitant was the mite *Sertitympanum separationis*. In addition to 110 females, 33 immatures and 16 males were found; this was the first report of males and immatures from this genus (Elsen et al. 1992). The three types were present in all three nests. The second most abundant inhabitant was *Androlaelaps fahrenholzi* (Laelapidae) (49 females, 24 males, and 32 immatures), a common ectoparasite of many different hosts. The third most abundant species was the flea, *Opisocrostis bruneri* (68, mostly larvae). Three species of mites of the genus *Bakerdania*, totalling 38 individuals and likely including at least 3 new species, were the third most abundant type of mite. Twenty-four nymphs and larvae of oribatid (soil) mites were found. Twenty-four glycyphagid protonymphs were observed, but were badly macerated and could not be identified. There were 13 individuals of *Stratiolaelaps scimitus* (Womersley). Five individuals of *Macrocheles mesochthonius*, a species described by Krantz & Whitaker (1988), were identified. Other mites included were *Alloparasitus* n.sp., *Dendrolaelaps* sp., *Proctolaelaps pygmaeus, Cheyletus eruditus*, and a chigger (unidentified).

Hypoaspis is a difficult and poorly known genus. Many species have been described, but many were later put in separate genera, including *Alloparasitus* and *Stratiolaelaps*. Evans & Till (1966) provided a key that we used to identify the subgenera (now all recognized as full genera) and other details on the *Hypoaspis* group. One that we had called *Hypoaspis* sp. was sent to E. E. Lindquist, who said it was "a distinctive new species," but it turned out to be in the genus *Alloparasitus*.

Other than mites, the flea *Opisocrostis bruneri* was the only other important species, and was the third most abundant nest inhabitant. Ten ants

and a staphylinid beetle, *Atheta* sp., were present, along with a few springtails, a spider, and an unidentified beetle. The other insects and spider were not identified.

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A SURVEY OF SPIDER DIVERSITY IN MORGAN-MONROE/ YELLOWWOOD STATE FOREST

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ABSTRACT. As both predators and prey, spiders are important components of forest ecosystems, yet there is a paucity of information about spider species assemblages in Indiana forests. Between 2014 and 2017, the Indiana Forest Alliance sponsored an extensive taxonomic survey, called an ecoblitz, in Morgan-Monroe/ Yellowwood State Forests in Indiana. During this ecoblitz, 128 spider species were collected. Of these species, 31 were new distribution records for Indiana. Of the total number of species collected, 62% were collected in the bottomland habitat, 60% on slopes, and 19% on ridges. Only 10% of the total species were found in all three habitats. In pair-wise comparisons of habitats, species composition differed between habitats even when species richness was similar. Likewise, collection of spider species during the day differed in composition from those collected at night with only 26% collected during both periods. These data emphasize the benefits of multi-year surveys, such as the ecoblitz, and the importance of sampling in multiple habitats as well as during the day and the night. The high number of new distribution records in our sample reinforces the premise that spiders as a group are underrepresented in scientific studies of forests in Indiana.

Keywords: Araneae, species richness, Indiana state forests, ecoblitz, spiders

INTRODUCTION

As conservation practitioners become increasingly aware of the importance of baseline taxonomic surveys, the "bioblitz" has become a popular means of rapid field assessment (Parker et al. 2018) as well as a means to engage and educate the public as "citizen scientists" (Lundmark 2003). In most cases, a bioblitz engages volunteers to document as many species as possible of a specific taxon within a narrow time window and location. Since the first bioblitz in 1996 organized by the U.S National Park Service, these surveys have collected baseline inventories for numerous natural history museums, parks, nature preserves, and land trusts. The collected information contributes to knowledge and decision-making for conservation managers (Ballard et al. 2017). The value of baseline information to conserving the biodiversity of our natural areas is becoming increasingly critical as we face increased loss of habitats and the inevitable environmental shifts through climate change (Bellard et al. 2012).

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While bioblitzes have been essential in documenting species inventories, they have been limited in that they are typically restricted to one or two days. The Indiana Forest Alliance (IFA) organized the first extensive and intensive taxonomic survey in an Indiana State Forest. This expanded version of a bioblitz – labeled the "ecoblitz" – was conducted in the Back Country Area (BCA) of Morgan-Monroe/Yellowwood State Forests in south-central Indiana. The survey covered 12 taxonomic groups and was led by experts with teams including 41 scientists, 45 students, and numerous volunteers over a four-year period. The ecoblitz also covered seasons from spring through fall and included natural history observations. As part of this ecoblitz effort, we surveyed the diversity of spiders from 2014–2017.

Spiders are important components in forest ecosystems as both predators and prey. As first-level consumers in the forest food web, spiders eat an array of insect species due to their varying lifestyles (Wise 1993). For example, some species are ambush or hunting spiders, while other species build webs varying from platforms to orbs (Cardoso et al. 2011). As generalist predators,

individual spiders are constrained by the size and abundance of available insect prey rather than by availability of specific insect species. Spiders vary in body size from minute cryptic species found in the leaf litter to large, active hunters found on tree bark, and they regulate insect populations from all forest microhabitats, e.g., leaf litter (Uetz 1979; Wise 2006).

Conversely, spiders are important prey items for numerous forest animals. Small mammals such as shrews eat ground spiders found in woody debris (Whitaker & Mumford 1972). Numerous insectivorous bird species snatch spiders from their webs, or glean spiders crawling on leaves in the canopy, and can have a significant effect on spider assemblages in forests (Gunnarsson 2007). Salamanders, toads, frogs, and insectivorous snakes and lizards also eat spiders. In fact, spiders are a component of the diet of all insect-eating animals, including predaceous insects and other spiders (Wise 2006).

In many biodiversity studies, spiders are considered robust indicators of arthropod diversity due to their spatial and temporal ranges, small size, and high relative abundance (New 1999). Because the distribution of spider species reflects characteristics of habitat structure (Uetz 1991), different forest successional stages contain characteristic species assemblages. Recent studies have suggested that spiders are good indicators of the effects of forest management, which typically alters habitat structure (Willett 2001; Pearce & Venier 2006).

Although there is much information in the literature about mammals and birds in Indiana forests, there is little knowledge about spider species despite their important ecological role. In fact, there is a paucity of information about spiders in Indiana for all habitats (Milne et al. 2016) and baseline data are sorely needed in a world where global biodiversity is rapidly diminishing.

METHODS

The ecoblitz area included the 900-acre (364 ha) Back Country Area of Morgan-Monroe/Yellowwood State Forests (Fig. 1) located along the border of Monroe County and Brown County in south-central Indiana. The two state forests represent a mature eastern deciduous forest within the maple-beech to oak-hickory transition zone. This area has ridge-ravine topography with a predominately closed canopy. Because of the deep ravines that dissect the forest, there is a wide range of microhabitats. Also, due to the maturity of the forest, there are numerous snags and a large amount of coarse woody debris, thick leaf litter on the forest floor, and sunny openings from tree fall. The bottomlands include intermittent creeks.

Spiders were collected as part of the ecoblitz in the Back Country Area for four years (2014–2017). During 2014–16, spiders were sampled both early in the season (June) and late (September) to obtain a representation of spiders with varying life histories. Both day and night sampling were done to include spiders with different activity periods. In 2017, samples were collected in only one survey period, which was at night in July. Each survey period included three habitats: dry ridge, mesic slope, and bottomland with a creek bed.

Our sampling methods included: 1) aerial search of leaves, branches, tree trunks, and the empty spaces between vegetation within arm's reach; 2) sweep netting to capture spiders in shrubby or herbaceous vegetation; 3) ground search on low vegetation, fallen logs, rocks, or steep banks; 4) beat-sheet method in which a square sheet was stretched under the edge of a tree branch or bush and, upon shaking the plant, spiders that fell onto the sheet were captured; and 5) litter sort method where handfuls of leaf litter was collected and dumped into a litter sorter. Material fell onto a white sheet and the small spiders living in this microhabitat were then captured.

Spiders were identified in the field when possible. Unknowns were collected in 70% ethanol for later identification under the microscope. Abundance data was not collected, so results reflect presence-absence only. Adult spiders were identified to species using *The Spiders of North America* by Ubick et al. (2005) and other taxonomic keys from the primary literature. The analysis and species list reflect data pooled from the four years of sampling.

The ecoblitz survey was authorized by permits from the Indiana Department of Natural Resources. Voucher specimens are located at the Indiana State University collection.

RESULTS

Species richness.—A total of 128 species from 28 families were identified (Table 1). Each year new species were added to the cumulative list (2014: 73 species; 2015: 100 total species; 2016: 122 total species; 2017: 128 total species), as well as representatives from new families.

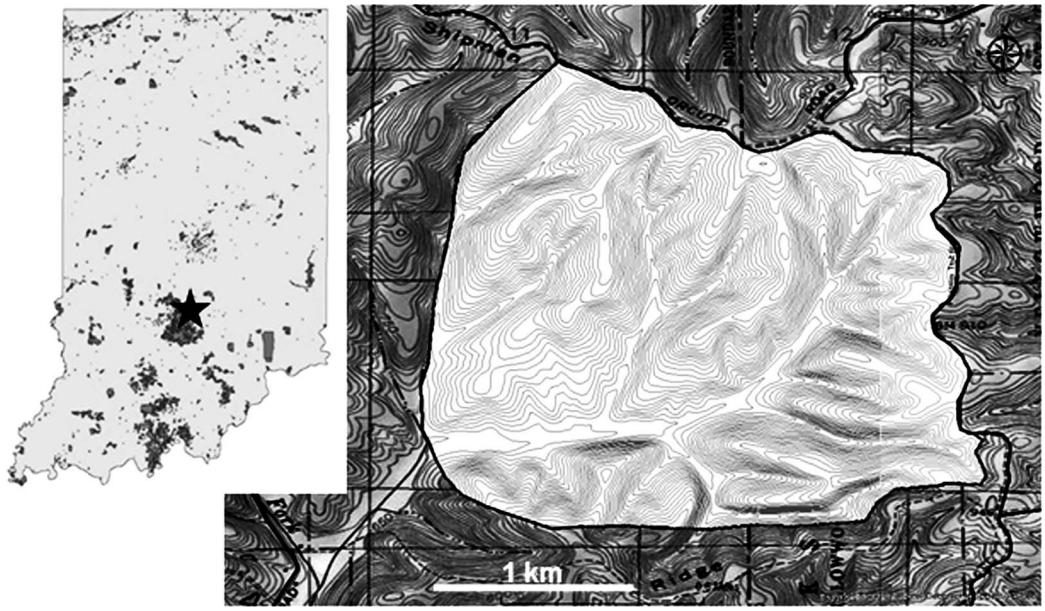

Figure 1.—Map illustrating the location of Morgan-Monroe/Yellowwood State Forest within Indiana (left; marked by star) and location of the 900-acre Ecoblitz area (right; light gray) within the Morgan Monroe/Yellowwood State Forest Back Country Area. Black lines depict roads. (*Map prepared by Rae Schnapp*)

For example, in 2016 our first representative species in the families Liocranidae, Mysmenidae, Titanoecidae, and Halonoproctidae were found. Our forest sample represents about 28% of the total spider species (454) documented for Indiana (Milne et al. 2016).

New distribution records.—In the four years of sampling, 31 species were collected that are new records for Indiana (Table 1, marked with *). All but six of these species are included in a recent report on Indiana spider distributions (Milne et al. 2016). The latest additions include: *Drapetisca alteranda*, a tree-trunk specialist sheet-web weaver in the family Linyphiidae; *Ocrepeira ectypa*, an orb weaver in family Araneidae; *Emblyna zaba*, a mesh-web builder in family Dictynidae; *Agyneta semipallida*, a small sheet-web builder in family Linyphiidae; *Maymena ambita,* a horiziontal web builder in the family Mysmenidae; and *Chinattus parvulus*, a jumping spider in the family Salticidae.

Habitat comparisons.—Of the 128 species collected, 62% were collected in the bottomland habitat, 60% on slopes, and 19% on ridges (Fig. 2). Only 10% of total species were found in all three habitats (13 species out of a total of 128). Even though the bottomland and slope habitats were similar in the number of species, their actual species composition was moderately dissimilar (Sørensen Coefficient = 0.44 comparing pooled habitat assemblages, Table 2). Species compositions in the bottomland and slope habitats were moderately dissimilar from the species composition on ridges (Sørensen Coefficient values < 0.40, Table 2).

Comparison of day versus night collections.—The data revealed that different assemblages of spider species were active during the day versus the night (Sørensen Coefficient = 0.40 comparing pooled data). Of the 128 species documented, 41% were found only during the day and 33% were found only during the night; 26% of the total species were present in both the day and night samples (Fig. 3).

DISCUSSION

The results illustrate several points about the value of an inventory that samples spiders over multiple years and seasons, in both daytime and nighttime, and across multiple habitats. First, the multi-year and multi-season ecoblitz allowed us to find a greater number and variety of species than the typical one-shot bioblitz. The typical bioblitz, or rapid assessment survey, takes place during a single designated time irrespective of weather

Table 1.—Spider species list by family from Morgan Monroe/Yellowwood State Forests Ecoblitz 2014–2017. Species marked with an "*" represent new distribution records (31) for Indiana. Spiders unable to be identified past the genus level but distinct species (in some cases due to immaturity) are indicated by "sp." Habitat: B = bottomland; R = dry ridge; S = mesic slope. Time of Collection: D = daytime only; N = nighttime only; B = both daytime and nighttime. (See Milne et al. (2016) to find more information on the biology and distributions of some of the species listed.)

Family and species	Habitat	Time of collection
Agelenidae		
**Agelenopsis emertoni*	B	D
Agelenopsis pennsylvanica	S	B
Agelenopsis potteri	S	N
Agelenopsis utahana	B	D
Coras juvenilis	B,S	B
Wadotes calcaratus	B,S	B
Wadotes hybridus	B,S,R	N
Anyphaenidae		
Anyphaena pectorosa	B,R	D
Wulfila saltabundus	B,S,R	B
Araneidae		
Acanthepeira stellata	S	D
Araneus bicentenarius	B	N
Araneus marmoreus	B,S	N
**Cyclosa conica*	B,S,R	D
Eustala anastera	B	B
Mangora maculata	B,S,R	B
Mangora placida	S	N
Metepeira labyrinthea	S	N
Micrathena gracilis	S	B
Micrathena mitrata	B,S	B
Micrathena sagittata	B	D
Neoscona arabesca	B,S	B
Neoscona crucifera	B	N
**Ocrepeira ectypa*	B	N
Verrucosa arenata	B	N
Cheiracanthiidae		
Cheiracanthium inclusum	B	D
Clubionidae		
Clubiona sp.	B,S	N
Elaver excepta	S	N
Corinnidae		
Castianeira cingulata	B,S,R	B
Ctenidae		
Anahita punctulata	B,S,R	B
Cybaeidae		
Cybaeus sp.	S	N
Dictynidae		
**Emblyna zaba*	B	N
**Lathys immaculata*	S,R	B
Gnaphosidae		
Drassodes neglectus	B	D
**Drassyllus fallens*	B	D
**Gnaphosa fontinalis*	B	D
Herpyllus ecclesiasticus	S	N
**Micaria longipes*	S	N
Hahniidae		
Cicurina arcuata	R	N
**Hahnia flaviceps*	S	D
Neoantistea agilis	B	N
Neoantistea magna	S	D
Halonoproctidae		
**Ummidia tuobita*	S	N
Linyphiidae		
**Agyneta barrowsi*	S	D
**Agyneta semipallida*	B	D
**Bathyphantes alboventris*	B,S	D
Bathyphantes pallidus	S	N
Centromerus latidens	S	N
Ceraticelus fissiceps	B,S	N
Ceraticelus sp.	S	B
**Drapetisca alteranda*	S	N
Frontinella pyramitela	S	D
Islandiana longisetosa	B	D
**Lepthyphantes turbatrix*	S	D
**Mermessus maculatus*	B,S	B
Pityohyphantes costatus	B	D
**Styloctetor purpurescens*	B,S	D
Liocranidae		
Agroeca sp.	R	D
Lycosidae		
Allocosa funerea	S	D
Gladicosa gulosa	B	N
**Gladicosa pulchra*	B	N
Pardosa lapidicina	B,S	D
Pardosa milvina	B,S	B
Pirata alachuus	B	B
Pirata sedentaris	B	N
Piratula insularis	B,S	B
Piratula minuta	B	B
**Schizocosa crassipes*	B,S,R	B
Schizocosa ocreata	B,S,R	B
Schizocosa saltatrix	S,R	N
Tigrosa aspersa	S	N
Mysmenidae		
**Maymena ambita*	R	D
Oxyopidae		
Oxyopes salticus	B	D
Philodromidae		
Philodromus imbecillus	B	D
Philodromus rufus vibrans	S	D
Tibellus sp.	B,S	D

Table 1.—Continued.

Family and species	Habitat	Time of collection
Phrurolithidae		
Phrurotimpus alarius	B,S,R	B
Phrurotimpus borealis	B,R	B
**Scotinella redempta*	B,S,R	B
Pisauridae		
**Dolomedes albineus*	B,S	N
Dolomedes tenebrosus	B,S	B
Dolomedes triton	B	N
Dolomedes vittatus	B,S,R	N
Pisaurina brevipes	B	D
Pisaurina mira	B,S	B
Salticidae		
**Chinattus parvulus*	S	D
**Colonus puerperus*	R	D
Colonus sylvanus	B	D
Eris militaris	B	D
Hentzia sp.	B	D
Maevia inclemens	B,S	D
Pelegrina galathea	R	D
Pelegrina proterva	S	D
Phidippus audax	S	D
Sassacus sp.	S	D
Zygoballus rufipes	B,S	D
Segestriidae		
Ariadna bicolor	S	N
Tetragnathidae		
Leucauge venusta	B,S,R	B
Tetragnatha elongata	S	N
Tetragnatha versicolor	B	D
Theridiidae		
Argyrodes sp.	S	B
Crustulina altera	S	D
Cryptachaea porteri	B	N
**Dipoena nigra*	S	N
**Enoplognatha caricis*	B	N
Faiditus globosus	B	D
Latrodectus variolus	S	N
Neospintharus trigonum	B	N
**Parasteatoda tabulata*	B	N
Pholcomma hirsutum	S	D
**Phylloneta pictipes*	S	D
**Robertus frontatus*	S	B
Steatoda triangulosa	B	N
Theridion albidum	B,S	D
**Theridion cheimatos*	B,S	D
Theridion fronduem	B,S	B
Theridion murarium	S	D
Theridula opulenta	B	N
**Thymoites marxi*	B	N
Thymoites unimaculata	B	N
Yunohamella lyrica	B,R	D
Theridiosomatidae		
Theridiosoma gemmosum	B,S	B

Table 1.—Continued.

Family and species	Habitat	Time of collection
Thomisidae		
Misumena vatia	B	N
Tmarus angulatus	S	D
Xysticus ferox	B,S,R	B
Xysticus fraternus	R	B
Titanoecidae		
Titanoeca brunnea	S,R	D
Uloboridae		
Hyptiotes sp.	S	D
Uloborus sp.	B	D

conditions. Spider activity, however, varies widely with weather conditions (Radai et al. 2017). For example, during a single bioblitz sample along waterways of Indianapolis, Milne found only 37 species of spiders (Holland et al. 2017) due to rain on the sampling day. For perspective, 78 species of spiders were collected during the one day bioblitz at Goose Pond in June 2016 (unpublished data). On the other hand, 128 species of spiders were found in the multi-year and multi-season ecoblitz. The ecoblitz made it possible for us to choose tentative sampling dates and, if the weather on a scheduled date was adverse to spider activity, to reschedule to take advantage of the best conditions for spider activity. Each subsequent year in the multi-year inventory yielded unique species (in 2014, 73 species collected; in 2015, an additional 27 species collected; in 2016, an additional 22 species collected, and in 2017, an additional 6 species collected). Furthermore, seasonality is important to the spider inventory (Toti et al. 2000). Since we are limited to species identification for adult spiders only (due to the role of genitalia in identification), it is imperative to sample spiders when adults are present. Some species overwinter

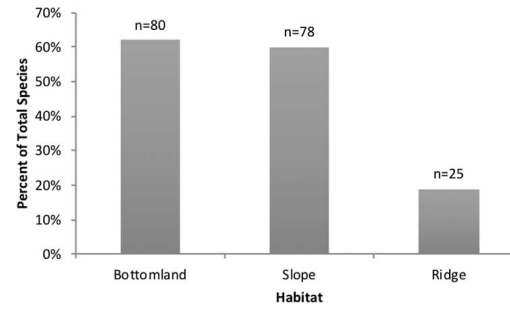

Figure 2.—Percent of total species found in each habitat: bottomland, slope, and ridge.

Table 2.—Sørensen Coefficients comparing the similarity in species composition between 1) habitats: bottomland (n = 80), slope (n = 78), and ridge (n = 25), and 2) time of sample: day (n = 84) vs. night (n = 78) using pooled presence-absence data (where 0.00 = no similarity, 1.00 = maximum similarity).

Bottomland vs slope	Bottomland vs ridge	Slope vs ridge	Day vs night
0.44	0.33	0.32	0.40

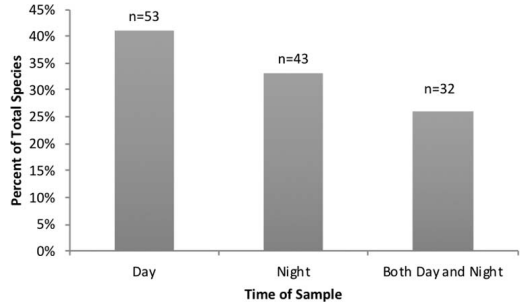

Figure 3.—Percent of total species found only during the day, only during the night, or found in both day and night.

as adults, and others do not reach maturity until fall (Wise 1993).

Second, the multi-habitat inventory allowed us to sample from a wide variety of spider species with different habitat requirements. The overlap of species assemblages between the moist bottomlands and slopes and the dry ridges was relatively low. This result was expected. Numerous studies demonstrate the effect of plant community physiognomy and the resulting microhabitats on the composition of the spider community (reviewed in Turnbull 1973; Uetz 1991; Halaj et al. 2000). Different spider species vary in their microhabitat requirements, and species composition will vary among herbaceous vegetation, shrubs, tree bark, rotting logs, stream banks, and creek beds (Uetz 1991). Many of the spiders collected were from the litter. The dominant tree species varied among habitats (e.g., black oak (*Quercus velutina* Lam.) on ridges, tulip poplar (*Liriodendron tulipifera* L.) on slopes, and American beech (*Fagus grandifolia* Ehrh.) in the bottomlands), and thus the type of litter varied as well. Leaf litter structure (Bultman & Uetz 1982), as well as litter depth (Wagner et al. 2003), can have significant effects on spider species composition.

Third, as found in several other studies (Coddington et al. 1996; Green 1999; Costello & Daane 2005), our results emphasize the importance of sampling spiders during both day and night. Many spiders such as orb weavers hide in retreats during their non-active period (Foelix 2011) and would be more difficult to find, whereas they would be visible during their active period (e.g., *Araneus bicentarius*). Many nocturnal hunting spiders withdraw under bark or other reclusive places during the day (e.g., *Dolomedes albineus*) (Cloudsley-Thompson 1987). Other spiders, such as the nursery spider (*Pisurina mira)* are present both day and night. In the absence of our night sample, 33% of the total species would have been missed (Fig. 3). In fact, the large fishing spider, *Dolomedes albineus* (Fig. 4) – one of the new distribution records for Indiana – would have been missed entirely if we had not sampled at night.

Even though the design of our ecoblitz allowed us to sample a large number and variety of spider species, the design has an important limitation. Because our survey was limited to spiders within our physical reach, our result on species richness likely under-estimates the total spider species of Morgan-Monroe/Yellowwood State Forests. Forest spiders occur on tree trunks and in the tree canopy beyond the reach of our sample (Szinetár & Horváth 2005). Several studies have shown that the forest canopy is a significant reservoir of spider diversity with distinct species assemblages associated with different forest horizontal layers as well as with the canopy of different tree species (Larrivée & Buddle 2009; Hsieh & Linsenmair 2011).

In conclusion, the 31 new distribution records for Indiana produced by the ecoblitz indicate that typical biodiversity surveys underestimate the diversity of spiders in Indiana forests. This underestimate is further highlighted by the fact that even the ecoblitz missed the spider species richness that occurs in forest canopies which is logistically difficult. Thus, all estimates of Indiana spider species to date are likely severe underestimates (Milne et al. 2016).

The ecoblitz design can be improved to capture a greater proportion of the spider species diversity that exists in the forests. For example, the addition of a third sampling period may yield additional species. Coddington et al. (1996) suggested May, July, and September as prime sampling periods for spiders. We also could have more systematically sampled the large woody

Figure 4.—A large female fishing spider, *Dolomedes albineus*, in the family Pisauridae, guarding an eggcase. An example of a new distribution record in Indiana found in the ecoblitz site of Morgan-Monroe/Yellowwood State Forest. (*Photo by Brian Foster*)

debris and standing dead trees or snags that provide habitat for additional assemblages of spiders. Buddle (2001) found spider diversity higher on woody debris than on the forest floor litter. Castro & Wise (2010) reported that distance from woody debris can affect the leaf-litter spider assemblage.

Yet, despite these limitations, sampling efforts like the ecoblitz – which rely heavily on volunteers – have encouraged surveys of under-represented groups, such as spiders and lichens (Lendemer 2017), and provide baseline data on species present in a relatively undisturbed mature forest such as the Morgan-Monroe/Yellowwood State Forest. Since state forests are managed for multiple uses, including timber harvest, baseline data in unlogged areas can serve as a reference for assessing the consequences of such practices on species diversity and the effectiveness of these management practices (Frelich et al. 2005). The current knowledge of spiders and other invertebrates, which are integral to forest ecosystem functioning, is minimal. Organized surveys such as ecoblitzes are invaluable strategies to improve basic understanding of species diversity.

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DAILY TEMPERATURE EXTREMES AND PRECIPITATION ACROSS INDIANA OVER A 120-YEAR PERIOD

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ABSTRACT. A database of daily maximum and minimum temperatures recorded at 17 sites across Indiana from 1 September 1897 through 31 August 2018 shows relatively warm and cool periods alternating over decadal timescales. Average values in all 17 stations were computed over 20-year intervals in order to smooth out year-to-year variability. These intervals reveal an underlying pattern in daily extreme temperatures. The spread from the coolest 20-year period to the warmest is 1.1–1.2° C for both the mean daily maximum and minimum temperatures. Relatively warm daily maxima and minima exist in the 40-year interval 1918–1958, followed by cooler temperatures during the next two decades ending in 1978. Annual mean maxima and minima over the years 1998–2018 are warmer than in the previous 20-year period, 1978–1998, but still cooler than the peak reached earlier in the 20th century. However, when viewed over three-month intervals, daily temperature minima for March-May and June-August during the most recent 20-year period are the warmest in the record. The same is true for daily temperature maxima in March through May. However, 20-year mean daily temperature maxima for June through August have remained stable over the past 60 years. Time-integrated precipitation amounts for the 17-station composite increased during the second half of the 20th century. Twenty-year mean annual precipitation for the period 1998–2018 is 12–13% above corresponding values for 1938–1978.

Keywords: Climate, precipitation, temperature, weather

INTRODUCTION AND OBJECTIVES

The concept of climate encompasses all of the quantities required to specify the state of the Earth's atmosphere (e.g., Wilson et al. 1971; Barry & Hall-McKim 2014). Among these quantities, temperature and precipitation have a clear influence on society. Some of the processes that influence climate operate on a planetary spatial scale. These include absorption of solar radiation, emission and absorption of longwave thermal radiation by polyatomic gases such as carbon dioxide and water vapor, and exchanges of latent heat when liquid water evaporates from the Earth's surface and condenses in the atmosphere to form clouds and precipitation (e.g., Frederick 2008). Considerable attention has focused on changes in climate with emphasis on the role of human activities, particularly as they influence temperature (IPCC 1996, 2013). Analysis of the surface temperature dataset provided by stations throughout the world (Jones et al. 2012; Osborn & Jones 2014) shows an increase in mean surface temperature from the start of the 20th century up to about 1945, a flattening or a small decrease until approximately 1975, and an increase thereafter. The rate of change during the first 10 to 15 years of the 21st century was smaller than during the period from 1975 to 2000. Based on these data the warming in global mean temperature from 1900 to 2015 was roughly 1° C (Met Office Hadley Centre 2018), although the change was not a simple linear trend over time. While global averages provide useful measures of large-scale climate, changes in the atmosphere need not be spatially uniform. For example, weather patterns that influence regional temperatures and precipitation respond to varying surface topography, properties of local ground cover such as reflectivity and moisture content, and proximity to large bodies of water (e.g., Sutton 1953). These factors mesh with large-scale variables such as the latitudinal temperature gradient to produce characteristic regional climates.

This work focuses on the regional climate of the state of Indiana using temperature and precipitation data collected over a period of 121 years. The geographic region of interest includes major agricultural activities and several metropolitan areas with populations greater than 100,000, including one with approximately two million people (STATS Indiana 2018). Agricultural

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Table 1.—Stations measuring daily temperature extremes and precipitation: percent data availability for the period 1 September 1897 through 31 August 2018.

Station (s): nearest town	Latitude (°N)	Longitude (°W)	T_{max} availability (%)	T_{min} availability (%)	Precipitation availability (%)
1. Mount Vernon	37.93	87.90	97.2	97.2	97.9
2. Princeton	38.36	87.57	98.2	98.2	98.8
3. Paoli	38.56	86.47	97.6	97.7	95.9
4. Vincennes	38.68	87.53	98.6	98.6	98.9
5. Madison	38.74	85.38	96.4	95.8	95.3
6. Oolitic	38.89	86.53	87.4	87.4	87.2
7. Columbus	39.12	85.92	99.4	99.4	99.2
8. Bloomington	39.17	86.53	96.7	97.5	97.3
9. Greensburg	39.35	85.48	97.9	97.6	96.5
10. Shelbyville	39.53	85.78	94.1	93.7	93.0
11. Rockville	39.76	87.23	97.2	97.1	97.0
12. Anderson	40.11	85.69	98.7	98.5	97.6
13. Farmland	40.25	85.13	83.3	83.3	93.9
14. Marion	40.57	85.66	99.5	99.5	98.8
15. Winamac	41.03	86.60	88.1	88.0	87.3
16. Angola	41.66	85.00	85.6	85.6	85.9
17. South Bend	41.71	86.25	96.4	96.4	96.4

productivity and water supplies depend on regional precipitation amounts, while air temperatures influence the length of the growing season and the demand for energy to heat or cool interior living space. Motivated by these issues, the objective of this work is to characterize changes that have occurred in daily maximum and minimum temperatures and in precipitation across Indiana over timescales of years to more than a century. This will provide a useful baseline against which to measure future behavior. Assessments of climate-related influences on activities throughout the state would benefit from knowledge of atmospheric changes that have actually occurred over the past several decades.

THE DATASETS AND DATA HANDLING

The datasets consist of daily maximum temperatures (T_{max}), daily minimum temperatures (T_{min}), and 24-hour integrated precipitation (P) at each of 17 sites across Indiana. The time frame covers 121 years from 1 September 1897 through 31 August 2018. All data were obtained via the web site of the NOAA National Centers for Environmental Information (CDO 2018). The stations are part of the Global Historical Climatology Network and have undergone established quality assurance checks before being made available for public access. Table 1 lists the stations from South to North across the state with their latitudes and longitudes. The number of stations that have made weather observations in Indiana since the latter part of the 20th century is far larger than the subset in Table 1. The 17 stations selected for this work are those that were in operation over most or all of the time from 1897 through the present. The requirement for ongoing observations throughout the entire 20th century greatly limits the number of sites available.

It is important that the datasets be as complete as possible. Yet, gaps ranging from three months to several years exist in the record acquired since the year 2000 at several sites. In these cases data from one or more stations, not listed in Table 1, were used to fill the gaps and create a continuous record through 31 August 2018. Details of these special cases appear in the Appendix. Data gaps prior to the year 2000, when the number of observing stations was relatively small, were treated as described later. Table 1 lists the percentage of days during the 121 years on which data exist, where these values include data merged from nearby sites. Only stations operating over the time frame of interest where entries exist on 83% or more of the days enter the analysis. The average data availabilities for T_{max}, T_{min}, and P, computed over all 17 sites, are 94.8%, 94.8%, and 95.1%, respectively.

Data handling: temperatures.—The original data consist of the quantities $T_{max}(s,y,d)$, $T_{min}(s,y,d)$, and $P(s,y,d)$ where s labels a site in Table 1, y labels a year, and d labels the day

number of the year, d=1, 2,..., 365 or 366 where d=1 is 1 January. The multi-year mean daily extreme temperatures for each site s and day d are:

$$T^{C}_{max}(s,d) = \left[\Sigma_y T_{max}(s,y,d)\right]/N_x(s,d) \quad (1)$$

$$T^{C}_{min}(s,d) = \left[\Sigma_y T_{min}(s,y,d)\right]/N_n(s,d) \quad (2)$$

where data are available at site s on day-of-year d during $N_x(s,d)$ years for T_{max} and $N_n(s,d)$ years for T_{min}. The summations extend over all years, a maximum of 121, where each one-year period begins on 1 September and y=1 labels 1 September 1897 through 31 August 1898. The quantities $T^{C}_{max}(s,d)$ and $T^{C}_{min}(s,d)$ expressed as functions of d define climatological annual cycles in the daily maximum and minimum temperatures and serve as references for defining relatively warm and cool periods. The daily "temperature deviations" are:

$$\delta T_{max}(s,y,d) = T_{max}(s,y,d) - T^{C}_{max}(s,d) \quad (3)$$

$$\delta T_{min}(s,y,d) = T_{min}(s,y,d) - T^{C}_{min}(s,d) \quad (4)$$

Equations 3 and 4 characterize daily deviations from the long-term climatology at each site, although such high temporal resolution is neither necessary nor desirable when the focus is on long-term behavior. It is useful to consider average values of $\delta T_{max}(s,y,d)$ and $\delta T_{min}(s,y,d)$ computed over 3-month-long seasons, corresponding to specific ranges of d, or over entire years, corresponding to all values of d. Then, further averaging over all sites, corresponding to all values of s, can reveal the annualized behavior of temperature and identify extended warm and cool periods on a statewide basis from the late 19th century to the summer of 2018.

This work divides a year into four "meteorological seasons" based on the day-number d. Meteorological Autumn, labelled by m=1, encompasses d=244–334 and coincides approximately with September through November. Winter, m=2, being approximately December-February, refers to d=335–365 or 366 plus d=1–60 of the following calendar year. Meteorological Spring, m=3, defined by d=61–151 approximately spans March-May, and Summer, m=4, refers to June-August with d=152–243. The average values of $\delta T_{max}(s,y,d)$ and $\delta T_{min}(s,y,d)$ over season m at each site are:

$$\delta T^{M}_{max}(s,y,m) = [\Sigma_d \delta T_{max}(s,y,d)]/M_x(s,y,m) \quad (5)$$

$$\delta T^{M}_{min}(s,y,m) = [\Sigma_d \delta T_{min}(s,y,d)]/M_n(s,y,m) \quad (6)$$

where the sums extend over the number of days in season m of year y on which data exist at site s, labelled as $M_x(s,y,m)$ and $M_n(s,y,m)$. When data are available on every day of the season $M_x(s,y,m) = M_n(s,y,m) = 91$ or 92. Alternately, annual-average values, labelled $\delta T^{A}{}_{max}(s,y)$ and $\delta T^{A}{}_{min}(s,y)$ result from extending the summations in Eqs. 5 and 6 over all values of d in each 12-month period and dividing by the appropriate number of days on which data exist. As used in this work, one year begins on d=244, 1 September in non-leap years, and extends through d=243, 31 August of the following calendar year, where time is labelled by the calendar year that contains September.

Finally, the mean over all sites of the seasonal or annual values gives a state-wide picture of the behavior of temperature extremes. The seasonal values are:

$$\delta T^{N}_{max}(y,m) = \left[\Sigma_s \delta T^{M}_{max}(s,y,m)\right]/S_{x1}(y,m) \quad (7)$$

$$\delta T^{N}_{min}(y,m) = \left[\Sigma_s \delta T^{M}_{min}(s,y,m)\right]/S_{n1}(y,m) \quad (8)$$

where m=1, 2, 3, 4, and the annualized values are:

$$\delta T^{S}_{max}(y) = \left[\Sigma_s \delta T^{A}_{max}(s,y)\right]/S_{x2}(y) \quad (9)$$

$$\delta T^{S}_{min}(y) = \left[\Sigma_s \delta T^{A}_{min}(s,y)\right]/S_{n2}(y) \quad (10)$$

where $S_{x1}(y,m)$ and $S_{n1}(y,m)$ are the number of sites, up to 17, that have seasonal mean data in season m of year y. Similarly, $S_{x2}(y)$ and $S_{n2}(y)$ are the number of sites that have annual mean data in year y.

If sites throughout the state experience relatively warm or relatively cool years, each of the $\delta T^{A}{}_{max}(s,y)$-values will have a significant positive correlation with the multi-station mean $\delta T^{S}{}_{max}(y)$ in Eq. 9. The same applies to the $\delta T^{A}{}_{min}(s,y)$ and $\delta T^{S}{}_{min}(y)$ in Eq. 10. If, however, the annual-mean deviations vary randomly from site-to-site, there will be a tendency for positive and negative values

Table 2.—Statistical properties of the temperature deviations δT_{max} and δT_{min}: correlations of annual means across stations and standard deviations of daily values.

Station (s): nearest town	Correlation coefficient r_{max}	Correlation coefficient r_{min}	Standard deviation σ_{max} (°C)	Standard deviation σ_{min} (°C)
1. Mount Vernon	0.84	0.86	5.4	4.9
2. Princeton	0.89	0.85	5.4	5.1
3. Paoli	0.91	0.84	5.4	5.6
4. Vincennes	0.93	0.87	5.5	5.1
5. Madison	0.80	0.77	5.3	4.9
6. Oolitic	0.88	0.82	5.5	5.3
7. Columbus	0.91	0.72	5.5	5.2
8. Bloomington	0.92	0.63	5.4	5.2
9. Greensburg	0.85	0.58	5.5	5.4
10. Shelbyville	0.96	0.92	5.4	5.2
11. Rockville	0.68	0.89	5.4	5.3
12. Anderson	0.93	0.72	5.5	5.3
13. Farmland	0.94	0.80	5.5	5.3
14. Marion	0.92	0.92	5.5	5.3
15. Winamac	0.91	0.84	5.4	5.3
16. Angola	0.94	0.81	5.3	5.0
17. South Bend	0.87	0.74	5.4	5.1

to cancel in the summations of Eqs. 9 and 10. Correlation coefficients $r_{max}(s)$ that relate $\delta T^S_{max}(y)$ to each of the $\delta T^A_{max}(s,y)$, s=1, 2,. . ., 17, measures the extent to which the entire state of Indiana experiences a similar temperature deviation in a given year. The analogous correlation coefficient relating $\delta T^S_{min}(y)$ and the $\delta T^A_{min}(s,y)$ is $r_{min}(s)$. Table 2 lists r_{max} and r_{min} for each site. All values are positive and lie in the range 0.58 to 0.96; the average values of r_{max} and r_{min} computed over the 17 stations are 0.89 and 0.80, respectively. This shows a high degree of spatial coherence in the annual-mean temperature deviations across Indiana.

The mathematical development given above is straightforward, but the summations assume that temperature data exist for each day of each 12-month period at each station. Yet, Table 1 shows that, on average, measurements are not available on about 5% of the days. This work applies two methods to account for gaps in the temperature record. Method 1 computes the means in Eqs. 1 and 2 using the existing measurements, with $N_{max}(s,d)$ and $N_{min}(s,d)$ being the number of years that actually enter the summations for each site and day-number. Subsequent calculations based on Eqs. 3–10 use all available data in the summations with appropriate adjustment of the divisors. Method 1 effectively assumes that missing data have no effect on the computed temperature deviations when averaged over a season, a year, or over multiple sites.

Method 2 for handling gaps in the record uses existing data to estimate values of missing temperatures. This approach is based on statistical regression and utilizes the fact that there is a significant positive correlation between temperature deviations across all stations, where the values in Table 2 illustrate this correlation on an annualized basis. For each day of the 121-year record, the average T_{max} and T_{min} values computed across all stations are:

$$T^S_{max}(y,d) = [\Sigma_s T_{max}(s,y,d)]/S_{x3}(y,d) \quad (11)$$

$$T^S_{min}(y,d) = [\Sigma_s T_{min}(s,y,d)]/S_{n3}(y,d) \quad (12)$$

where s=1, 2,. . ., $S_{x3}(y,d)$ in Eq. 11 and s=1, 2,. . ., $S_{n3}(y,d)$ in Eq. 12. Here $S_{x3}(y,d)$ and $S_{n3}(y,d)$ are the number of stations, up to 17, where values of T_{max} and T_{min}, respectively, exist on day number d of year y. Each day of the entire 121-year data record has a computed value of $T^S_{max}(y,d)$ and of $T^S_{min}(y,d)$; no gaps exist. The next step in Method 2 involves relating the daily values from each site to the multi-station means. This is done by determin-

ing the regression coefficients $a_0(s)$, $a_1(s)$, $b_0(s)$, and $b_1(s)$ in the expressions:

$$T_{max}(s,y,d) = a_0(s) + a_1(s)T^S_{max}(y,d) + \varepsilon_{max}(s,y,d) \quad (13)$$

$$T_{min}(s,y,d) = b_0(s) + b_1(s)T^S_{min}(y,d) + \varepsilon_{min}(s,y,d) \quad (14)$$

where $\varepsilon_{max}(s,y,d)$ and $\varepsilon_{min}(s,y,d)$ are residuals which approximate normal distributions with means of 0.0. The regression coefficients for each site, derived by minimizing the sum of squares of the residuals, have very high statistical significance. The t-statistics for a_1 and b_1, defined as the ratio of the estimated value to its standard error, are near 1000. Equation 13 typically explains 94–98% of the variance in the $T_{max}(s,y,d)$, with similar results for $T_{min}(s,y,d)$ in Eq. 14. Equations 13 and 14, with the derived regression coefficients and residuals set to 0.0, are the basis for estimating values of $T_{max}(s,y,d)$ and $T_{min}(s,y,d)$ on all days when measurements are not available. The percentage variance explained by Eqs. 13 and 14 provides high confidence in the estimates provided by the approach. These computed values then replace the gaps in the daily record for each site.

The 121-year data record covers 44,194 days at each of 17 stations for a total of 751,298 station-days, and actual temperature measurements exist on 94.8% of these. Methods 1 and 2 are different approaches to cope with the 5.2% of station-days when data are missing, although the quantity of estimated data is substantial for Oolitic, Farmland, Winamac, and Angola. Method 2 captures day-to-day fluctuations in temperature that extend over the entire state via use of $T^S_{max}(y,d)$ and $T^S_{min}(y,d)$, while the regressions scale this behavior to conditions encountered at each individual station. Furthermore, when missing data are filled in using Method 2 the final datasets that enter the various averages are effectively "complete" with no remaining gaps. Given its relative sophistication, this work regards Method 2 as the preferred approach to account for missing data. However, differences between the seasonal and annual-mean temperature deviations produced by Methods 1 and 2 can provide an index of the accuracy of the results.

Data handling: precipitation.—The precipitation data for each site consist of total daily liquid precipitation values. For site s in year y on day d this is P(s,y,d) expressed in cm. As with temperature deviations, the year is divided into four meteorological seasons labelled by m. The total precipitation for season m in year y at site s is the sum over the corresponding daily values:

$$P^M(s,y,m) = \Sigma_d P(s,y,d) \quad (15)$$

where the summation extends over all day-of-year numbers encompassed by the season. A summation analogous to Eq. 15 over all days of each 12-month period, extending from 1 September through the next 31 August, produces annual total precipitation values, $P^A(s,y)$, for site s and year y. Application of Eq. 15 requires that one account for data gaps, and as with temperature, this must involve certain assumptions. Table 1 shows that, depending on the site, data were missing on from 0.8% (Columbus) to 14.1% (Angola) of the days from 1 September 1897 through 31 August 2018. This work considers two methods for handling the data gaps and examines the sensitivity of the conclusions to the different approaches.

Method 1 makes the extreme assumption that $P(s,y,d) = 0.0$ on each day when no measurement exists. This produces a seasonal sum for each station and 12-month period via Eq. 15 that is less than or equal to the true total precipitation, where the magnitude of the error depends on the number of days on which precipitation actually occurred but for which the data are missing.

Method 2 for filling data gaps is based on regression and is analogous to that used with the temperature datasets. Let $P_S(y,d)$ be the multi-station mean precipitation for day-number d of year y computed via:

$$P_S(y,d) = [\Sigma_s P(s,y,d)]/N_{ps}(y,d) \quad (16)$$

where s ranges from 1 to $N_{ps}(y,d)$ and where $N_{ps}(y,d)$ is the number of stations with data on day-number d of year y. Each day of the entire 121-year time frame has a value of $P_s(y,d)$. The multi-station mean computed via Eq. 16 is the basis for estimating values of missing data at individual stations. Consider station s whose precipitation record has gaps to be filled in. The existing data from station s and the $P_S(y,d)$-values for the same days are the basis of a regression:

$$P(s,y,d) = c_0(s) + c_1(s)P_S(y,d) + \varepsilon_p(s,y,d) \quad (17)$$

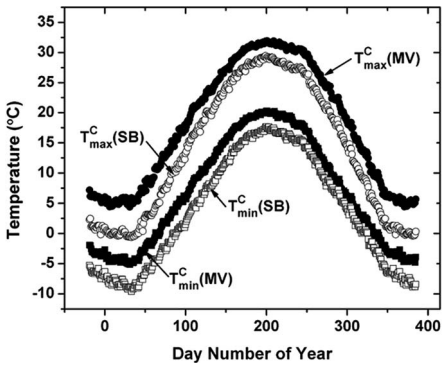

Figure 1.—Climatological annual cycles in daily maximum T^C_{max} and daily minimum T^C_{min} temperatures for Mount Vernon (MV, solid symbols) and South Bend (SB, open symbols). Days 0 and 365 refer to 31 December in adjacent non-leap years. The horizontal scale extends from 20 days before Day 0 to 20 days after Day 365.

where $\varepsilon_p(s,y,d)$ is the residual, and the best-fit coefficients $c_0(s)$ and $c_1(s)$ for the site are derived by least-squares methods. In practice, $c_0(s)$ varied from -0.020 (Oolitic) to +0.041 (South Bend), while $c_1(s)$ varied from 0.525 (South Bend) to 1.243 (Oolitic). All values of $c_1(s)$ had very high statistical significance, with the t-statistic in the range t = 93 to 250. However, the percent variances explained by Eq. 17 are much smaller than the corresponding regressions for temperature, ranging from only 16.4% for Oolitic to 61.3% at South Bend with an average over all stations of 44.1%. This indicates a larger degree of spatial and temporal variability in precipitation than in temperature. Equation 17 with the residual set to 0.0 allows estimating missing P(s,y,d)-values for each station. On days when $P_m(y,d) = 0.0$, missing data were filled in with $P(s,y,d) = 0.0$ regardless of the value of $c_0(s)$. Method 2 has the advantage of using information from multiple sites to fill in missing data, where the regression coefficients customize the estimates to each individual station. Method 2 should produce accurate estimates of total precipitation over a season or a year, although the daily estimates will contain larger errors than in the case of temperature.

RESULTS: TEMPERATURE

Figure 1 presents the climatological annual cycles in daily extreme temperatures, $T^C_{max}(s,d)$ and $T^C_{min}(s,d)$ as functions of d for the southernmost and northernmost stations in Table 1, Mount Vernon and South Bend, respectively. These curves illustrate the magnitude of the latitudinal gradient across Indiana and the contrast between stations. The warmest daily maxima occur in the period 12–28 July, several weeks after the summer solstice. The coldest daily minima appear over an extended period from approximately 2 January to 12 February. Averaged over the annual cycle, the daily maximum at Mount Vernon is 4.1° C warmer than at South Bend, while the daily minimum averages 3.3° C warmer. The relevant quantities for this work are temperature deviations defined relative to a climatological annual cycle, as in Fig. 1, computed separately for each station. Variations in regional climate that affect the entire state of Indiana will appear as systematic changes in the temperature deviations δT_{max} and δT_{min} imposed on a background that includes latitudinal variations and site-specific influences.

The temperature deviations defined by Eqs. 3 and 4 are well-approximated by normal distributions with means of 0.0 and standard deviations that vary little with location. Figure 2 presents histograms of $\delta T_{max}(s,y,d)$ and $\delta T_{min}(s,y,d)$ for Shelbyville where the solid lines indicate best-fit normal distributions. The standard deviations are 5.4° C for δT_{max} and 5.2° C for δT_{min}. Small deviations from a normal distribution appear as small positive values of δT_{min}, but the wings of both distributions are well-represented by a Gaussian curve. The distributions of δT_{max} and δT_{min} for all stations are very similar to those for Shelbyville; Table 2 lists the standard deviations of the best-fit normal distributions to δT_{max} and δT_{min} for each site, labelled σ_{max} and σ_{min}, respectively. All of the standard deviations lie in the range σ_{max}=5.3–5.6° C and σ_{min}=4.9–5.6° C. The following analyses are based on spatial averages of the original $\delta T_{max}(s,y,d)$ and $\delta T_{min}(s,y,d)$ over all 17 stations as well as temporal averages over seasons or the entire year.

Figure 3 presents the multi-station annual mean values, $\delta T^S_{max}(y)$ and $\delta T^S_{min}(y)$, computed via Eqs. 9 and 10, versus year for a 120-year period beginning in 1898. Data gaps were filled using the regression-based Method 2 described previously. Each point refers to 12 months beginning on 1 September and ending with 31 August of the following year. Horizontal line segments are means of these annual results over six 20-year periods beginning with 1 September of year y_1 and ending with 31 August of year y_2 extending from $(y_1,y_2) = (1898, 1918)$ to $(y_1,y_2) = (1998, 2018)$.

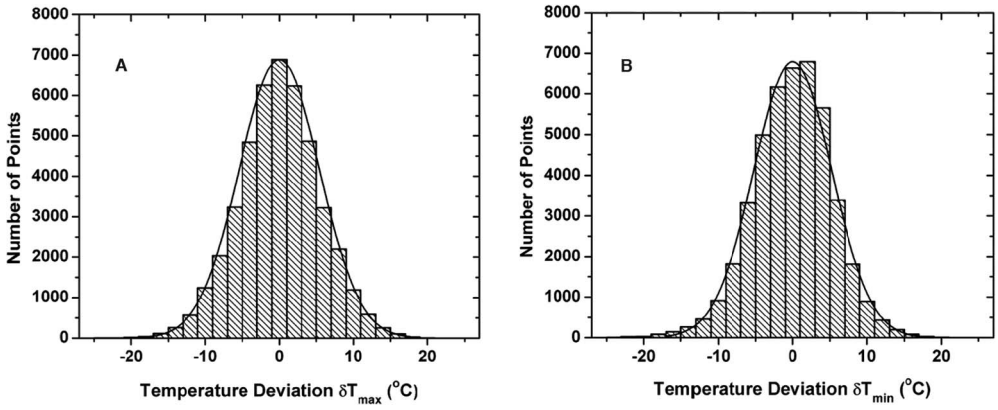

Figure 2.—Histograms of daily temperature deviations for Shelbyville over the period 1 September 1897 through 31 August 2018. The smooth curve indicates the best-fit normal distribution. (A) Deviations of daily maximum temperature δT_{max}; (B) Deviations of daily minimum temperature δT_{min}.

Results for individual years span the range $\delta T^{S}_{max}(y) = -2.1°$ C to $+2.3°$ C and $\delta T^{S}_{min}(y) = -2.0°$ C to $+2.1°$ C, but the erratic year-to-year variations lead to a substantial degree of cancellation in the 20-year means. The 20-year means of $\delta T^{S}_{max}(y)$ vary from a minimum of $-0.57°$ C for 1978–1998 to a maximum of $+0.55°$ C for 1918–1938. Corresponding 20-year means of $\delta T^{S}_{min}(y)$ are $-0.70°$ C for 1958–1978 and $+0.48°$ C for 1918–1938. The differences among the 20-year means are quite small with a spread from coldest to warmest being only 1.1–1.2° C for both the daily maxima and minima.

Figure 3 shows drops in the 20-year mean temperature deviations from 1938–1958 to 1958–1978 for both the maximum and minimum temperatures. The data imply a cooling between 0.9° C and 1.0° C, and these are the largest changes between two consecutive 20-year periods in the record. This raises the concern that an instrument-related shift might have influenced multiple sensors somewhere near the boundary between the two periods. Instrument history files provided with the datasets show that sensors were replaced at more than half of the sites between 1952 and 1956, although the old sensors were exchanged for new ones of the same type. An examination of annual-mean data from the individual stations shows no obvious shift in the records that coincide in time with the instrument changes. In most

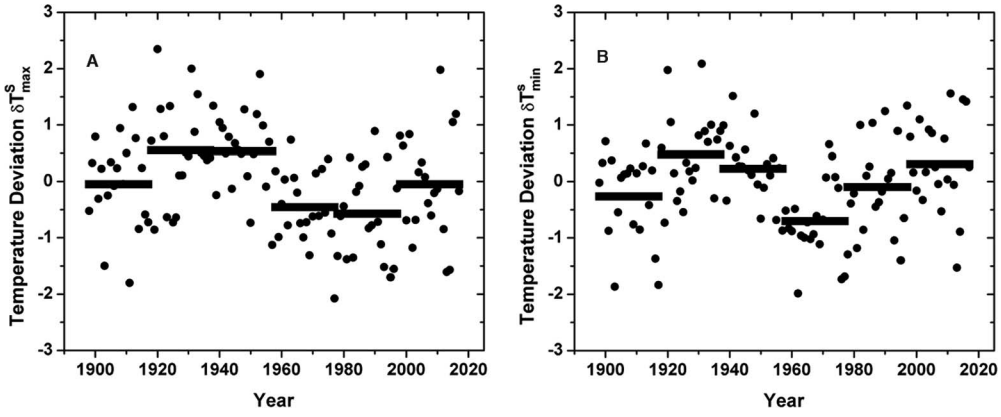

Figure 3.—Mean temperature deviations for one-year intervals extending from 1 September through 31 August for 1898 through 2018. Points refer to 12-month means averaged over 17 sites. Line segments are averages of 20 consecutive 12-month periods. (A) Deviations of daily maximum temperature δT^{S}_{max}; (B) Deviations of daily minimum temperature δT^{S}_{min}.

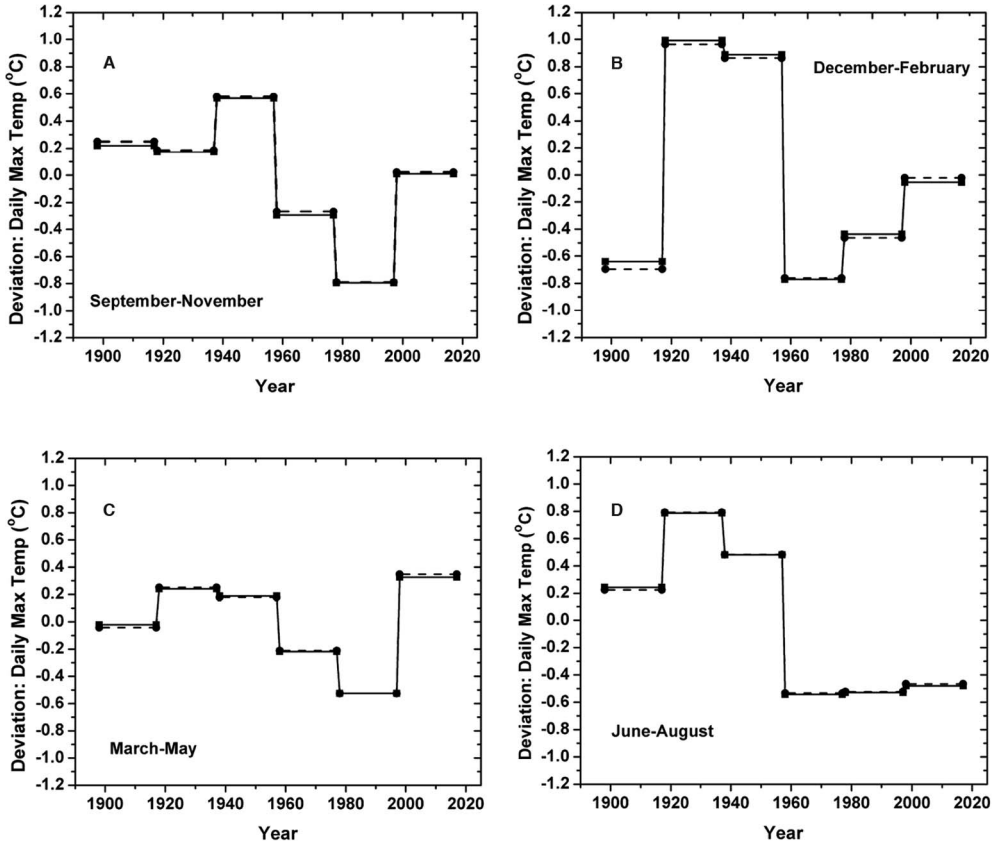

Figure 4.—Deviations in daily maximum temperature averaged over 17 sites and 20-year periods from 1 September 1898 through 31 August 2018. The panels refer to different meteorological seasons. (A) Autumn, September-November; (B) Winter, December-February; (C) Spring, March-May; and (D) Summer, June-August. Dashed lines use Method 1 for filling in missing data; solid lines use Method 2.

cases, no immediate change appeared or the decline in temperature did not develop until several years after a change in sensor. Based on the information available, there is no compelling basis to claim that the apparent cooling across Indiana from 1938–1958 to 1958–1978 is an artifact of the instrumentation, although a detailed assessment of events that occurred roughly 60 years ago is not possible. Any attempt to alter the archived data to remove hypothesized biases must involve subjective assumptions and would be open to serious criticism. Clearly, a geophysical origin for the patterns shown in Fig. 3 is predicated on a stable long-term data record. It is relevant to note that datasets published by IPCC (2013) show a slight decline in global-mean temperature between approximately 1955 and 1975 after reaching a peak in the 1940s. This global behavior is qualitatively consistent with that observed across Indiana, although changes in the globally-averaged data are less pronounced.

Figure 4 presents 20-year means values of $\delta T^{N}_{max}(y,m)$ computed using Eq. 7 for A = Autumn, m=1, B=Winter, m=2, C=Spring, m=3, and D = Summer, m=4, where these meteorological seasons are defined according to the day-numbers of the year given previously. The data record extends from September 1898 through August 2018. Solid lines account for data gaps via Method 2, while the dashed lines use Method 1. No significant differences exist between results based on these two different approaches, and this consistency supports the claim that missing data have negligible impact on the derived temporal behavior. A feature common to all seasons is a drop in δT^{N}_{max} from 1938–1958 to 1958–1978, although the change in Spring is less prominent than in other seasons. As described above, there is

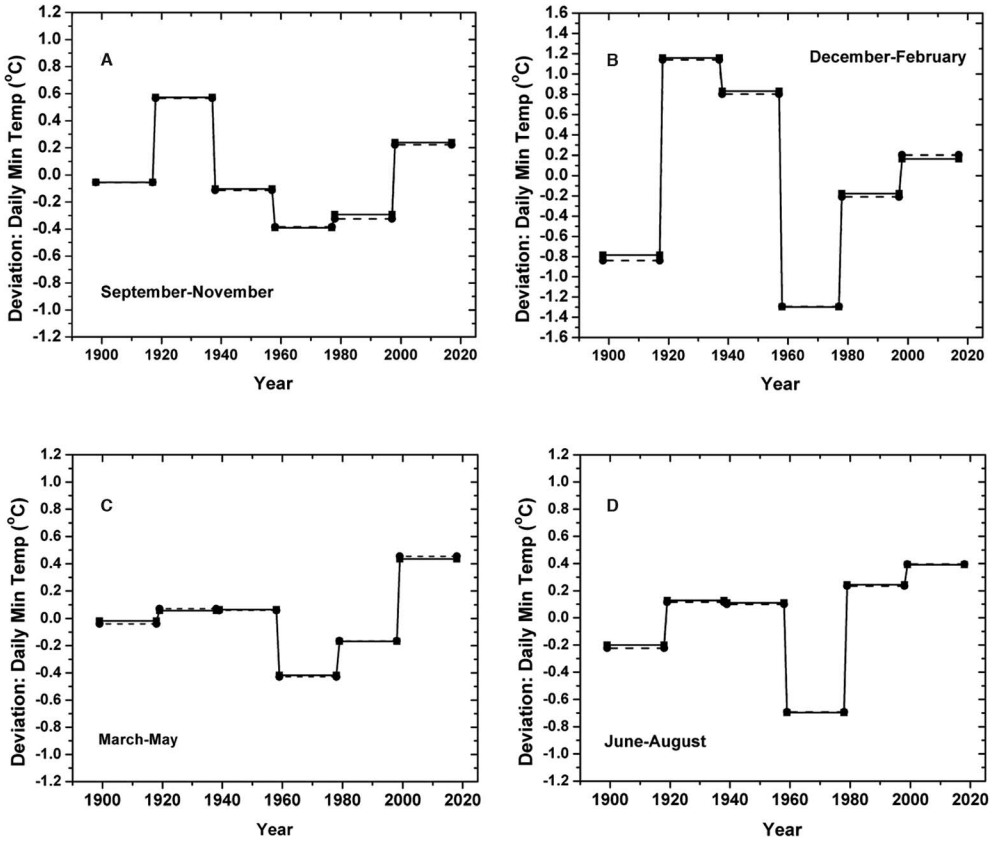

Figure 5.—Deviations in daily minimum temperature averaged over 17 sites and 20-year periods from 1 September 1898 through 31 August 2018. The panels refer to different meteorological seasons: (A) Autumn, September-November; (B) Winter, December-February; (C) Spring, March-May; and (D) Summer, June-August. Dashed lines use Method 1 for filling in missing data; solid lines use Method 2.

no convincing evidence in the historical records that this behavior is an instrument-related artifact.

The 20-year mean δT^{N}_{max}-values are smallest in 1978–1998 for Autumn and Spring and in 1958–1978 for Winter and Summer, while larger values existed earlier in the 20th century. The daily maximum temperature deviations increase from 1978–1998 to the most recent interval, 1998–2018, in all four seasons, although the change in the summer maxima is negligible over the past 60 years. The most recent 20-year interval had the highest δT^{N}_{max}-values of the 120-year record during Spring, while the most recent 20-year interval for Autumn, Winter and Summer ranked as the fourth, third, and fourth highest δT^{N}_{max} of the six intervals for these seasons respectively. Although the temporal patterns in Fig. 4 are of interest, all of the values lie in the narrow range -1° C to +1° C. When averaged over 20-year periods, changes in daily maximum temperatures across Indiana have been small. The greatest temporal change appears in meteorological winter where the contrast between the smallest and largest values in Fig. 4B is 1.7–1.8° C.

Figure 5 presents 20-year mean values of $\delta T^{N}_{min}(y,m)$ computed via Eq. 8 for each meteorological season. Note that the vertical scale for Winter in Fig. 5B is expanded relative to those for other seasons. As with Fig. 4, the dashed lines based on Method 1 for filling data gaps differ insignificantly from the solid lines based on Method 2. A drop in the 20-year mean δT^{N}_{min} from 1938–1958 to 1958–1978 occurs in all seasons, but the change in Spring is much less than in Winter and Summer. The corresponding change in Autumn is very small and, by itself, would not arouse concern over a possible

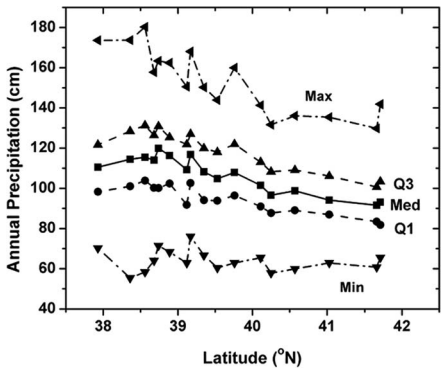

Figure 6.—Measures of annual total precipitation at each station expressed as functions of latitude based on 121 years of data. Curves denote the median (Med), the upper limit of the smallest quartile of values (Q1), the lower limit of the largest quartile of values (Q3), the minimum (Min) and the maximum (Max) at each site. Missing data were filled in using Method 2.

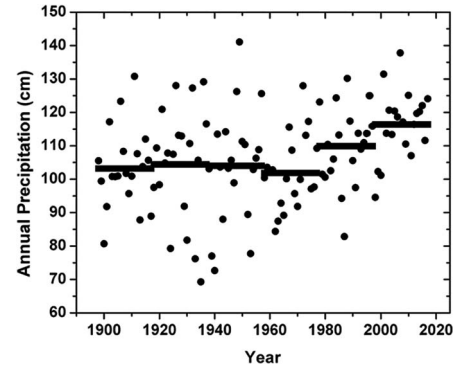

Figure 7.—Total annual precipitation for 12-month intervals from 1 September 1898 through 31 August 2018. Points refer to 12-month totals averaged over 17 stations. Line segments are averages of 20 consecutive 12-month periods. Missing data were filled in using Method 2.

instrument artifact. All seasons show a monotonic increase in δT^{N}_{min} over the most recent 60 years, from 1958–1978 to 1998–2018. Autumn and Winter of the most recent 20-year period have the second warmest δT^{N}_{min}-values of the 120 years, while Spring and Summer of the most recent period have the highest δT^{N}_{min}-values in the record. As with the δT^{N}_{max}-values in Fig. 4, Winter is the most variable season for which the largest 20-year mean δT^{N}_{min}-value is 2.4–2.5° C warmer than the minimum.

RESULTS: PRECIPITATION

Figure 6 summarizes the total 12-month precipitation received at each site over the 121-year period 1 September 1897 – 31 August 2018 with values expressed as functions of latitude across Indiana. Missing data were filled in using Method 2. The quantities presented are the median annual precipitation, the upper limit of the smallest quartile of values, the lower limit of the largest quartile, the smallest annual precipitation recorded at each site and the largest. One fourth of the annual precipitation amounts recorded at a site lie between each pair of adjacent curves. The annual median values cover the range from 91.6 cm for Angola to 119.8 cm at Madison. Mean precipitation amounts lie from 3.6 cm below to 2.3 cm above the medians depending on location. There is an obvious dependence on latitude across the state with smallest annual precipitation amounts occurring at the most northern locations. The six southernmost sites have median amounts in the range 110–120 cm, while values for the five northernmost stations lie between 90–100 cm. The minimum annual precipitation over the period is typically 55–65% of the median value while the maximum is 135–150% of the median.

The next issue involves temporal changes in precipitation over the state on time scales of years to over a century. Figure 7 presents values of total annual precipitation averaged over all 17 stations in Table 1, with data gaps filled via Method 2. The annual multi-station composites show a large scatter from year-to-year around a long-term average of 106.6 cm, varying from a minimum of 69.3 cm in 1935 to a maximum of 141.1 cm in 1949. The horizontal line segments in Fig. 7 denote 20-year averages beginning on 1 September 1898 and ending with 31 August 2018. Despite the large interannual variability, the 20-year means are remarkably stable over the first 80 years of the observing period followed by an increase in recent years. The 20-year mean annual value for the period 1 September 1958 – 31 August 1978 was 101.8 cm, rising to a maximum of 116.4 cm for 1 September 1998 – 31 August 2018.

Figure 8 presents 20-year mean seasonal precipitation amounts for A = Autumn, B = Winter, C = Spring, and D = Summer. Dashed lines denote results obtained using Method 1 which sets daily precipitation to 0.0 on days when data are missing; solid lines use the regression-based Method 2 for filling the gaps. The only major disagreement between results from the two

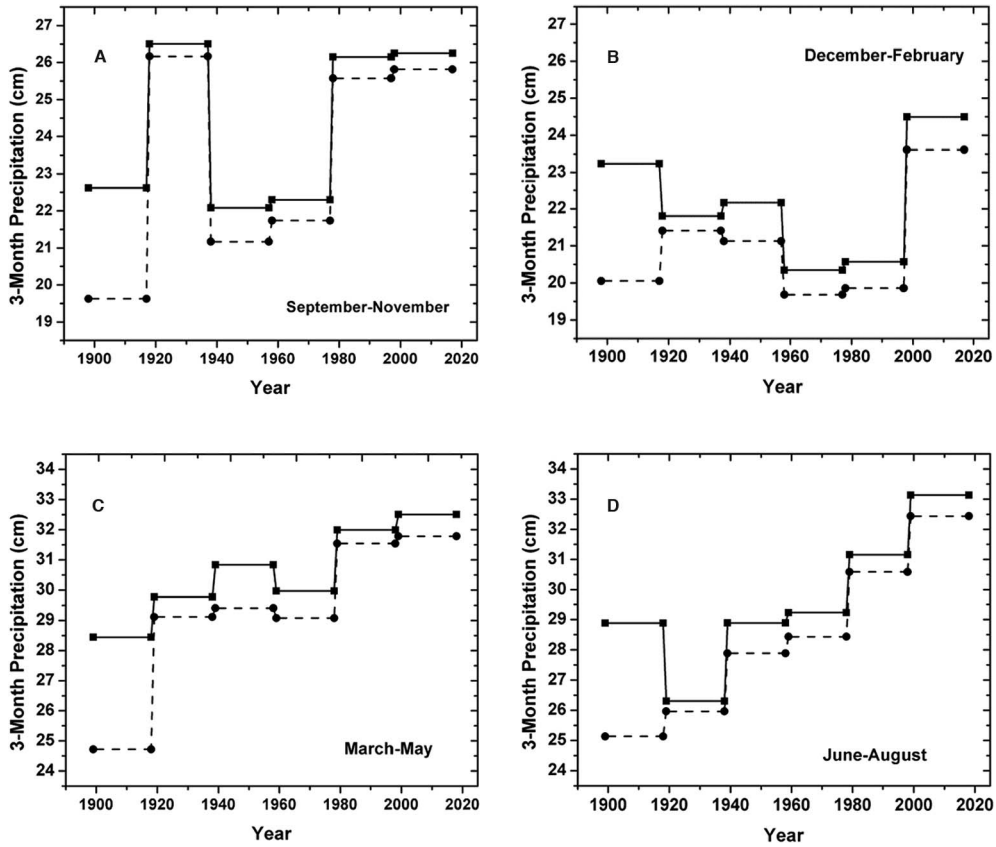

Figure 8.—Total precipitation for three-month periods averaged over 17 sites and 20-year intervals from 1 September 1898 through 31 August 2018. The panels refer to different meteorological seasons: (A) Autumn, September-November; (B) Winter, December-February; (C) Spring, March-May; and (D) Summer, June-August. Dashed lines use Method 1 for filling in missing data; solid lines use Method 2.

approaches appears in the earliest interval, 1 September 1898 – 31 August 1918, during which the datasets were less complete than in later decades. In this case, Method 1 provides a substantial underestimate of the true precipitation. Still, as of 1 September 1918 and afterwards, the same temporal pattern appears regardless of the method used to fill in missing data. All values quoted below are based on Method 2.

The largest annual precipitation may occur in Spring or Summer. Note the wider range of precipitation in the y-axis for those seasons. When averaged over the entire record, amounts received in Spring are slightly larger than in Summer, 30.6 cm versus 29.6 cm. Smaller long-term amounts occur in Autumn and Winter, 24.3 cm and 22.1 cm, respectively. The most striking feature of Fig. 8 is the growth in precipitation over time. Monotonic increases occur over the three most recent 20-year periods in Winter and Spring, over the last four periods in Autumn, and over the last five 20-year periods in Summer. Twenty-year-mean annual precipitation for 1998–2018 exceeded that for 1958–1978 by 17.7% in Autumn, 20.4% in Winter, 8.5% in Spring, and 13.3% in Summer. In three of the four seasons, the largest total precipitation exists in the most recent 20-year period, while in Autumn the total for 1998–2018 is negligibly less, 0.25 cm, than the record set earlier in the 20th century.

DISCUSSION

The 12-month mean temperature deviations $\delta T^{S}_{max}(y)$ and $\delta T^{S}_{min}(y)$ averaged over all stations vary in the range -2.1° C to +2.3° C over the 121-year period 1 September 1897 through 31 August 2018. This interannual variation is largely random, and the long-term behavior is not well-

described by a simple trend line. However, when averaged over 20-year intervals a pattern consisting of alternating warmer and cooler periods emerges, where the range from coolest to warmest is less than 1.2° C. The 20-year-mean δT^{S}_{max} and δT^{S}_{min} values both have maxima in the interval 1 September 1918 to 31 August 1938, with δT^{S}_{max} dropping to relatively low values in the 40-year span 1 September 1958 to 31 August 1998. The minimum for δT^{S}_{min} is in the earlier of these two 20-year intervals. The two most recent 20-year periods for δT^{S}_{max} and the three most recent for δT^{S}_{min} show monotonic increases over time, although the 20-year mean temperature deviations for the period 1998–2018 do not reach the levels that prevailed for 1918–1938. Given the small magnitude of the 20-year-mean temperature changes, the observing network must provide a long-term dataset stable to a tolerance of less than 1° C in order to detect variations of geophysical origin unambiguously.

A similar overall pattern in the temperature deviations emerges when results are sorted according to meteorological seasons, although there are differences in detail. All seasons show a monotonic increase in δT^{N}_{min} over the past three 20-year periods. In Spring and Summer the largest δT^{N}_{min}-values of the record appear in the most recent 20-year period, indicating a tendency toward warmer daily minimum temperatures which typically occur near sunrise. The temperature minima depend on prevailing atmospheric water vapor amounts which modulate surface heating by longwave radiation and control latent heat release in condensation (e.g., Petterssen 1940). In the case of δT^{N}_{max}, the value for Spring shows a maximum in the most recent 20-year interval. In contrast, the mean value of δT^{N}_{max} for Summer has remained nearly flat over the most recent three 20-year periods. These maximum values typically occur in mid-afternoon and are coupled to the prevailing level of sunlight as modified by cloudiness.

The 17-station composite implies an increase in seasonal and annual precipitation over time. In Winter, Spring, and Summer the largest seasonally-integrated precipitation values on record occurred in the most recent 20-year period, while in Autumn the most recent 20-year result was only 0.2–0.3 cm below the maximum established early in the 20th century. Viewed in an annualized sense, yearly total precipitation in the most recent 20-year period was 12–13% above the average value for 1 September 1938 to 31 August 1978. The measurement of precipitation is straightforward and is not subject to issues of long-term stability that potentially influence the temperature record.

Long-term changes in daily maximum and minimum temperatures across Indiana show a temporal pattern similar to, but not identical to, global mean temperatures presented by IPCC (2013). A period of relative global-scale warmth existed early in the 20th century, followed by a period of constant-to-lower temperatures. A warming from the 1970s into the 21st century is the most recent feature of the global data. Globally-averaged data of necessity obscure day-night contrasts and regional-scale effects that can be substantial. The temperatures and precipitation experienced in the state are functions of prevailing weather patterns which include cloudiness, water vapor amounts, and air flow from other latitudes. Imposed on this regional variability are small systematic changes in the surface radiation balance in response to the buildup of long-lived greenhouse gases. Viewed on a year-to-year basis or as 20-year means, the archived meteorological records are likely to reflect regional influences more than the global-scale effects. There is no expectation that long-term changes confined to Indiana will precisely mimic those derived for a much larger geographic area or the entire globe.

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Just prior to publication, the author passed away.

APPENDIX: THE TREATMENT OF GAPS IN DATA ACQUIRED SINCE 2000

The datasets from Vincennes, Madison, and Winamac contain gaps after the year 2000 in which no data exist for periods of three continuous months or longer. Such extended gaps prior to the year 2000 were treated by Methods 1 and 2 as described in the text. However, the enlarged number of stations in recent years allows one to substitute measured values taken from locations close to, or at least at the same latitude as, the long-established sites in Table 1. Such an approach was not possible in the early years of the data records due to the relative sparsity of stations.

For the period 1 July 2013 through 31 August 2018 temperature and precipitation data associated with the station near Vincennes in Table 1 came from the site

labelled "Vincennes 5NE" by the National Oceanic and Atmospheric Administration. This substitute location is at latitude 38.74°N, about 8 km northeast of Vincennes, Indiana. Similarly, for the three-month period 1 June through 31 August 2018, temperature and precipitation data associated with the site at Winamac in fact came from Woodburn, Indiana at latitude 41.16°N, essentially the same latitude as Winamac. The assembly of a complete dataset for Madison covering recent years proved more difficult than for Vincennes and Winamac. Temperature measurements for Madison ended in the summer of 2011, while daily precipitation measurements continued through 30 June 2018. Temperature data taken from nearby Big Oaks Wildlife Refuge, latitude 38.93°N, served as a substitute during the period 1 June 2011 through 31 July 2018, when this dataset ended. The final month of substitute temperatures, 1–31 August 2018, came from Crittenden, Kentucky, latitude 38.77°N. No precipitation data exist for either Big Oaks or Crittenden during the period 1 July through 31 August 2018. The final summer of precipitation data used for Madison comes from measurements at Seymour, Indiana, latitude 38.96°N, about 50 km to the northwest. These substitutions have negligible effect on the 17-station composite values on which the conclusions of this work are based.

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ATHERTON ISLAND, PARKE AND VIGO COUNTIES, INDIANA

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The mission of Ouabache Land Conservancy (OLC) is to set aside land in perpetuity, both for the owners and for the population at large. Recently, on "Atherton Island" near the ridge just northeast of Lyford, Indiana, 76 ha (188 acres) in three separate donations were made to OLC. It has been named the "Atherton Island Natural Area," and is on the south central portion of the island. The town of Atherton, and Atherton Island itself, are named for a geologist named Atherton (Baker 1995).

Few people locally know about Atherton Island which is located mostly in western Parke County but includes 8–10 km^2 (3–4 mi^2) of northern Vigo County. The village of Atherton, located 0.8 km ($\sim$0.5 mi) east of Route 41 on the Vigo/Parke County line, is at the western edge of the Island. Atherton Island begins about 3.2 km (2 mi) south of Atherton and approximately 6.4 km (4 mi) north of Markle's Dam on the eastern edge of North Terre Haute (Fig. 1). These hills extend northward for about 19 km (12 mi), ultimately reaching approximately 4 km (2.5 mi) north of Mecca. As an interesting historical aside, in 1816, the year Indiana became a state and when Markle's Dam was built, the Atherton Island area remained Indian country.

It is possible to drive around the boundary of most of the Island via the following route: take Rt. 41 northbound from Terre Haute towards Lyford. Route 41 lies on the immediate west side of the Island until it turns sharply eastward toward Big Raccoon River Valley. Instead of turning to the east on Rt. 41, follow 600W northward. This traces the immediate western edge of Atherton Island. About 6.5 km (4 mi) north of the Rt. 41/600W junction and 0.4 km (0.25 mi) south of Raccoon Creek, turn right (east) on Coxville Road (originally Armiesburg Road). This skirts the northern edge of Atherton Island. In roughly 0.8 km (0.5 mi) the road turns south, and in another 0.4 km (0.25 mi) reaches the northeast corner of the island. Coxville Road continues south through Mecca and Coxville and outlines the immediate east edge of Atherton Island. Rosedale lies south of the most southeastern projection of Atherton Island.

Why is it called an Island when it is not surrounded by water? Approximately ten thousand plus years ago the Wisconsinan glaciers were melting to the north forming a much larger Wabash River than today. The glacial Wabash flowed south on both sides of Atherton Island, the main part of the river to the west, and a smaller part to the east where the northern section of Raccoon Creek now flows. Raccoon Creek originally flowed southwest from the Bridgeton area and entered the Wabash River south of Atherton Island. However, the area northeast of North Terre Haute became blocked so that Raccoon Creek could no longer flow south. It was forced to divert its course to the north for about 16 km (10 mi) and enter the Wabash River north of the Island. Although there are several ideas about how this blockage and reversal came about (Dryer 1913; Bartle 1924; Fidlar 1948), no single hypothesis adequately explains the chain of events (Wayne 1966). It is likely that the blocking of the valley northeast of Terre Haute was initially by ice. Outwash gravels and till filled this area later thus permanently blocking it. Finally, sand settled upon this area; deposited by water first and then by wind resulting in the formation of dunes.

As the glaciers thawed, the Wabash River eventually lowered to its present level and Atherton Island was no longer surrounded by water. It is now a raised hilly area with Raccoon Creek on the east and flowing north, the Wabash River some two miles to the west flowing south. You might find it interesting to take a trip around Atherton Island as described above.

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[2] Produced the map.

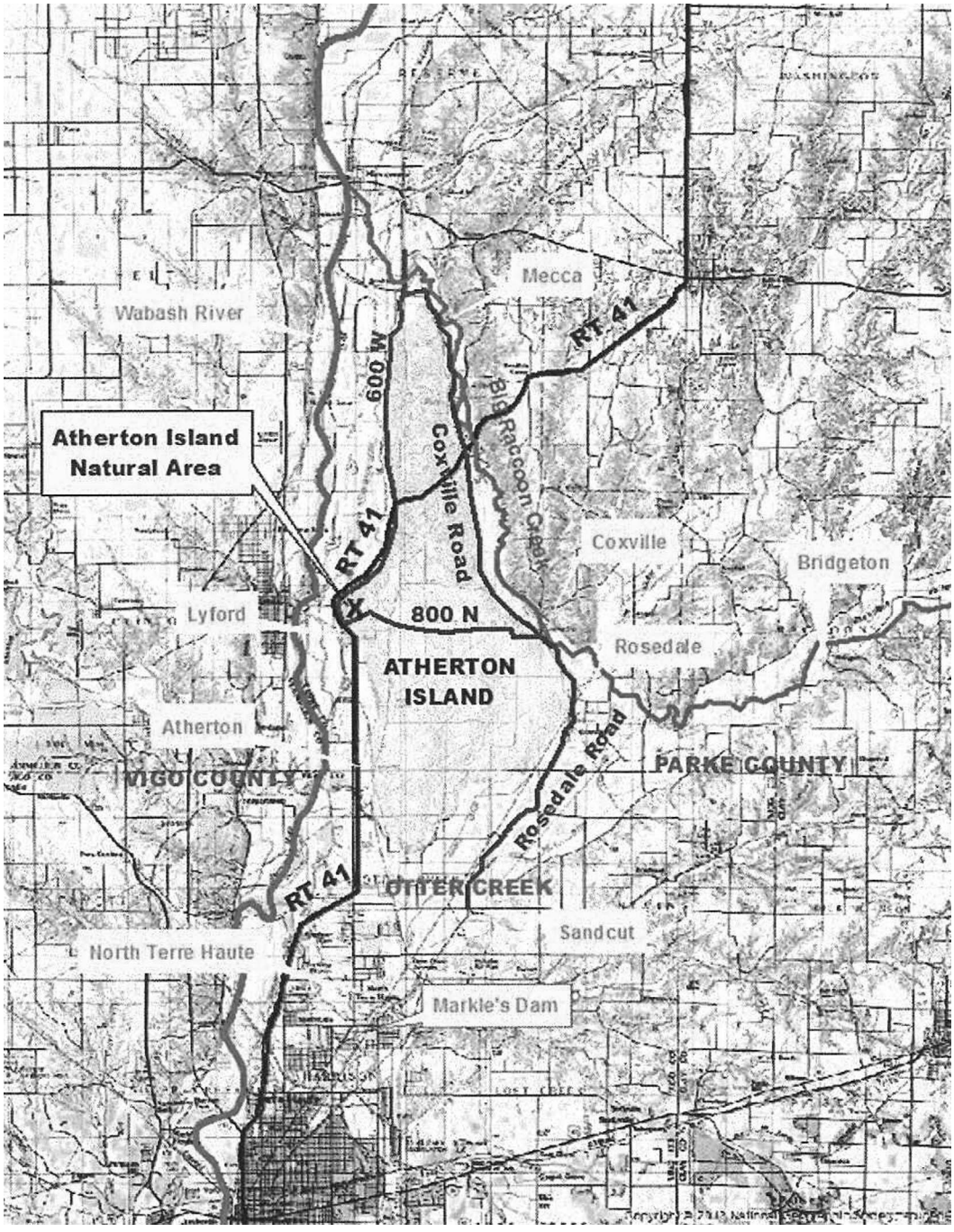

Figure 1.—Map of Atherton Island and surrounding area in Parke and Vigo counties. (Map by Linda Castor)

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INDIANA ACADEMY OF SCIENCE
2018 Year End Financial Report

	Balance 1-Jan-18	Revenues	Expenses	Balance 31-Dec-18
OPERATING FUND				
Dues		$34,320.00		
Interest		$740.54		
Misc. Income		$3,172.00		
Contributions				
Annual Meeting		$49,975.00		
Foundation Support		$176,055.57		
Officer's Expenses			$118,919.90	
Operating Expenses			$22,883.87	
Financial Expenses			$16,686.65	
Newsletter Expenses			$0.00	
Annual Meeting			$75,763.21	
Academy Store		$168.97	$72.00	
Web Site Expenses			$31,282.30	
Operating Funds Total	**$31,663.44**	**$264,263.11**	**$265,607.93**	**$30,318.62**
RESTRICTED FUNDS				
Proceedings	$27,533.66	$15,480.49	$23,228.38	$19,785.77
Special Publications	($31,857.49)	$16,541.83	$38,794.25	($54,109.91)
Research Grants*	$8,665.32	$46,227.06	$67,388.40	($12,496.02)
Lilly Library	$6,756.47	$0.00	$0.00	$6,756.47
Welch Fund	$6,108.56	$0.00	$0.00	$6,108.56
Life Member's Fund	$14,343.61	$0.00	$0.00	$14,343.61
Past President's Fund	$8,599.17	$0.00	$0.00	$8,599.17
Special Projects	$3,140.55	$17,100.00	$22,100.00	($1,859.45)
Total Restricted Funds	**$43,289.85**	**$95,349.38**	**$151,511.03**	**($12,871.80)**
TOTAL FUNDS	**$74,953.29**	**$359,612.49**	**$417,118.96**	**$17,446.82**
FUNDS ON DEPOSIT				
Checking Account	$36,568.70	$426,549.50	$446,260.12	$16,857.98
Money Market Savings Account	$54,838.64	$11.50	$53,500.00	$1,350.14
Cert. of Deposit	$13,588.75	$31.21	$13,619.96	$0.00
TOTAL FUNDS DEPOSITED	**$104,996.09**			**$18,208.12**

*** Provided support for 22 senior member grants and 10 high school grants**

ACADEMY FOUNDATION FUNDS			
TOTAL ACADEMYFOUNDATION FUNDS	**$9,615,750.04**		**$8,775,201.62**

Foundation Funding Used For	
Operating Fund	$176,055.57
Proceedings	$15,480.49
Publications	$3,910.00
Grants	$36,956.22
Special Projects	$17,100.00
Total	**$249,502.28**

Michael S. Finkler
Treasurer

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